

# 1 A survey of spiking activity reveals a functional hierarchy of mouse 2 corticothalamic visual areas

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33 **Abstract**  
34 The mammalian visual system, from retina to neocortex, has been extensively studied at both  
35 anatomical and functional levels. Anatomy indicates the cortico-thalamic system is hierarchical,  
36 but characterization of cellular-level functional interactions across multiple levels of this  
37 hierarchy is lacking, partially due to the challenge of simultaneously recording activity across  
38 numerous regions. Here, we describe a large, open dataset (part of the *Allen Brain Observatory*)  
39 that surveys spiking from units in six cortical and two thalamic regions responding to a battery of  
40 visual stimuli. Using spike cross-correlation analysis, we find that inter-area functional  
41 connectivity mirrors the anatomical hierarchy from the *Allen Mouse Brain Connectivity Atlas*.  
42 Classical functional measures of hierarchy, including visual response latency, receptive field  
43 size, phase-locking to a drifting grating stimulus, and autocorrelation timescale are all correlated  
44 with the anatomical hierarchy. Moreover, recordings during a visual task support the behavioral  
45 relevance of hierarchical processing. Overall, this dataset and the hierarchy we describe provide  
46 a foundation for understanding coding and dynamics in the mouse cortico-thalamic visual  
47 system.

## 48 **Introduction**

49  
50 Mammalian vision is the most widely studied sensory modality. Probing its cellular substrate has  
51 yielded insights into how the stream of photons impinging onto the retina leads to conscious  
52 perception and visuo-motor behaviors. Yet the vast majority of our knowledge of physiology at  
53 the cellular level derives from small-scale studies subject to substantial uncontrolled variation,  
54 uneven coverage of neurons, and selective usage of stimuli. The field's ability to validate models  
55 of visual function has been hampered by the absence of large-scale, standardized, and open *in*  
56 *vivo* physiology datasets (Olshausen & Field 2004; Carandini et al 2005). To address this  
57 shortcoming, we previously developed a 2-photon optical physiological pipeline to  
58 systematically survey visual responses (de Vries et al., 2019). Calcium imaging facilitates the  
59 monitoring of activity in genetically defined cell populations over the course of many sessions.  
60 However, it lacks high temporal resolution and single-spike sensitivity, and doesn't easily allow  
61 distributed, simultaneous recordings from cortical and deep subcortical structures. We therefore  
62 developed a complementary pipeline that leverages Neuropixels probes to measure spiking  
63 activity in six cortical visual areas as well as two visual thalamic nuclei, LGN and LP.

64  
65 The concept of hierarchy has informed ideas about the architecture of the mammalian visual  
66 system for more than 50 years (Hubel and Wiesel, 1962), and has inspired powerful multi-  
67 layered computational networks (Fukushima, 1980; Krizhevsky et al., 2012; Riesenhuber and  
68 Poggio, 1999). This hierarchy has been investigated most extensively in the macaque, from the  
69 LGN and primary visual cortex (V1) into frontal eye fields and beyond (Bullier, 2001;  
70 Chaudhuri et al., 2015; Felleman and Van Essen, 1991; Murray et al., 2014; Rockland and  
71 Pandya, 1979; Schmolesky et al., 1998; Yamins and DiCarlo, 2016).

72  
73 The existence of such a hierarchy in the mouse, with its far smaller brain and dense cortical  
74 graph (Gămănuț et al., 2018), is less clear (D'Souza and Burkhalter, 2017; Glickfeld and Olsen,  
75 2017; Wang et al., 2012; Wang and Burkhalter, 2007). Yet given the utility of the laboratory  
76 mouse as a model organism, understanding the presence and extent of a hierarchy is of crucial  
77 importance. Harris, Mihalas et al applied multi-graph connectivity analysis to the *Allen Mouse*  
78 *Brain Connectivity Atlas* and inferred a shallow hierarchy in the full cortico-thalamic network,  
79 based on more than 1000 viral tracer injections aligned to a high-resolution 3D coordinate  
80 system (Harris et al., 2019). This analysis revealed a hierarchical ordering of visual areas in the  
81 mouse, with LGN at the bottom, and cortical area AM at the top. We sought to investigate  
82 whether this anatomical hierarchy is reflected in the spiking activity and functional properties of  
83 these visual areas.

84  
85 Here, we describe a large-scale and systematic electrophysiological survey of spiking activity  
86 across visual cortico-thalamic structures in awake, head-fixed mice viewing diverse artificial and  
87 natural stimuli. We used Neuropixels silicon probes (Jun et al., 2017) to simultaneously record  
88 the electrical activity of hundreds of neurons with high spatial and temporal resolution (Allen et  
89 al., 2019; Steinmetz et al., 2018; Stringer et al., 2019). This dataset complements our previously  
90 released survey using optical recordings of calcium-evoked fluorescent activity in 60,000 cortical  
91 neurons (de Vries et al., 2019). Both datasets are part of the *Allen Brain Observatory*, a pipeline  
92 of animal husbandry, surgical procedures, equipment, and standard operating procedures (SOPs),  
93 coupled to strict, activity- and operator-independent quality control (QC) measures. All  
94 physiological data passing QC is made freely and publicly available via [brain-map.org](http://brain-map.org) and the  
95 AllenSDK. Our initial characterizations of these pipelines treat each dataset independently; a

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96 detailed comparison of the results obtained via calcium imaging and electrophysiology is  
97 forthcoming.

98  
99 Here, we have studied to what extent the flow of spikes follows the anatomical hierarchy by  
100 mining a *functional* dataset and relating it to a *structural* dataset. We first perform cross-  
101 correlation analysis between pairs of neurons to determine the relative timing of spiking activity  
102 across areas. We then demonstrate that a variety of functional metrics previously used to identify  
103 hierarchical processing support the cortico-thalamic hierarchy found neuroanatomically. Finally,  
104 recordings during active behavior suggest that one role of the hierarchy may be to amplify  
105 responses to behaviorally relevant stimulus changes (Brincat et al., 2018; Issa et al., 2018;  
106 Vinken et al., 2017).

### 107 108 **A survey of visually evoked spiking activity**

109  
110 Each mouse in the study proceeds through an identical series of steps, carried out by highly  
111 trained staff according to a set of SOPs (Figs. 1A and S1; see [http://help.brain-](http://help.brain-map.org/display/observatory/Documentation)  
112 [map.org/display/observatory/Documentation](http://help.brain-map.org/display/observatory/Documentation)). On the day of the experiment, we use cortical area  
113 maps derived from intrinsic signal imaging (ISI) to simultaneously target up to six Neuropixels  
114 probes to V1 and five higher-order visual cortical areas (LM, AL, RL, PM, AM) (Fig. 1B–D). As  
115 probes are inserted up to 3.5 mm into the brain, we regularly obtained concurrent recordings  
116 from two thalamic regions: the lateral geniculate nucleus (LGN) and the lateral posterior nucleus  
117 (LP, making up the visual pulvinar) (Fig. 1E), in addition to hippocampus and other areas  
118 traversed by the silicon probes. This configuration allowed us to sample the mouse visual system  
119 with unprecedented coverage, creating cellular-resolution activity maps across up to 8  
120 corticothalamic visual areas at once, while also obtaining physiological measurements from  
121 nearby regions, such as hippocampus (Fig. 1F). The bulk of these recordings were made in  
122 C57BL/6J wildtype mice ( $N = 30$ ), supplemented by recordings in three transgenic lines ( $N = 8$   
123 Pvalb-IRES-Cre x Ai32,  $N = 12$  Sst-IRES-Cre x Ai32, and  $N = 8$  Vip-IRES-Cre x Ai32), to  
124 facilitate the identification of genetically-defined inhibitory cell types via opto-tagging (Lima et  
125 al., 2009).

126  
127 We implemented QC procedures to ensure consistent data across experiments (Fig. S2 and  
128 Methods), reducing the number of completed and analyzed experiments from 87 to 58.  
129 Extracellularly recorded units were identified and sorted via the automated Kilosort2 algorithm  
130 (Pachitariu et al., 2016; Stringer et al., 2019) followed by QC to remove units with artifactual  
131 waveforms, yielding a total of 99,180 units across experiments (Fig. S3). A variety of quality  
132 metrics were calculated to assess unit contamination and completeness, which are used to select  
133 units for further analysis (Fig. S4). Units were mapped to structures in the Common Coordinate  
134 Framework Version 3 (CCFv3) by imaging fluorescently labeled probe tracks with optical  
135 projection tomography (Fig. S5). After filtering units based on quality metrics, we  
136 simultaneously recorded from a mean of  $682 \pm 144$  units per experiment,  $128 \pm 51$  units per  
137 probe, and  $56 \pm 30$  units per cortico-thalamic visual area (Fig. 1G). We sampled from a mean of  
138  $6.1 \pm 1.1$  cortico-thalamic visual areas in each experiment, with a subset of experiments  
139 including units from 8 visual areas simultaneously (Fig. 1H).

140  
141 During each recording session, mice passively viewed a battery of natural and artificial stimuli  
142 (Fig. 2A and S6). In this study, we focused our analysis on a subset of these: drifting gratings  
143 (Fig. 2B), full-field flashes (Fig. 2C), and local Gabor patches (which are used to map spatial

144 receptive fields) (Fig. 2D). Overall, units recorded in all 8 cortico-thalamic visual areas were  
145 highly visually responsive, with 60% displaying significant receptive fields (Fig. 2E and Fig. S7,  
146 categorical  $\chi^2$  test,  $P < 0.01$ ). As a control, we searched for significant receptive fields in  
147 simultaneously recorded hippocampal regions (CA1, CA3, and dentate gyrus), and only found  
148 them in 1.4% of units. Mapping recorded units with a significant receptive field to their location  
149 within the CCFv3 and aggregating over experiments, we recapitulated the previously described  
150 retinotopic map organization in each area (Fig. 2F) (Bennett et al., 2019; Garrett et al., 2014;  
151 Román Rosón et al., 2019), supporting the accuracy of spatial registration in the CCFv3.

152

### 153 **A functional hierarchy of visual areas**

154

155 The work of (Harris, Mihalas, et al. 2019) assigned a hierarchy score to each of the visually  
156 responsive areas from which we recorded. This score is derived using an optimization algorithm  
157 that considers the set of distinct axonal termination patterns of connectivity between areas—  
158 deeming each as either feedforward versus feedback connections—and finds the most self-  
159 consistent network architecture (Fig. 3A; Supplementary Methods). The LGN sits at the bottom  
160 of the hierarchy, followed by its major target structure, V1. Four higher-order visual areas (LM,  
161 RL, LP, and AL) reside at intermediate levels, with areas PM and AM occupying the top level of  
162 the areas we studied here (Fig. 3A,D).

163

164 To compare this anatomical hierarchy to a possible functional hierarchy measured in the spike  
165 recordings, we computed a directional metric of functional connectivity, the spike cross-  
166 correlogram (CCG), between units (Jia et al., 2013; Smith and Kohn, 2008; Zandvakili and  
167 Kohn, 2015). This analysis focused on activity during periods of drifting grating presentation.  
168 For each pair of recorded units, we identified significant functional interactions as determined by  
169 a short latency ( $<10$  ms) sharp peak in the jitter-corrected CCG (Fig. 3B), which removed slow  
170 timescale correlations larger than the jitter window (25 ms). This selection criterion yielded  
171 56,874 pairs of units out of 12,908,146 total possible pairs (0.44%). These fast-timescale spiking  
172 interactions can provide a measure of the functional hierarchical relationship between areas,  
173 based on the measured relative spike timing between pairs of units (Fig. 3B example pair, peak  
174 offset = 3 ms). If units in one region tend to lead the spiking activity of higher regions, the  
175 distribution of peak offsets would deviate from 0. For example, the peak offset distribution of  
176 CCG pairs between V1 and LM showed a significant positive delay when compared to V1–V1  
177 distribution (example mouse, Fig 3C;  $N = 30$  pairs,  $P = 2.6e-8$ , Wilcoxon Rank-sum test),  
178 indicating V1 neurons spike earlier than LM and thus are lower in the hierarchy. As we had only  
179 limited experiments with measurable sharp CCG peaks between thalamic and cortical areas  
180 (LGN–cortex: 1 mouse; LP–cortex: 2 mice), we restricted our CCG-based hierarchy analysis to  
181 cortico-cortical interactions.

182

183 We quantified the distribution of sharp CCG peak time lags for all functionally connected pairs  
184 of units across each pair of cortical areas in each mouse and combined the median of peak offset  
185 distributions across mice (Fig. 3E;  $N = 25$  mice; see Fig. S8 for complete peak offset  
186 distributions between all areas across all mice). V1 units consistently fired action potentials  
187 earlier than units in other areas (Fig. 3E, left column). In contrast, area AM consistently fired  
188 later than other regions, indicating this area resides at the uppermost levels of the hierarchy (Fig.  
189 3E, right column). Quantifying the functional delay between all pair-wise sets of areas revealed  
190 an organization remarkably similar to the anatomical hierarchy (Fig. 3D). The correlation  
191 between the anatomical hierarchy score and the median temporal delay between all regions was

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192 high (Fig. 3F; Pearson's  $r = 0.81$ ,  $P = 2e-9$ ; Spearman's  $r = 0.78$ ,  $P = 2e-8$ ), indicating that unit  
193 spiking activity follows a functional hierarchy closely mirroring the anatomical structure of the  
194 visual cortico-thalamic system.

195  
196 We next assessed how this ordering of areas correlated with four classical measures of functional  
197 hierarchy in primates (Bullier, 2001; Chaudhuri et al., 2015; Schmolesky et al., 1998). First, we  
198 quantified the temporal latency of responses to full-field flashes. Whereas cells in each area have  
199 broadly distributed onsets (Fig. 4A,B), consistent with primate results (Schmolesky et al., 1998),  
200 the mean visual latency of each area was correlated with its anatomical hierarchy score (Fig. 4C;  
201 Pearson's  $r = 0.95$ ,  $P = 0.00025$ ). Statistical testing revealed significantly different latencies for  
202 all pairs of areas, except for LGN-V1, RL-LP, LP-AL, and AM-PM (Fig. S9A,B).

203  
204 Second, the size of spatial receptive fields typically increases when ascending the visual  
205 processing stream (Freeman et al., 2013; Hubel, 1988; Lennie, 1998; Wang and Burkhalter,  
206 2007), likely due to the pooling of convergent inputs from neurons in lower regions. We  
207 measured receptive fields using a localized Gabor stimulus (Figure 4D), and found a systematic  
208 increase in receptive field size with anatomical hierarchy score (Fig. 4D-F; Pearson's  $r = 0.97$ ,  $P$   
209  $= 8.3e-5$ ). Statistical testing revealed significantly different receptive field sizes for all pairs of  
210 areas, except for LM-RL (Fig. S9C,D).

211  
212 Third, Hubel and Wiesel (1962) described 'simple' and 'complex' cells in V1. Complex cells are  
213 thought to result from the integration of inputs from simple cells with different preferred spatial  
214 phases, and can therefore be identified by the lack of phase-dependent responses to a drifting  
215 grating stimulus (Hubel and Wiesel, 1965, 1962; Matteucci et al., 2019; Riesenhuber and Poggio,  
216 1999). The fraction of cells with phase-dependent grating responses, which are common in the  
217 retina and decrease up the visual hierarchy, is a useful measure of hierarchical level. We  
218 quantified this with a modulation index (MI) that robustly reflects phase-dependent responses to  
219 drifting gratings (Matteucci et al., 2019; Wypych et al., 2012). MI measures the difference in  
220 power of the visually evoked response at a unit's preferred stimulus frequency versus the average  
221 power spectrum.  $MI > 3$  corresponds to strong modulation of spiking at the stimulus frequency  
222 (indicative of simple-cell-like responses), whereas smaller MI values indicate less modulation by  
223 stimulus temporal frequency (indicative of complex-cell-like responses) (Matteucci et al., 2019).  
224 Compatible with more simple-like processing, MI was higher in LGN and V1, whereas the  
225 higher order areas showed considerably less phase-dependent modulation (Figure 4G-I,  
226 Pearson's  $r = -0.89$ ,  $P = 0.003$ ). Statistical testing revealed significantly different modulation  
227 indices for all pairs of areas, except for RL-AL and AM-PM (Fig. S9E,F).

228  
229 Finally, previous work in the primate brain demonstrated that the 'timescale' of neural activity  
230 increases at higher levels of the cortical hierarchy (Chen et al., 2015; Murray et al., 2014). We  
231 quantified the temporal scale for each area in our study by fitting an exponential decay to its  
232 mean spike-count autocorrelation function following a full-field flash stimulus (Fig. 4J). We  
233 found that higher-order areas had a longer timescale and thus integrate over longer temporal  
234 windows than lower stages, an important signature of multi-layer processing (Fig. 4J-L;  
235 Pearson's  $r = 0.77$ ,  $P = 0.026$ ). Statistical testing revealed significantly distinct timescales for  
236 LGN-V1, LGN-RL, and for AM and PM vs. all other areas (Fig. S9G,H).

237  
238 Together, these four response metrics and the functional connectivity analysis support the  
239 existence of a functional hierarchy spanning the mouse corticothalamic visual system. These

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240 metrics are not dependent on overall firing rate, which does not correlate with hierarchy score  
241 (Fig. S9I-J).

242  
243 The role of this hierarchical structure should ultimately be related to the behavioral and cognitive  
244 operations implemented by the system. Higher levels of the hierarchy are positioned to  
245 increasingly integrate sensory input with behavioral goals. To test whether the hierarchy we  
246 found correlates with behaviorally relevant processing, we performed additional experiments  
247 beyond our passive viewing survey to measure spiking activity across the visual hierarchy while  
248 mice actively performed a visual change detection task (4527 units from 14 mice) (Garrett et al.,  
249 2019). In this go/no-go task, mice report when a visual stimulus (here, natural scenes; Fig. 5A)  
250 changes identity by licking a reward spout.

251  
252 During the Neuropixels recordings, mice performed the task with high hit rates and low false  
253 alarm rates (mean hit rate = 0.70, mean false alarm rate = 0.12, mean  $d' = 1.9 \pm 0.2$ ; Fig. 5B).  
254 Units recorded during the task had clear visually evoked spiking responses to the flashed visual  
255 stimuli and showed greater evoked spike rates when the visual stimulus changed identity (from A  
256 to B at  $t = 0$  in Fig. 5C-D). As in the passive viewing mice, latency to first spike in response to  
257 the stimulus was correlated with hierarchy score (Fig. 5E). These latencies during active  
258 behavior were highly correlated with latencies measured in the passive viewing condition  
259 (Pearson's  $r = 0.95$ ,  $P = 0.003$ ). Since this task requires mice to detect stimulus changes, for each  
260 unit we computed a 'change modulation index' to capture the differential response to repeated  
261 versus changed images (this metric varies from -1 to 1 with 0 representing no modulation). The  
262 mean change modulation was positive for each area, indicating that a change in image identity  
263 elicits stronger responses compared to the same image presentation. Importantly, change  
264 modulation systematically increased along the hierarchy from LGN to AM (Fig. 5F; Pearson's  $r$   
265 = 0.82,  $P = 0.013$ ). Other aspects of neural activity during the task, such as the pre-change spike  
266 rate, the change response spike rate, and the baseline firing rate were not correlated with  
267 hierarchy score (Fig. S10E-G). Statistical testing revealed that all pairs of areas have  
268 significantly different change modulation indices, except for LM-AL, RL-PM, RL-AM, and  
269 PM-AM (Fig. S10D). This suggests that change-related signals are amplified at higher levels of  
270 the visual hierarchy.

271

272

## 273 Discussion

274

275 One long-term goal of the Allen Institute is to systematically survey neuronal activity in the  
276 visual corticothalamic complex, responding to a battery of commonly used visual stimuli in  
277 awake mice in a way that is minimally biased, maximally reproducible, and freely accessible to  
278 all (Koch and Reid, 2012). We previously presented one such survey, based on two-photon  
279 calcium imaging, that captured cellular activity in six cortical regions in various transgenic  
280 animals (de Vries et al., 2019). We here complement this *Allen Brain Observatory* database at  
281 [www.brain-map.org](http://www.brain-map.org) with a survey of spiking activity measured using high-density silicon  
282 Neuropixels probes (Jun et al., 2017). We recorded from the same cortical regions as in the two-  
283 photon imaging survey, in addition to thalamic visual areas LGN and LP. (We also recorded  
284 units from hippocampus and nearby areas due to the 3.5 mm span of our Neuropixels recordings;  
285 these also are included in our open data release.) In agreement with the foundational studies of  
286 mouse visual cortex (Andermann et al., 2011; Marshel et al., 2011; Niell and Stryker, 2008), we

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287 find a plethora of units across the 8 regions with robust visual responses, with 60% of units  
288 displaying significant visual receptive fields (Fig. 2E).

289  
290 For this first report on our electrophysiological survey, we focused on one important aspect of  
291 this rich dataset – exploiting the dynamic flow of spikes between brain areas to infer functional  
292 hierarchical processing in the visual cortico-thalamic system and relating this to quantitative  
293 measures of its anatomical hierarchy. Based on anterograde viral tracing with Cre-dependent  
294 AAV in 1,256 experiments in 50 distinct mouse lines, Harris et al (2019) derived anatomical  
295 rules describing cortico-cortical, cortico-thalamic, and thalamo-cortical projections into and out  
296 of 37 cortical and 24 thalamic regions via their layer-specific axonal termination patterns. Using  
297 an optimization approach that labels connections as either feedforward or feedback to find the  
298 most self-consistent network, the algorithm assigns a hierarchy score to every region (Fig. 3A).  
299 The study clearly demonstrates that the full corticothalamic system of the mouse is hierarchically  
300 organized, but the difference between the lowest and highest rungs is only a few full levels, due  
301 to parallel and short-cut projections among areas; thus, this system is organized as a ‘shallow’  
302 hierarchy.

303  
304 From the complete corticothalamic anatomical hierarchy of Harris et al (see Fig. 6d in their  
305 paper), we extracted the six cortical and two thalamic visual areas we targeted with Neuropixels  
306 probes. Our analysis indicates these areas represent at least seven distinct levels starting with  
307 LGN and V1, followed by LM/RL, LP, AL, and finally PM and AM at the highest rungs (Fig.  
308 3A), consistent with previous anatomical hierarchy schemes in rodents (Coogan and Burkhalter,  
309 1993; D’Souza et al., 2016). It follows that neurons at a lower level of this hierarchy should  
310 spike earlier than neurons at higher levels (this will only be true on average as there are many  
311 feedback pathways, neurons with a diversity of time constants, and other sources of  
312 heterogeneity). Accordingly, for those pairs of units in any two regions with overlapping  
313 receptive fields, we compute the CCG to identify short latency peaks and extract the relative  
314 spike timing between pairs of neurons (Fig. 3E; Fig. S8); note that the range of temporal lags  
315 involved,  $\pm 10$  ms, may include both mono- and multi-synaptic connections. This uncovers a  
316 striking correspondence between the anatomical and functional network organization—the  
317 bigger the difference in the anatomical hierarchy score of two areas, the larger the median time  
318 lag of spikes between these regions (Fig. 3F).

319  
320 We quantified visual responses in the ascending areas of this visual hierarchy by computing four  
321 previously used measures of hierarchical processing – response latency, receptive field size,  
322 degree of phase modulation by a drifting grating stimulus, and autocorrelation timescale. All four  
323 measures either increased or decreased systematically across these eight visual regions, and  
324 pairwise statistical tests suggest that each of these measures can independently differentiate  
325 between distinct hierarchical levels (Fig. 4; Fig. S9).

326  
327 Even though these functional metrics are correlated with the anatomical hierarchy score, it is  
328 important to note that describing the system with a single variable is a simplification, meant only  
329 to reflect a first-order characterization of its organization. While we have simultaneously  
330 recorded spiking activity from more mouse visual areas than any previous study, we only  
331 sampled six of the 16 mouse cortical visual areas (Zhuang et al., 2017). Moreover, there is  
332 substantial overlap in the distribution of response properties across areas—for example, many  
333 units in AM spike earlier than the slowest units in LGN (Fig. 4B). This suggests much  
334 processing occurs in parallel, in addition to the general hierarchical sequence in response to

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335 sensory drive. The primate visual system is organized into distinct streams (Maunsell, 1992;  
336 Ungerleider and Mishkin, 1982), and there is anatomical and functional evidence for parallel  
337 streams in mice (Smith et al., 2017; Wang et al., 2012). Future studies can uncover how  
338 distributed activity dynamics emerge from connectivity within these hierarchical and parallel  
339 circuits.

340  
341 The functional hierarchy we establish provides a general framework for investigating how it is  
342 used to solve behaviorally relevant tasks. As a first step in this direction, we carried out  
343 experiments to assess whether this same hierarchy is visible in measures reflecting signal  
344 processing during behavior (Fig 5.). We found that the relative response to a rewarded changed  
345 image increased systematically from LGN to AM (Fig. 5F). This increase in change-related  
346 signals suggests that unexpected stimuli are amplified by successive levels of the hierarchy,  
347 consistent with evidence from an oddball paradigm in rat higher-order cortex (Vinken et al.,  
348 2017). These results are compatible with general theories of hierarchical predictive processing,  
349 which posit that unexpected signals are preferentially passed to higher processing stages  
350 (Dürschmid et al., 2016; Grimm et al., 2011; Issa et al., 2018; Keller and Mrsic-Flogel, 2018).

351  
352 A major challenge in neuroscience is to understand how spiking activity flows through  
353 distributed brain networks to mediate cognition and behavior. The cortex is widely and densely  
354 connected (Gămănuț et al., 2018; Oh et al., 2014), with diverse cell-type-specific anatomical  
355 pathways. The concept of hierarchy is one important first-order organizing principle for  
356 understanding form and function in the brain (Sporns, 2010). Reinforcing the anatomical and  
357 functional evidence for a mouse cortical hierarchy, cell type composition and gene expression  
358 systematically change across the global hierarchy in the mouse cortex (Fulcher et al., 2019; Kim  
359 et al., 2017; Tasic et al., 2018). Nonetheless, the cortex also displays additional levels of  
360 organization including functional sub-modules and parallel processing streams. These aspects  
361 must be incorporated to establish a more complete mapping between cortical structure and  
362 function (Han et al., 2018; Lennie, 1998; Ungerleider and Mishkin, 1982; Wang et al., 2012).  
363



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365 encouragement and support.

366

### 367 **Author contributions**

368 Conceptualization: C.K., S.R.O, J.H.S., X.J., S.G., C.B., S.M., D.D., S.dV., M.B., C.R.

369 Supervision: C.K., S.R.O, J.H.S., P.A.G., C.R., C.F., S.M., H.Z., S.D.

370 Investigation, validation, methodology, and formal analyses:

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374 D.S., J.S., D.W., A.W., R.A., D.B., M.C., E.L., K.R., K.N., B.S., E.J., K.J., J.M., K.N., M.G.,

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379 Project administration: C.T., S.N., L.C., L.E., N.H., J.W.P.

380 Visualization: J.H.S, X.J., S.G, C.B., H.C., S.D.

381 Original draft written by C.K., S.R.O., J.H.S., X.J. with input and editing from S.M., H.C., C.B.,

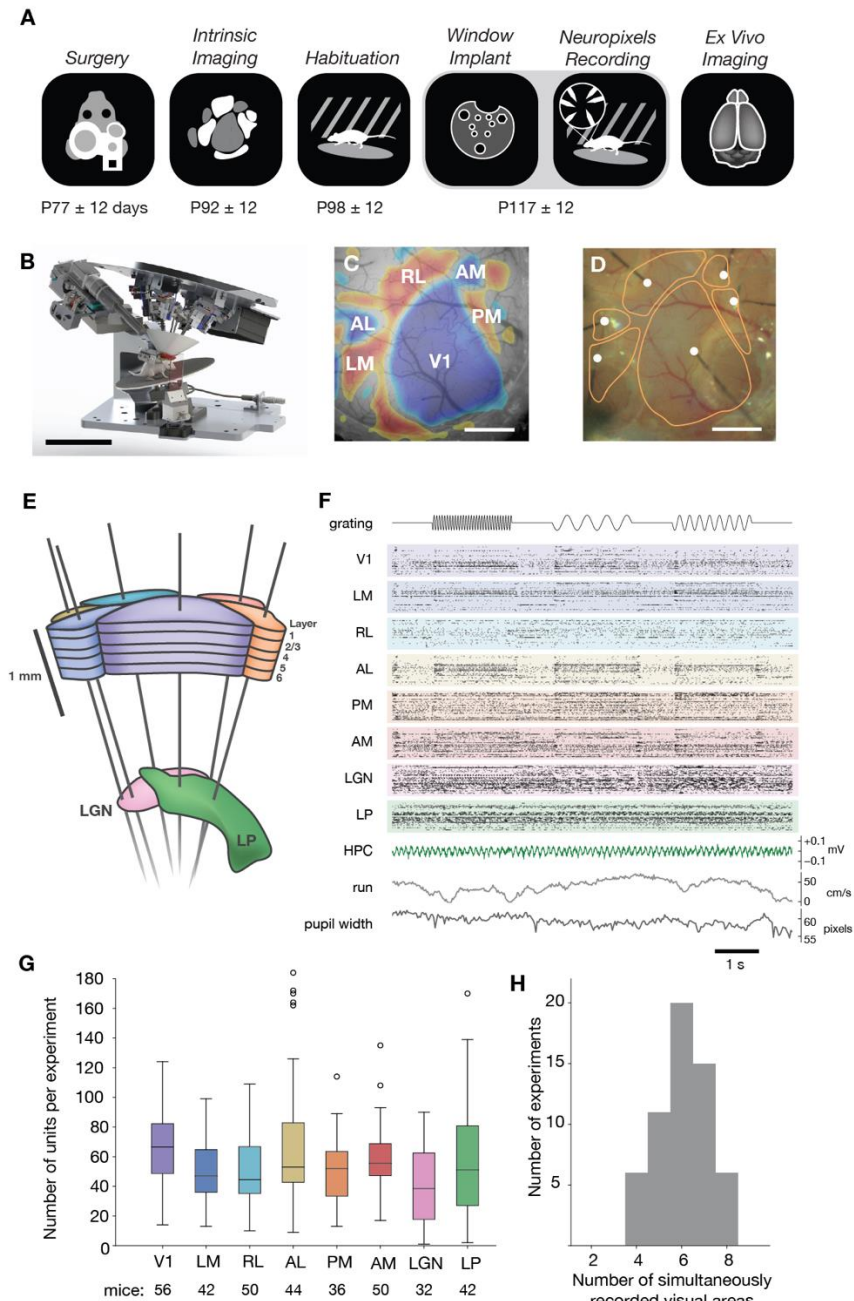
382 S.G.

383 All co-authors reviewed the manuscript.

384

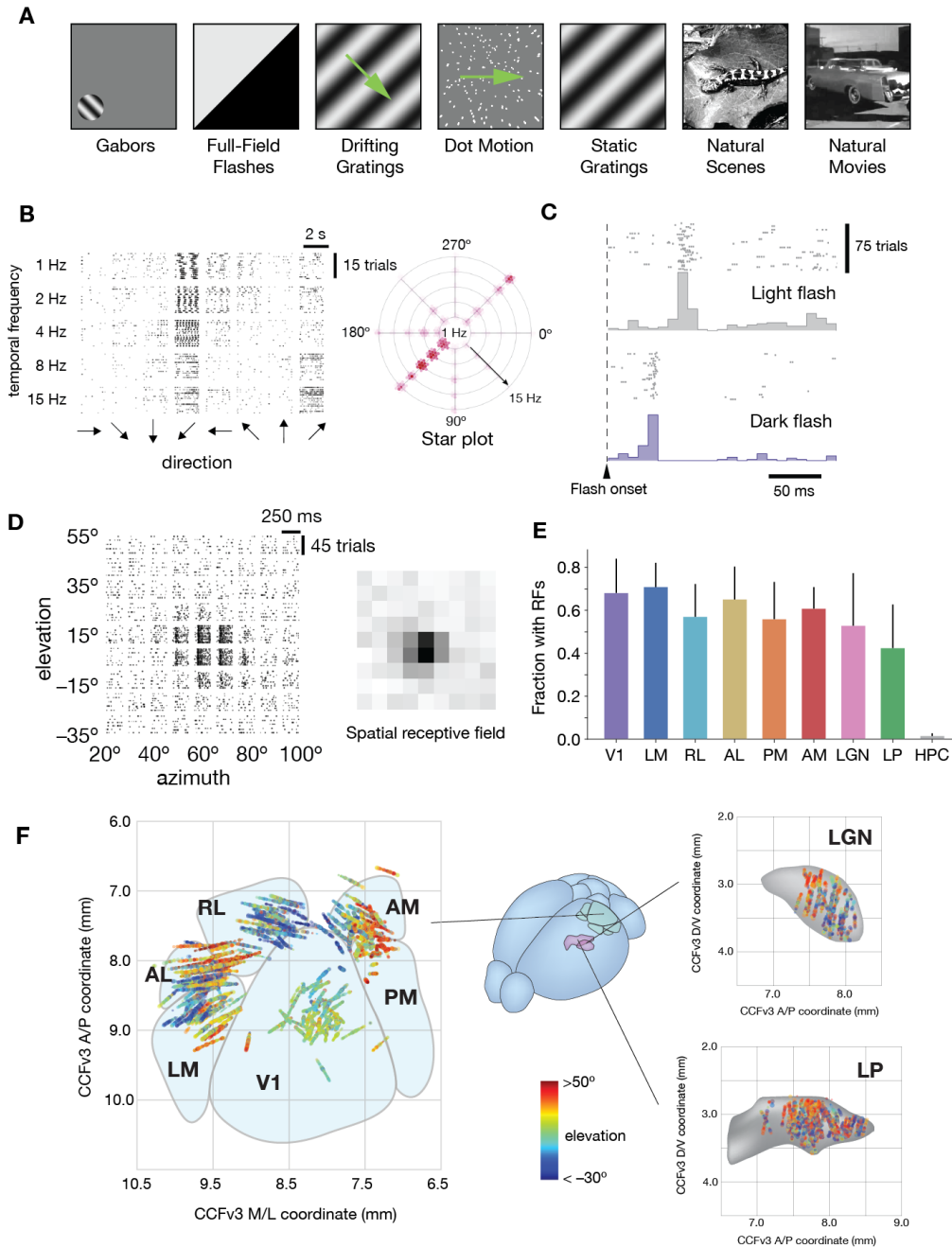
385 **Competing interests** The authors declare no competing interests.

Neuropixels Survey of the Mouse Visual System



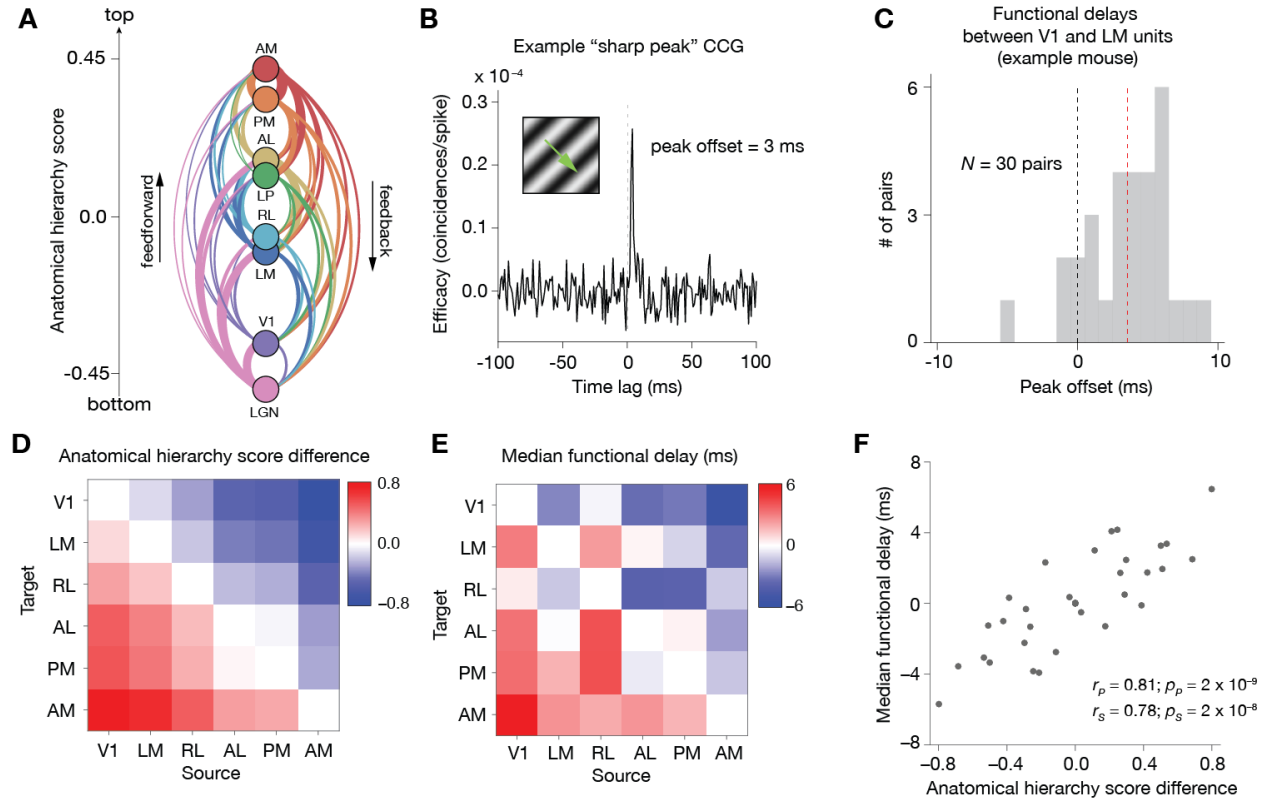
386  
 387 **Figure 1. A standardized pipeline for extracellular electrophysiology in the mouse corticothalamic**  
 388 **visual system. (A)** Icons representing six major steps in the data collection pipeline, with the average age  
 389 of mice at each step indicated below. **(B)** Rig for parallel recording from six Neuropixels probes. Scale  
 390 bar = 10 cm. **(C)** Example retinotopic map used for targeting probes to six cortical visual areas. Scale  
 391 bar = 1 mm. **(D)** Image of Neuropixels probes during an experiment, with area boundaries from **(C)**  
 392 overlaid in orange. Probe tips are marked with white dots. Scale bar = 1 mm. **(E)** Schematic of target probe  
 393 insertion trajectories through cortex into two thalamic visual areas, LGN and LP. **(F)** Example raster plot  
 394 of 405 simultaneously recorded units from 8 visual areas. The phase of the drifting grating visual stimulus  
 395 (15 Hz, 2 Hz, or 4 Hz), hippocampal local field potential, mouse running speed, and pupil width are also  
 396 shown. **(G)** Box plot of the number of units recorded per area per experiment, after filtering based on ISI  
 397 violations (<0.5), amplitude cutoff (<0.1), and presence ratio (>0.95) (see Methods and Figure S4 for  
 398 quality metric definitions and distributions). **(H)** Histogram of the number of simultaneously recorded  
 399 corticothalamic visual areas per experiment.

## Neuropixels Survey of the Mouse Visual System



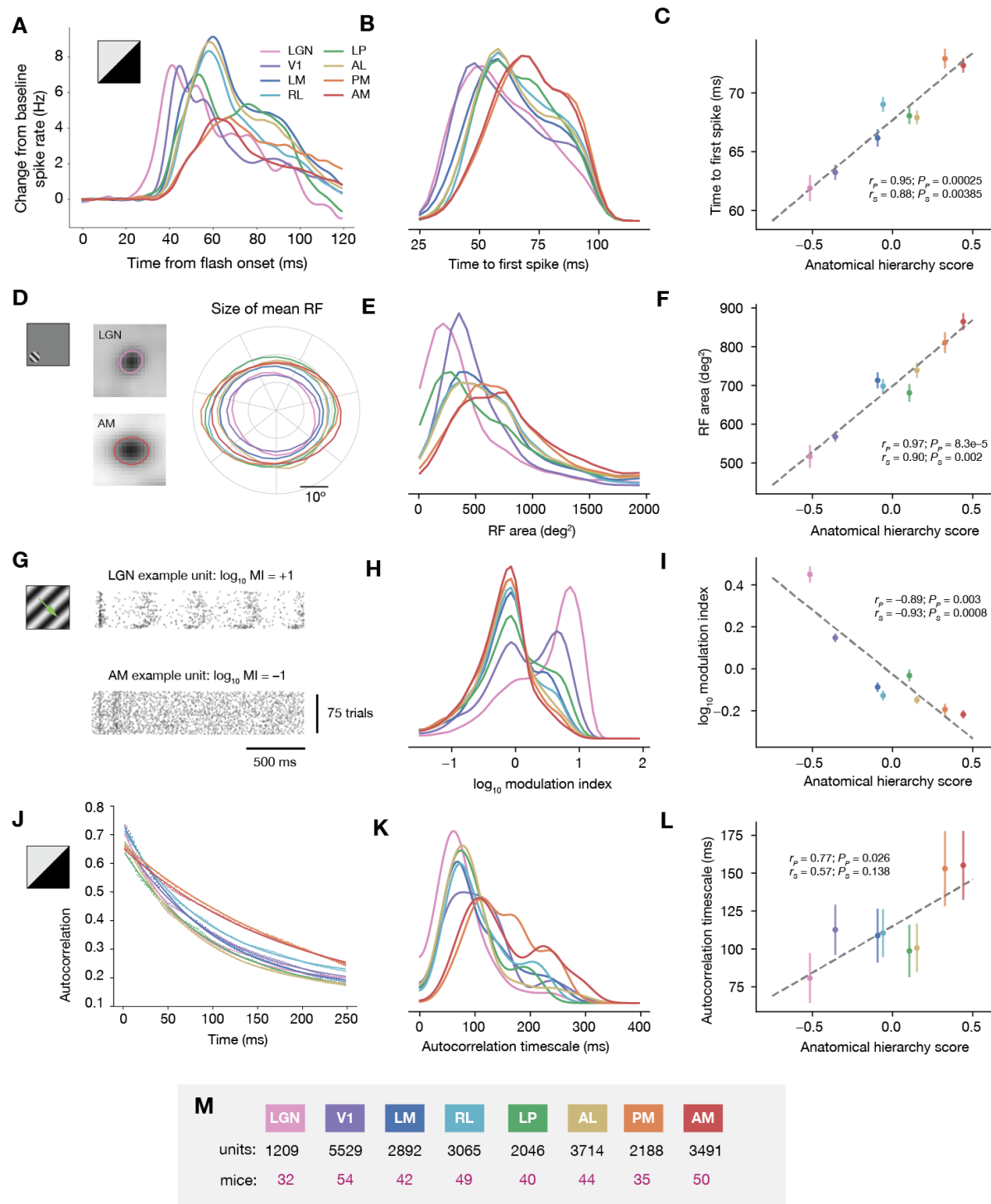
400  
401 **Figure 2. High-throughput mapping of visual response properties.** (A) During Neuropixels  
402 recordings, mice are exposed to up to seven types of natural and artificial stimuli. (B) Raster plots of  
403 spike times for 40 unique conditions of a drifting grating stimulus, for an example V1 unit. The single-  
404 trial responses are used to construct a “star plot,” which efficiently summarizes the unit’s tuning  
405 properties. (C) Raster plot of spike times for two conditions of the full-field flash stimulus, for the same  
406 unit in B. A peri-stimulus time histogram summarizes the response across trials. (D) Raster plot of spike  
407 times for 81 conditions of the Gabor stimulus for the same unit as in B and C. Summing the spike counts  
408 across trials at each location produces a spatial receptive field, shown on the right. (E) Mean fraction of  
409 units with significant receptive fields (RFs) across 8 visual areas, with hippocampus included as a control.  
410 Error bars represent standard deviation across experiments. (F) Each unit with a significant receptive field  
411 is represented by a dot at its spatial location in the mouse Common Coordinate Framework. Color  
412 represents the elevation of the receptive field center, revealing smoothly varying maps of visual space  
413 when aggregating across experiments. Area boundaries are approximate.

## Neuropixels Survey of the Mouse Visual System



414  
 415 **Figure 3. Functional connectivity recapitulates the anatomical hierarchy.** (A) Relative anatomical  
 416 hierarchy scores from Harris, Mihalas et al. (2019). Each area's hierarchy score is based on the ratio of  
 417 feed-forward vs. feedback projection patterns (colored lines) between itself and the other 7 areas. (B)  
 418 Method for measuring cross-area spiking interactions between pairs of units. "Sharp peaks" in the jitter-  
 419 corrected CCG are those with a peak amplitude  $>7x$  the standard deviation of the flanks. The peak offset,  
 420 or functional delay, is defined as the difference between the CCG peak time and the CCG center (at 0  
 421 ms). (C) Distribution of functional delay between V1 and LM reflected by pairwise CCG peak offset in  
 422 one example mouse ( $N = 30$  pairs; median = 3.9ms). (D) Re-plotting of anatomical hierarchy scores from  
 423 (A), showing the difference in score between all pairs of cortical areas. Statistical testing (Wilcoxon  
 424 Rank-sum test) revealed that all areas have significant different hierarchical score, except for RL and LM.  
 425 (E) Combined median of functional delay across mice ( $N = 25$  mice in total) for each pair of cortical  
 426 areas. Statistical testing (Wilcoxon Rank-sum test) revealed that the peak offset distribution of  
 427 neighboring-areas were significantly different from within-area, except for AL-PM. (F) Correlation  
 428 between the median functional delay and the difference in hierarchy scores, indicating a link between  
 429 structure and function (Pearson's  $r = 0.81$ ,  $P = 2e-9$ ).  
 430

## Neuropixels Survey of the Mouse Visual System

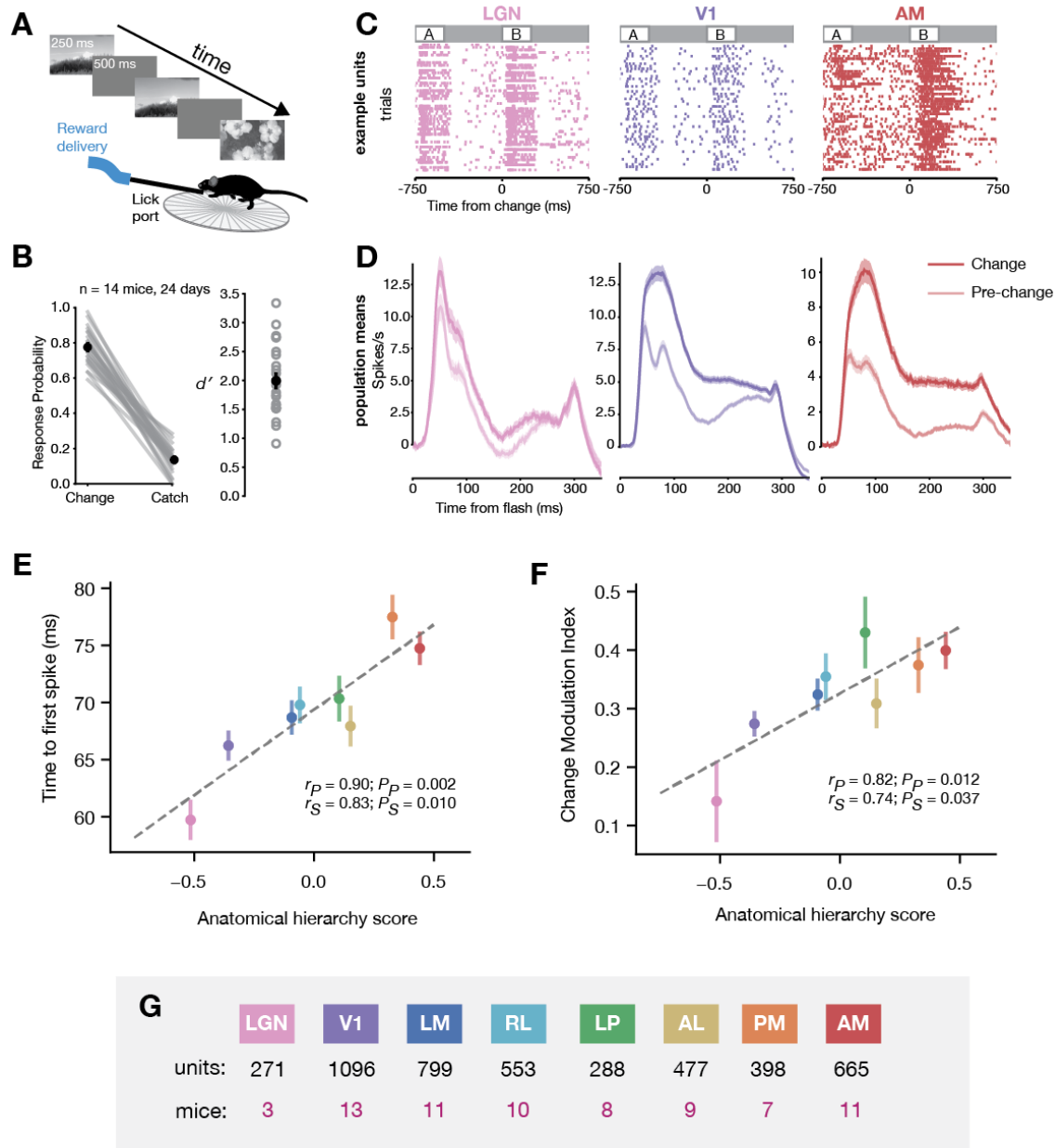


431  
 432 **Figure 4. Four measures of hierarchical processing applied to the mouse visual system.** (A) Mean  
 433 peri-stimulus time histogram (PSTH) of the spiking response to a full-field flash stimulus across 2  
 434 thalamic and 6 cortical regions, with the baseline firing rate subtracted for each area. (B) Distribution of  
 435 first spike times in response to the flash stimulus across all units in each of 8 areas. (C) Correlation  
 436 between mean time to first spike and hierarchy score obtained from anatomical tracing studies. (D)  
 437 Outlines of the extent of the mean receptive field (RF) for each area, at 50% of the peak firing rate.  
 438 Exemplar mean receptive fields for LGN and AM are shown on the left. (E) Distribution of receptive  
 439 field sizes across all units in each of 8 areas. (F) Correlation between mean receptive field size and

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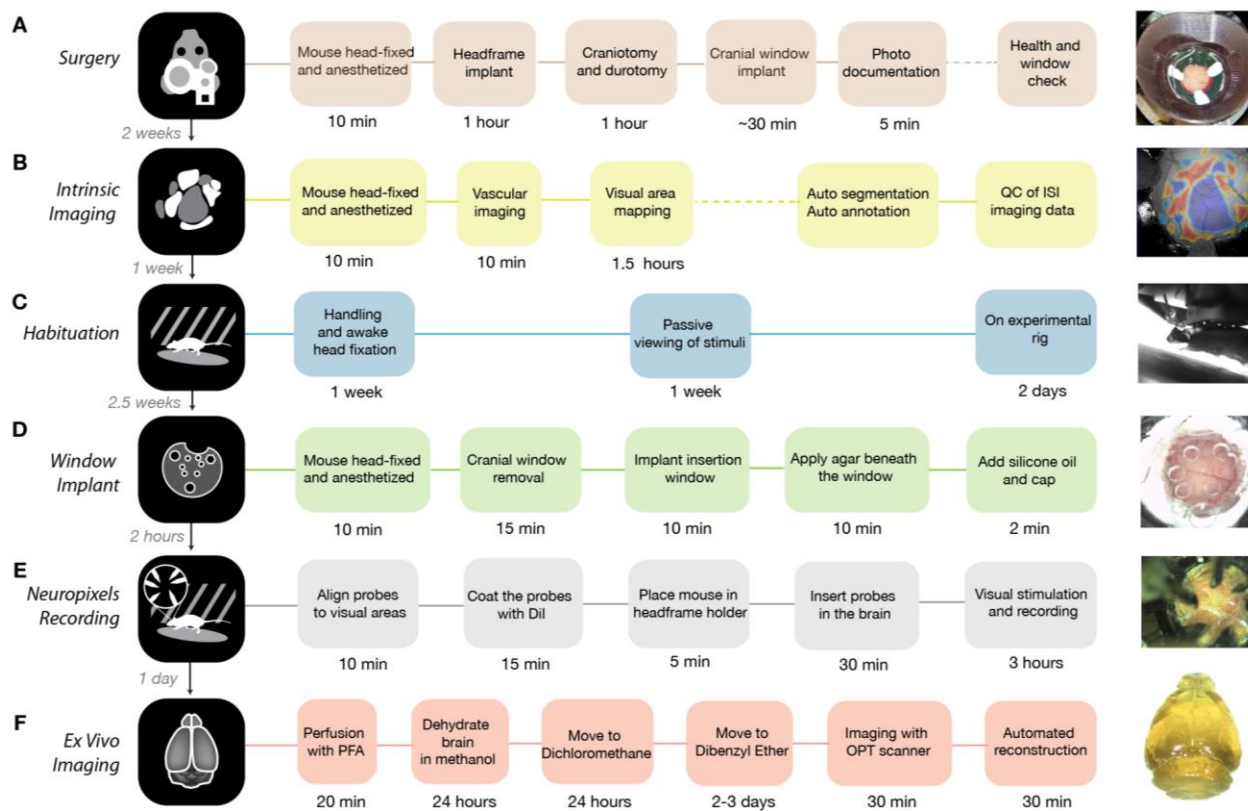
440 anatomical hierarchy score. **(G)** Raster plots showing the response of exemplar units in LGN and AM to a  
441 2 Hz drifting grating stimulus. Modulation index (MI) is higher in units that fire at the same temporal  
442 frequency as the grating. **(H)** Distribution of MI across all units in each of 8 areas. **(I)** Correlation  
443 between mean MI and anatomical hierarchy score. **(J)** Mean autocorrelation values for 8 areas in the 250  
444 ms period following the onset of a full-field flash stimulus. An exponential fit is used to determine the  
445 autocorrelation timescale. **(K)** Distribution of autocorrelation timescales for all units in each of 8 areas.  
446 **(L)** Correlation between mean autocorrelation timescale and anatomical hierarchy score. **(M)** Figure  
447 legend, indicating color of each area, total number of units per area, and total number of mice per area.  
448 All error bars represent 95% bootstrap confidence intervals. See Fig S4B for unit selection criteria.

Neuropixels Survey of the Mouse Visual System



449  
 450 **Figure 5. Higher-order areas signal behaviorally relevant changes in image identity more strongly**  
 451 **than lower-order areas.** (A) Mice were rewarded when they correctly detected change in the identity of  
 452 a natural scene shown for 250 ms, separated by 500 ms blank screen. (B) After training, 10 mice had high  
 453 hit rates and low false alarm rates, with an average  $d'$  of 1.9 +/- 0.2. (C) Rasters showing spiking of  
 454 exemplar units from LGN, V1, and AM during 50 trials of the change detection task. (D) Population peri-  
 455 stimulus time histograms averaged over all units in LGN, V1, and AM. For each area, the response to the  
 456 change image is shown as a darker line, while the response to the pre-change image is shown as a lighter  
 457 line. The change modulation index is defined as the normalized difference between the firing rate during  
 458 the pre-change image and the change image. (E) Correlation between mean time to first spike and  
 459 anatomical hierarchy score across all 8 areas during the change detection task. (F) Correlation between  
 460 mean change modulation index and anatomical hierarchy score across all 8 areas. Responses to the  
 461 stimulus change are always greater than the pre-change flash (CMI > 0) and this difference increases from  
 462 LGN to AM. (G) Figure legend, indicating color of each area, total number of units per area, and total  
 463 number of mice per area. Error bars in E and F represent 95% bootstrap confidence intervals.

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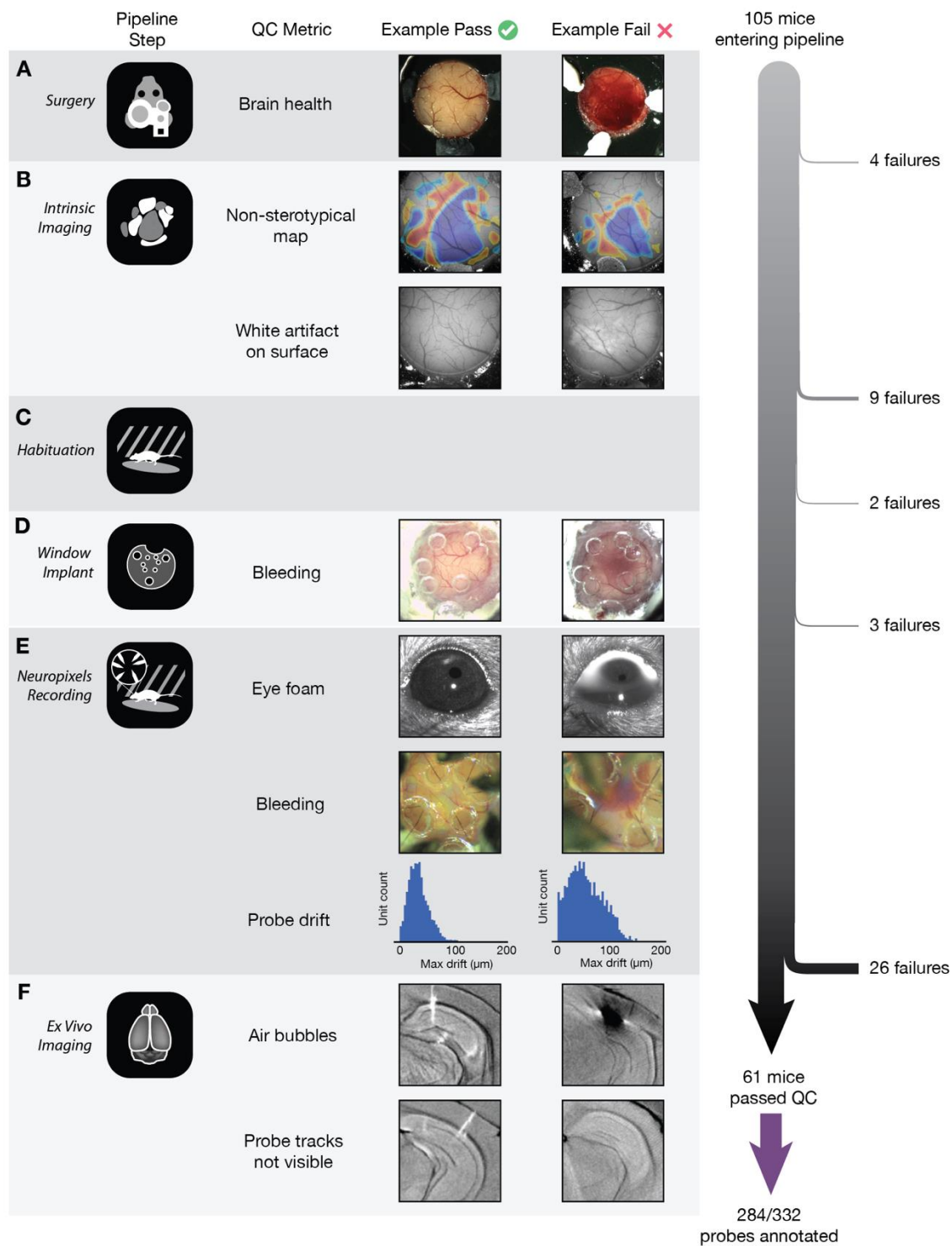


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**Supplementary Figure 1. Pipeline procedures.** Summary of procedures involved in each step of the pipeline.

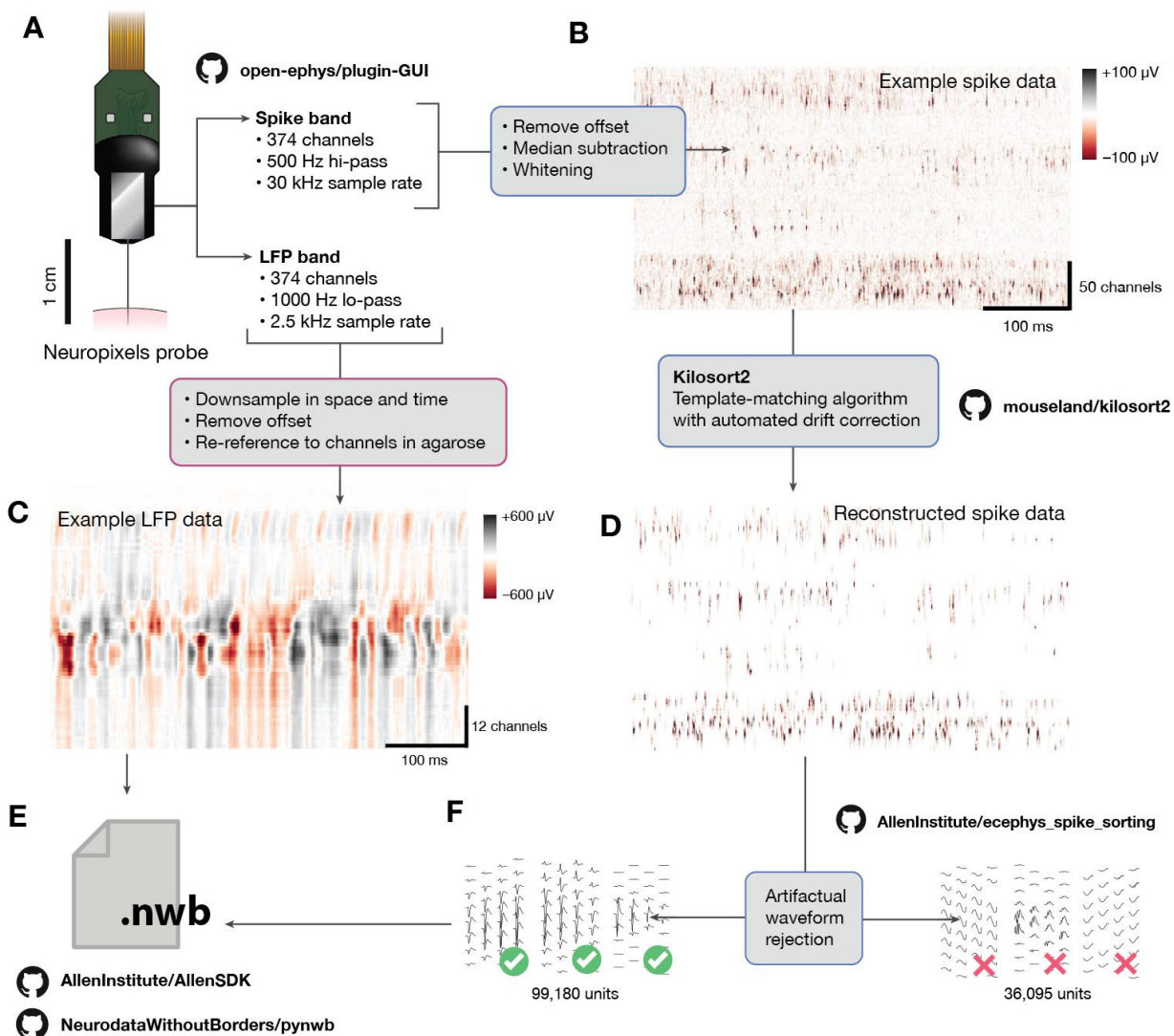


Neuropixels Survey of the Mouse Visual System



467  
 468 **Supplementary Figure 2. Pipeline quality control.** Major QC metrics for each pipeline step, with  
 469 examples of passing and failing experiments. The number of mice failing QC at each stage is shown on  
 470 the right.  
 471

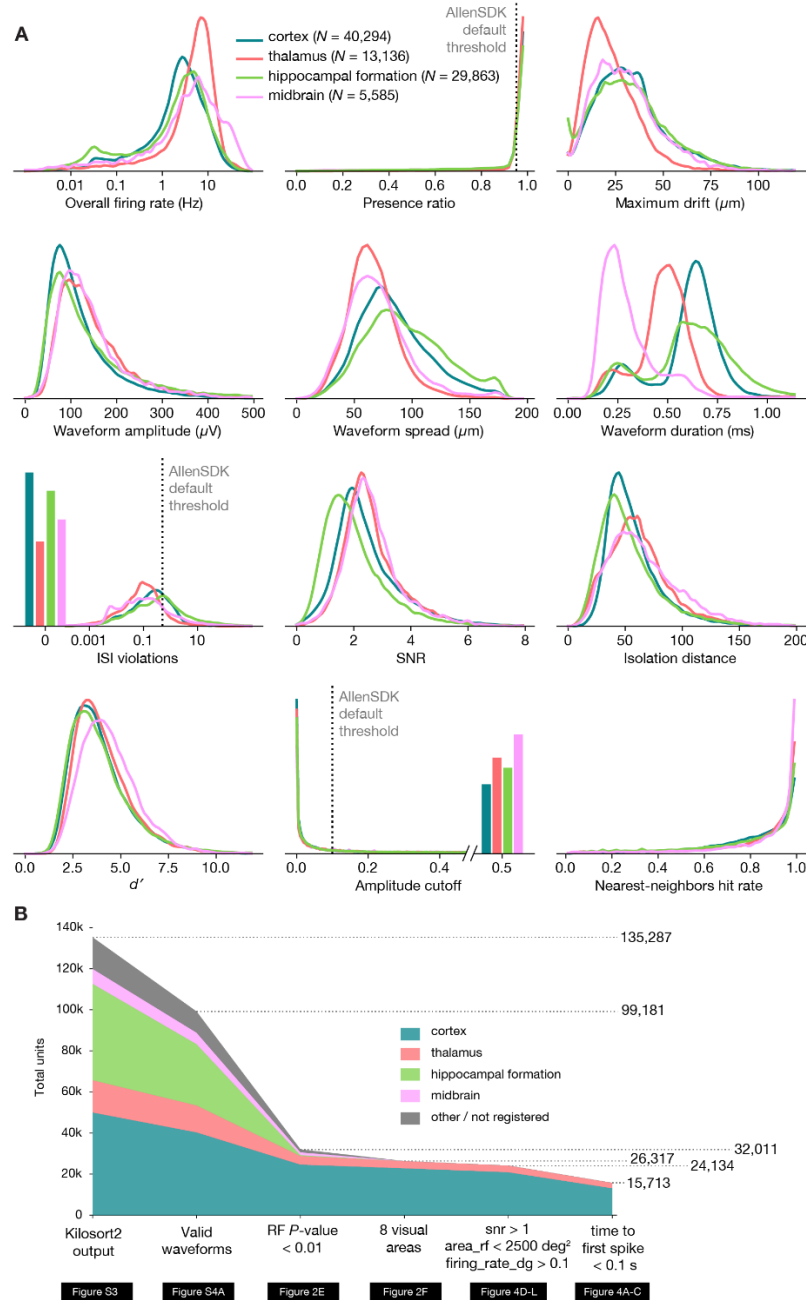
## Neuropixels Survey of the Mouse Visual System



472  
473 **Supplementary Figure 3. Data processing pipeline.** (A) Data from the Neuropixels probe is split at the  
474 hardware level into two separate streams for each electrode: spike band and LFP band. (B) The spike  
475 band passes through offset subtraction, median subtraction, and whitening steps prior to sorting. The  
476 resulting data can be viewed as an image, with dimensions of time and channels, and colors  
477 corresponding to voltage levels. (C) The LFP data is down-sampled to 1.25 kHz and 40  $\mu\text{m}$  channel  
478 spacing prior to packaging. (D) We use the Kilosort2 to match spike templates to the raw data. The output  
479 of this algorithm can be used to reconstruct the original data using information about template shape,  
480 times, and amplitudes. (E) The spike and LFP data are packaged into Neurodata Without Borders (NWB)  
481 files. (F) The outputs of Kilosort2 are passed through a semi-automated QC procedure to remove units  
482 with artifactual waveforms. Only units with obvious spike-like characteristics are used for further  
483 analysis.  
484

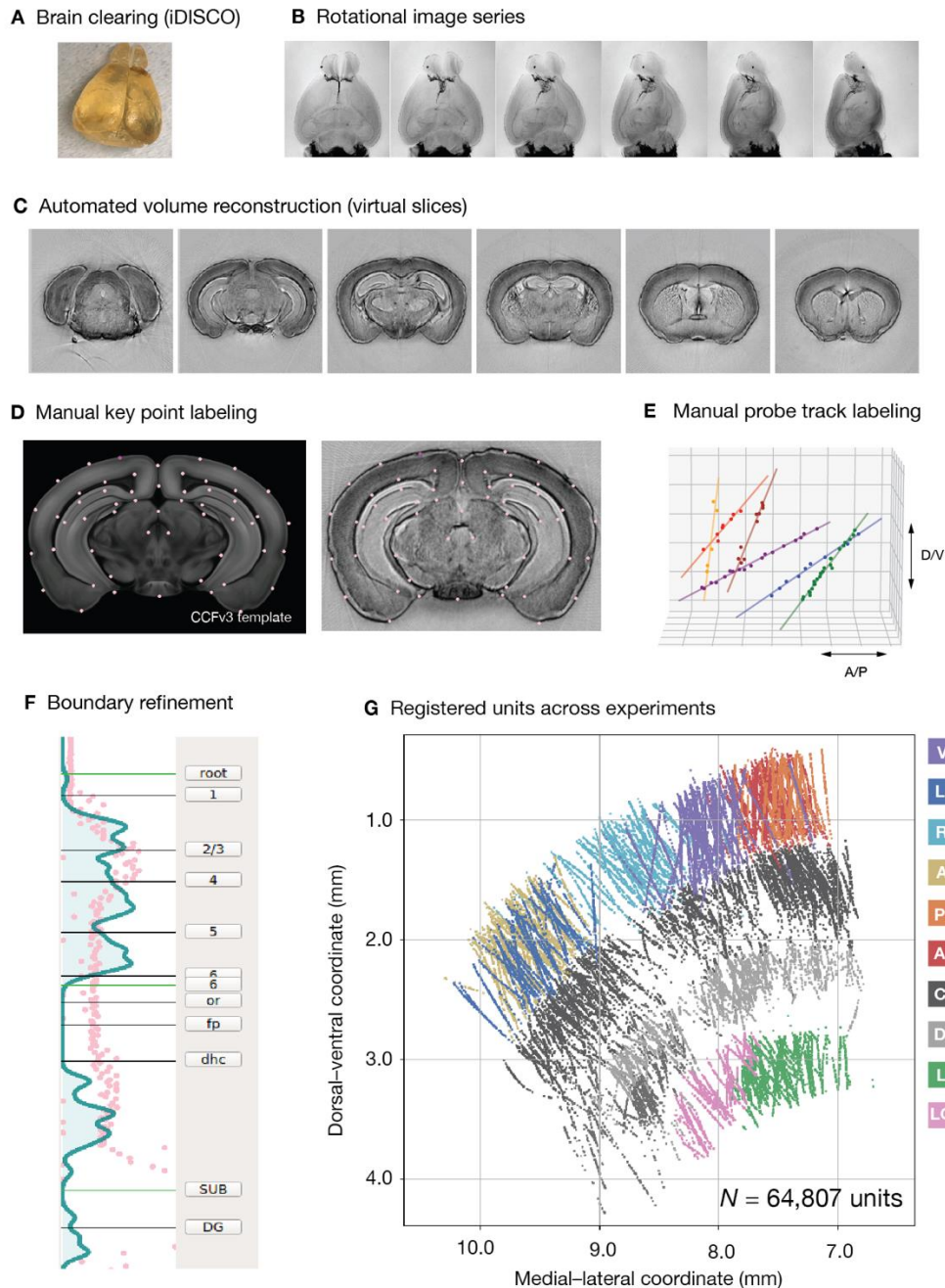
## Neuropixels Survey of the Mouse Visual System

Supplementary Figure 4



485  
486 **Supplementary Figure 4. Unit quality metrics.** (A) Density functions for 12 unit QC metrics, plotted  
487 for units in cortex, hippocampus, thalamus, and midbrain, aggregated across experiments. Default  
488 AllenSDK thresholds are shown as dotted lines. (B) Unit selection flowchart for generating manuscript  
489 figures. Note that we do not use the default AllenSDK filters in this work, but instead use an RF  $P$ -value  
490 of 0.01 as the primary metric for selecting units for analysis. CCFv3 structure labels used for region  
491 identification are as follows: cortex (VISp, VISl, VISrl, VISam, VISpm, VISal, VISmma, VISmmp,  
492 VISli, VIS), thalamus (LGd, LD, LP, VPM, TH, MGm, MGv, MGd, PO, LGv, VL, VPL, POL, Eth, PoT,  
493 PP, PIL, IntG, IGL, SGN, VPL, PF, RT), hippocampal formation (CA1, CA2, CA3, DG, SUB, POST,  
494 PRE, ProS, HPF), midbrain (MB, SCig, SCiw, SCsg, SCzo, SCop, PPT, APN, NOT, MRN, OP, LT,  
495 RPF), other / nonregistered (CP, ZI, grey).

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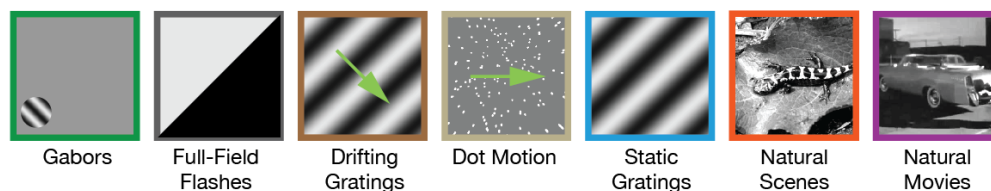


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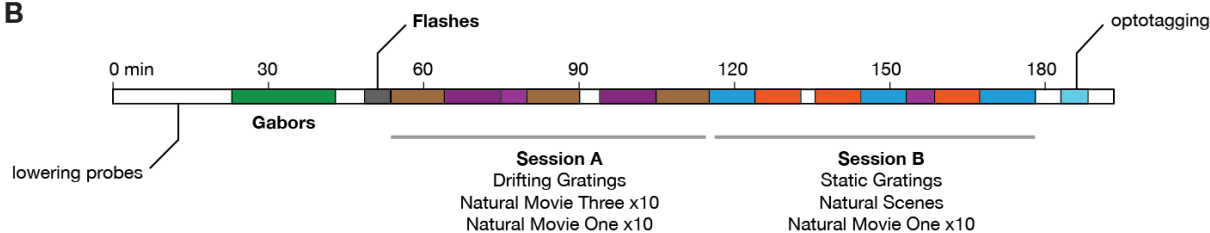
498 **Supplementary Figure 5. Aligning units with the Common Coordinate Framework (CCFv3).** (A)  
499 Following each experiment, the brain is removed and cleared using a variant of the iDISCO method. (B)  
500 The cleared brain is imaged at 400 rotational angles using a custom-built optical projection tomography  
501 microscope. (C) We generated an isotropic 3D volume from rotational images using a computational  
502 tomography algorithm. (D) Key points from the CCFv3 template brain are manually identified in each  
503 individual brain. (E) Points along each fluorescently labeled probe track are manually identified in the  
504 volume. Using the key points from D, we define a warping function to translate points along the probe  
505 axis into the Common Coordinate Framework. (F) We then align the regional boundaries to boundaries in  
506 the physiological data, primarily the decrease in unit density at the border between cortex and  
507 hippocampus, and between hippocampus and thalamus. (G) Finally, units in the database are mapped to a  
508 3D location in the CCFv3, and are assigned a structure label.

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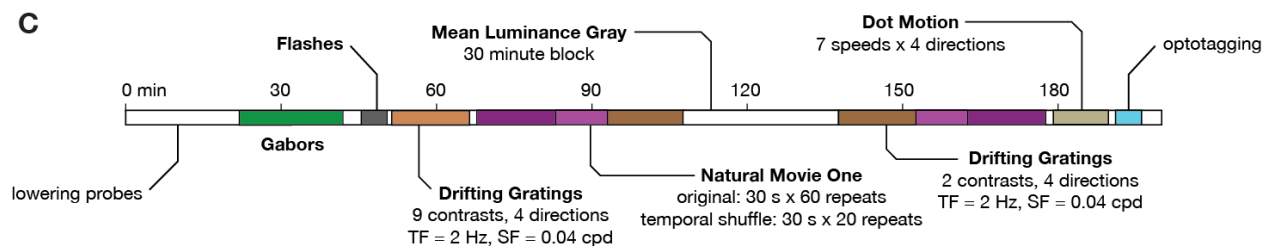
**A**



**B**

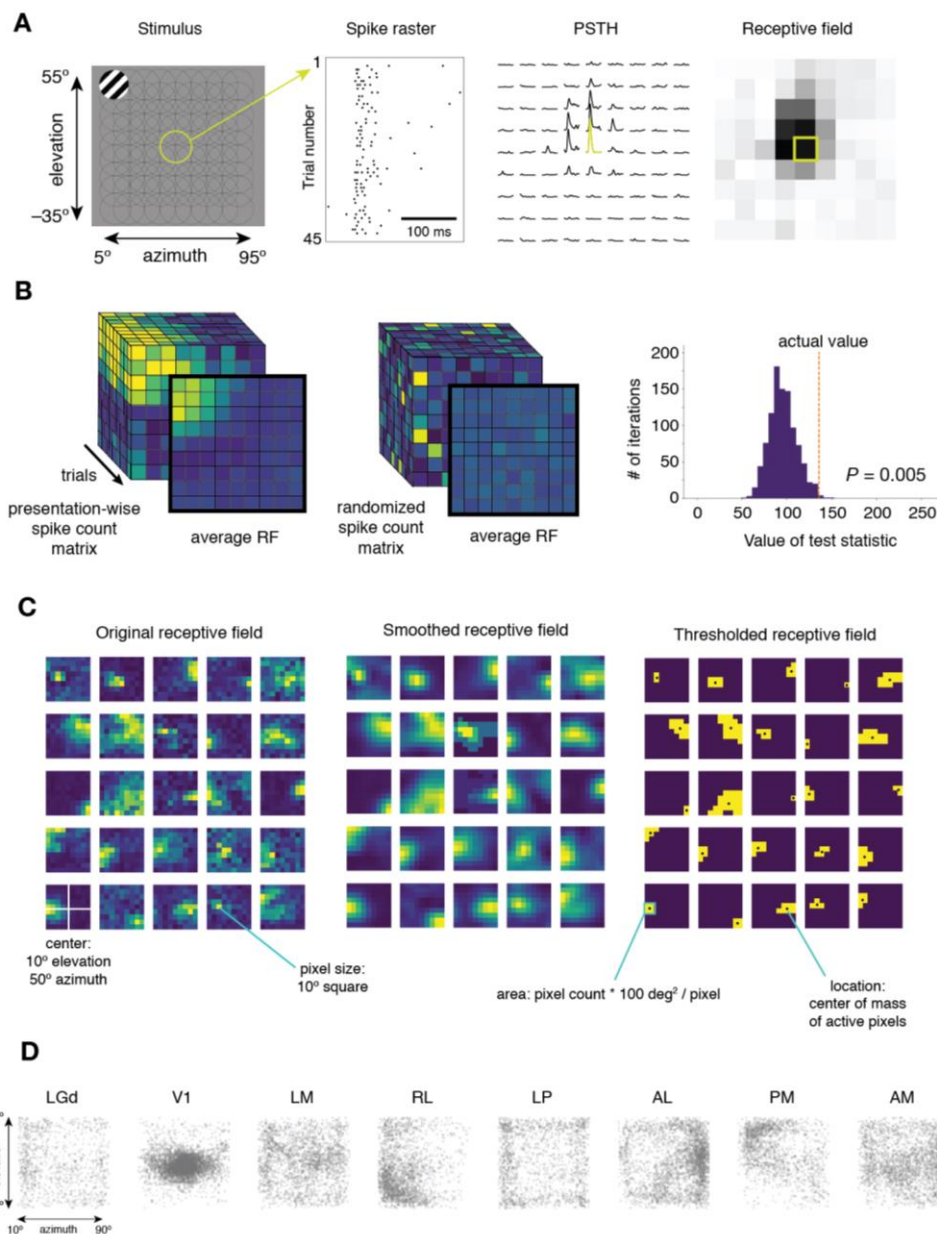


**C**



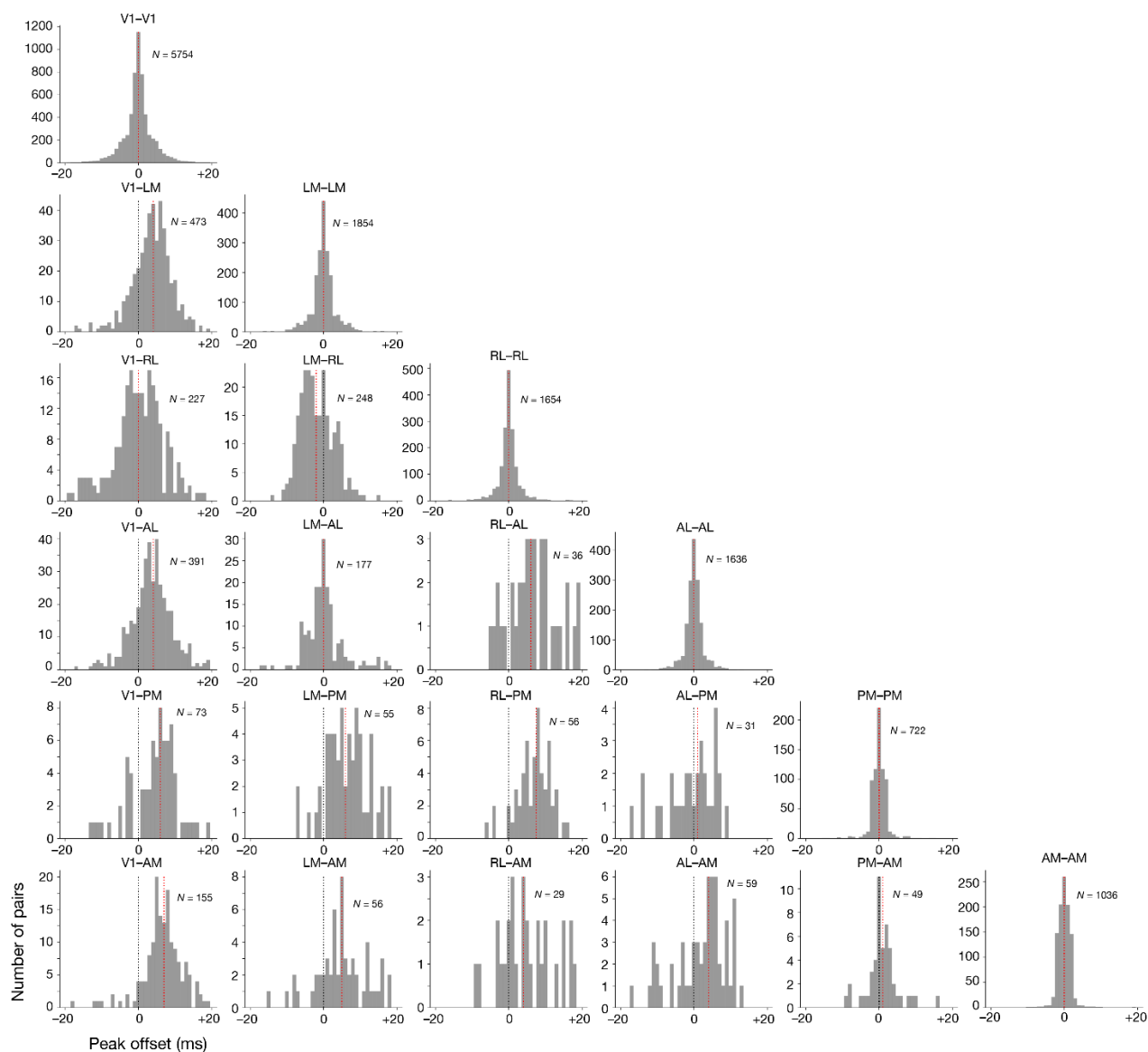
509  
 510 **Supplementary Figure 6. Details of the visual stimulus set.** (A) Example frames from each type of  
 511 stimulus. (B) Timing diagram for visual stimulus set #1, known as “Brain Observatory 1.1.” (C) Timing  
 512 diagram for visual stimulus set #2, known as “Functional Connectivity.”

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 514 **Supplementary Figure 7. Receptive analysis and unit selection.** (A) Our receptive field mapping  
 515 procedure consists of flashing 20° diameter drifting gratings for 250 ms in each of 81 randomized  
 516 locations on the screen. For each unit, we can construct a spike raster showing the timing of spikes on  
 517 each of 45 trials with the stimulus at a particular location. Collapsing over trials yields a peri-stimulus  
 518 time histogram (PSTH) for each location. Collapsing over time yields a spike count for each spatial bin. A  
 519 matrix of spike counts represents the receptive field for this unit. (B) We use a categorical  $\chi^2$  test to  
 520 determine which units have significant receptive fields. We compare the actual matrix of presentation-  
 521 wise spike counts (left) to a series of randomized spike count matrices (center) to determine the  
 522 probability that the receptive field could have occurred by chance (right). (C) To calculate receptive field  
 523 properties, we first smooth the receptive field with a Gaussian filter, then select all pixels above a  
 524 threshold value. The center of mass of the above-threshold pixels indicates the receptive field location,  
 525 while the total number of above-threshold pixels indicates the area. These processing steps are shown for  
 526 25 receptive fields randomly chosen from one experiment. (D) Receptive field locations for all units in  
 527 our analysis (RF  $P$ -value < 0.01).

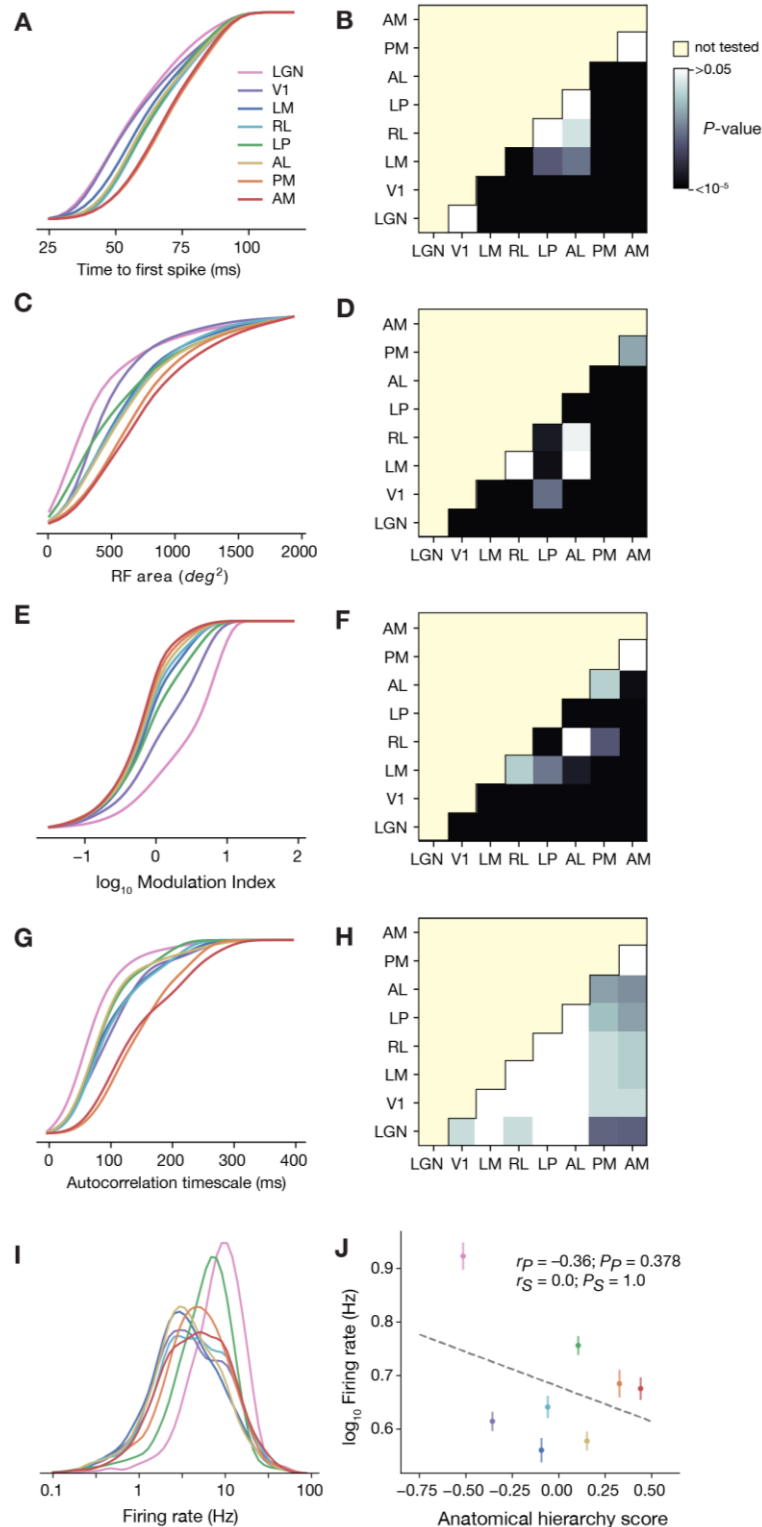
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**Supplementary Figure 8. Distributions of pairwise functional delays.** Histograms of CCG peak offsets for all pairs of units included in Figure 3.

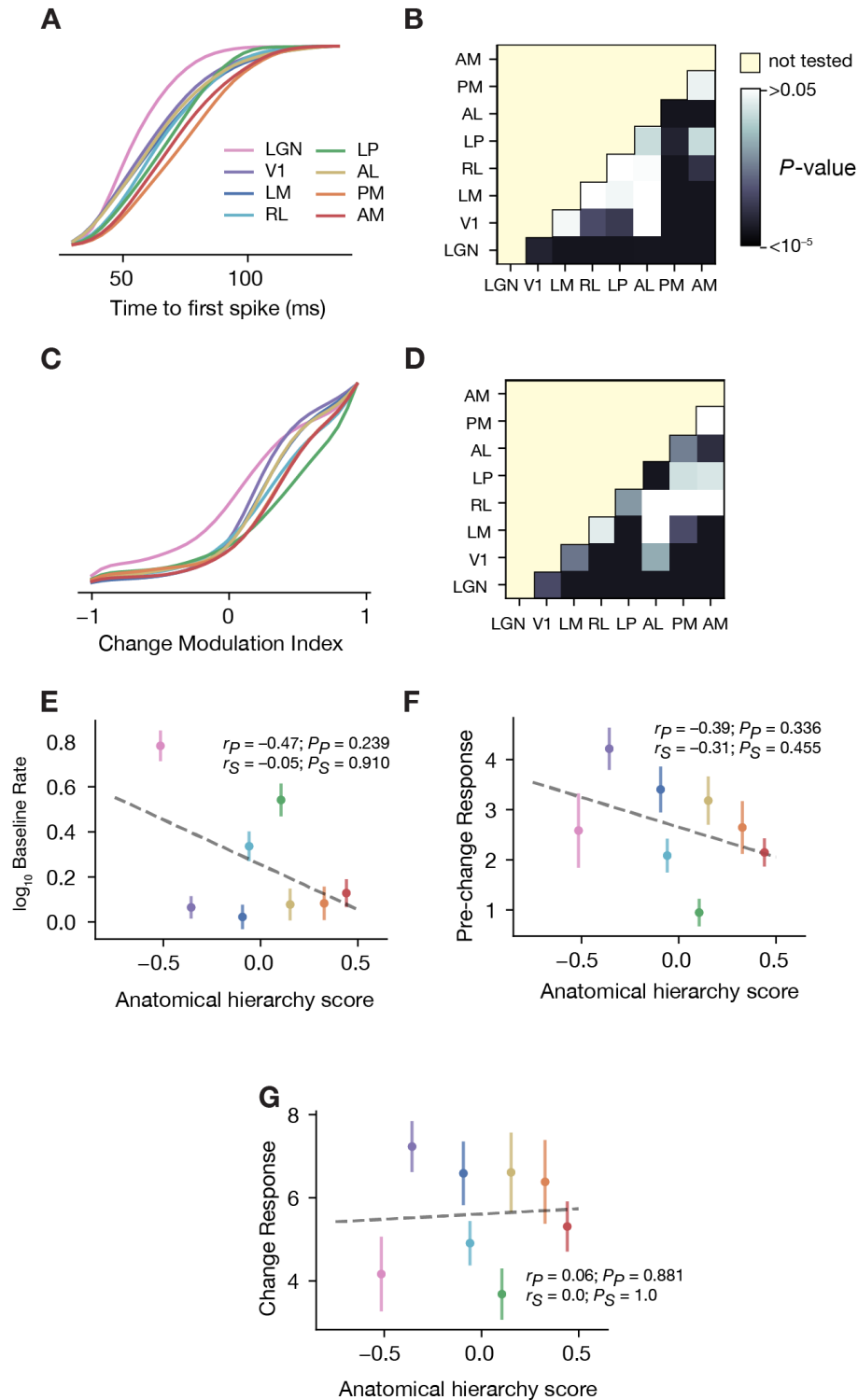
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**Supplementary Figure 9. Extended data for Figure 4.** (A) Cumulative probability function for time to first spike (same data as Figure 4B). (B) P-values for pairwise comparisons of time to first spike between areas (Wilcoxon rank-sum test with Benjamini-Hochberg False Discovery Rate correction). (C) Cumulative probability function for receptive field area (same data as Figure 4E). (D) P-values for pairwise comparisons of receptive field size between areas. (E) Cumulative probability function for modulation index (same data as Figure 4H). (F) P-values for pairwise comparisons of modulation index between area. (G) Cumulative probability function for autocorrelation timescale (same data as Figure 4K). (H) P-values for pairwise comparisons of autocorrelation timescale between areas. (I) Distribution of overall firing rates for all units in each area. (J) Correlation between mean firing rate and anatomical hierarchy score.



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563  
564 **Supplementary Figure 10. Extended data for Figure 5.** (A) Cumulative probability function for time  
565 to first spike (same data as Figure 5E). (B) *P*-values for pairwise comparisons of time to first spike  
566 between areas (Wilcoxon rank-sum test with Benjamini-Hochberg False Discovery Rate correction). (C)  
567 Cumulative probability function for change modulation index (same data as Figure 5F). (D) *P*-values for  
568 pairwise comparisons of change modulation index between areas (E) Correlation between mean baseline  
569 firing rate and hierarchy score. (F) Correlation between mean pre-change response spike rate and  
570 hierarchy score. (G) Correlation between mean change response spike rate and hierarchy score.  
571

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## 748 **Materials and Methods**

### 749 **1. Mice**

750 Mice were maintained in the Allen Institute for Brain Science animal facility and used in  
751 accordance with protocols approved by the Allen Institute's Institutional Animal Care and Use  
752 Committee.

753 Wild-type C57BL/6J mice were purchased from Jackson Laboratories at age P25-50. For  
754 experiments involving opto-tagging of inhibitory cells, Pvalb-IRES-Cre, Vip-IRES-Cre, and Sst-  
755 IRES-Cre mice were bred in-house and crossed with an Ai32 channelrhodopsin reporter line  
756 (Madisen et al., 2012). Pvalb-IRES-Cre;Ai32 breeding sets (pairs and trios) consisted of  
757 heterozygous Pvalb-IRES-Cre mice crossed with either heterozygous or homozygous  
758 Ai32(RCL-ChR2(H134R)\_EYFP) mice. Pvalb-IRES-Cre is expressed in the male germline. To  
759 avoid germline deletion of the stop codon in the LoxP-STOP-LoxP cassette, Pvalb-IRES-  
760 Cre;Ai32 mice were not used as breeders. Sst-IRES-Cre;Ai32 breeding sets (pairs and trios)  
761 consisted of heterozygous Sst-IRES-Cre mice crossed with either heterozygous or homozygous  
762 Ai32(RCL-ChR2(H134R)\_EYFP) mice. Vip-IRES-Cre;Ai32 breeding sets (pairs and trios)  
763 consisted of heterozygous Vip-IRES-Cre mice crossed with either heterozygous or homozygous  
764 Ai32(RCL-ChR2(H134R)\_EYFP) mice. Cre<sup>+</sup> cells from Ai32 lines are highly photosensitive,  
765 due to expression of Channelrhodopsin-2 (Zhang et al., 2006).

766 Following surgery, all mice were single-housed and maintained on a reverse 12-hour light cycle.  
767 All experiments were performed during the dark cycle. For passive viewing experiments,  
768 animals were given ad libitum access to food and water. For behavioral experiments, mice were  
769 given an amount of water required to maintain 85% of their initial body weight, with ad libitum  
770 access to food.

### 771 **2. Surgery**

772

#### 773 **2.1. Headframe design**

774 To enable co-registration across surgery, intrinsic signal imaging, and electrophysiology rigs,  
775 each animal was implanted with a 304 stainless steel headframe that provides access to the brain  
776 via a cranial window and permits head fixation in a reproducible configuration (de Vries et al.,  
777 2019). The cranial window angle was at 23 degrees of roll and 6 degrees of pitch, referenced to a  
778 plane passing through lambda and bregma and the mediolateral axis. Use of this headframe  
779 allowed the 5 mm craniotomy to be repeatably centered at  $x = -2.8$  mm and  $y = 1.3$  mm (origin  
780 at lambda).

781

782 The headframe was glued to a well made of black acrylic photopolymer that served four  
783 functions: (1) shielding the craniotomy and probes during the experiment, (2) providing a surface  
784 for precisely aligning the insertion window, (3) routing the animal ground to an exposed gold  
785 pin, and (4) holding threads for a plastic cap that protects the craniotomy before and after the  
786 experiment.

787

#### 788 **2.2. Surgical procedures**

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789 A pre-operative injection of dexamethasone (3.2 mg/kg, S.C.) was administered 3 h before  
790 surgery. Mice were initially anesthetized with 5% isoflurane (1-3 min) and placed in a  
791 stereotaxic frame (Model# 1900, Kopf), and isoflurane levels were maintained at 1.5-2.5% for  
792 surgery. Body temperature was maintained at 37.5°C. Carprofen was administered for pain  
793 management (5-10 mg/kg, S.C.). Atropine was administered to suppress bronchial secretions and  
794 regulate hearth rhythm (0.02-0.05 mg/kg, S.C.). An incision was made to remove skin, and the  
795 exposed skull was levelled with respect to pitch (bregma-lambda level), roll, and yaw. The  
796 headframe was placed on the skull and fixed in place with White C&B Metabond (Parkell). Once  
797 the Metabond was dry, the mouse was placed in a custom clamp to position the skull at a rotated  
798 angle of 20°, to facilitate creation of the craniotomy over visual cortex. A circular piece of skull 5  
799 mm in diameter was removed, and a durotomy was performed. The brain was covered by a 5 mm  
800 diameter circular glass coverslip, with a 1 mm lip extending over the intact skull. The bottom of  
801 the coverslip was coated with a layer of silicone to reduce adhesion to the brain surface. The  
802 coverslip was secured to the skull with Vetbond (Patterson Veterinary) (Goldey et al., 2014).  
803 Kwik-Cast (World Precision Instruments) was added around the coverslip to further seal the  
804 implant, and Metabond bridges between the coverslip and the headframe well were created to  
805 hold the Kwik-Cast in place. At the end of the procedure, but prior to recovery from anesthesia,  
806 the mouse was transferred to a photodocumentation station to capture a spatially registered  
807 image of the cranial window (**Supplementary Figure 1A**).

### 808 **2.3. Surgery quality control**

809 In cases of excessive bleeding or other complications, the surgical procedure was aborted, and  
810 the mouse was euthanized. Mice that completed surgery entered a 7-10 day recovery period that  
811 included regular checks for overall health, cranial window clarity, and brain health. If mice failed  
812 the first health check, they received another one the following week. Mice that exhibited signs of  
813 deteriorating health or damaged brain surface vasculature were not passed on to the next step.  
814 Out of 105 mice entering the surgery step, 4 were removed from the pipeline due to QC failures  
815 at this stage (**Supplementary Figure 2A**).

## 816 **3. Intrinsic Signal Imaging**

817 Intrinsic signal imaging (ISI) measures the hemodynamic response of the cortex to visual  
818 stimulation across the entire field of view. This technique can be used to obtain retinotopic maps  
819 representing the spatial relationship of the visual field (or, in this case, coordinate position on the  
820 stimulus monitor) to locations within each cortical area. This mapping procedure was used to  
821 delineate functionally defined visual area boundaries to enable targeting of Neuropixels probes to  
822 retinotopically defined locations in primary and secondary visual areas (Juavinett et al., 2017).

### 823 **3.1. Data acquisition**

824 Mice were lightly anesthetized with 1-1.4% isoflurane administered with a SomnoSuite (model  
825 #715; Kent Scientific) and vital signs were monitored with a PhysioSuite (model # PS-MSTAT-  
826 RT). Eye drops (Lacri99 Lube Lubricant Eye Ointment; Refresh) were applied to maintain  
827 hydration and clarity of eyes during anesthesia. Imaging sessions began with a vasculature image  
828 acquired under green illumination (527 nm LEDs; Cree Inc., C503B-GCN-CY0C0791). Next the  
829 imaging plane was defocused and the hemodynamic response to a visual stimulus was imaged

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830 under red light (635 nm LEDs; Avago Technologies, HLMP-EG08-Y2000) with an Andor Zyla  
831 5.5 10 tap sCMOS camera. The stimulus consisted of an alternating checkerboard pattern (20°  
832 wide bar, 25° square size) moving across a mean luminance gray background. On each trial, the  
833 stimulus bar was swept across the four cardinal axes 10 times in each direction at a rate of 0.1 Hz  
834 (Kalatsky and Stryker, 2003). Up to 10 trials were performed on each mouse.

### 835 **3.2. Data processing**

836 A minimum of three trials were averaged to produce altitude and azimuth phase maps, calculated  
837 from the discrete Fourier transform of each pixel. A “sign map” was produced from the phase  
838 maps by taking the sine of the angle between the altitude and azimuth map gradients. In the sign  
839 maps, each cortical visual area appears as a contiguous red or blue region (Garrett et al., 2014).  
840 These maps are used to confirm the cortical area identity of each probe insertion, using the  
841 vasculature as fiducial markers (**Figure 1C-D, Supplementary Figure 1B**).

842 The altitude and azimuth maps were also used to create a map of eccentricity from the center of  
843 visual space (the intersection of 0° altitude and 0° azimuth). Because the actual center of gaze  
844 will vary from mouse to mouse, the eccentricity map was shifted to align with the screen  
845 coordinates at the center of V1 (which maps to the center of the retina). This V1-aligned  
846 eccentricity map was used for probe targeting, to ensure that recorded neurons represent a  
847 consistent region on the retina, approximately at the center of the right visual hemifield.

### 848 **3.3. ISI quality control**

849 The quality control process for the ISI-derived maps included four distinct inspection steps:

850 3.3.1. The brain surface and vasculature images were inspected post-acquisition for clarity,  
851 focus, and position of the cranial window within the field of view.

852  
853 3.3.2. Individual trials were inspected for visual coverage range and continuity of phase  
854 maps, localization of the signal from the amplitude maps and stereotypical  
855 organization of sign maps. Only trials respecting these criteria were included in the  
856 final average, and a minimum of 3 trials were required.

857  
858 3.3.3. Visual area boundaries were delineated using automated segmentation, and maps  
859 were curated based on stringent criteria to ensure data quality. The automated  
860 segmentation and identification of a minimum of six visual areas including V1, LM,  
861 RL, AL, AM and PM was required. A maximum of three manual adjustments were  
862 permitted to compensate for algorithm inefficiency.

863  
864 3.3.4. Each processed retinotopic map was inspected for coverage range (35-60° altitude  
865 and 60-100° azimuth), bias (absolute value of the difference between max and min  
866 of altitude or azimuth range; <10°), alignment of the center of retinotopic  
867 eccentricity with the centroid of V1 (<15° apart), and the area size of V1 (>2.8 cm<sup>2</sup>).

868 If QC was not passed after the first round of ISI mapping, the procedure was repeated up to two  
869 more times to obtain a passing map. In addition to the QC procedures carried out on the ISI-



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870 derived maps, the vasculature images were also examined for the presence of white artifacts on  
871 the brain surface. White artifacts, an indicator of potential brain damage, were grounds for  
872 failing the mouse out of the pipeline. Out of 101 mice entering ISI, 9 did not pass onto  
873 habituation due to QC failures during this step (**Supplementary Figure 2B**).

## 874 **4. Habituation and Behavior Training**

875

### 876 **4.1. Habituation for passive viewing experiments**

877 Mice underwent two weeks of habituation in sound-attenuated training boxes containing a  
878 headframe holder, running wheel, and stimulus monitor (**Supplementary Figure 1C**). Each  
879 mouse was trained by the same operator throughout the 2-week period. During the first week, the  
880 operator gently handles the mice, introduces them to the running wheel, and head-fixes them  
881 with progressively longer durations each day. During the second week, mice run freely on the  
882 wheel and are exposed to visual stimuli for 10 to 50 min per day. The following week, mice  
883 undergo habituation sessions of 75 minutes and 100 minutes on the recording rig, in which they  
884 view a truncated version of the same stimulus that will be shown during the experiment.

### 885 **4.2. Behavior training**

886 A subset of mice were trained to perform a change detection task in which one of 8 natural  
887 images was continuously flashed (250 ms image presentation followed by 500 ms gray screen)  
888 and mice were rewarded for licking when the image identity changed (**Figure 5A**). The change  
889 detection task is described in detail by (Garrett et al., 2019). Briefly, for each trial the time of  
890 image change was drawn from an exponential distribution with a minimum of 5 image flashes  
891 (3.75 s) and a maximum of 11 flashes (8.25 s). Licking before the image change restarted the  
892 trial. Trials in which the mouse licked within 750 ms of image change were “hits,” while licks  
893 within 750 ms of non-change catch trials (occurring at the same distribution of times since the  
894 last change as change trials) were classified as false alarms (**Figure 5B**). Mice must perform the  
895 task with a  $d'$  above 1 and have at least 100 contingent (non-aborted) trials for 3 consecutive  
896 days prior to moving to the recording rig.

897

### 898 **4.3. Habituation quality control**

899 Upon completion of the second week of habituation, mice received an assessment of overall  
900 stress levels that reflected observations made by the trainer, including coat appearance,  
901 components of the mouse grimace scale, and overall body movements. Out of 92 mice entering  
902 habituation for passive viewing experiments, 2 did not pass on to the insertion window implant  
903 step (**Supplementary Figure 2C**)

## 904 **5. Insertion Window Implant**

905

### 906 **5.1. Window generation**

907 Following the completion of a successful ISI map, a custom insertion window was generated for  
908 each mouse. First, six insertion targets were manually drawn on the V1-aligned eccentricity map  
909 using a web-based annotation tool. Targets were positioned at the center of retinotopy of V1,

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910 LM, AL, AM, and PM; because the retinotopic center of RL often lies on the boundary between  
911 RL and S1 barrel cortex, the target location was adjusted to be closer to the geometric center of  
912 this area. The coordinates of each target were used to automatically generate the outlines of the  
913 insertion window, which was subsequently laser-cut out of 0.5 mm clear PETG plastic (Ponoko).  
914 When seated in the headframe well, the window facilitates access to the brain via holes over each  
915 of the six visual areas. A solidified agarose/ACSF mixture injected between the brain and the  
916 window stabilizes the brain during the recording.

### 917 **5.2. Surgical procedure**

918  
919 On the day of recording, the cranial coverslip was removed and replaced with an insertion  
920 window containing holes aligned to six cortical visual areas. First, the mouse was anesthetized  
921 with isoflurane (3%–5% induction and 1.5% maintenance, 100% O<sub>2</sub>) and eyes were protected  
922 with ocular lubricant (I Drop, VetPLUS). Body temperature was maintained at 37.5°C (TC-1000  
923 temperature controller, CWE, Incorporated). Metabond bridges were removed from the glass  
924 cranial window, followed by the sealing layer of Kwik-Cast. Using a 2 mm silicone suction cup,  
925 the cranial window was gently lifted to expose the brain. The insertion window was then placed  
926 in the headframe well and sealed with Metabond. An agarose mixture was injected underneath  
927 the window and allowed to solidify. The mixture consisted of 0.4 g high EEO Agarose (Sigma-  
928 Aldrich), 0.42 g Certified Low-Melt Agarose (Bio Rad), and 20.5 mL ACSF (135.0 mM NaCl,  
929 5.4 mM KCl, 1.0 mM MgCl<sub>2</sub>, 1.8 mM CaCl<sub>2</sub>, 5.0 mM HEPES). This mixture was optimized to  
930 be firm enough to stabilize the brain with minimal probe drift, but pliable enough to allow the  
931 probes to pass through without bending. A layer of silicone oil (30,000 cSt, Aldrich) was added  
932 over the holes in the insertion window to prevent the agarose from drying (**Supplementary**  
933 **Figure 1D**). A 3D-printed plastic cap was screwed into the headframe well to keep out cage  
934 debris. At the end of this procedure, mice were returned to their home cages for 1-2 hours.

935

### 936 **5.3. Insertion window implant quality control**

937 3 out of 90 mice did not pass through to the recording step due to procedure failures during  
938 insertion window implantation. These failures were caused by the headframe coming loose from  
939 the skull or excessive bleeding after cranial window removal, after which the mice were  
940 euthanized (**Supplementary Figure 2D**).

## 941 **6. Neuropixels Recordings**

942

### 943 **6.1. Probes**

944 All neural recordings were carried out with Neuropixels probes (Jun et al., 2017). Each probe  
945 contains 960 recording sites, a subset of 374 (“Neuropixels 3a”) or 383 (“Neuropixels 1.0”) of  
946 which can be configured for recording at any given time. The electrodes closest to the tip were  
947 always used, providing a maximum of 3.84 mm of tissue coverage. The sites are oriented in a  
948 checkerboard pattern on a 70 μm wide x 10 mm long shank. Neural signals are routed to an  
949 integrated base containing amplification, digitization, and multiplexing circuitry. The signals  
950 from each recording site are split in hardware into a spike band (30 kHz sampling rate, 500 Hz  
951 highpass filter) and an LFP band (2.5 kHz sampling rate, 1000 Hz lowpass filter). Due to their  
952 dense site configuration (20 μm vertical separation along the entire length of the shank), each

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953 probe has the capacity to record hundreds of neurons at the same time. Our goal was to insert 6  
954 probes/mouse. Overall, we achieved a penetration success of 5.7 probes/mouse, with failures due  
955 to dura regrowth, collisions with the protective cone or opto-tagging fiber optic cable, or probe  
956 breakage during manipulation.

957 The base of each probes contains 32 10-bit analog-to-digital converters (ADCs), each of which  
958 are connected to 12 spike-band channels and 12 LFP-band channels via multiplexers. A full  
959 cycle of digitization requires 156 samples: 12 samples from each of 12 spike-band channels, and  
960 1 sample from each of 12 LFP-band channels. Each ADC serves a contiguous bank of odd or  
961 even channels, so ADC 1 digitizes channels [1,3,5,...,23], ADC 2 digitizes channels [2,4,6,...,24],  
962 ADC 3 digitizes channels [25,27,29,...,47], etc. Because of the need for interleaved sampling,  
963 common-mode noise will be shared across all channels that are acquired simultaneously, e.g.  
964 [1,2,25,26,49,50,...,361,362].

### 965 **6.2. Experimental rig**

966 The experimental rig (**Figure 1B**) was designed to allow six Neuropixels probes to penetrate the  
967 brain approximately perpendicular to the surface of visual cortex. Each probe is mounted on a 3-  
968 axis micromanipulator (New Scale Technologies, Victor, NY), which are in turn mounted on a  
969 solid aluminum plate, known as the probe cartridge. The cartridge can be removed from the rig  
970 using a pair of pneumatic tool-changers, to facilitate probe replacement and maintenance.

### 971 **6.3. Workflow Sequencing Engine**

972 The experimental procedure was guided by a Work Sequencing Engine (WSE), a custom GUI  
973 written in Python. This software ensured that all experimental steps were carried out in the  
974 correct order, reducing trial-to-trial variability and optimizing operator efficiency. The GUI  
975 logged the operator ID, mouse ID, and session ID, and ensured that all hardware and software  
976 were properly configured. The WSE was also used to start and stop the visual stimulus, the body  
977 and eyetracking cameras, and Neuropixels data acquisition.

### 978 **6.4. Probe alignment**

979  
980 The tip of each probe was aligned to its associated opening in the insertion window using a  
981 coordinate transformation obtained via a prior calibration procedure. The XY locations of the six  
982 visual area targets were supplied by the Workflow Sequencing Engine (WSE), and these values  
983 were translated into XYZ coordinates for each 3-axis manipulator using a custom Python script.  
984 The operator then moved each probe into place with a joystick, with the probes fully retracted  
985 along the insertion axis.

### 986 **6.5. Application of CM-DiI**

987  
988  
989 CM-DiI (1 mM in ethanol; ThermoFisher Product #V22888) was used to localize probes during  
990 the *ex vivo* imaging step because its fluorescence is maintained after brain clearing, and it has a  
991 limited diffusion radius. The probes were coated with CM-DiI before each recording by  
992 immersing them one by one into a well filled with dye.  
993

994 **6.6. Head fixation**

995  
996 The mouse was placed on the running wheel and fixed to the headframe clamp with three set  
997 screws. Next, the plastic cap was removed from the headframe well and an aluminum cone with  
998 3D-printed wings was lowered to prevent the mouse's tail from contacting the probes. An IR  
999 dichroic mirror was placed in front the right eye to allow the eyetracking camera to operate  
1000 without interference from the visual stimulus. A black curtain was then lowered over the front of  
1001 the rig, placing the mice in complete darkness except for the visual stimulus monitor.  
1002

1003 **6.7. Grounding**

1004  
1005 A 32 AWG silver wire (A-M Systems) was cemented to the skull during the initial  
1006 headframe/cranial window surgery. This wire becomes electrically conductive with the brain  
1007 surface following the application of the ACSF/agarose mixture beneath the insertion window.  
1008 The wire was pre-soldered to a gold pin embedded in the headframe well, which mates with a  
1009 second gold pin on the protective cone. The cone pin was soldered to 22 AWG hook-up wire  
1010 (SparkFun Electronics), which was connected to both the behavior stage and the probe ground.  
1011 Prior to the experiment, the brain-to-probe ground path was checked using a multimeter.  
1012

1013 The reference connection on the Neuropixels probes was permanently soldered to ground using a  
1014 silver wire, and all recordings were made using an external reference configuration. The  
1015 headstage grounds (which are contiguous with the Neuropixels probe grounds) were connected  
1016 together with 36 AWG copper wire (Phoenix Wire). For Neuropixels 3a, two probes had a direct  
1017 path to animal ground, and the others were wired up serially. All probes were also connected to  
1018 the main ground via the data cable (a dual coaxial cable). For Neuropixels 1.0, all probes were  
1019 connected in parallel to animal ground, and were not connected to the main ground through the  
1020 data cable (a single twisted pair cable).  
1021

1022 **6.8. Probe insertion**

1023  
1024 The probe cartridge was initially held approximately 30 cm above the mouse. Once the mouse  
1025 was secured in the headframe, the cartridge was lowered so the probe tips were approximately  
1026 2.5 mm above the brain surface. The probes were then manually lowered one by one to the brain  
1027 surface until spikes were visible on the electrodes closest to the tip. After the probes penetrated  
1028 the brain to a depth of ~100 microns, they were inserted automatically at a rate of 200  $\mu\text{m}/\text{min}$   
1029 (total of 3.5 mm or less in the brain) to avoid damage caused by rapid insertion (Fiáth et al.,  
1030 2019). Once the probes reached their targets, they were allowed to settle for 5 to 10 min. Photo-  
1031 documentation was taken with the probes fully retracted, after the probes reached the brain  
1032 surface (**Supplementary Figure 1E**), and again after the probes were fully inserted.  
1033

1034 **6.9. Data acquisition and synchronization**

1035  
1036 Neuropixels data was acquired at 30 kHz (spike band) and 2.5 kHz (LFP band) using the Open  
1037 Ephys GUI (Siegle et al., 2017). Gain settings of 500x and 250x were used for the spike band  
1038 and LFP band, respectively. Each probe was either connected to a dedicated FPGA streaming  
1039 data over Ethernet (Neuropixels 3a) or a PXIe card inside a National Instruments chassis  
1040 (Neuropixels 1.0). Raw neural data was streamed to a compressed format for archiving which  
1041 was extracted prior to analysis.

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1042  
1043 Videos of the eye and body were acquired at 30 Hz. The angular velocity of the running wheel  
1044 was recorded at the time of each stimulus frame, at approximately 60 Hz. Synchronization  
1045 signals for each frame were acquired by a dedicated computer with a National Instruments card  
1046 acquiring digital inputs at 100 kHz, which was considered the master clock. A 32-bit digital  
1047 “barcode” was sent with an Arduino Uno (SparkFun DEV-11021) every 30 s to synchronize all  
1048 devices with the neural data. Each Neuropixels probe has an independent sample rate between  
1049 29,999.90 Hz and 30,000.31 Hz, making it necessary to align the samples offline to achieve  
1050 precise synchronization. The synchronization procedure used the first matching barcode between  
1051 each probe and the master clock to determine the clock offset, and the last matching barcode to  
1052 determine the clock scaling factor. If probe data acquisition was interrupted at any point during  
1053 the experiment, each contiguous chunk of data was aligned separately. Because one LFP band  
1054 sample was always acquired after every 12th spike band sample, these data streams could be  
1055 synchronized automatically once the spike band clock rate has been determined.

1056  
1057 To synchronize the visual stimulus to the master clock, a silicon photodiode (PDA36A,  
1058 Thorlabs) was placed on the stimulus monitor above a “sync square” that flips from black to  
1059 white every 60 frames. The analog photodiode signal was thresholded and recorded as a digital  
1060 event by the sync computer. Individual frame times were reconstructed by interpolating between  
1061 the photodiode on/off events.

### 1062 1063 **6.10. Stimulus monitor**

1064  
1065 Visual stimuli were generated using custom scripts based on PsychoPy (Peirce, 2007) and were  
1066 displayed using an ASUS PA248Q LCD monitor, with 1920 x 1200 pixels (21.93 in wide, 60 Hz  
1067 refresh rate). Stimuli were presented monocularly, and the monitor was positioned 15 cm from  
1068 the mouse’s right eye and spanned 120° x 95° of visual space prior to stimulus warping. Each  
1069 monitor was gamma corrected and had a mean luminance of 50 cd/m<sup>2</sup>. To account for the close  
1070 viewing angle of the mouse, a spherical warping was applied to all stimuli to ensure that the  
1071 apparent size, speed, and spatial frequency were constant across the monitor as seen from the  
1072 mouse’s perspective.

### 1073 1074 **6.11. Stimuli for passive viewing experiments**

1075  
1076 All experiments began with a receptive field mapping stimulus consisting of 2 Hz, 0.04 cycles  
1077 per degree drifting gratings with a 20° circular mask. These Gabor patches randomly appeared at  
1078 one of 81 locations on the screen (9 x 9 grid) for 250 ms at a time, with no blank interval. The  
1079 receptive field mapping stimulus was followed by a series of dark or light full-field flashes,  
1080 lasting 250 ms each and separated by a 2 second inter-trial interval.

1081  
1082 Next, mice were shown one of two possible stimulus sets. The first, called “Brain Observatory  
1083 1.1” is a concatenation of two sessions from the Two-Photon Imaging Brain Observatory (de  
1084 Vries et al., 2019) (**Supplementary Figure 6B**). Drifting gratings were shown with a spatial  
1085 frequency of 0.04 cycles/deg, 80% contrast, 8 directions (0°, 45°, 90°, 135°, 180°, 225°, 270°,  
1086 315°, clockwise from 0° = right-to-left) and 5 temporal frequencies (1, 2, 4, 8, and 15 Hz), with  
1087 15 repeats per condition. Static gratings were shown at 6 different orientations (0°, 30°, 60°, 90°,  
1088 120°, 150°, clockwise from 0° = vertical), 5 spatial frequencies (0.02, 0.04, 0.08, 0.16, 0.32  
1089 cycles/degree), and 4 phases (0, 0.25, 0.5, 0.75); they are presented for 0.25 seconds, with no

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1090 intervening gray period. The Natural Images stimulus consisted of 118 natural images taken from  
1091 the Berkeley Segmentation Dataset (Martin et al., 2001), the van Hateren Natural Image Dataset  
1092 (van Hateren and van der Schaaf, 1998), and the McGill Calibrated Colour Image Database  
1093 (Olmos and Kingdom, 2004). The images were presented in grayscale and were contrast  
1094 normalized and resized to 1174 x 918 pixels. The images were presented in a random order for  
1095 0.25 seconds each, with no intervening gray period. Two natural movie clips were taken from the  
1096 opening scene of the movie Touch of Evil (Welles, 1958). Natural Movie One was a 30 second  
1097 clips repeated 20 times (2 blocks of 10), while Natural Movie Three was a 120 second clip  
1098 repeated 10 times (2 blocks of 5). All clips were contrast normalized and were presented in  
1099 grayscale at 30 fps.

1100  
1101 The second stimulus set, called “Functional Connectivity,” consisted of a subset of the stimuli  
1102 from the Brain Observatory 1.1 set shown with a higher number of repeats (**Supplementary**  
1103 **Figure 6C**). Drifting gratings were presented at 4 directions and one temporal frequency (2 Hz)  
1104 with 75 repeats. A contrast-tuning stimulus consisting of drifting gratings at 4 directions (0°, 45°,  
1105 90°, 135°, clockwise from 0° = left-to-right) and 9 contrasts (0.01, 0.02, 0.04, 0.08, 0.13, 0.2,  
1106 0.35, 0.6, 1.0) was also shown. The Natural Movie One stimulus was presented a total of 60  
1107 times, with an additional 20 repeats of a temporally shuffled version. Last, a dot motion stimulus  
1108 consisting of approximately 200 1.5° radius white dots on a mean-luminance gray background  
1109 moving at one of 7 speeds (0°/s, 16°/s, 32°/s, 64°/s, 128°/s, 256°/s, 512°/s) in four different  
1110 directions (0°, 45°, 90°, 135°, clockwise from 0° = left-to-right) at 90% coherence was shown.

1111

### 1112 **6.12. Stimuli for behavioral experiments**

1113 Mice carried out one hour of a change detection task as described in (Garrett et al., 2019).  
1114 Following the behavior session, the lick spout was retracted and receptive field mapping stimuli  
1115 and full-field flashes were presented for 25 minutes, with the same parameters as those used in  
1116 the passive viewing experiments. All other aspects of the rig, including the running wheel,  
1117 stimulus monitor, and electrophysiological recordings were the same as for the passive viewing  
1118 experiments.

### 1119 **6.13. Probe removal and cleaning**

1120 Once the stimulus set was over, probes were retracted from the brain at a rate of 1 mm/s, after which the  
1121 probe cartridge was raised to its full height. The protective cap was screwed into the headframe well, then  
1122 mice were removed from head fixation and returned to their home cages overnight. Probes were  
1123 immersed in a well of 1% Tergazyme for ~24 hours, which was sufficient to remove tissue and silicone  
1124 oil prior to the next recording session.

### 1125 **6.14. Quality control for the Neuropixels recording session**

1126 Neuropixels recording sessions were subjected to the following QC criteria (**Supplementary**  
1127 **Figure 2E**):

1128 6.14.1. *Eye foam*. If white buildup around the eye obscures the pupil, the experiment is  
1129 cancelled and the session is failed (8 mice).

1130

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- 1131 6.14.2. *Bleeding*. If bleeding resulting from the window implant or the probe insertion  
1132 obscures the vasculature, the session is failed (4 mice).  
1133
- 1134 6.14.3. *Probe insertion*. If fewer than four probes successfully enter the brain, the session is  
1135 failed (1 mouse).  
1136
- 1137 6.14.4. *Dropped frames*. If the stimulus monitor photodiode measures >60 delayed frames,  
1138 the session is failed (1 mouse).  
1139
- 1140 6.14.5. *Missing files*. If any critical files are overwritten, the session is failed (2 mice).  
1141
- 1142 6.14.6. *Noise levels*. If high RMS noise levels in the spike band persist after median  
1143 subtraction, the session is failed (4 mice).  
1144
- 1145 6.14.7. *Probe drift*. If one or more probes exhibit >80 microns of drift over the course of the  
1146 experiment, the session is failed (6 mice). Typical drift levels are around 40 microns,  
1147 and drift levels are highly correlated across probes.
- 1148 In total, out of 87 mice entering the recording step, 61 passed session-level QC.

## 1149 **7. Ex Vivo Imaging**

### 1150 1151 **7.1. Tissue clearing**

1152 Mice were perfused with 4% paraformaldehyde (PFA) (after induction with 5% isoflurane and 1 L/min of  
1153 O<sub>2</sub>). The brains were preserved in 4% PFA, rinsed with 1x phosphate buffered saline (PBS) the next  
1154 morning, and stored at 4°C in PBS. Next, brains were run through a tissue clearing process based on the  
1155 iDISCO method (Renier et al., 2014). This procedure uses different solvents which dehydrate and  
1156 delipidate the tissue. The first day, the brains were immersed in different concentrations of methanol (20,  
1157 40, 60) for an hour each, then overnight in 80% methanol. On the second day, they were dipped into  
1158 100% methanol (twice for one hour) and then into a mixture of 1/3 methanol and 2/3 dichloromethane  
1159 overnight. On the third day, the brains were moved from pure dichloromethane (2 x 20 min) to pure  
1160 dibenzyl ether, where they remained for 2 days until clearing was complete (**Supplementary Figure**  
1161 **5A**).

### 1162 **7.2. Optical projection tomography (OPT)**

1163 Whole-brain 3D imaging was accomplished with optical projection tomography (OPT) (Nguyen et al.,  
1164 2017; Sharpe, 2002; Wong et al., 2013). The OPT instrument consisted of collimated light sources for  
1165 transmitted illumination (on-axis white LED, Thorlabs MNWHL4 with Thorlabs SM2F32-A lens and  
1166 Thorlabs DG20-600 diffuser) or fluorescence excitation (off-axis Thorlabs M530L3, with Thorlabs  
1167 ACL2520U-DG6-A lens and Chroma ET535/70m-2P diffuser), a 0.5x telecentric lens (Edmund Optics  
1168 62-932) with emission filter (575 nm LP, Edmund Optics 64-635), and a camera (IDS UI-3280CP). The  
1169 specimen was mounted on a rotating magnetic chuck attached to a stepper motor, which positioned the  
1170 specimen on the optical axis and within a glass cuvette filled with dibenzyl ether. The stepper motor and  
1171 illumination triggering were controlled with an Arduino Uno (SparkFun DEV-11021) and custom shield

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1172 including a Big Easy Driver (SparkFun ROB-12859). Instrument communication and image capture was  
1173 accomplished with MicroManager (Edelstein et al., 2014).

1174 A series of 400 images were captured with transmitted LED illumination with each image captured with  
1175 the specimen rotated 0.9 degrees relative to the previous position. This series of 400 images was repeated  
1176 with the fluorescence excitation LED. Each channel was stored as a separate OME-TIFF dataset before  
1177 extracting individual planes and metadata required for reconstruction using a custom Python script  
1178 (**Supplementary Figure 5B**).

1179 Isotropic 3D volumes were reconstructed from these projection images using NRecon (Bruker). The  
1180 rotation axis offset and region-of-interest bounds were set for each image series pair using the transmitted  
1181 channel dataset, then the same values applied to the fluorescence channel dataset. A smoothing level of 3  
1182 using a Gaussian kernel was applied to all images. Reconstructions were exported as single-plane 16-bit  
1183 TIFF images taken along the rotation axis with final voxel size of 7.9  $\mu\text{m}$  per side (**Supplementary**  
1184 **Figure 5C**).

### 1185 **7.3. Registering Probes to the Common Coordinate Framework**

1186 Reconstructed brains were downsampled to 10  $\mu\text{m}$  per voxel and roughly aligned to the Allen Institute  
1187 Common Coordinate Framework (CCFv3) template brain using an affine transform. The volume was  
1188 then cropped to a size of 1023 x 1024 x 1024 and converted to Drishti format  
1189 (<http://sf.anu.edu.au/Vizlab/drishti>). Next, 6-54 registration points were marked in up to 14 coronal slices  
1190 of the individual brain by comparing to the CCFv3 template brain, obtained from (Shamash et al., 2018)  
1191 (**Supplementary Figure 5D**). Fluorescent probe tracks were manually labeled in coronal slices of the  
1192 individual brain, and the best-fit line was found using singular value decomposition (**Supplementary**  
1193 **Figure 5E**). The registration points were used to define a 3D nonlinear transform (VTK  
1194 thinPlateSplineTransform), which was used translate each point along the probe track into the CCFv3  
1195 coordinate space. Each CCFv3 coordinate corresponds to a unique brain region, identified by its structure  
1196 acronym (e.g., CA3, LP, VISp, etc.). A list of CCFv3 structure acronyms along each track was compared  
1197 to the physiological features measured by each probe (e.g., unit density, LFP theta power;  
1198 **Supplementary Figure 5F**). The location of major structural boundaries were manually adjusted to align  
1199 the CCFv3 labels with the physiology data, and each recording channel (and its associated units) was  
1200 assigned to a unique CCFv3 structure (**Supplementary Figure 5G**). White matter structures were not  
1201 included; any units mapped to a white matter structure inherited the gray matter structure label that was  
1202 immediately ventral along the probe axis.

### 1203 **7.4. Identification of cortical visual area targets**

1204  
1205 To confirm the identity of the cortical visual areas, images of the probes taken during the  
1206 experiment were compared to images of the brain surface vasculature taken during the ISI  
1207 session. Vasculature patterns were used to overlay the visual area map on an image of the brain  
1208 surface with the probes inserted. When done in custom software, key points were selected along  
1209 the vasculature on both images and a perspective transform (OpenCV) was performed to warp  
1210 the insertion image to the retinotopic map. When done manually, the overlap of both images was  
1211 done in Photoshop or Illustrator (Adobe Suite). In both cases, the probe entry points were  
1212 manually annotated. Finally, an area was assigned to each probe. Overall, successful targeting of  
1213 the 6 target visual areas occurred at the following rates: 89% for AM, 72% for PM, 98% for V1,



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1214 85% for LM, 79% for AL, and 90% for RL. A small subset of penetrations were mapped to LI,  
1215 MMA, or MMP (Zhuang et al., 2017). Penetration points that could not be unambiguously  
1216 associated with a particular visual area were classified as “VIS.” If the cortical area label  
1217 obtained via CCFv3 registration did not match the area identified in the insertion image overlay,  
1218 the insertion image overlay took precedence.

1219

### 1220 **7.5. *Ex vivo* imaging quality control**

#### 1221 **7.6.**

1222 QC was performed on a probe-by-probe, rather than a mouse-by-mouse basis. Some probes were  
1223 not visible in the OPT images due to faint CM-DiI signal or reconstruction artifacts caused by air  
1224 bubbles in the tissue (**Supplementary Figure 2F**). In total, 284 out of 332 probes were mapped  
1225 to the CCFv3. Probes that failed the *ex vivo* imaging step were not excluded from further  
1226 analysis, but only included structure labels for channels in cortex (with the bottom of cortex  
1227 identified based on the drop in unit density between cortex and hippocampus).

## 1228 **8. Spike Sorting**

1229

### 1230 **8.1. Data pre-processing**

1231

1232 Data was written to disk in a format containing the original 10-bit samples from each ADC.  
1233 These files were backed up to a tape drive, then extracted to a new set of files that represent each  
1234 sample as a 16-bit integer, scaled to account for the gain settings on each channel. Separate data  
1235 files were generated for the LFP band and spike band, along with additional files containing the  
1236 times of synchronization events. The extracted files consume approximately 36% more disk  
1237 space than the originals.

1238

1239 Prior to spike sorting, the spike-band data passed through 4 steps: offset removal, median  
1240 subtraction, filtering, and whitening. First, the median value of each channel was subtracted to  
1241 center the signals around zero. Next, the median across channels was subtracted to remove  
1242 common-mode noise. While Neuropixels have been measured to have a spike-band RMS noise  
1243 levels of 5.1  $\mu\text{V}$  in saline (Jun et al., 2017), this cannot be achieved in practice when recording in  
1244 vivo. The signals become contaminated by background noise in neural tissue; movement artifacts  
1245 associated with animal locomotion, whisking, and grooming; and electrical noise introduced by  
1246 the additional wiring required to support multiple probes on one rig. To remove noise sources  
1247 that are shared across channels, the median was calculated across channels that are sampled  
1248 simultaneously, leaving out adjacent (even/odd) channels that are likely measuring the same  
1249 spike waveforms, as well as reference channels that contain no signal. For each sample, the  
1250 median value of channels N:24:384, where  $N = [1, 2, 3, \dots, 24]$ , was calculated, and this value was  
1251 subtracted from the same set of channels. This method rejects high-frequency noise more  
1252 effectively than subtracting the median of all channels, at the cost of leaving a residual of  $\sim 2 \mu\text{V}$   
1253 for large spikes, visible in the mean waveforms. Given that this value is well below the RMS  
1254 noise level of the Neuropixels probes under ideal conditions, it should not affect spike sorting.  
1255 The original data is over-written with the median-subtracted version, with the median value of  
1256 each block of 16 channels saved separately, to allow reconstruction of the original signal if  
1257 necessary. The median-subtracted data file is sent to the Kilosort2 Matlab package  
1258 (<https://github.com/mouseland/kilosort2>, commit 2fba667359dbdabb0e52e67fa848f197e44cf5ef,

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1259 April 8, 2019), which applies a 150 Hz high-pass filter, followed by whitening in blocks of 32  
1260 channels. The filtered, whitened data is saved to a separate file for the spike sorting step.

1261

### 1262 **8.2. Kilosort2**

1263

1264 Kilosort2 was used to identify spike times and assign spikes to individual units (Stringer et al.,  
1265 2019). Traditional spike sorting techniques extract snippets of the original signal and perform a  
1266 clustering operation after projecting these snippets into a lower-dimensional feature space. In  
1267 contrast, Kilosort2 attempts to model the complete dataset as a sum of spike "templates." The  
1268 shape and locations of each template is iteratively refined until the data can be accurately  
1269 reconstructed from a set of  $N$  templates at  $M$  spike times, with each individual template scaled by  
1270 an amplitude,  $a$ . A critical feature of Kilosort2 is that it allows templates to change their shape  
1271 over time, to account for the motion of neurons relative to the probe over the course of the  
1272 experiment. Stabilizing the brain using an agarose-filled plastic window has virtually eliminated  
1273 probe motion associated with animal running, but slow drift of the probe over ~3-hour  
1274 experiments is still observed. Kilosort2 is able to accurately track units as they move along the  
1275 probe axis, eliminating the need for the manual merging step that was required with the original  
1276 version of Kilosort (Pachitariu et al., 2016). The spike-sorting step runs in approximately real  
1277 time (~3 hours per session) using a dual-processor Intel 4-core, 2.6 GHz workstation with an  
1278 NVIDIA GTX 1070 GPU.

1279

### 1280 **8.3. Removing putative double-counted spikes**

1281

1282 The Kilosort2 algorithm will occasionally fit a template to the residual left behind after another  
1283 template has been subtracted from the original data, resulting in double-counted spikes. This can  
1284 create the appearance of an artificially high number of ISI violations for one unit or artificially  
1285 high zero-time-lag synchrony between nearby units. To eliminate the possibility that this  
1286 artificial synchrony will contaminate data analysis, the outputs of Kilosort2 are post-processed to  
1287 remove spikes with peak times within 5 samples (0.16 ms) and peak waveforms within 5  
1288 channels (~50 microns). This process removes >10 within-unit overlapping spikes from  $2.5 \pm$   
1289  $1.8\%$  of units per session. It removes  $2.05 \pm 0.65\%$  of spikes in total, after accounting for  
1290 between-unit overlapping spikes.

1291

### 1292 **8.4. Removing units with artifactual waveforms**

1293

1294 Kilosort2 generates templates of a fixed length (2 ms) that matches the time course of an  
1295 extracellularly detected spike waveform. However, there are no constraints on template shape,  
1296 which means that the algorithm often fits templates to voltage fluctuations with characteristics  
1297 that could not physically result from the current flow associated with an action potential. The  
1298 units associated with these templates are considered "noise," and are automatically filtered out  
1299 based on 3 criteria: spread (single channel, or >25 channels), shape (no peak and trough, based  
1300 on wavelet decomposition), or multiple spatial peaks (waveforms are non-localized along the  
1301 probe axis). The automated algorithm removed 94% of noise units, or 26% of total units. A final  
1302 manual inspection step was used to remove an additional 2140 noise units across all experiments  
1303 (**Supplementary Figure 3**).

1304

### 1305 **8.5. Spike sorting quality control**

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1306 All units not classified as noise are packaged into Neurodata Without Borders (NWB) files for  
1307 potential further analysis. Because different analyses may require different quality thresholds for  
1308 defining inclusion criteria, we calculate a variety of metrics that can be used to filter units. These  
1309 metrics are based on both the physical characteristics of the units' waveforms, or their isolation  
1310 with respect to other units from the same recording (**Supplementary Figure 4A**).

1311 8.5.1. *Firing rate*:  $N/T$ , where  $N$  = number of spikes in the complete session and  $T$  = total  
1312 time of the recording session in seconds.

1313  
1314 8.5.2. *Presence ratio*: The session was divided into 100 equal-sized blocks; the presence  
1315 ratio is defined as the fraction of blocks that include 1 or more spikes from a  
1316 particular unit. Units with a low presence ratio are likely to have drifted out of the  
1317 recording, or could not be tracked by Kilosort2 for the duration of the experiment.

1318  
1319 8.5.3. *Maximum drift*: To compute the maximum drift for one unit, the peak channel was  
1320 calculated from the top principal components of every spike. Next, the peak channel  
1321 values are binned in 51 s intervals, and the median value is calculated across all  
1322 spikes in each bin (assuming at least 10 spikes per bin). The maximum drift is  
1323 defined as the difference between the maximum peak channel and the minimum  
1324 peak channel across all bins. The average maximum drift across all units is used to  
1325 identify sessions with a high amount of probe motion relative to the brain.

1326  
1327 8.5.4. *Waveform amplitude*: The difference (in microvolts) between the peak and trough of  
1328 the waveform on a single channel.

1329  
1330 8.5.5. *Waveform spread*: Spatial extent (in microns) of channels where the waveform  
1331 amplitude exceeds 12% of the peak amplitude.

1332  
1333 8.5.6. *Waveform duration*: Difference (in ms) of the time of the waveform peak and trough  
1334 on the channel with maximum amplitude.

1335  
1336 8.5.7. *Inter-spike-interval (ISI) violations*: This metric searches for refractory period  
1337 violations that indicate a unit contains spikes from multiple neurons. The ISI  
1338 violations metric represents the relative firing rate of contaminating spikes. It is  
1339 calculated by counting the number of violations  $<1.5$  ms, dividing by the amount of  
1340 time for potential violations surrounding each spike, and normalizing by the overall  
1341 spike rate. It is always positive (or 0), but has no upper bound. See (Hill et al., 2011)  
1342 for more details.

1343  
1344 8.5.8. *Signal-to-noise ratio (SNR)*: After selecting 1000 individual spike waveforms on the  
1345 channel with maximum amplitude, the mean waveform on that channel was  
1346 subtracted. SNR is defined the ratio between the waveform amplitude and 2x the  
1347 standard deviation of the residual waveforms (Suner et al., 2005). Because this  
1348 definition of SNR assumes that waveforms remain stable over time, changes in a  
1349 unit's waveform as a result of probe motion will cause this metric to be inaccurate.  
1350 In addition, because it is only calculated for the peak channel, this metric does not  
1351 necessarily reflect the overall isolation quality of a unit when taking into account all

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1352 available information.

1353

1354 8.5.9. *Isolation distance*: The square of the Mahalanobis distance required to find the same  
1355 number of “other” spikes as the total number of spikes for the unit in principal  
1356 component space (Schmitzer-Torbert et al., 2005). Similarly to SNR, isolation  
1357 distance is not tolerant to electrode drift, and changes in waveform shape over time  
1358 can reduce the isolation distance calculated over the entire session.

1359

1360 8.5.10. *d'*: Linear discriminant analysis is used to find the line of maximum separation in PC  
1361 space. *d'* indicates the separability of the unit of interest from all other units. See  
1362 (Hill et al., 2011) for more information. This metric is not tolerant to electrode drift,  
1363 and changes in waveform shape over time can reduce the value of *d'* calculated over  
1364 the entire session.

1365

1366 8.5.11. *Amplitude cutoff*: This metric provides an approximation of a unit’s false negative  
1367 rate. First, a histogram of spike amplitudes is created, and the height of the  
1368 histogram at the minimum amplitude is extracted. The percentage of spikes above  
1369 the equivalent amplitude on the opposite side of the histogram peak is then  
1370 calculated. If the minimum amplitude is equivalent to the histogram peak, the  
1371 amplitude cutoff is set to 0.5 (indicating a high likelihood that >50% of spikes are  
1372 missing). This metric assumes a symmetrical distribution of amplitudes and no drift,  
1373 so it will not necessarily reflect the true false negative rate.

1374

1375 8.5.12. *Nearest neighbors hit rate*: For each spike belonging to the unit of interest, the four  
1376 nearest spikes in principal-component space are identified. The “hit rate” is defined  
1377 as the fraction of these spikes that belong to the unit of interest. This metric is based  
1378 on the “isolation” metric from (Chung et al., 2017). Again, electrode drift that alters  
1379 waveform shape can negatively impact this metric without necessarily changing the  
1380 isolation quality of a unit at any given timepoint.

1381 Filtering of units based on quality metrics and other criteria is illustrated in **Supplementary**  
1382 **Figure 4B**.

## 1383 **9. Data analysis**

1384

### 1385 **9.1. Receptive field analysis**

1386 The receptive field for one unit is defined as the 2D histogram of spike counts at each of 81  
1387 locations of the Gabor stimulus (9 x 9 pixels, 10° separation between pixel centers,  
1388 **Supplementary Figure 7A**).

1389 A chi-square test for independence was used to assess the presence of a significant receptive  
1390 field. A chi-square test statistic was computed  $\chi^2 = \sum_{i=0}^n \frac{(E_i - O_i)^2}{E_i}$ , where  $O_i = \frac{1}{m_i} \sum_{j=0}^{m_i} R_{i,j}$  is the  
1391 observed average response (R) of the unit over m presentations of the Gabor stimulus at location  
1392 i, and  $E_i = \frac{\sum_i^n \sum_j^{m_i} R_{i,j}}{\sum_i^n m_i}$  is the expected (grand average) response per stimulus presentation. A P-

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1393 value was then calculated for each unit by comparing the test statistic against a null distribution  
1394 of 1,000 test statistics, each computed from the unit's responses after shuffling the locations  
1395 across all presentations (**Supplementary Figure 7B**).

1396 To compute the receptive field area and center location, each receptive field was first smoothed  
1397 using a Gaussian filter ( $\sigma = 1.0$ ). The smoothed receptive field was thresholded at  $\max(\text{RF})$   
1398  $- \text{std}(\text{RF})$ , a value that provided good agreement with the qualitative receptive field boundaries.  
1399 The receptive field center location was calculated based on the center of mass of the largest  
1400 contiguous area above threshold, and its area was equivalent to its pixel-wise area multiplied by  
1401  $100 \text{ degrees}^2$  (**Supplementary Figure 7C**).

### 1402 **9.2. Cross-correlation analysis**

1403 To measure the functional interactions between pairs of units, cross-correlograms (CCGs) were  
1404 used (Gerstein and Perkel, 1972; Jia et al., 2013; Smith and Kohn, 2008). The CCG is defined as:

$$1405 \text{ CCG}(\tau) = \frac{\frac{1}{M} \sum_{i=1}^M \sum_{t=1}^N x_1^i(t) x_2^i(t+\tau)}{\theta(\tau) \sqrt{\lambda_1 \lambda_2}}$$

1406 where  $M$  is the number of trials,  $N$  is the number of bins in the trial,  $x_1^i$  and  $x_2^i$  are the spike trains  
1407 of the two units on trial  $i$ ,  $\tau$  is the time lag relative to reference spikes, and  $\lambda_1$  and  $\lambda_2$  are the  
1408 mean firing rates of the two units. The CCG is essentially a sliding dot product between two  
1409 spike trains.  $\theta(\tau)$  is the triangular function which corrects for the overlap time bins caused by the  
1410 sliding window. To correct for firing rate dependency, we normalized the CCG by the geometric  
1411 mean spike rate. An individually normalized CCG is computed separately for each drifting  
1412 grating orientation and averaged across orientation to obtain the CCG for each pair of units.

1413 A jitter correction method (Harrison and Geman, 2009; Smith and Kohn, 2008) was used to  
1414 remove stimulus-locked correlations and slow temporal correlations from the original CCG.

$$1415 \text{ CCG}_{\text{jitter\_corrected}} = \text{CCG}_{\text{original}} - \text{CCG}_{\text{jittered}}$$

1416 The jitter corrected CCG is created by subtracting the CCG calculated from a jittered spike train  
1417 where spike times within a small time window are randomly shuffled across trials within that  
1418 window. For our measurement, a 25 ms jitter window was chosen based on previous studies (Jia  
1419 et al., 2013; Zandvakili and Kohn, 2015).

1420 The jitter-corrected CCG was deemed to be significant sharp peak if the CCG peak occurred  
1421 within a 10 ms time lag and the magnitude of CCG peak was at least 7-fold larger than the  
1422 standard deviation of the CCG flanks ( $\pm 50$  to 100 ms).

1423 A Wilcoxon rank sum test was used to compare the distribution of CCG peak offsets between  
1424 neighboring areas (defined by the anatomical hierarchical score) and the distribution of CCG  
1425 peak offset within an area. The significance test was performed within each mouse, and the  $P$ -  
1426 values were combined across 25 mice using Fisher's method. V1-LM vs. V1-V1,  $P = 0$ ; LM-  
1427 RL vs. LM-RL,  $P = 1.9\text{e-}5$ ; RL-AL vs. RL-RL,  $P = 2.4\text{e-}5$ ; AL-PM vs. AL-AL,  $P = 0.081$ ;

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1428 PM–AM vs. PM–PM,  $P = 3.2e-4$ . All between-area distributions are significantly different from  
1429 the within-area distributions at the 5% confidence level, except for AL–PM.

### 1430 **9.3. Response latency**

1431 Response latency is calculated as the time to first spike (TTF). TTF is estimated in each trial by  
1432 looking for the time of first spike 30 ms after stimulus onset. If no spike is detected within 250  
1433 ms after stimulus onset, that trial is not included. The overall latency for each unit is defined as  
1434 the median TTF across trials.

### 1435 **9.4. Modulation index**

1436 The stimulus modulation index reflects how spiking activity of each unit is modulated by the  
1437 temporal frequency of the drifting grating stimulus (Matteucci et al., 2019; Wypych et al., 2012).  
1438 It is defined as:

1439 
$$\left| \frac{PS(f_{\text{pref}}) - \langle PS \rangle_f}{\sqrt{\langle PS^2 \rangle_f - \langle PS \rangle_f^2}} \right|$$

1440 where PS indicates the power spectral density of the peristimulus time histogram (PSTH), and  
1441 denotes the averaged power over all frequencies;  $f_{\text{pref}}$  is the preferred temporal frequency of the  
1442 unit. This metric quantifies the difference between spiking response power at each unit's  
1443 preferred frequency and the total power. The power spectrum was computed using Welch's  
1444 method on the 10 ms-binned PSTH for each unit's preferred condition.

### 1445 **9.5. Autocorrelation timescale**

1446 We calculated the autocorrelation for each unit during the 250 ms presentation period of the full-  
1447 field flash stimulus. We estimated autocorrelation timescale in each mouse by calculating a mean  
1448 of autocorrelation across units within each area, and then fitting an exponential decay to estimate  
1449 the timescale.

### 1450 **9.6. Analysis of neural responses during the change detection task**

1451 For each unit, spike density functions (SDFs) were calculated by convolving spike times relative  
1452 to each image change or the image flash preceding image change (“pre-change”) with a causal  
1453 exponential filter (decay time constant = 5 ms). The firing rate during a baseline window 250 ms  
1454 immediately preceding each image change or pre-change flash was subtracted from each SDF.  
1455 Mean SDFs were then calculated by averaging across all image change or pre-change flashes.  
1456 Units were included in further analysis if their mean firing rate was greater than 0.1 spikes/s and  
1457 the peak of the mean SDF (during a response window from 30 to 280 ms following image  
1458 change) was greater than 5 times the standard deviation of the mean SDF during the baseline  
1459 window.

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1460 Responses to image change and pre-change were calculated as the mean baseline-subtracted  
1461 firing rate during the response window. We defined the change modulation index for each unit as  
1462 the difference between the mean response to image change and pre-change divided by their sum  
1463 (**Figure 5D**).

### 1464 **9.7. Eye and pupil tracking**

1465  
1466 A single, universal eye tracking model was trained in DeepLabCut (Mathis et al., 2018), a  
1467 ResNET-50 based network, to recognize up to twelve tracking points each around the perimeter  
1468 of the eye, the pupil, and the corneal reflection. A published numerical routine (Halir and  
1469 Flusser, 1998) was used to fit ellipses to each set of tracking points. For each ellipse, the  
1470 following parameters were calculated: center coordinates, half-axes, and rotation angle. Fits were  
1471 performed on each frame if there at least six tracked points and a confidence of  $l > 0.8$  as  
1472 reported by the output of DeepLabCut. For frame where there were less than 6 tracked points  
1473 above the confidence threshold, the ellipse parameters were set to not-a-number (NaN).

1474 The training data set contained two sources of hand-annotated data: (1) 3 frames from each of 40  
1475 randomly selected movies. On each frame, 8 points from were annotated around the eye and  
1476 pupil. The center of the corneal reflection was annotated with a single point. (2) 4150 frames  
1477 with the pupil and corneal reflections annotated with ellipses.

### 1478 **9.8. Anatomical hierarchy analysis**

1479  
1480 A detailed description of the unsupervised construction of a data-driven anatomical hierarchy is  
1481 available in (Harris et al., 2019). Here we provide a summary of how the anatomical hierarchy of  
1482 the six visual cortical areas (V1, LM, AL, RL, PM, AM) and two thalamic nuclei (LGN, LP) was  
1483 constructed based on the anatomical connectivity. Specifically, the anatomical hierarchy was  
1484 uncovered based on cortical lamination patterns of the structural connections among the cortical  
1485 and thalamic regions of interest, obtained from Cre-dependent viral tracing experiments.

1486 To classify laminar patterns of cortico-cortical (CC) and thalamo-cortical (TC) connections and  
1487 to assign a direction to each cluster of laminar patterns, we used a large-scale dataset on cell  
1488 class-specific connectivity among all 37 cortical areas and 24 thalamic nuclei defined using 15  
1489 Cre driver transgenic lines (849 cortical and 81 thalamic experiments; 7063 unique source-target-  
1490 Cre line combinations), available in Harris et al (2019). For each transgenic line, the strength and  
1491 layer termination pattern of the connections were quantified based on *relative layer density*, the  
1492 fraction of the total projection signal in each layer scaled by the relative layer volumes in that  
1493 target. For the connections above a threshold ( $10^{-1.5}$ ), unsupervised clustering of the layer  
1494 termination patterns was performed, yielding nine clusters of distinct cortical layer termination  
1495 patterns of CC and TC connections. See Figure 5A,B of Harris et al (2019) for a schematic of the  
1496 nine types of cortical target lamination patterns.

1497 Following the classification of the nine clusters of the laminar patterns, an unsupervised method  
1498 was employed to simultaneously assign a direction to a cluster type and to construct a hierarchy  
1499 by maximizing the self-consistency of the obtained hierarchy. The mapping function  $M_{CC}$  maps a  
1500 type of CC connection cluster ( $C_{T_{i,j}} \in \{1, \dots, 9\}$ , where  $C_{T_{i,j}}$  denotes the layer termination pattern  
1501 of the connection from area  $j$  to area  $i$  for Cre-line  $T$ ) to either feedforward ( $M_{CC} = 1$ ) or

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1502 feedback ( $M_{CC} = -1$ ) type, i.e.,  $M_{CC}: \{1, \dots, 9\} \rightarrow \{-1, 1\}$ . Similarly, the mapping function  $M_{TC}$  of  
 1503 the thalamocortical layer termination types to either direction is defined as  $M_{TC}: \{1, \dots, 9\} \rightarrow$   
 1504  $\{-1, 1\}$ . By constructing the hierarchy of all 37 cortical areas and 24 thalamic nuclei, Harris et al  
 1505 (2019) found the optimal mapping function that maximizes the self-consistency measured by the  
 1506 *global hierarchy score* (Refer to Eq 5 and Eq 10 of Harris et al (2019) to see how the global  
 1507 hierarchy score was defined for CC and TC connections, respectively.). Specifically, the optimal  
 1508 mapping for CC connections assigns connections of cluster 2, 6, and 9 to one direction (feedback)  
 1509 and 1, 3, 4, 5, 7, and 8 to the opposite direction (feedforward). For TC connections, the most self-  
 1510 consistent hierarchy that maximizes the global hierarchy score is obtained when connections of  
 1511 cluster 2 and 6 correspond to feedback and the rest to feedforward patterns (Figure 6A of Harris  
 1512 et al (2019)).

1513 With these mapping functions  $M_{CC}$  and  $M_{TC}$  obtained from the construction of the all-area  
 1514 hierarchy (Figure 6A of Harris et al (2019)), the hierarchical organization of the six visual  
 1515 cortical areas (V1, LM, AL, RL, PM, AM) and the two thalamic nuclei (LGN, LP) was  
 1516 constructed using only the connections among these 8 regions. We first uncovered the cortical  
 1517 hierarchy using the intra-cortical connections among the six cortical areas: V1, LM, AL, RL,  
 1518 PM, and AM (240 unique “source-target-Cre line” combinations). The initial hierarchical  
 1519 position of a cortical area is defined as:

$$1520 \quad H_i^0 = \frac{1}{2} \left( \langle M_{CC} (C_{T_{i,j}}) \cdot \text{conf}(T) \rangle_j - \langle M_{CC} (C_{T_{j,i}}) \cdot \text{conf}(T) \rangle_j \right), \quad (1)$$

1521 where the first term describes the average direction of connections to area  $i$ , and thus represents  
 1522 the hierarchical position of the area as a target. The second term on the other hand, represents the  
 1523 average direction of connections from area  $i$ , depicting the hierarchical position of the area as a  
 1524 source. To account for the Cre-line-specific bias, the Cre-dependent confidence measure,  
 1525  $\text{conf}(T) = 1 - | \langle M_{CC}(C_{T_{i,j}}) \rangle_{i,j} |$  is included. The initial hierarchy score ( $H_i^0$ ) of each area  $i$  then  
 1526 is iterated using a two-step iterative scheme until the fixed point is reached:

$$1527 \quad H_i^{n-1/2} = \frac{1}{2} \left\{ \langle H_j^{n-1} + M_{CC} (C_{T_{i,j}}) \rangle_j - \langle -H_j^{n-1} + M_{CC} (C_{T_{j,i}}) \rangle_j \right\} \quad (2-1)$$

$$1528 \quad H_i^n = H_i^{n-1/2} - \langle H_j^{n-1/2} \rangle_j \quad (2-2)$$

1529 where  $n$  refers to iterative steps.

1530 After hierarchical positions of cortical areas are found based on CC connections, the hierarchical  
 1531 positions of LGN and LP relative to the cortical areas were computed by including TC  
 1532 connections from LGN and LP to the six visual cortical areas (25 unique “source-target-Cre line”  
 1533 combinations). Since thalamic areas are always the source in TC connections, the initial  
 1534 hierarchy score of each thalamic area  $i$  is defined by the average direction of connections from  
 1535 the area:

$$1536 \quad H_i^0 = - \langle M_{TC} (C_{T_{j,i}}) \cdot \frac{\min(N_{ff}, N_{fb})}{N_{ff} + N_{fb}} \rangle_j \quad (3)$$

1537 The parameters  $N_{ff}$  and  $N_{fb}$  refer to the numbers of feedforward and feedback thalamocortical  
 1538 connections, respectively. Once the initial positions of the thalamic areas in the hierarchy are  
 1539 obtained using Eq 3, hierarchy scores of thalamic and cortical areas are iterated until the fixed  
 1540 points are reached, using a full mapping function  $M_{CC+TC}$  that combines  $M_{CC}$  and  $M_{TC}$ , as done  
 1541 with the cortical hierarchy based on CC connections only (Eq 2).



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1542 To test the significance of the hierarchy levels of these areas, we generated 100 sampled  
1543 connectivity data of the same size via bootstrapping, and computed the hierarchy scores of the  
1544 eight regions using the bootstrapped connectivity data. We performed Wilcoxon paired signed  
1545 rank sum tests on these scores, showing that hierarchy levels of LM and RL cannot be  
1546 meaningfully distinguished ( $P = 0.08$ ) but the rest of the areas are at significantly distinct  
1547 hierarchical positions, with the 5% confidence level.

### 1548 **9.9. Other statistical methods**

1549 To quantify the correlation between the mean value of each metric and the anatomical hierarchy  
1550 score, both the Pearson correlation coefficient (`scipy.stats.pearsonr`) and Spearman's rank  
1551 correlation coefficient (`scipy.stats.spearmanr`) were used.

1552  
1553 To test for significant differences between pairs of areas, a Wilcoxon rank-sum statistic was  
1554 used (`scipy.stats.ranksum`). For time to first spike, receptive field size, modulation index, and  
1555 firing rate, each unit was considered an independent sample. For autocorrelation timescale,  
1556 which is computed across all units in one area, each area for one mouse was considered an  
1557 independent sample. Correction for multiple comparisons was performed using the Benjamini-  
1558 Hochberg False Discovery Rate (`statsmodels.stats.multitest.multipletests`).

### 1559 **10. Data processing pipeline**

1560 Data for each session was uploaded to the Allen Institute Laboratory Information Management  
1561 System (LIMS). Each dataset was run through the same series of processing steps using a set of  
1562 project-specific workflows. Out of 61 sessions entering the processing pipeline, 58 resulted in  
1563 successful NWB file generation. The 3 processing failures were due to mismatches in session  
1564 identifiers or expected file structures that prevented the workflow from completing.

### 1565 **11. Data and code availability**

1566 The data from all 58 passive viewing experiments used to generate main text Figures 1 through 4  
1567 is available for download in Neurodata Without Borders format via the AllenSDK. Example  
1568 Jupyter Notebooks for accessing the data can be found at  
1569 [https://allensdk.readthedocs.io/en/latest/visual\\_coding\\_neuropixels.html](https://allensdk.readthedocs.io/en/latest/visual_coding_neuropixels.html).

1570 The metrics table used to generate Figure 5E–F and Supplementary Figure 10 is available in the  
1571 GitHub repository for this manuscript (see below). The remaining data for the active behavior  
1572 experiments will be made available upon request.

1573 Code is available in the following repositories:

<b>Purpose</b>	<b>GitHub Repository</b>
Generating manuscript figures	AllenInstitute/neuropixels_platform_paper
Data pre-processing and unit metrics	AllenInstitute/ecephys_spike_sorting
Spike sorting	mouseland/Kilosort2
OPT post-processing and CCF registration	AllenInstitute/AIBSOPT
Calculating stimulus metrics	AllenInstitute/AllenSDK

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Data acquisition

open-ephys/plugin-GUI  
open-ephys-plugins/neuropixels-3a  
open-ephys-plugins/neuropixels-PXI

1574

## 1575 **12. Open-source software libraries**

1576 NumPy (van der Walt et al., 2011)  
1577 SciPy (Jones et al., 2001)  
1578 IPython (Pérez and Granger, 2007)  
1579 Matplotlib (Hunter, 2007)  
1580 Pandas (McKinney, 2010)  
1581 xarray (Hoyer and Hamman, 2017)  
1582 scikit-learn (Pedregosa et al., 2012)  
1583 VTK (Schroeder et al., 2006)  
1584 DeepLabCut (Mathis et al., 2018; Nath et al., 2019)  
1585 statsmodels (Seabold and Perktold, 2010)  
1586 allenCCF (Shamash et al., 2018)  
1587 tiff file - <https://pypi.org/project/tiff file/>  
1588 Jupyter - <https://jupyter.org/>  
1589 pynwb - <https://pynwb.readthedocs.io/en/stable/>

1590

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