

1 **A large-scale, standardized physiological survey reveals 2 higher order coding throughout the mouse visual cortex**

3 Saskia E. J. de Vries^{1, #,*}, Jerome Lecoq^{1, #,*}, Michael A. Buice^{1, #,*}, Peter A.
4 Groblewski¹, Gabriel K. Ocker¹, Michael Oliver¹, David Feng¹, Nicholas Cain¹, Peter
5 Ledochowitsch¹, Daniel Millman¹, Kate Roll¹, Marina Garrett¹, Tom Keenan¹, Leonard
6 Kuan¹, Stefan Mihalas¹, Shawn Olsen¹, Carol Thompson¹, Wayne Wakeman¹, Jack
7 Waters¹, Derric Williams¹, Chris Barber¹, Nathan Berbesque¹, Brandon Blanchard¹,
8 Nicholas Bowles¹, Shiella Caldejon¹, Linzy Casal¹, Andrew Cho¹, Sissy Cross¹, Chinh
9 Dang¹, Tim Dolbeare¹, Melise Edwards¹, John Galbraith¹, Nathalie Gaudreault¹, Fiona
10 Griffin¹, Perry Hargrave¹, Robert Howard¹, Lawrence Huang¹, Sean Jewell², Nika Keller¹,
11 Ulf Knoblich¹, Josh Larkin¹, Rachael Larsen¹, Chris Lau¹, Eric Lee¹, Felix Lee¹, Arielle
12 Leon¹, Lu Li¹, Fuhui Long¹, Jennifer Luviano¹, Kyla Mace¹, Thuyanh Nguyen¹, Jed
13 Perkins¹, Miranda Robertson¹, Sam Seid¹, Eric Shea-Brown^{1,2}, Jianghong Shi², Nathan
14 Sjoquist¹, Cliff Slaughterbeck¹, David Sullivan¹, Ryan Valenza¹, Casey White¹, Ali
15 Williford¹, Daniela Witten², Jun Zhuang¹, Hongkui Zeng¹, Colin Farrell¹, Lydia Ng¹, Amy
16 Bernard¹, John W. Phillips¹, R. Clay Reid¹, Christof Koch¹

17

18

19 ¹Allen Institute for Brain Science, Seattle, WA, USA

20 ²University of Washington, Seattle, WA, USA

21 *These authors contributed equally to this work

22 #correspondence should be addressed to S.E.J.dV. (saskiad@alleninstitute.org), J.L.
23 (jeromele@alleninstitute.org), and M.A.B. (michaelbu@alleninstitute.org).

24

25

26 **Summary**

27 To understand how the brain processes sensory information to guide behavior, we
28 must know how stimulus representations are transformed throughout the visual cortex.
29 Here we report an open, large-scale physiological survey of neural activity in the awake
30 mouse visual cortex: the Allen Brain Observatory Visual Coding dataset. This publicly
31 available dataset includes cortical activity from nearly 60,000 neurons collected from 6
32 visual areas, 4 layers, and 12 transgenic mouse lines from 221 adult mice, in response to
33 a systematic set of visual stimuli. Using this dataset, we reveal functional differences
34 across these dimensions and show that visual cortical responses are sparse but
35 correlated. Surprisingly, responses to different stimuli are largely independent, e.g.
36 whether a neuron responds to natural scenes provides no information about whether it
37 responds to natural movies or to gratings. We show that these phenomena cannot be
38 explained by standard local filter-based models, but are consistent with multi-layer
39 hierarchical computation, as found in deeper layers of standard convolutional neural
40 networks.

41 **Introduction**

42 Traditional understanding, based on several decades of research, suggests that
43 neurons early in the visual pathway are broadly responsive and become more selective
44 and specialized through a series of hierarchical processing stages¹⁻⁴. However, the
45 computations and mechanisms required for such transformations remain unclear. A key
46 challenge results from the fact that our understanding of the mammalian visual system is
47 the result of many small studies, recording responses from different stages in the circuit,
48 using different stimuli and different analyses.⁵ The inherent experimental selection biases
49 and lack of standardization of this approach introduce additional obstacles to creating a
50 cohesive understanding of cortical function. To address these differences, we conducted a
51 survey of visual responses across multiple layers and areas in the mouse visual cortex,
52 using a diverse set of visual stimuli. This survey was executed in pipeline fashion, with
53 standardized equipment and protocols and with strict quality control measures not
54 dependent upon stimulus-driven activity (**see Methods, Supplemental Figures 1-8**).

55 Previous work in mouse has revealed functional differences among cortical areas in
56 layer 2/3 in terms of the spatial and temporal frequency tuning of the neurons in each
57 area.^{6,7} However, it is not clear how these differences extend across layers and across
58 diverse neuron populations. Here we extend such functional studies to include 12 Cre-
59 defined neuron populations, including excitatory populations across 4 cortical layers (from
60 layer 2/3 to layer 6), and two inhibitory populations (Vip and Sst). Further, it is known that
61 stimulus statistics affect visual responses, such that responses to natural scenes cannot
62 be well predicted by responses to noise or grating stimuli⁸⁻¹¹. To examine the extent of this
63 discrepancy in the mouse visual cortex, and whether it varied across areas and layers, we
64 designed a stimulus set that included both artificial (gratings and noise) and natural
65 (scenes and movies) stimuli. While artificial stimuli can be easily parameterized and
66 interpreted, natural stimuli are likely to be closer to what is ethologically relevant to the
67 mouse. Finally, as recording modalities have enabled recordings of larger and larger
68 populations of neurons, it has become clear that populations might code visual and
69 behavioral activity in a way that is not apparent by considering single neurons alone.¹²
70 Here we imaged populations of neurons (mean 118 ± 82 , st. dev, for excitatory
71 populations) to explore both single neuron and population coding properties.

72 We find that 74% of neurons in the mouse visual cortex respond to at least one of
73 these visual stimuli, many showing classical tuning properties, such as orientation and
74 direction selective responses to gratings. These tuning properties reveal functional
75 differences across cortical areas and Cre lines. The responses to all stimuli are highly
76 sparse, both in terms of lifetime and population sparseness. We demonstrate that for all
77 cells the visual responses are better fit by a quadratic “complex cell” model than by a
78 linear-nonlinear “simple cell” model. Importantly, we find that the responsiveness to
79 different stimuli is largely independent, i.e. cells that respond to natural movies do not
80 necessarily respond to natural scenes. These properties are not consistent with a
81 traditional Gabor-style spatio-temporal wavelet basis, but rather are to be expected in
82 deeper layers of a multi-layer hierarchical network.

84 **Results**

85 Using adult C57BL/6 mice (mean age 108 ± 17 days st. dev) that expressed a
86 genetically encoded Ca²⁺ sensor (GCaMP6f) under the control of specific Cre-line drivers
87 (10 excitatory lines, 2 inhibitory lines, **Supplemental Figure 7**), we imaged the activity of
88 neurons in response to a battery of diverse visual stimuli. Data was collected from 6
89 different cortical visual areas (V1, LM, AL, PM, AM, and RL) and 4 different cortical layers.
90 Visual responses of neurons at the retinotopic center of gaze were recorded in response
91 to drifting gratings, flashed static gratings, locally sparse noise, natural scenes and natural
92 movies (**Figure 1f**), while the mouse was awake and free to run on a rotating disc. In total,
93 59,526 neurons were imaged from 410 experiments, each consisting of three one-hour
94 imaging sessions (**Table 1**).

95 In order to systematically collect physiological data on this scale, we built data
96 collection and processing pipelines (**Figure 1, Supplemental Figures 1-5**). The data
97 collection workflow progressed from surgical headpost implantation and craniotomy to
98 retinotopic mapping of cortical areas using intrinsic signal imaging, *in vivo* two-photon
99 calcium imaging of neuronal activity, brain fixation, and histology using serial two-photon
100 tomography (**Figure 1a,b,c**). To maximize data standardization across experiments, we
101 developed multiple hardware and software tools to regulate systematic data collection
102 (**Figure 1d**). One of the key components was the development of a registered coordinate
103 system that allowed an animal to move from one data collection step to the next, on
104 different experimental platforms, and maintain the same experimental and brain coordinate
105 geometry (**see Methods, Supplemental Figure 1**). In addition to such hardware
106 instrumentation, formalized standard operating procedures and quality control metrics
107 were crucial for the collection of these data (**Figure 1e**).

108 Following data collection, movies of fluorescence associated with calcium influx
109 were motion corrected, normalized, and regions of interest (ROIs) were segmented using
110 automated algorithms (**see Methods, Supplemental Figure 9**). Signals from overlapping
111 ROIs were demixed, and contamination from surrounding neuropil was subtracted
112 (**Supplemental Figure 10**). Segmented ROIs were matched across imaging sessions and
113 ROIs were filtered to remove apical dendrites and other processes, with the aim of only
114 including somatic ROIs. For each ROI, events were detected from $\Delta F/F$ using an L0
115 regularized deconvolution algorithm (**see Methods, Supplemental Figure 11**), which
116 deconvolves pointwise events assuming a linear calcium response for each event and
117 penalizes the total number of events included in the trace.

118 For each neuron, we computed the mean response to each stimulus condition
119 using the detected events, and parameterized its tuning properties. Many neurons showed
120 robust responses, exhibiting orientation-selective responses to gratings, localized spatial
121 receptive fields, and reliable responses to natural scenes and movies (**Figure 2a-f**,
122 **Supplemental Figure 13**). For each neuron and each categorical stimulus (i.e. drifting
123 gratings, static gratings, and natural scenes), the preferred stimulus condition was
124 identified as the condition that evoked the largest mean response for that stimulus (e.g.
125 the orientation and temporal frequency with the largest mean response for drifting
126 gratings). For each trial of the stimulus, the neural activity of the neuron was compared to

127 a distribution of activity for that neuron taken during the epoch of spontaneous activity, and
128 a p-value was computed. If at least 25% of the trials of the neuron's preferred condition
129 had a significant difference from the distribution of spontaneous activities ($p < 0.05$), the
130 neuron was deemed to be responsive to that stimulus (see **Methods** for responsiveness
131 criteria for locally sparse noise and natural movies).

132 In total, 74% of neurons were responsive to at least one of the visual stimuli
133 presented (**Figure 2g**). The percent of responsive neurons depended on area and
134 stimulus, such that V1 and LM showed the highest number of visually responsive neurons.
135 This dropped in other higher visual areas and was lowest in RL where only 31% of
136 neurons responded to any of the visual stimuli. Natural movies elicited responses from the
137 most neurons, while static gratings elicited responses from the fewest (**Figure 2h**). In
138 addition to varying by area, the percent of responsive neurons was also specific to Cre
139 lines and layers, suggesting functional differences across these dimensions
140 (**Supplemental Figures 14-18**). Note that the retinotopic location of the center of gaze is
141 close the border of RL and somatosensory cortex, which could result in the imaging of
142 non-visual neurons and cause the low rate of responsiveness in this area.

143 For responsive neurons, visual responses were parameterized by computing several
144 metrics, including preferred spatial frequency, preferred temporal frequency, direction
145 selectivity, and receptive field size (see **Methods**). Comparing these metrics across these
146 areas, layers, and Cre lines, we find evidence of functional differences across these
147 dimensions (**Figure 3, Supplemental Figures 19, 20**).

148 We included several Cre lines that label specific sub-populations of neurons. For
149 instance, Rorb, Scnn1a-Tg3, and Nr5a1 label distinct layer 4 populations, and exhibit
150 distinct tuning properties. For all the computed parameters, Rorb and Scnn1a-Tg3 show
151 significant differences (KS test, **Supplemental Figure 20**) suggesting distinct channels of
152 feedforward information. In layer 5, on the other hand, Tlx3 and Fezf2, which label cortico-
153 cortico and cortico-thalamic projecting neurons respectively, do not show significant
154 differences, implying more homogenous feedback signals. These data also provide the
155 first broad survey of visually evoked responses of both Vip and Sst inhibitory neurons.
156 Responses to drifting gratings support the model of mutual inhibition between these
157 inhibitory populations^{13,14}, wherein nearly all Sst cells respond reliably to the grating
158 stimulus while the Vip cells are nearly all unresponsive, and possibly even suppressed
159 (**Supplemental Figure 14**). Interestingly, receptive fields mapped using locally sparse
160 noise reveal that Vip neurons have remarkably large receptive field areas, larger than both
161 Sst and excitatory neurons in V1 (**Figure 3f**). The visual responses of these two
162 populations add important details to the inhibitory cortical circuit.

163 Comparisons across areas and layers reveal that direction selectivity is highest in
164 layer 4 of V1 (**Figure 3b**). In superficial layers, the differences across areas indicate that
165 V1, LM, and AL show significantly higher direction selectivity than PM, AM, and RL
166 (**Supplemental Figure 19**). This pattern in single neuron selectivity was reflected in our
167 ability to decode the visual stimulus from single-trial population vector responses, using all
168 cells, responsive and unresponsive. We used a K-nearest-neighbors classifier to predict
169 the grating direction. Matching the tuning properties, areas V1, AL, and LM showed higher

170 decoding performance than AM, PM, and RL, and these differences were more
171 pronounced in superficial layers than in deeper layer (**Figure 3c**). However, there are
172 cases where this relationship between population decoding and direction selectivity is
173 broken. For example, Nr5a1 neurons in V1 show the highest median direction selectivity,
174 but the lowest population decoding performance of excitatory neurons. Even matching
175 population size, Nr5a1 continues to show lower decoding performance than other Cre
176 lines (**Supplemental Figure 21**). Destroying trial-to-trial correlations by shuffling trials, we
177 found a slight increase in decoding performance, indicating that noise correlations do not
178 improve the discriminability of population responses to different stimuli (**Supplemental**
179 **Figure 21**). This result is in contrast to the impact of noise correlations on population
180 coding in the mammalian retina^{15,16}, suggesting a transformation of population coding
181 strategies across the visual pathway.

182 Across all areas, layers, and stimuli, visual responses in mouse cortex were highly
183 sparse. Among responses to natural scenes, we found that most neurons responded to a
184 very small number of scenes. The sparseness of individual neurons was measured using
185 lifetime sparseness, which captures the selectivity of a neuron's mean response to
186 different stimulus conditions^{17,18} (**see Methods**). A neuron that responds strongly to only a
187 few scenes will have a lifetime sparseness close to 1, whereas a neuron that responds
188 broadly to many scenes will have a lower lifetime sparseness (**Figure 4a**). Excitatory
189 neurons had a median lifetime sparseness of 0.71 in response to natural scenes. While
190 Sst neurons were comparable to excitatory neurons (median 0.78), Vip neurons exhibited
191 low selectivity (median 0.35). Lifetime sparseness did not increase outside of V1;
192 Responses did not become more selective in the higher visual areas. (**Figure 4b**,
193 **Supplemental Figures 22,23**). Lifetime sparseness is high for all stimuli (data not shown).
194 A complement to the sparseness of an individual neuron is the population sparseness - a
195 measurement of how many neurons respond to each stimulus condition. Like lifetime
196 sparseness, population sparseness is also high in these data for excitatory and Sst
197 neurons (**Figure 4c**), across all areas.

198 Such sparse activity could underlie a form of sparse coding to reduce redundancy
199 and increase efficiency, such that neurons with similar tuning preferences do not respond
200 at the same time.^{19,20} This makes a specific prediction: Similarly tuned neurons should
201 have negatively correlated trial-by-trial activity. Contrary to this prediction of "explaining
202 away," we found that similarly tuned neurons exhibited positively correlated trial-by-trial
203 fluctuations in almost all experiments in this dataset (**Figure 4e,f, Supplemental Figure**
204 **24**). This result is consistent with reports in other sensory systems and recording
205 methods,²¹ suggesting that sparse single-neuron responses underlying dense population
206 codes are a common feature of cortical representations at the level of rates.

207 In addition to sparsity in responses across stimulus conditions, the visually evoked
208 responses throughout the mouse cortex showed a large amount of trial-to-trial variability.
209 Indeed, the percent of responsive trials for most neurons at their preferred conditions was
210 low — the median is less than 50% (**Figure 5a, Supplemental Figure 25**). This means
211 that the majority of neurons in the mouse visual cortex are usually unresponsive, even
212 when presented with the stimulus condition that elicits their largest average response. We

213 also calculated a more complete measurement of response reliability, defined as the
214 square of the expected correlation between the trial averaged response to the true,
215 unmeasured, mean response²² (**see Methods**). A neuron that responds precisely the
216 same way on each trial to a set of stimuli will have a reliability of 1, while a completely
217 random neuron will have a reliability of 0. We find that neurons had higher reliability for
218 natural stimuli than for the artificial stimuli across all areas and layers (**Figure 5b,c**,
219 **Supplemental Figure 25**). Altogether, responsive neurons had a mean reliability of $0.62 \pm$
220 0.2 (st. dev) for natural scenes and 0.46 ± 0.2 (st. dev) for drifting gratings.

221 One possible source of trial-to-trial variability could be the locomotor activity of the
222 mouse. Previous studies have shown that visual responses in the mouse cortex are
223 modulated by the animal's running activity.²³⁻²⁷ The mice in our experiments were free to
224 run on a disc during the experiment and animals showed a range of running behaviors
225 (**Supplemental Figure 26**). For experiments in which the animals spent enough time
226 running such that there were sufficient stimulus trials when the mouse was both stationary
227 and running (at least 10% of trials for each), we compared the responses in these two
228 states. Consistent with previous reports, many neurons show modulated response (**Figure**
229 **5d,e**). While most neurons show enhanced responses when running, for many neurons
230 the difference between stationary and running is not significant (only 13% and 37% of
231 neurons show significant modulation of their responses to drifting gratings and natural
232 scenes respectively, using a KS test).

233 To examine whether the locomotor activity could be a source of trial-to-trial
234 variability, we compared the reliability of neurons' visual responses to the fraction of time
235 the animal spent running. We found that reliability is higher when the mouse runs
236 consistently, but this increase is modest from a baseline of reliability when the mouse is
237 either completely stationary or shows mixed running behavior (**Figure 5f**). This effect on
238 stimulus response reliability is consistent across different stimuli, both natural and artificial.
239 Thus locomotor activity does contribute to the variability of visual responses, but is unlikely
240 to fully explain the amount of variability found in these data.

241 We asked whether a standard modeling approach could capture the observed
242 stimulus responses and variability. We used a generalized linear model (GLM) to predict
243 extracted events, smoothed with a Gaussian window, from time series input of the stimuli
244 along with the binary running state of the mouse (**Figure 6a, see Methods**). Only neurons
245 that were matched in all three imaging sessions were used for modeling (~19,000
246 neurons), and all neurons were modeled regardless of whether they met our
247 responsiveness criteria. The basis functions for the GLM are two spatiotemporal wavelet
248 pyramids: one a standard linear basis and another that squares the basis functions before
249 summation, approximating a "complex" neuron receptive field. While the model captures
250 the activity of some neurons very well (**Figure 6b**), the median prediction, r , for natural
251 stimuli is ~0.2-0.3 across areas (**Figure 6c,d**), suggesting a large amount of variation
252 unaccounted for by the stimulus with this model. We computed a complexity ratio by
253 comparing the total weight of the quadratic basis functions to the total weights for each
254 model, and found that almost all neurons are mostly complex, with complexity ratios near

255 1 (**Figure 6e**). This means that no neuron is better described by a "simple" linear-nonlinear
256 model than the "complex" quadratic model.

257 For each neuron, we trained the model separately using natural stimuli (natural
258 scenes and natural movies) and artificial stimuli (drifting gratings, static gratings and
259 locally sparse noise). Comparing the models' performances, we found that the overall
260 distribution of performance for models trained and tested with natural stimuli was much
261 higher than the corresponding models for artificial stimuli (**Figure 6c**). This was true even
262 for neurons that met our responsiveness criteria for gratings but not natural scenes.
263 Further, models trained on natural stimuli predicted responses to artificial stimuli better
264 than vice versa, although the cross-stimulus prediction was worse than the within-stimulus
265 prediction, consistent with previous reports⁹⁻¹¹.

266 Surprisingly, whether a neuron responded to one stimulus (e.g. natural scenes,
267 drifting gratings, etc.) was largely, though not completely, independent of whether it
268 responded to another stimulus. Unlike the examples shown in Figure 2, which were
269 chosen to highlight responses to all stimuli, most neurons were responsive to only a
270 subset of the stimuli presented (**Figure 7a**). The overlap of the set of neurons that
271 responds to each pairwise combination of stimuli was computed for each experiment and
272 compared to the maximum and minimum amount of overlap possible given the fraction of
273 responsive neurons to each stimulus (**Figure 7b, Supplemental Figure 28**). There is
274 above chance overlap for all presentations of natural movies — particularly for natural
275 movie one, which is repeated in each imaging session (**Figure 7c**). There is also above
276 chance overlap for responses to static gratings and natural scenes. However, natural
277 movies and all other stimuli showed overlap close to the level of chance. That is, whether
278 a neuron responded to natural scenes is independent of whether it responded to natural
279 movies. Notably, locally sparse noise showed the least amount of overlap with other
280 stimuli, and even below chance overlap with some, such as static gratings. These results
281 are consistent across all visual areas.

282 The independence of whether a neuron responded to two stimuli is also reflected
283 in the correlation between the reliability of neurons' responses to those two stimuli (**Figure**
284 **7d,e**). For neurons that responded to two stimuli, we computed the Pearson correlation
285 between the reliability of responses to each stimulus. We found the same structure in
286 cross-stimulus comparisons such that the reliabilities of natural movie responses were
287 highly correlated, but most stimulus pairs had low correlations. Thus, whether a neuron
288 responds to two stimuli is largely independent, and even when it does respond to both, the
289 reliability of those responses remains largely independent.

290 Independence between responses to ostensibly similar stimuli is a striking feature of
291 the data and one not predicted by the classical model of the early visual system (namely
292 spatiotemporal Gabor-type wavelets). This observation, together with the fact that neural
293 activity is sparse in both a lifetime and population sense, and finally that the "simple"
294 linear-nonlinear wavelet based GLM accounted for so little of the explainable variance, all
295 point to the idea that much of the neural activity is driven by relatively higher order
296 features. We quantified this by comparing the population level neural responses to
297 standard deep convolutional networks (CNNs; **Figure 8**). This is an interesting comparison

298 because the original inspiration for these model architectures was the important and early
299 set of results describing “simple” and “complex” neurons in Area 17 of anesthetized cat
300 visual cortex¹.

301 Units in CNN models (such as VGG16²⁸) are optimally driven by progressively higher
302 order features in deeper layers of the model (**Figure 8a**). The first pooling layer contains
303 many units that appear as coarse edge detectors, while the second pooling layer contains
304 more complex features, with a small subset consisting of oriented gratings similar to
305 traditional V1 receptive fields. By the third pooling layer, there are no such simple looking
306 features, but even more complex shapes and textured patterns. As a natural consequence
307 of this increasing specificity, we see the lifetime and population sparsity in response to
308 natural scenes increase through the pooling layers (**Figure 8b**). This trend is consistent
309 across multiple CNNs; It is not specific to VGG16 (not shown).

310 Units in VGG16 also display the independence of stimuli observed in the data
311 (**Figure 8c**). We compared the units that respond to each of the flashed stimuli (locally
312 sparse noise, static gratings and natural scenes) for each pooling layer of VGG16. For the
313 lower layers, as expected, there is a high degree of overlap in populations that respond to
314 different stimuli. Moving through deeper layers of the network, the degree of independence
315 increases. The last pooling layer shows nearly complete independence of stimuli.

316 We used similarity-of-similarity matrix (SSM) analysis²⁹ to compare the neural
317 responses with responses at different pooling layers of VGG16 in order to quantify how
318 similar the two representations are (**Figure 8d**). A similarity matrix is constructed by
319 computing the correlation of the trials average population responses to pairs of scenes.
320 We then computed the correlation of similarity matrices between each pooling layer of
321 VGG16 and each cortical area, layer and Cre line in these data. Because the network has
322 a degree of similarity to itself, we only compare pooling layers as the model layers
323 between pooling layers are highly correlated (see **Methods**).

324 The highest correlations are for pooling layer 3 of VGG16 for most cortical areas and
325 layers (**Figure 8d**). Superficial layers in V1 map to the middle layers most strongly
326 whereas LM, PM, and AL in those layers tend to map to slightly higher layers, suggesting
327 a potential hierarchy, albeit a shallow one³⁰. As a comparative baseline, we compute the
328 SSM metric for a linear Gabor wavelet basis (**Figure 8d**), which is highest in the input
329 layer and falls deeper into the network. These results support the view that throughout the
330 mouse visual cortex, neurons exhibit responses to more complex and sophisticated stimuli
331 than the classical model suggests.^{5,31}

332 **Discussion**

333 Data standardization and experimental reproducibility is both a challenge and an
334 opportunity for the field of systems neuroscience. *In vivo* neuronal recordings are
335 notoriously difficult experiments that require an in-depth expertise in many scientific fields
336 and multiple years of training. As such, these experiments are difficult to scale up. Despite
337 these challenges, large cohesive datasets for systems neuroscience offer an opportunity
338 to address fundamental issues of standardization and reproducibility. Here we combined

339 standardized operating procedures with integrated engineering tools to address these
340 long-standing difficulties. We demonstrated data collection in over an order of magnitude
341 more animals (221 mice) than is typically performed in the field and maintained tight
342 standardization across three years of continuous data collection.

343 We have reduced critical experimental biases by separating quality control of data
344 collection from response characterization. Historically, the field has been dominated by
345 single-neuron electrophysiological recordings in which electrodes were advanced until a
346 neuron was found that responded to a test stimulus. The stimulus was then optimized to
347 elicit the strongest reliable response from that neuron. The experiment proceeded using
348 manipulations around this stimulus condition that had been tuned to drive the strongest
349 response. Such studies have discovered many characteristic response properties, but may
350 fail to capture the variability of responses, the breadth of features that elicit a neural
351 response, and the breadth of features that do not elicit a response. Recently, calcium
352 imaging and denser electrophysiological recordings have enabled large populations of
353 neurons to be recorded simultaneously. By combining calcium imaging with strong quality
354 control and standardization, we have created an unprecedented survey of mouse visual
355 cortex using a standard and well-studied but diverse set of stimuli while limiting the
356 selection bias towards those stimuli.

357 Under the canonical model, V1 sits at the initial stages of a processing hierarchy
358 where neurons respond to low-level features, specifically with spatially localized receptive
359 fields with spatial and temporal frequency preferences.^{1–4} Neural responses become
360 increasing specialized in the higher areas moving away from V1, reaching extremes in
361 which cells show very selective responses to specific objects and even faces.^{4,32}

362 The field has a growing body of evidence showing that the canonical model needs to
363 be enhanced to support more sophisticated visual computation.^{5,31} For instance, neurons
364 in mouse V1 show complex visual responses previously associated with higher cortical
365 areas, including pattern selectivity for plaid stimuli³³ Furthermore, the emergence of the
366 rodent as a prominent model of the visual system in recent years has revealed evidence of
367 non-visual computation, including behavioral responses such as reward timing and
368 sequence learning³⁴, as well as modulation by multimodal sensory stimuli^{35,36} and motor
369 signals.^{23,24,37–39}

370 We expected this survey to provide strong evidence for low-level responses that
371 become progressively higher order throughout the higher visual areas of mouse cortex.
372 Instead, neurons throughout the mouse cortex show highly variable, sparse responses,
373 best fit by “complex” models. Further, responsiveness to different stimuli is largely
374 independent. Rather than support the canonical model, these results provide evidence of
375 higher order coding wherein neurons exhibit specialized responses to a set of sparse and
376 higher level features of the visual field.

377 Neurons tuned to low-level features will not, as a whole, show the property of
378 independence that we observe in these data. Such neurons should be, by and large,
379 equally mappable using noise stimuli, grating stimuli, and natural stimuli – with some
380 stimulus specific modifications in the resulting receptive field.^{8,9,11} While we observe

381 individual examples of neurons that behave exactly this way, this is not a general feature
382 of the population of responses (**Figure 7, Supplemental Figure 28**). Computationally, we
383 can consider how a system that responds to low-order features will behave by examining
384 either the early layers of a CNN (**Figure 8c,d**) or a wavelet basis (not shown), where we
385 see strong dependence and correlation of responses across stimuli, contrary to what is
386 observed in the dataset. Strikingly, the fact that none of the neurons in the dataset are
387 better fit by the “simple” model in our GLM wavelet basis model (**Figure 6**) further supports
388 our finding that neurons are not tuned to low-level features.

389 Neurons that respond to higher-order features, on the other hand, result in
390 responses that are sparse in both a population and lifetime sense, as we observe here. In
391 a CNN, the network develops features during training that allow it to correctly classify
392 images. Whereas the early units of these networks tend to be more general and low-order,
393 as described above, the intermediate units become increasingly specialized for features
394 that are necessary for the trained task. As a result, the CNN shows a greater degree of
395 stimulus independence with depth (**Figure 8c**). Our data, throughout the mouse visual
396 cortex, shows a degree of independence that is similar to that observed in the third pooling
397 layer of VGG16 (**Figure 8c**). This is consistent with a comparison of sparsity, both lifetime
398 and population, between the dataset and VGG16, as well as the representation mapping
399 using SSM-analysis that shows most layers and areas are more similar to the middle
400 pooling layers, while a wavelet basis is most similar to the input and early layers (**Figure**
401 **8b,d**). These results are also consistent with an alternative methodology, SVCCA⁴⁰ (not
402 shown). Taken together, these results reveal that neurons throughout the mouse visual
403 cortex exhibit higher order coding, revealing that they are specialized for high-level
404 features.

405 This is not to say that there are not plenty of cells in the early visual cortical areas
406 that show Gabor-type receptive fields. VGG16, at the second pooling layer, for example,
407 has units with optimal stimuli that closely resemble Gabors, but they are the minority.
408 Additionally, probing such networks with stimuli such as linear gratings or noise stimuli, or
409 with approaches such as spike triggered averaging, will result in responses that can be
410 characterized with Gabor-type receptive fields even though this is not the optimal stimulus
411 condition that drives such units. We posit that the same phenomenon is almost certainly
412 at play in the mouse visual cortex. Specialized, higher-order visual neurons have been
413 known to exist, either high in the visual hierarchy or as particular exceptions (e.g. looming
414 detectors, motion pattern cells). By including a broad range of stimuli and reducing
415 stimulus bias in our data collection and analysis, we have revealed that such higher order
416 cells are closer to the rule than the exception in the mouse visual cortex. Given that much
417 of the existing literature describes the visual system of cat and primate, it is interesting to
418 speculate as to whether these results might generalize to other species.

419 Identifying the exact response characteristics of the population of cells remains an
420 open problem. The optimal stimuli of units in a CNN are the result of optimization for an
421 object recognition task on natural stimuli. Such a “task” appropriate to define the response
422 characteristics of the mouse visual system remains unclear. Understanding the

423 computation of the mouse visual circuit will require identifying the features and stimuli that
424 are ethologically relevant to the mouse.

425 The Allen Brain Observatory Visual Coding dataset is an openly available dataset,
426 accessible via a dedicated web portal (<http://observatory.brain-map.org/visualcoding>), with
427 a custom Python-based Application Programming Interface, the AllenSDK
428 (<http://alleninstitute.github.io/AllenSDK/>). We believe these data will be a valuable
429 resource to the systems neuroscience community as a testbed for theories of cortical
430 computation and a benchmark for experimental results. Already, these data have been
431 used by other researchers to develop image processing methods,^{41,42} to examine stimulus
432 encoding and decoding,⁴³⁻⁴⁷ and to test models of cortical computations.⁴⁸ Ultimately, we
433 expect these data will seed as many questions as they answer, fueling others to pursue
434 both new analyses and further experiments to unravel how cortical circuits represent and
435 transform sensory information.

436

437 **References**

438

- 439 1. Hubel, D. & Wiesel, T. Receptive fields of single neurones in the cat's striate cortex.
440 *J. Physiol.* **148**, 574–591 (1959).
- 441 2. Hubel, D. H. & Wiesel, T. N. Receptive fields, binocular interaction and functional
442 architecture in the cat's visual cortex. *J. Physiol.* **160**, 106–154.2 (1962).
- 443 3. Felleman, D. J. & Van Essen, D. C. Distributed Hierarchical Processing in the
444 Primate Cerebral Cortex.
- 445 4. DiCarlo, J. J., Zoccolan, D. & Rust, N. C. How does the brain solve visual object
446 recognition? *Neuron* (2012). doi:10.1016/j.neuron.2012.01.010
- 447 5. Olshausen, B. & Field, D. What is the other 85 % of V1 doing? *Probl. Syst.* ... 1–29
448 (2004). doi:10.1093/acprof:oso/9780195148220.003.0010
- 449 6. Andermann, M. L., Kerlin, A. M., Roumis, D. K., Glickfeld, L. L. & Reid, R. C.
450 Functional specialization of mouse higher visual cortical areas. *Neuron* **72**, 1025–
451 1039 (2011).
- 452 7. Marshel, J. H., Garrett, M. E., Nauhaus, I. & Callaway, E. M. Functional
453 specialization of seven mouse visual cortical areas. *Neuron* **72**, 1040–1054 (2011).
- 454 8. Fournier, J., Monier, C., Pananceau, M. & Frégnac, Y. Adaptation of the simple or
455 complex nature of V1 receptive fields to visual statistics. *Nat. Neurosci.* **14**, 1053–
456 60 (2011).
- 457 9. David, S., Vinje, W. & Gallant, J. L. Natural Stimulus Statistics Alter the Receptive
458 Field Structure of V1 Neurons. *J. Neurosci.* **24**, 6991–7006 (2004).
- 459 10. Talebi, V. & Baker, C. L. Natural versus Synthetic Stimuli for Estimating Receptive
460 Field Models: A Comparison of Predictive Robustness. *J. Neurosci.* **32**, 1560–1576
461 (2012).
- 462 11. Yeh, C.-I., Xing, D., Williams, P. & Shapley, R. Stimulus ensemble and cortical layer
463 determine V1 spatial receptive fields. *Proc. Natl. Acad. Sci.* **106**, 14652–14657
464 (2009).
- 465 12. Averbeck, B. B., Latham, P. E. & Pouget, A. Neural correlations, population coding
466 and computation. *Nature Reviews Neuroscience* (2006). doi:10.1038/nrn1888
- 467 13. Pfeffer, C. K., Xue, M., He, M., Huang, Z. J. & Scanziani, M. Inhibition of inhibition in
468 visual cortex: the logic of connections between molecularly distinct interneurons.
469 *Nat. Neurosci.* **16**, 1068–1076 (2013).
- 470 14. Fu, Y. *et al.* A cortical circuit for gain control by behavioral state. *Cell* **156**, 1139–
471 1152 (2014).
- 472 15. Franke, F. *et al.* Structures of Neural Correlation and How They Favor Coding.
473 *Neuron* (2016). doi:10.1016/j.neuron.2015.12.037
- 474 16. Zylberberg, J., Cafaro, J., Turner, M. H., Shea-Brown, E. & Rieke, F. Direction-
475 Selective Circuits Shape Noise to Ensure a Precise Population Code. *Neuron*
476 (2016). doi:10.1016/j.neuron.2015.11.019
- 477 17. Rolls, E. T. & Tovee, M. J. Sparseness of the neuronal representation of stimuli in
478 the primate temporal visual cortex. *J. Neurophysiol.* **73**, 713–726 (1995).

479 18. Vinje, W. E. & Gallant, J. L. Sparse Coding and Decorrelation in Primary Visual
480 Cortex During Natural Vision. *Science* (80-). **287**, 1273–1276 (2000).

481 19. Olshausen, B. A. & Field, D. J. Sparse coding with an overcomplete basis set: A
482 strategy employed by V1 ? *Vis. Res.* **37**, 3311–3325 (1997).

483 20. Barlow, H. Possible principles underlying the transformation of sensory messages.
484 *Sens. Commun.* 217–234 (1961). at
485 <<http://www.trin.cam.ac.uk/horacebarlow/21.pdf>>

486 21. Cohen, M. R. & Kohn, A. Measuring and interpreting neuronal correlations. *Nature
487 Neuroscience* (2011). doi:10.1038/nn.2842

488 22. Schoppe, O., Harper, N. S., Willmore, B. D. B., King, A. J. & Schnupp, J. W. H.
489 Measuring the Performance of Neural Models. *Front. Comput. Neurosci.* **10**, 1–11
490 (2016).

491 23. Niell, C. M. & Stryker, M. P. Modulation of visual responses by behavioral state in
492 mouse visual cortex. *Neuron* **65**, 472–9 (2010).

493 24. Saleem, A., Ayaz, A., Jeffery, K., Harris, K. & Carandini, M. Integration of visual
494 motion and locomotion in mouse visual cortex. *Nat. Neurosci.* **16**, 1864–1869
495 (2013).

496 25. Dadarlat, M. C. & Stryker, M. P. Locomotion enhances neural encoding of visual
497 stimuli in mouse V1. *J. Neurosci.* **37**, 3764–3775 (2017).

498 26. Dipoppa, M. *et al.* Vision and locomotion shape the interactions between neuron
499 types in mouse visual cortex. *bioRxiv* 058396 (2016). doi:10.1101/058396

500 27. Polack, P. O., Friedman, J. & Golshani, P. Cellular mechanisms of brain state-
501 dependent gain modulation in visual cortex. *Nat. Neurosci.* **16**, 1331–1339 (2013).

502 28. Simonyan, K. & Zisserman, A. Very Deep Convolutional Networks for Large-Scale
503 Image Recognition. 1–14 (2014). doi:10.1016/j.infsof.2008.09.005

504 29. Kriegeskorte, N., Mur, M. & Bandettini, P. Representational similarity analysis -
505 connecting the branches of systems neuroscience. *Front. Syst. Neurosci.* **2**, 4
506 (2008).

507 30. Harris, J. A. *et al.* The organization of intracortical connections by layer and cell
508 class in the mouse brain. *bioRxiv* 292961 (2018). doi:10.1101/292961

509 31. Masland, R. H. & Martin, P. R. The unsolved mystery of vision. *Curr. Biol.* **17**, R577-
510 82 (2007).

511 32. Quiroga, Q., Reddy, L., Kreiman, G., Koch, C. & Fried, I. Invariant visual
512 representation by single neurons in the human brain. *Nature* **435**, 1102–1107
513 (2005).

514 33. Palagina, G., Meyer, J. F. & Smirnakis, S. M. Complex Visual Motion
515 Representation in Mouse Area V1. *J. Neurosci.* **37**, 164–183 (2017).

516 34. Gavornik, J. P. & Bear, M. F. Higher brain functions served by the lowly rodent
517 primary visual cortex. *Learn. Mem.* **21**, 527–533 (2014).

518 35. Bieler, M. *et al.* Rate and Temporal Coding Convey Multisensory Information in
519 Primary Sensory Cortices. *Environ. Neurosci.* **4**, ENEURO.0037-17.2017 (2017).

520 36. Ibrahim, L. A. *et al.* Cross-Modality Sharpening of Visual Cortical Processing

521 through Layer-1-Mediated Inhibition and Disinhibition. *Neuron* **89**, 1031–1045
522 (2016).

523 37. Keller, G. B., Bonhoeffer, T. & Hübener, M. Sensorimotor mismatch signals in
524 primary visual cortex of the behaving mouse. *Neuron* **74**, 809–15 (2012).

525 38. Stringer, C. *et al.* Spontaneous behaviors drive multidimensional, brain-wide
526 population activity. *bioRxiv* 306019 (2018). doi:10.1101/306019

527 39. Musall, S., Kaufman, M. T., Gluf, S. & Churchland, A. Movement-related activity
528 dominates cortex during sensory-guided decision making. *bioRxiv* 308288 (2018).
529 doi:10.1101/308288

530 40. Raghu, M., Gilmer, J., Yosinski, J. & Sohl-Dickstein, J. SVCCA: Singular Vector
531 Canonical Correlation Analysis for Deep Learning Dynamics and Interpretability. 1–
532 17 (2017). doi:1706.05806

533 41. Petersen, A., Simon, N. & Witten, D. SCALPEL: Extracting Neurons from Calcium
534 Imaging Data. 1–31 (2017). at <<http://arxiv.org/abs/1703.06946>>

535 42. Sheintuch, L. *et al.* Tracking the Same Neurons across Multiple Days in
536 Ca2+Imaging Data. *Cell Rep.* **21**, 1102–1115 (2017).

537 43. Ellis, R. J. *et al.* High-accuracy Decoding of Complex Visual Scenes from Neuronal
538 Calcium Responses. 1–32 (2018).

539 44. Cai, L., Wu, B. & Ji, S. Neuronal Activities in the Mouse Visual Cortex Predict
540 Patterns of Sensory Stimuli. (2018).

541 45. Zylberberg, J. Untuned but not irrelevant: A role for untuned neurons in sensory
542 information coding. *bioRxiv* 1–18 (2017). doi:10.1101/134379

543 46. Christensen, A. J. & Pillow, J. W. Running reduces firing but improves coding in
544 rodent higher- order visual cortex. *bioRxiv* 1–14 (2017).

545 47. Esfahany, K., Siergiej, I., Zhao, Y. & Park, I. M. Organization of Neural Population
546 Code in Mouse Visual System. *bioRxiv* 1–16 (2017). doi:10.1101/220558

547 48. Sweeney, Y. & Clopath, C. Population coupling predicts the plasticity of stimulus
548 responses in cortical circuits. (2018). doi:10.1101/265041

549

550 **Acknowledgements**

551

552 We thank the Animal Care, Transgenic Colony Management and Lab Animal Services for
553 mouse husbandry. We thank Daniel Denman, Josh Siegle, Yazan Billeh and Anton
554 Arkhipov for critical feedback on the manuscript. This work was supported by the Allen
555 Institute, and in part by NIH 2R90DA033461, NSF DMS-1514743, Falconwood
556 Foundation, Center for Brains, Minds & Machines funded by NSF Science and Technology
557 Center Award CCF-1231216, Natural Sciences and Engineering Research Council of
558 Canada, NIH Grant DP5OD009145, and NSF CAREER Award DMS-1252624. We thank
559 Allan Jones for providing the critical environment that enabled our large scale team effort.
560 We thank the Allen Institute founder, Paul G Allen, for his vision, encouragement, and
561 support.

562

563

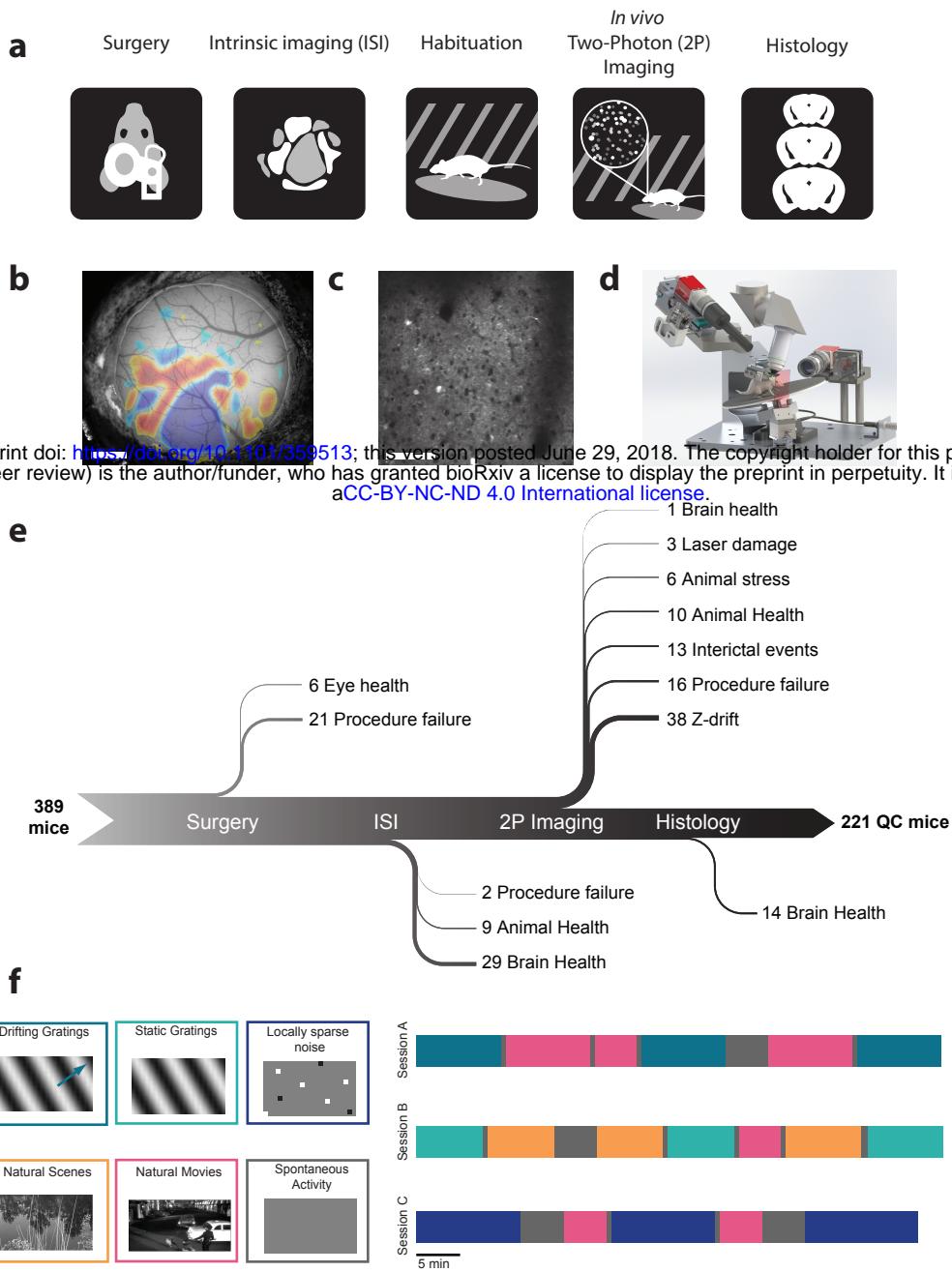
564 **Author Contributions**

565

566 SEJdV, MAB, KR, MG, TK, SM, SO, JW, CD, LN, AB, JWP, RCR, and CK conceived of
567 and designed the experiment. JL, TK, PH, AL, CS, DS, and CF built and maintained the
568 hardware. SEJdV, JL, MAB, GKO, DF, NC, LK, WW, DW, RV, CB, BB, TD, JG, SJ, NK,
569 CL, FL, FL, JP, NS, DW, JZ, and LN developed algorithms and software, including the
570 SDK and website. KR, NB, NB, SC, LC, AC, SC, ME, NG, FG, RH, LH, UK, JL, RL, EL,
571 LL, JL, KM, TN, MR, SS, CW, and AW collected data. JL and PAG managed the pipeline.
572 SEJdV, JL, MAB, GKO, MO, NC, PL, DM, and RV analyzed data. SEJdV, JL, and MAB
573 wrote the paper with input from PAG, GKO, MO, NC, PL, DM, RCR, and MG.

574

Figure 1



575 **Figure 1: A standardized systems neuroscience data pipeline to map**
576 **visual responses**

577 (a) Schematic describing the experimental workflow followed by each mouse going
578 through a large scale data pipeline. (b) Example intrinsic imaging map labelling individual
579 visual brain areas. Scale bar = 1mm. (c) Example averaged two photon imaging field of
580 view (400 μ m x 400 μ m) showcasing neurons labeled with Gcamp6f. Scale bar = 100 μ m.
581 (d) Custom design apparatus to standardize the handling of mice in two photon imaging.
582 We engineered all steps of the pipeline to co-register data and tools, enabling reproducible
583 data collection and a standardized experimental process (see Supplementary Figure 1-4).
584 (e) Number of mice passing Quality Control (QC) criteria established by Standardized
585 Operating Procedures (SOPs) at each step of the data collection pipeline with their
586 recorded failure reason. The data collection pipeline is closely monitored to maintain
587 consistently high data quality. (f) Standardized experimental design of sensory visual
588 stimuli to map responses properties of neurons across the visual cortex. 6 blocks of
589 different stimuli were presented to mice (left) and were distributed into 3 separate imaging
590 session called session A, session B and session C (right).

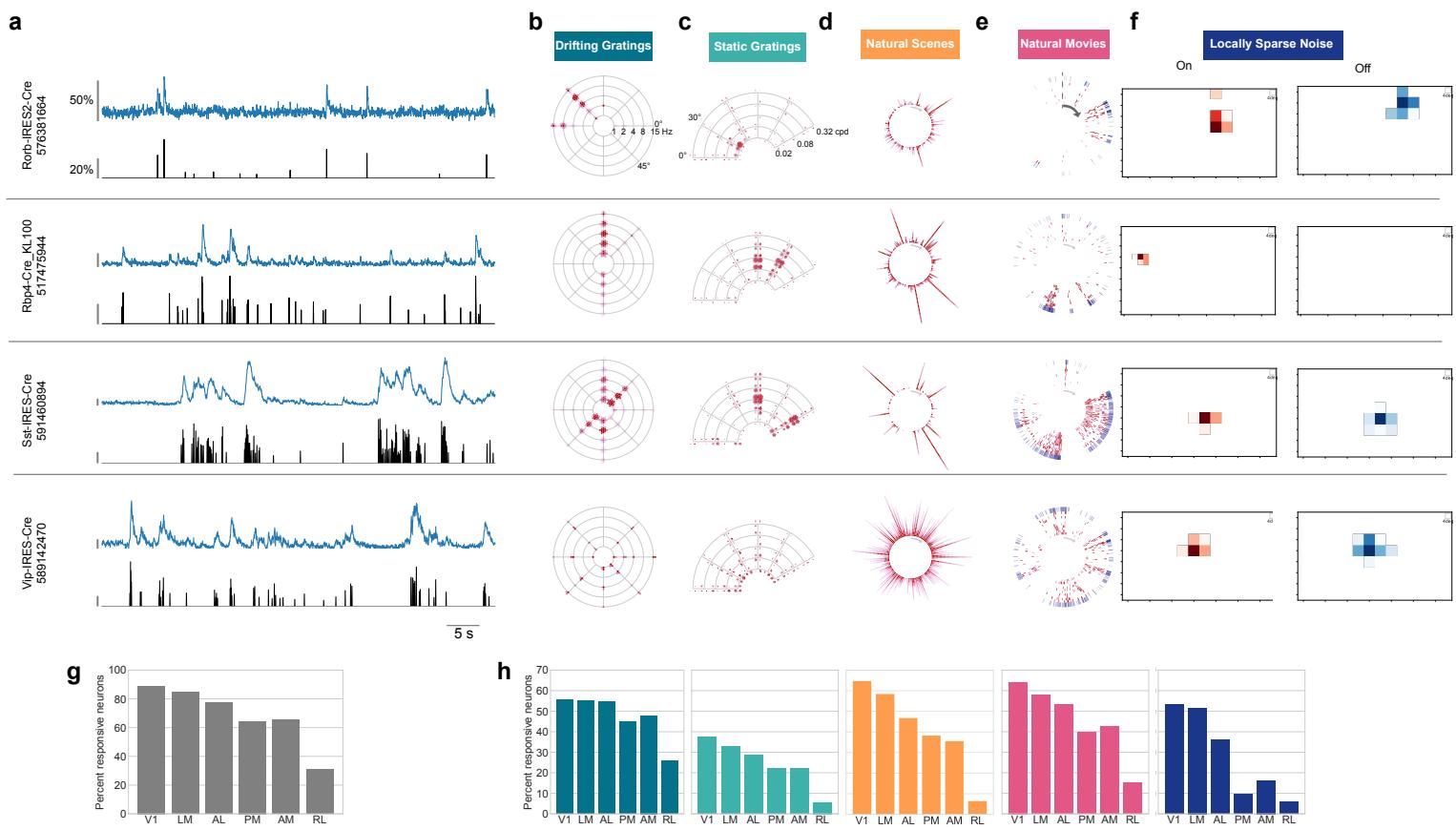
Table 1

| Cre line | Layers | E/I | V1 | LM | AL | PM | AM | RL |
|-------------------------------------|-----------|-----|-----------|-----------|-----------|-----------|-----------|----------|
| Emx1-IRES-Cre; Camk2a-tTA; Ai93 | 2/3, 4, 5 | E | 3096 (9) | 2098 (8) | 1787 (7) | 835 (4) | 457 (3) | 3011 (9) |
| Slc17a7-IRES2-Cre; Camk2a-tTA; Ai93 | 2/3, 4, 5 | E | 1864 (13) | 1864 (10) | 374 (2) | 1202 (10) | 235 (2) | 322 (3) |
| Cux2-CreERT2; Camk2-tTA; Ai93 | 2/3, 4 | E | 5168 (15) | 3845 (13) | 3037 (12) | 2987 (14) | 1611 (10) | 1370 (9) |
| Rorb-IRES2-Cre; Camk2a-tTA; Ai93 | 4 | E | 2218 (8) | 1191 (6) | 1242 (6) | 593 (6) | 735 (8) | 1757 (6) |
| Scnn1a-Tg3-Cre; Camk2a-tTA; Ai93 | 4 | E | 1873 (9) | | | | | |
| Nr5a1-Cre; Camk2a-tTA; Ai93 | 4 | E | 702 (8) | 416 (6) | 172 (4) | 331 (7) | 171 (6) | 1318 (5) |
| Rbp4-Cre_KL100; Camk2a-tTA; Ai93 | 5 | E | 531 (8) | 640 (8) | 490 (7) | 590 (7) | 355 (8) | 136 (5) |
| Fezf2-CreER;Ai148 | 5 | E | 490 (5) | 981 (5) | | | | |
| Tlx3-Cre_PL56;Ai148 | 5 | E | 1181 (6) | 946 (3) | | | | |
| Ntsr1-Cre_GN220;Ai148 | 6 | E | 331 (4) | 210 (2) | | 330 (3) | | |
| Sst-IRES-Cre;Ai148 | 4, 5 | I | 449 (18) | 413 (16) | 200 (2) | 608 (17) | | 46 (2) |
| Vip-IRES-Cre;Ai148 | 2/3, 4 | I | 247 (16) | 280 (15) | | 320 (15) | | |

591 **Table 1: Visual coding dataset.**

592 The number of cells (and experiments) imaged for each Cre line in each cortical visual
593 area. In total, 59,526 cells imaged in 410 experiments in 221 mice are included in this
594 dataset.

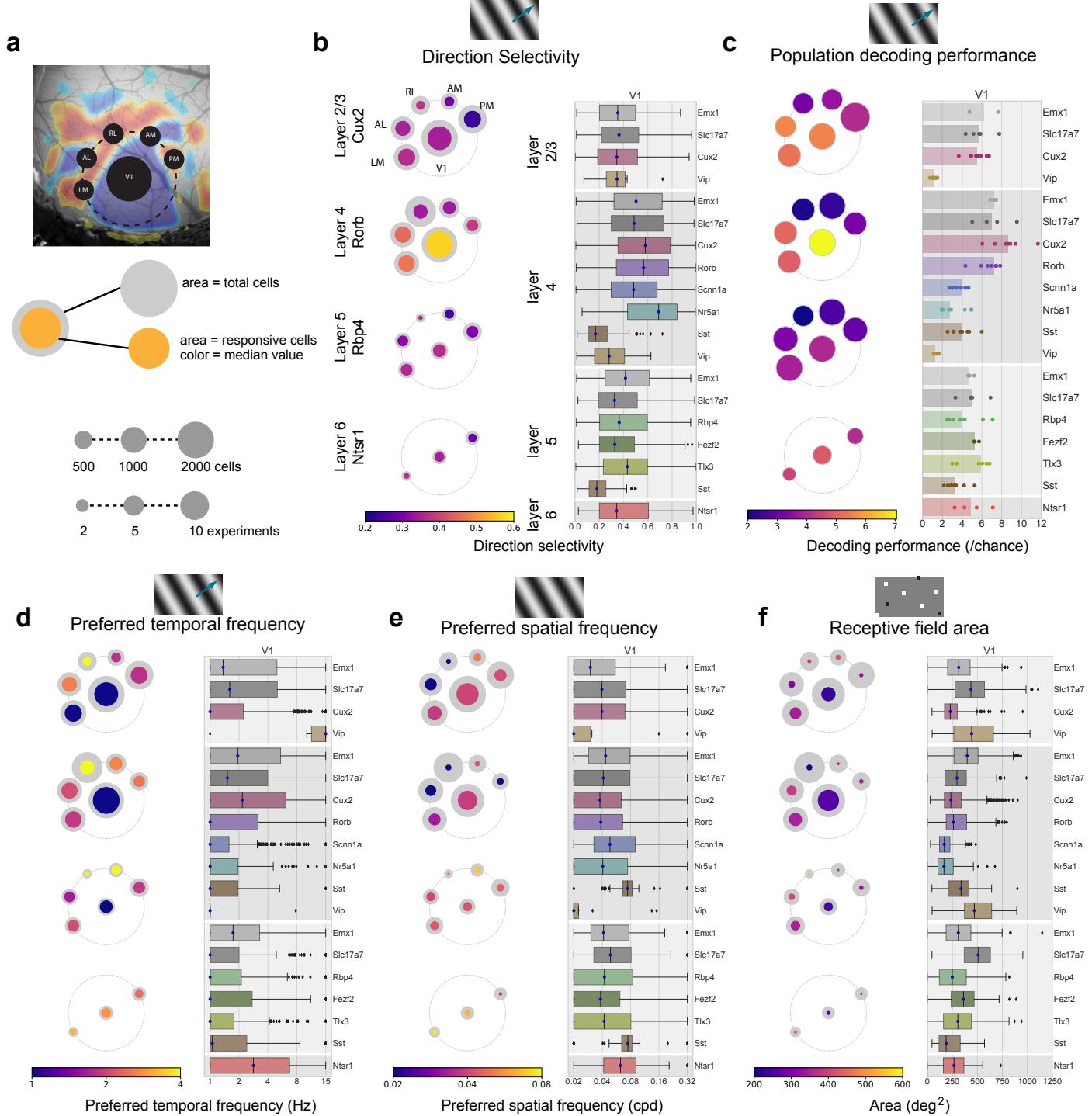
Figure 2



595 **Figure 2: Visual responses to diverse visual stimuli.**

596 (a) Activity for four example neurons, two excitatory neurons (Rorb, layer 4, Rbp4, layer 5)
597 and two inhibitory neurons (Sst layer 4, and Vip layer 2/3). $\Delta F/F$ (top, blue) and extracted
598 events (bottom, black) for each cell. (b) Star plot summarizing orientation and temporal
599 frequency tuning for responses to the drifting gratings stimulus (For details on response
600 visualizations see Supplemental Figure 13). (c) Fan plot summarizing orientation and
601 spatial frequency tuning for responses to static gratings. (d) Corona plot summarizing
602 responses to natural scenes. (e) Track plot summarizing responses to natural movies. (f)
603 Receptive field subunits mapped using locally sparse noise. (g) Percent of neurons that
604 responded to at least one stimulus across cortical areas. (h) Percent of neurons that
605 responded to each stimulus across cortical areas. Colors correspond to the labels at the
606 top of the figure.

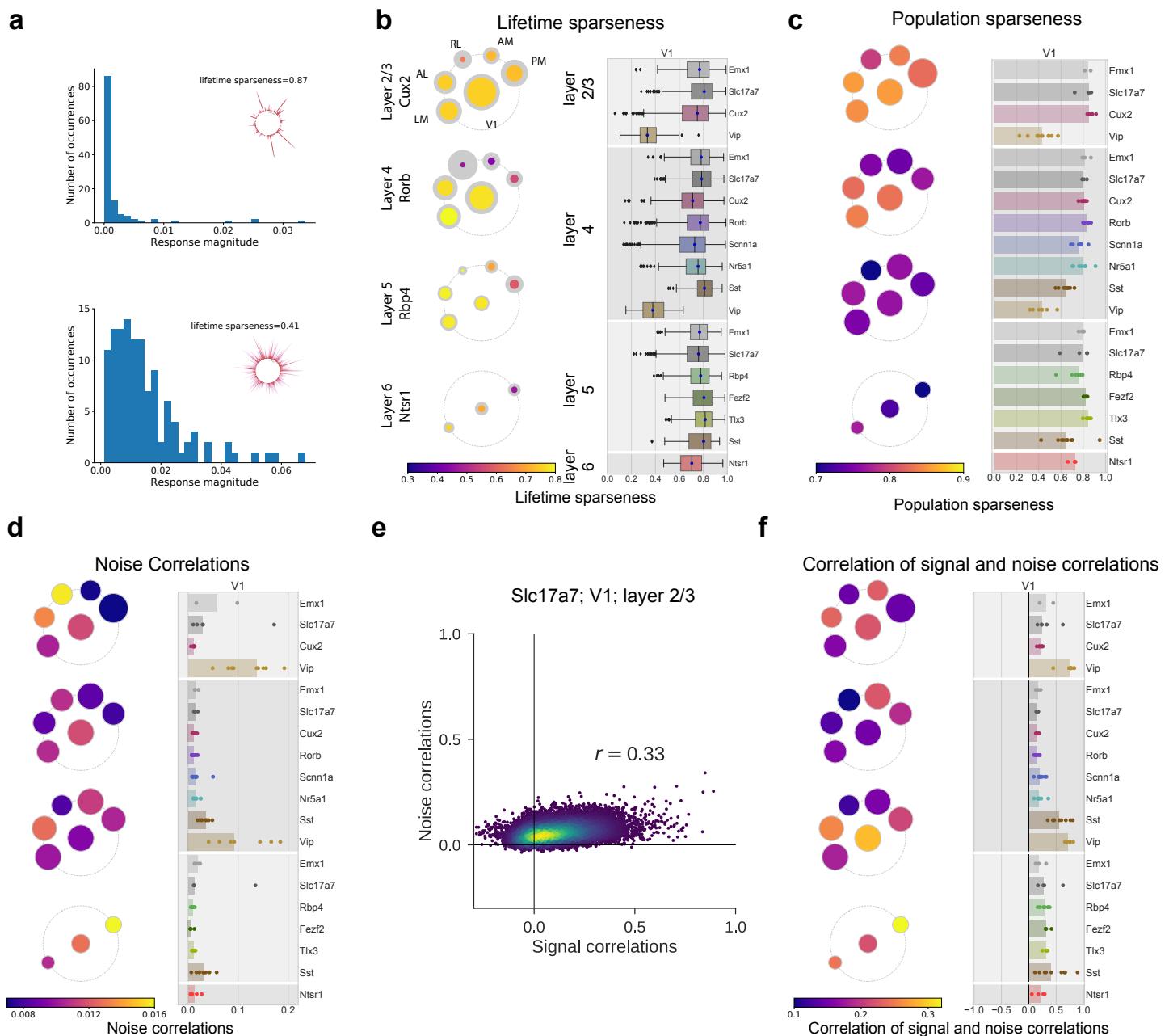
Figure 3



607 **Figure 3: Tuning properties reveal functional differences across areas**
608 **and Cre lines.**

609 (a) Pawplot visualization summarizes median value of a tuning metric across visual areas.
610 Top, each visual area is represented as a circle, with V1 in the center and the higher visual
611 areas surrounding it according to their location on the surface of the cortex. Center, each
612 paw-pad (visual area) has two concentric circles. The area of the larger circle represents
613 the number of cells imaged at that layer and area. The area of the inner, colored, circle
614 represents the number of responsive cells for that layer and area. The color of the inner
615 circle reflects the median value of the metric for the responsive cells, indicated by the
616 colorscale at the bottom of the plot. Bottom, scale of circle area for single cell metrics and
617 for population metrics. In contrast to single-cell metrics, for population metrics (e.g. Fig 3c)
618 each paw-pad (visual area) has only one circle, and the area represents the number of
619 datasets. For a metric's summary plot, four pawplots are shown, one for each layer. Only
620 data from one Cre line is shown for each layer. For each panel, a pawplot is paired with a
621 box plot or a strip plot (for single cell and population metrics respectively) showing the full
622 distribution for each Cre line and layer in V1. Data is assigned to cortical layers based on
623 both the Cre line and the imaging depth. Data collected above 275 μ m from the surface is
624 considered to be in layer 2/3. Data collected between 275 μ m and 375 μ m is considered to
625 be in layer 4. Data collected between 375 μ m and 500 μ m is considered to be in layer 5.
626 Data collected at 550 μ m is considered to be in layer 6. The box shows the quartiles of the
627 data, and the whiskers extend to 1.5 times the interquartile range. Points outside this
628 range are shown as outliers. For other cortical areas, see Supplemental Figure 19. (b)
629 Pawplot and box plot summarizing direction selectivity. (c) Pawplot and strip plot
630 summarizing decoding performance for drifting grating direction using K-nearest
631 neighbors. Each dot represents the mean five-fold cross-validated decoding performance
632 of a single experiment, with the median performance for a Cre-line/layer represented by
633 bar. (d) Pawplot and box plot summarizing preferred temporal frequencies. (e) Pawplot
634 and box plot summarizing preferred spatial frequencies. (f) Pawplot and box plot
635 summarizing receptive field area.

Figure 4

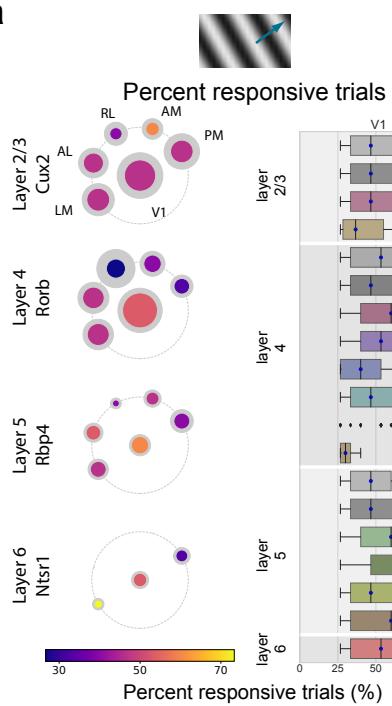


636 **Figure 4: Visual responses are sparse, but coding is dense.**

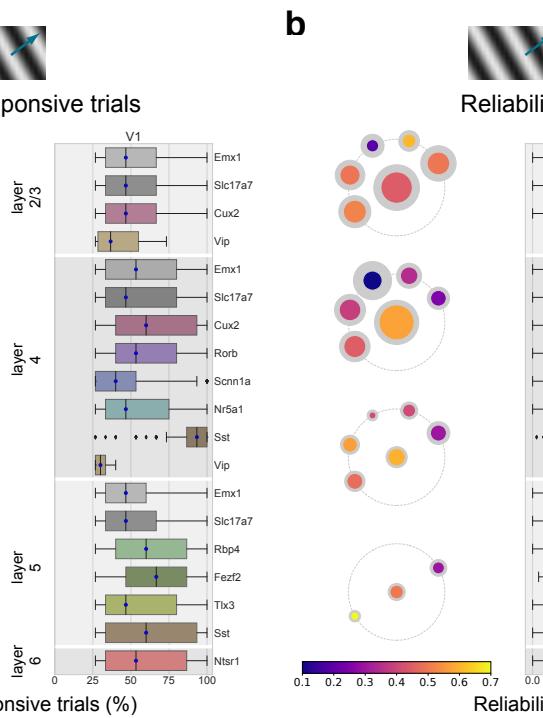
637 (a) Distribution of evoked responses for two example cells showing either high lifetime
638 sparseness (top) and low lifetime sparseness (bottom). The corona plot for each cell is
639 inset in the plot. (b) Pawplot and box plots summarizing lifetime sparseness of the
640 responses to natural scenes. (c) Pawplot and strip plot summarizing the population
641 sparseness of responses to natural scenes. (d) Pawplot and strip plot summarizing the
642 mean noise correlation of responses to natural scenes. (e) Correlation (spearman's rho)
643 between noise correlations and signal correlations for one experiment (Slc17a7, layer 2/3
644 of V1). (f) Pawplot and strip plot summarizing the correlation of signal correlations and
645 noise correlations.

Figure 5

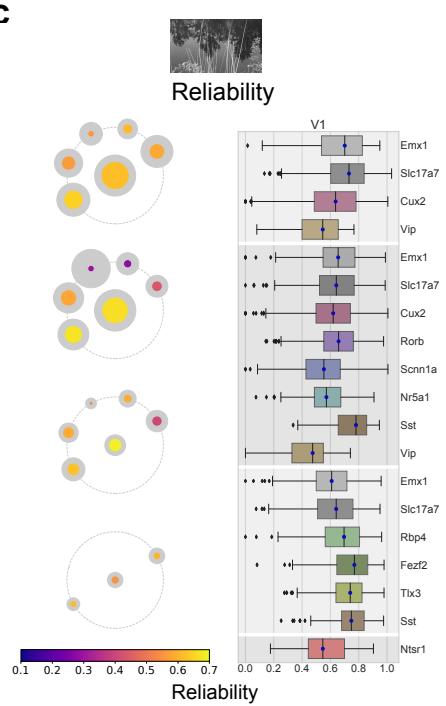
a



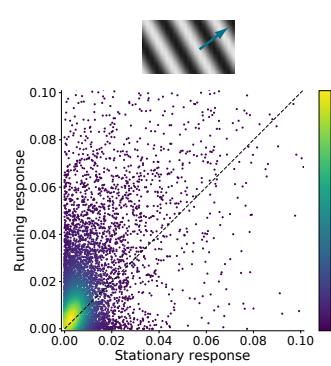
b



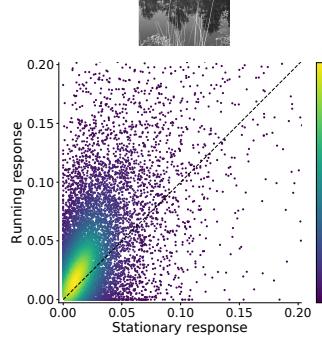
c



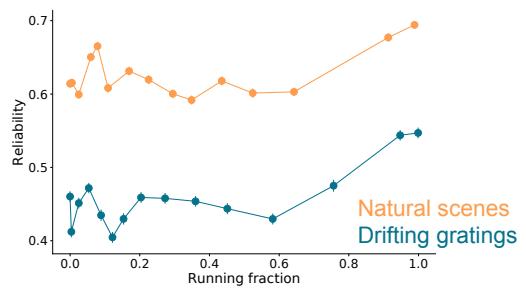
d



e



f

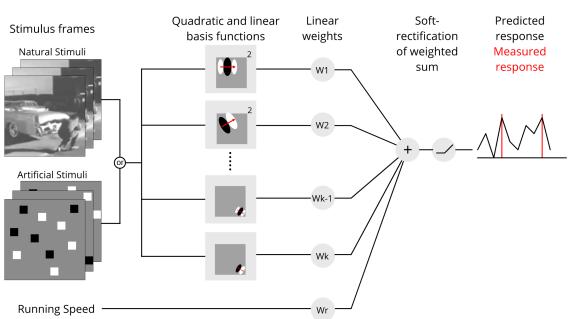


646 **Figure 5: Neural variability is only weakly explained by locomotor**
647 **activity.**

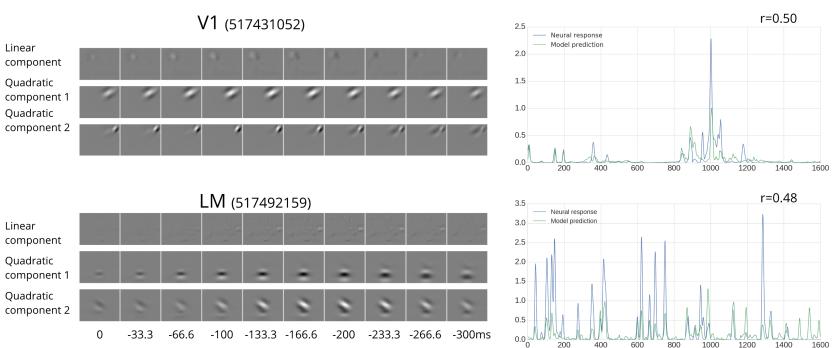
648 (a) Paw plot and box plot summarizing the percent of responsive trials for drifting gratings,
649 the percent of trials that have a significant response for each neurons preferred grating
650 condition. The responsiveness criteria is that a neuron responded to 25% of the trials,
651 hence the low end is capped at 25%. (b) Paw plot and box plot summarizing the reliability
652 of responses for drifting gratings. (c) Paw plot and box plot summarizing the reliability of
653 responses for natural scenes. (d) Evoked response to a neuron's preferred drifting grating
654 condition when the mouse is running (running speed > 1 cm/s) compared to when it is
655 stationary, shown as a density plot. (e) Evoked response to a neuron's preferred natural
656 scene when the mouse is running (running speed > 1 cm/s) compared to when it is
657 stationary, shown as a density plot. (f) Reliability as a function of running fraction, data
658 binned into equally sized bins, for drifting gratings and natural scenes.

Figure 6

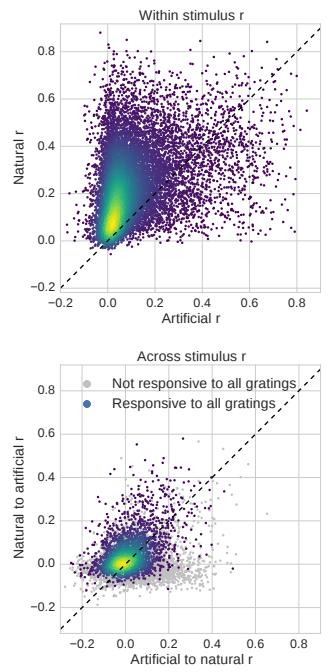
a



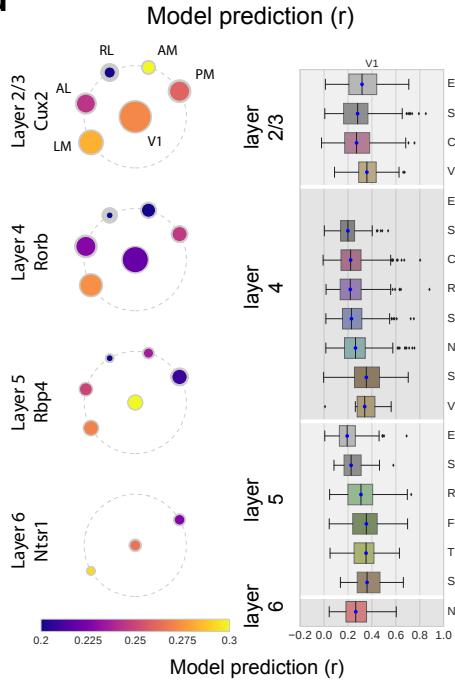
b



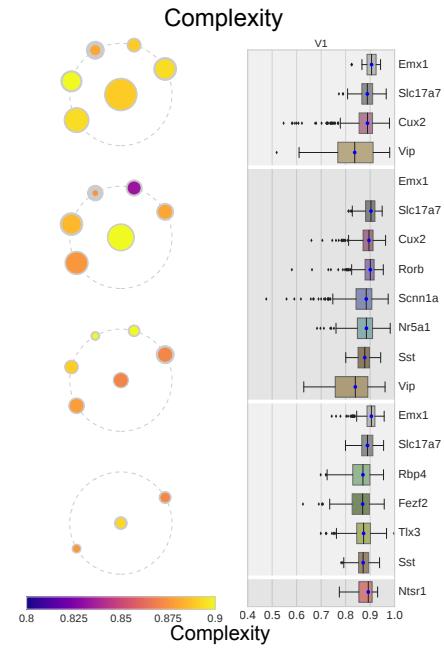
c



d



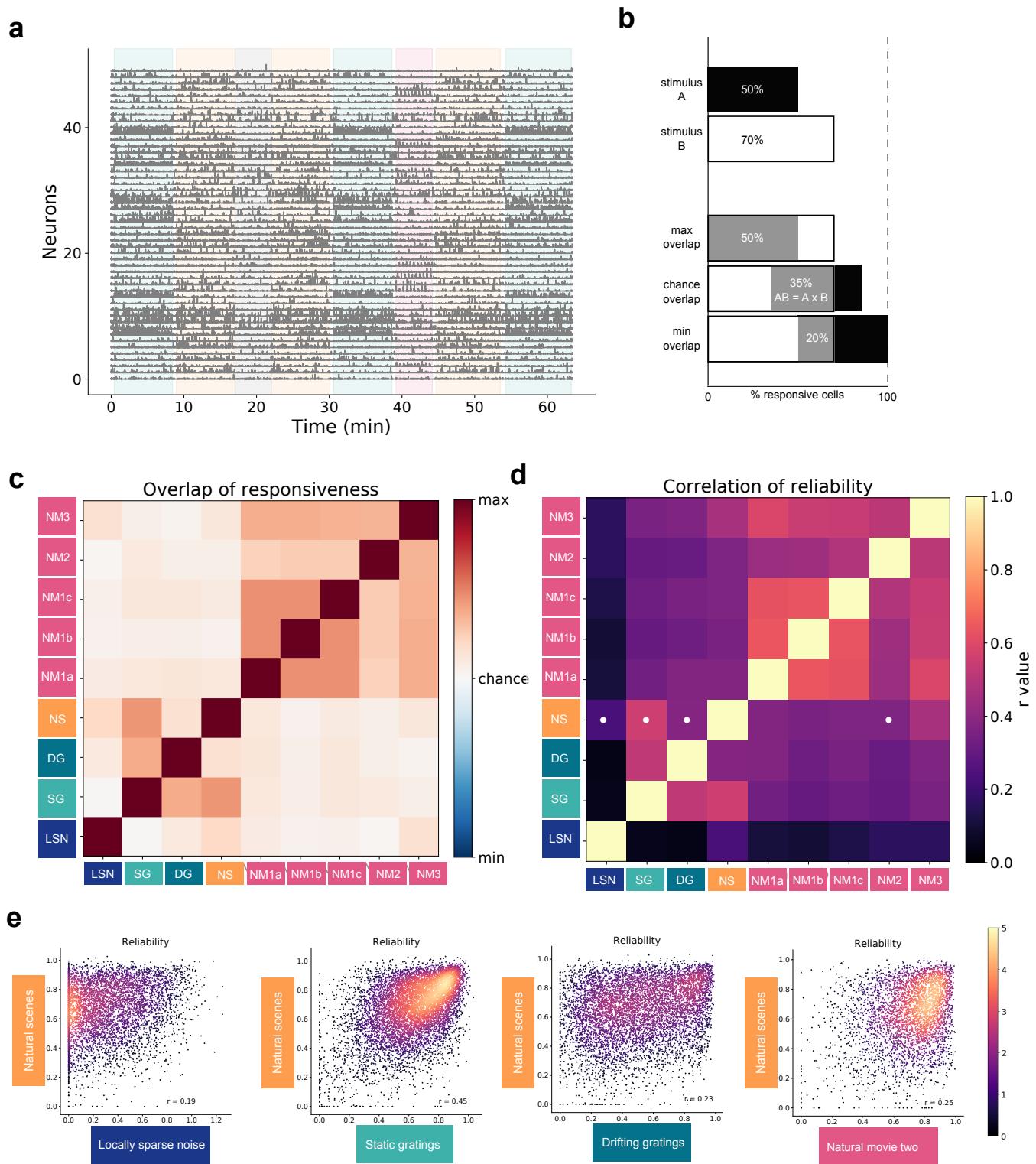
e



659 **Figure 6: All cells show a high degree of complexity and are better fit**
660 **with natural stimuli**

661 (a) Schematic for the GLM. The models are trained on either natural or artificial stimuli,
662 converted into a 30Hz time series and spatially downsampled. The time series input is
663 filtered with spatio-temporal Gabor wavelet pyramids, one of which is linearly combined,
664 the other of which is squared before components are combined. These weighted sums are
665 passed through a soft-rectification to predict the detected calcium events, which have
666 been smoothed with a Gaussian filter. (b) (left) Example filters for two cells from the
667 dataset, showing the linear filter as well as two (of many) quadratic components. (right)
668 Predicted response compared with smoothed calcium events for those example cells. (c)
669 Density plot comparing the mean r values for models trained on natural vs. artificial stimuli
670 for all modeled cells (top). Density plot showing cross stimulus performance of models
671 trained on one stimulus type and tested on the other (bottom). (d) Pawplot and box plot
672 summarizing the r values for the dataset. (e) Pawplot and box plot summarizing the
673 complexity across the dataset.

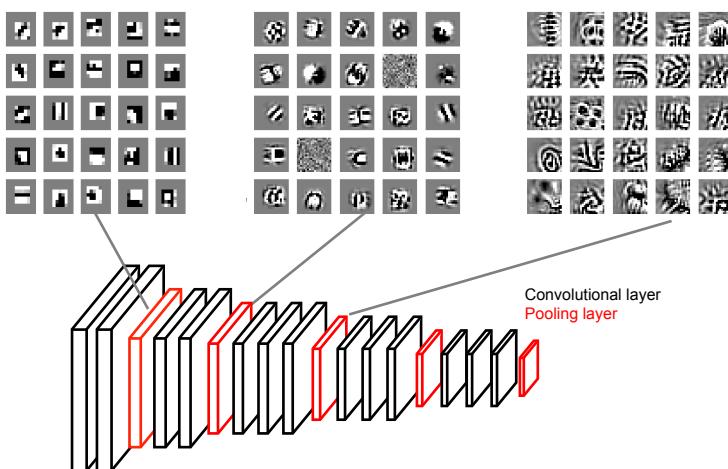
Figure 7



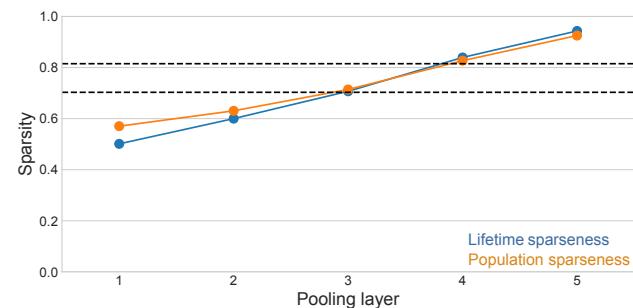
674 **Figure 7: Responses to different stimuli are largely independent.**

675 (a) Responses of 50 neurons during one imaging session (Cux2, V1, layer 2/3) with
676 stimulus epochs shaded using stimulus colors from Figure 1. (b) Schematic of overlap
677 analysis. If 50% of cells in an experiment respond to stimulus A and 70% of the cells
678 respond to stimulus B, chance overlap would be 35%. Maximum overlap would be 50%,
679 and minimum overlap would be 20%. The overlap between each pair of stimuli was
680 computed, and z-scored. (c) Median overlap z-score for each pair of stimuli for all
681 experiments. (d) The correlation of response reliability for cells responses to each pair of
682 stimuli. White dots indicate the combinations that are shown in panel d (e) Comparison of
683 the reliability of responses for natural scenes with locally sparse noise, static gratings,
684 drifting gratings and natural movie three (left to right).

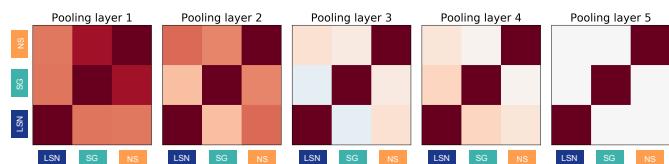
a



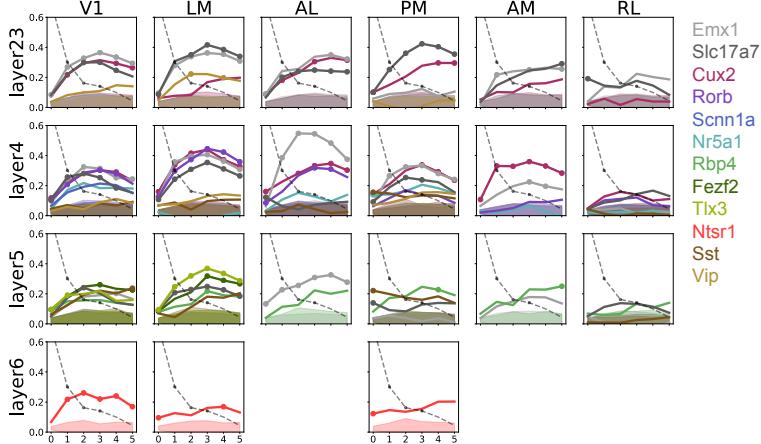
b



c



d



685 **Figure 8: Mouse visual cortex maps to mid-to-high levels of a**
686 **Convolutional Neural Network.**

687 (a) Schematic of VGG16 showing convolutional (black) and pooling (red) layers. Above,
688 example optimal stimuli for sample units found at the first three pooling layers. (b) Median
689 lifetime and population sparseness for each pooling layer of VGG16 in response to the
690 natural scenes stimulus used for this dataset. Dashed lines indicate the limits of median
691 lifetime sparseness for natural scenes found in V1 (see Figure 4b). (c) Stimulus overlap
692 (for the flashed stimuli from the data set) for the pooling layers of VGG16. (d) Similarity of
693 similarity matrix correlation between neural data from each Cre line, area, layer and each
694 pooling layer of VGG16 (see Methods). Shaded region is the null distribution for
695 significance at one standard deviation. Dashed line indicates the SSM correlation with a
696 spatial wavelet pyramid.