

# 1 **Gamma-band resonance of visual cortex to** 2 **optogenetic stimulation**

3 Abbreviated title: Gamma-band resonance of visual cortex

4 Jianguang Ni (倪劍光),<sup>1,2,\*</sup> Christopher Murphy Lewis,<sup>1,\*</sup> Thomas Wunderle,<sup>1</sup> Patrick  
5 Jendritza,<sup>1,2</sup> Ilka Diester,<sup>1</sup> and Pascal Fries<sup>1,3</sup>

6 <sup>1</sup>Ernst Strüngmann Institute (ESI) for Neuroscience in Cooperation with Max Planck Society,  
7 Deutschordenstraße 46, 60528 Frankfurt, Germany

8 <sup>2</sup>International Max Planck Research School for Neural Circuits, Max-von-Laue-Straße 4,  
9 60438 Frankfurt, Germany

10 <sup>3</sup>Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Kapittelweg 29,  
11 6525 EN Nijmegen, Netherlands.

12 \*These authors contributed equally.

13 Correspondence should be addressed to Dr. Pascal Fries, Ernst Strüngmann Institute (ESI) for  
14 Neuroscience in Cooperation with Max Planck Society, Deutschordenstraße 46, 60528 Frankfurt,  
15 Germany. E-mail: pascal.fries@esi-frankfurt.de.

16 Number of pages: 22. Number of figures: 9

17 Number of words in abstract: 213, introduction: 590, discussion: 1474

18 The authors declare no competing financial interests.

19 This work was supported by DFG (SPP 1665, FOR 1847, FR2557/5-1-CORNET to P.F.; EXC 1086,  
20 DI 1908/5-1, DI 1908/6-1 to I.D.), BMBF (01GQ1301 to I.D.), EU (HEALTH-F2-2008-200728-  
21 BrainSynch, FP7-604102-HBP, FP7-600730-Magnetropes to P.F.; ERC Starting Grant OptoMotorPath  
22 to I.D.), a European Young Investigator Award to P.F., the FENS-Kavli Network of Excellence to I.D.,  
23 National Institutes of Health (1U54MH091657-WU-Minn-Consortium-HCP to P.F.), the LOEWE  
24 program (NeFF to P.F. and I.D.). Present address of I.D.: Optophysiology, Bernstein Center and  
25 BrainLinks-BrainTools, University of Freiburg, Albertstraße 23, 79104 Freiburg, Germany.

26 Author contributions: J.N., C.M.L., T.W., P.F. designed research; J.N., C.M.L., T.W., P.J., I.D., P.F.  
27 performed experiments; J.N., C.M.L., T.W. analyzed data; J.N., P.F. wrote the paper.

## 28 **Abstract**

29 Activated visual cortex typically engages in neuronal synchronization in the gamma-  
30 frequency band (30-90 Hz). Gamma-band synchronization is related to cognitive  
31 functioning, and its mechanisms have been extensively investigated, predominantly  
32 through in-vitro studies. To further elucidate its mechanisms in-vivo, we performed  
33 simultaneous optogenetic stimulation and electrophysiological recordings of visual  
34 cortical areas 17 and 21a in the anesthetized cat. Viral transfection with AAV1 or AAV9  
35 under a CamKII $\alpha$  promoter led to robust Channelrhodopsin-2 (ChR2) expression.  
36 Immunohistochemical analysis showed that all ChR2-expressing neurons were  
37 negative for Parvalbumin, consistent with predominant or exclusive expression in  
38 excitatory neurons. Optogenetic stimulation used primarily surface illumination directly  
39 above the transfected and recorded cells. Stimulation with constant light led to strong  
40 and sustained gamma-band synchronization with strength and bandwidth similar to  
41 visually induced gamma. Rhythmic stimulation with light-pulse trains or sinusoidal light  
42 modulation revealed strongest resonance for gamma-band frequencies. Gamma  
43 resonance was confirmed by optogenetic white-noise stimulation. White-noise  
44 stimulation allowed the quantification of the transfer function between the optogenetic  
45 stimulation and the local field potential response. This transfer function showed a  
46 dominant peak in the gamma band. Thus, we find that visual cortical circuits resonate  
47 most strongly to gamma-band components in their input. This resonance renders both  
48 the sensitivity to input, and the output of these circuits, selectively tuned to gamma.

## 49 **Significance Statement**

50 Activated groups of cortical neurons often display rhythmic synchronization in the  
51 gamma-frequency band (30-90 Hz). Gamma-band synchronization is particularly well  
52 studied in visual cortex. We used optogenetics to control visual cortex neurons with  
53 light. Different optogenetic stimulation protocols, using constant light, rhythmically  
54 modulated light or white-noise modulated light, all demonstrated that the investigated  
55 circuits predominantly resonate to stimulation in the gamma band. The observed  
56 gamma-band resonance renders visual cortical circuits most sensitive to gamma-  
57 rhythmic synaptic inputs. This in turn renders their spike output and the ensuing  
58 interareal synchronization gamma rhythmic.

## 59 Introduction

60 When visual cortex of an awake or lightly anesthetized subject is activated by  
61 appropriate stimuli, its neurons typically synchronize their activity in the gamma-  
62 frequency band, between 30 and 90 Hz (Gray et al., 1989; Kreiter and Singer, 1996;  
63 Hoogenboom et al., 2006). Very similar gamma-band synchronization has also been  
64 found outside visual cortex, e.g. in somatosensory and auditory cortex (Brosch et al.,  
65 2002; Bauer et al., 2006), motor cortex (Brown et al., 1998; Schoffelen et al., 2005;  
66 Ball et al., 2008), parietal and frontal cortex (Pesaran et al., 2002; Gregoriou et al.,  
67 2009; Lundqvist et al., 2016) and in hippocampus (Csicsvari et al., 2003; Colgin et al.,  
68 2009). Studies on gamma-band synchronization have investigated both its functional  
69 consequences and its mechanisms.

70 Studies on the functional role of visual cortical gamma-band synchronization have  
71 used in-vivo approaches to investigate relations to visual stimulation, task  
72 requirements and behavior. These studies suggest that visual gamma subserves,  
73 among other functions, object segmentation (Gray et al., 1989) and perceptual and  
74 attentional stimulus selection (Fries et al., 2002; Womelsdorf et al., 2006; Bosman et  
75 al., 2012; Grothe et al., 2012).

76 Experimental studies on the mechanisms underlying gamma-band synchronization  
77 have partly used in-vivo approaches. For example, intracellular recordings in  
78 anesthetized cat visual cortex revealed a type of cell, denoted “chattering cell”, that  
79 intrinsically generates gamma-rhythmic bursts when depolarized by current injection  
80 and that exhibits pronounced oscillations when visually stimulated (Gray and  
81 McCormick, 1996). Also, several in-vivo studies demonstrated that excitatory neurons  
82 lead inhibitory neurons during the gamma cycle by a few milliseconds (Csicsvari et al.,  
83 2003; Hasenstaub et al., 2005; Vinck et al., 2013). Yet, most experimental  
84 investigations of gamma mechanisms used in-vitro approaches, because they more  
85 readily allow intracellular recordings and current injections, as well as pharmacological  
86 manipulations (Whittington et al., 2000; Buzsáki and Wang, 2012; Salkoff et al., 2015).

87 Optogenetics, using Channelrhodopsin or related opsins, provides novel opportunities  
88 to investigate the mechanisms underlying the generation of rhythms, including the  
89 gamma rhythm. While a majority of optogenetic studies have exploited pathway or cell-  
90 type specific opsin expression to elucidate particular neural circuits, several studies  
91 capitalized on the excellent temporal resolution of optogenetic stimulation to  
92 investigate mechanisms underlying different neuronal rhythms. For example, the  
93 rhythmic optogenetic stimulation of mouse hippocampal or neocortical parvalbumin-  
94 positive (PV) interneurons leads to the indirect induction of theta resonance (Stark et  
95 al., 2013).

96 One seminal study used optogenetics in mouse somatosensory cortex to stimulate  
97 either PV interneurons or excitatory neurons (Cardin et al., 2009). When optogenetic  
98 pulse trains were given to PV interneurons, the network showed local field potential  
99 (LFP) resonance in a gamma-frequency band peaking around 40-50 Hz. By contrast,

100 when the same pulse trains were delivered to excitatory neurons, resonance was  
101 strongest at 8 Hz and declined monotonically for increasing frequencies to vanish  
102 above 24 Hz.

103 We used optogenetics to investigate the resonance properties of visual cortex to  
104 provide further insights into mechanisms behind gamma-band synchronization among  
105 visual cortical neurons in vivo. We used visual cortex of the lightly anesthetized cat, a  
106 classical model system for research on vision and gamma-band synchronization. First,  
107 we tried three viral vectors and found that AAV5 fails to provide expression in the cat,  
108 whereas both AAV1 and AAV9 lead to robust expression. Constant optogenetic  
109 stimulation induced strong and sustained gamma-band activity. Rhythmic stimulation  
110 with pulse trains or sine waves at frequencies between 5 and 80 Hz revealed network  
111 resonance at 40 Hz or above. To investigate this resonance with greater spectral  
112 resolution, we applied optogenetic white noise stimulation, which confirmed resonance  
113 with a peak at 40-60 Hz.

## 114 **Materials and Methods**

115 Eight adult domestic cats (*felis catus*; four females) were used in this study. All  
116 procedures complied with the German law for the protection of animals and were  
117 approved by the regional authority (Regierungspräsidium Darmstadt). After an initial  
118 surgery for the injection of viral vectors and a 4-6 week period for opsin expression,  
119 recordings were obtained during a terminal experiment under general anesthesia.

### 120 **Viral vector injection**

121 For the injection surgery, anesthesia was induced by intramuscular injection of  
122 ketamine (10 mg/kg) and dexmedetomidine (0.02 mg/kg), cats were intubated, and  
123 anesthesia was maintained with N<sub>2</sub>O:O<sub>2</sub> (60/40%), isoflurane (~1.5%) and remifentanyl  
124 (0.3 µg/kg/min). Four cats were injected in area 17 and another four cats in area 21a.  
125 Rectangular craniotomies were made over the respective areas (Area 17: AP  
126 [0, -7.5] mm; ML: [0, 5] mm; area 21a: AP [0,-8] mm, ML [9, 15] mm). The areas were  
127 identified by the pattern of sulci and gyri, and the dura mater was removed over part  
128 of the respective areas. Three to four injection sites were chosen, avoiding blood  
129 vessels, with horizontal distances between injection sites of at least 1 mm. At each  
130 site, a Hamilton syringe (34 G needle size; World Precision Instruments) was inserted  
131 with the use of a micromanipulator and under visual inspection to a cortical depth of  
132 1 mm below the pia mater. Subsequently, 2 µl of viral vector dispersion was injected  
133 at a rate of 150 nl/min. After each injection, the needle was left in place for 10 min  
134 before withdrawal, to avoid reflux. Upon completion of injections, the dura opening  
135 was covered with silicone foil and a thin layer of silicone gel, the trepanation was filled  
136 with dental acrylic, and the scalp was sutured.

137 In one cat, area 17 in the left hemisphere was injected with AAV1-CamKIIα-  
138 hChR2(H134R)-eYFP (titer  $8.97 \cdot 10^{12}$  GC/ml) and area 17 in the right hemisphere with

139 AAV9-CamKII $\alpha$ -Chr2-eYFP (titer  $1.06 \times 10^{13}$  GC/ml). In two cats, area 17 of the left  
140 hemisphere was injected with AAV1-CamKII $\alpha$ -hChr2(H134R)-eYFP (titer:  
141  $1.22 \times 10^{13}$  GC/ml). In one cat, area 17 of the left hemisphere was injected with AAV5-  
142 CamKII $\alpha$ -Chr2-eYFP (titer  $4 \times 10^{13}$  GC/ml). In four cats, area 21a of the left  
143 hemisphere was injected with AAV9-CamKII $\alpha$ -hChr2(H134R)-eYFP (titer:  
144  $1.06 \times 10^{13}$  GC/ml). AAV1 and AAV9 viral vectors were obtained from Penn Vector Core  
145 (Perelman School of Medicine, University of Pennsylvania, USA), AAV5 viral vectors  
146 from UNC Vector Core (UNC School of Medicine, University of North Carolina, USA).

## 147 **Neurophysiological recordings**

148 For the recording experiment, anesthesia was induced and initially maintained as  
149 during the injection surgery, only replacing intubation with tracheotomy and  
150 remifentanyl with sufentanil. After surgery, during recordings, isoflurane concentration  
151 was lowered to 0.6%-1.0%, eye lid closure reflex was tested to verify narcosis, and  
152 vecuronium (0.25mg/kg/h i.v.) was added for paralysis during recordings. Throughout  
153 surgery and recordings, Ringer solution plus 10% glucose was given (20 ml/h during  
154 surgery; 7 ml/h during recordings), and vital parameters were monitored (ECG, body  
155 temperature, expiratory gases).

156 Each recording experiment consisted of multiple sessions. For each session, we  
157 inserted either single or multiple tungsten microelectrodes ( $\sim 1$  M $\Omega$  at 1 kHz; FHC), or  
158 three to four 32-contact probes (100  $\mu$ m inter-site spacing,  $\sim 1$  M $\Omega$  at 1 kHz;  
159 NeuroNexus or ATLAS Neuroengineering) in area 21a and area 17. Standard  
160 electrophysiological techniques (Tucker Davis Technologies, TDT) were used to  
161 obtain multi-unit activity (MUA) and LFP recordings. For MUA recordings, the signals  
162 were filtered with a passband of 700 to 7000 Hz, and a threshold was set to retain the  
163 spike times of small clusters of units. For LFP recordings, the signals were filtered with  
164 a passband of 0.7 to 250 Hz and digitized at 1017.1 Hz.

## 165 **Photo-stimulation**

166 Optogenetic stimulation was done with a 473 nm (blue) laser or with a 470 nm (blue)  
167 LED (Omicron Laserage). A 594 nm (yellow) laser was used as control. Laser light  
168 was delivered to cortex through a 100  $\mu$ m or a 200  $\mu$ m diameter multimode fiber  
169 (Thorlabs), LED light through a 2 mm diameter polymer optical fiber (Omicron  
170 Laserage). Fiber endings were placed just above the cortical surface, immediately next  
171 to the recording sites with a slight angle relative to the electrodes. Laser waveform  
172 generation used custom circuits in TDT, and timing control used Psychtoolbox-3, a  
173 toolbox in MATLAB (MathWorks) (Brainard, 1997).

174 For white-noise stimulation, the laser was driven by normally distributed white noise,  
175 with light intensities updated at a frequency of 1017.1 Hz. The total output intensity  
176 varied between sessions, with values in the range of 15-80 mW (46 recording sites in  
177 area 17 of 3 cats).

## 178 **Histology**

179 After conclusion of recordings, approximately five days after the start of the terminal  
180 experiment and still under narcosis, the animal was euthanized with pentobarbital  
181 sodium and transcardially perfused with phosphate buffered saline (PBS) followed by  
182 4% paraformaldehyde. The brain was removed, post-fixed in 4% paraformaldehyde  
183 and subsequently soaked in 10%, 20% and 30% sucrose-PBS solution, respectively,  
184 until the tissue sank. The cortex was sectioned in 50  $\mu$ m thick slices, which were  
185 mounted on glass slides, coverslipped with an antifade mounting medium, and  
186 subsequently investigated with a confocal laser scanning microscope (CLSM, Nikon  
187 C2 90i, Nikon Instruments) for eYFP-labelled neurons.

188 *Immunohistochemistry.* In two cats, one with injections in area 17 and one with  
189 injections in area 21a, slices were processed as described above and additionally  
190 stained for parvalbumin (PV). To this end, slices were preincubated in 10% normal  
191 goat serum (Sigma Aldrich) with 1% bovine serum albumin (BSA) and 0.5% Triton X-  
192 100 in phosphate buffer (PB) for 1 h at room temperature to block unspecific binding  
193 sites. Slices were subsequently incubated in 3% normal goat serum with 1% BSA,  
194 0.5% Triton X-100 and the primary antibody (rabbit anti-Parvalbumin, NB 120-11427,  
195 Novus Biologicals) over night at room temperature. After washing two times 15 min in  
196 PB, the slices were incubated with the secondary antibody (goat anti-rabbit Alexa Fluor  
197 647, A-21244, Thermo Fisher Scientific) in 3% normal goat serum, 1% BSA and 0.5%  
198 Triton X-100 for 1 h at room temperature. Finally, slices were again washed in PB,  
199 coverslipped and imaged with a Zeiss CLSM, using a 25X water immersion objective.

## 200 **Data analysis**

201 All data analysis was performed using custom code and the Fieldtrip toolbox  
202 (Oostenveld et al., 2011), both written in MATLAB (MathWorks).

203 *Spike densities, spike autocorrelation histograms, LFP power spectra, and MUA-LFP*  
204 *PPCs.* MUA rate was smoothed with a Gaussian (for constant light stimulation: SD =  
205 12.5 ms; for stimulation with pulse trains and sine waves: SD = 1.25 ms; in each case  
206 truncated at  $\pm 2$  SD) to obtain the spike density. To measure the MUA rhythmicity  
207 during optogenetic stimulation with pulse trains or sine waves of frequency  $f$ , the F1  
208 component of the MUA was calculated as the amplitude of the Fourier transform at  
209 frequency  $f$ . To give F1 values of the different animals equal impact on the grand  
210 average, F1 values were z-scored within each animal by subtracting the mean and  
211 dividing by the SD of all F1 values of that animal.

212 The spike autocorrelation histogram (ACH) was calculated at 1 ms resolution with  
213 maximum time lag of 250 ms. Subsequently, the ACH was normalized by the triangle  
214 function  $\text{tri}(t)$  and the MUA rate, such that the ACH is expressed in units of  
215 coincidence/spike:

216 
$$ACH_{norm}(t) = \frac{ACH(t)}{tri(t) \cdot r}$$

217 The triangle function is defined as

218 
$$tri(t) = \max(l - |t|, 0), t = [-\tau_m: 1: \tau_m]$$

219 , with  $l$  being the spike train length in seconds, and  $r$  being the mean spike rate in  
220 Hertz.

221 The ACH was smoothed with a Gaussian (SD = 0.5 ms, truncated at  $\pm 1.5$  SD), and  
222 the F1 component of the ACH was calculated and normalized as for the MUA spike  
223 train.

224 LFP power spectra were calculated for data epochs that were adjusted for each  
225 frequency to have a length of 4 cycles and moved over the data in a sliding-window  
226 fashion in 1 ms steps. Each epoch was multiplied with a Hann taper, Fourier  
227 transformed, squared and divided by the window length to obtain power density per  
228 frequency. For the different stimulation frequencies  $f$ , LFP power is shown as ratio of  
229 power during stimulation versus pre-stimulation baseline (-0.5 s to -0.2 s relative to  
230 stimulation onset).

231 MUA-LFP locking was quantified by calculating the MUA-LFP PPC (pairwise phase  
232 consistency), a metric that is not biased by trial number, spike count or spike rate  
233 (Vinck et al., 2010). Spike and LFP recordings were always taken from different  
234 electrodes. For each spike, the surrounding LFP was Hann tapered and Fourier  
235 transformed. Per spike and frequency, this gave the MUA-LFP phase, which should  
236 be similar across spikes, if they are locked to the LFP. This phase similarity is  
237 quantified by the PPC as the average phase difference across all possible pairs of  
238 spikes. To analyze PPC as a function of frequency and time (Fig. 4 and 9), the LFP  
239 around each spike in a window of  $\pm 2$  cycles per frequency was Hann tapered and  
240 Fourier transformed. PPC was then calculated for epochs of 100 ms length, i.e. using  
241 the phases of spikes in those epochs, moved over the data in a sliding-window fashion  
242 in 1 ms steps. To analyze PPC with higher spectral resolution (Fig. 5), the LFP around  
243 each spike in a window of  $\pm 0.5$  s (Fig. 5F, lower frequencies) or  $\pm 0.25$  s (Fig. 5F,  
244 higher frequencies) was Hann tapered and Fourier transformed to obtain the spike  
245 phase. For a given MUA channel, MUA-LFP PPC was calculated relative to all LFPs  
246 from different electrodes and then averaged.

247 *Estimation of transfer function from recordings during white-noise stimulation.* For  
248 each recording site and channel, the transfer function between the uniform white noise  
249 time series driving the laser and the simultaneously acquired local field potential was  
250 determined. The transfer function was estimated by Welch's average periodogram  
251 method, separately per recording site and trial. It is the ratio of the cross spectral  
252 density between the input (laser) and the output (LFP) time series, and the power  
253 spectral density of the input (laser). To determine the white-noise driven resonance

254 spectrum, the magnitude of the transfer function was computed for each recording  
255 site. The values from one such estimate demonstrate the transfer function for a single  
256 example recording site (Fig. 9D). In order to estimate the average transfer function  
257 across all recording sites, these magnitudes were normalized to equalize the total  
258 power. The normalized values across all recording sites were averaged to calculate  
259 the average spectrum (Fig. 9E).

260 *Statistical testing.* High-resolution spectra of LFP power changes and MUA-LFP PPC  
261 were compared between stimulation with blue light and control stimulation with yellow  
262 light (Fig. 5E,F). We calculated paired t-tests between spectra obtained with blue and  
263 yellow light, across recording sites. Statistical inference was not based directly on the  
264 t-tests (and therefore corresponding assumptions will not limit our inference), but the  
265 resulting t-values were merely used as a difference metric for the subsequent cluster-  
266 based non-parametric permutation test. For each of 10,000 permutations, we did the  
267 following: 1) We made a random decision per recording site to either exchange the  
268 spectrum obtained with blue light and the spectrum obtained with yellow light or not;  
269 2) We performed the t-test; 3) Clusters of adjacent frequencies with significant t-values  
270 ( $p < 0.05$ ) were detected, and t-values were summed over all frequencies in the cluster  
271 to form the cluster-level test statistic. 4) The maximum and the minimum cluster-level  
272 statistic were placed into maximum and minimum randomization distributions,  
273 respectively. For the observed data, clusters were derived as for the randomized data.  
274 Observed clusters were considered significant if they fell below the 2.5<sup>th</sup> percentile of  
275 the minimum randomization distribution or above the 97.5<sup>th</sup> percentile of the maximum  
276 randomization distribution (Maris and Oostenveld, 2007). This corresponds to a two-  
277 sided test with correction for the multiple comparisons performed across frequencies  
278 (Nichols and Holmes, 2002).

## 279 **Results**

### 280 **Transfection of cat visual cortex neurons by AAV1, AAV5 and AAV9**

281 Recombinant adeno-associated virus (AAV) vectors are widely used as gene delivery  
282 tools (Vasileva and Jessberger, 2005). AAV-mediated expression of  
283 Channelrhodopsin-2 (ChR2) has been used in several mammalian species, including  
284 mice, rats and non-human primates (Diester et al., 2011; Gerits et al., 2015;  
285 Scheyltjens et al., 2015). In this study, three pseudo-typed AAVs (AAV1, AAV5 and  
286 AAV9) were tested in visual cortex of the domestic cat (*felis catus*). We injected AAVs  
287 carrying the gene for the expression of hChR2(H134R)-eYFP under the control of the  
288 Calcium/calmodulin-dependent protein kinase type II alpha chain (CamKII $\alpha$ ) promoter.  
289 Injections targeted either area 17, the cat homologue of primate area V1, or area 21a,  
290 the cat homologue of primate area V4 (Payne, 1993).

291 AAV5 was injected into area 17 of one cat and this did not result in visible ChR2-eYFP  
292 expression (Figure 1A). This failure of AAV5 expression is consistent with a previous  
293 AAV5 transduction study in cat cerebral cortex (Vite et al., 2003). By contrast, AAV1



294 and AAV9 injections into area 17, and AAV9 injections into area 21a resulted in robust  
295 ChR2-eYFP expression (Figure 1B-D). For both, AAV1 and AAV9, fluorescence  
296 showed a dependence on cortical depth, being strong in superficial layers, of medium  
297 strength in deep layers and relatively weak in middle layers (Figure 1B,C). Higher  
298 magnification revealed labeling of individual neurons (Figure 1B-D, right panels).

299 As described below, we find that optogenetic stimulation of the transfected neurons  
300 leads to network resonance in the gamma-band range. The generation of gamma-  
301 band activity has been linked to Parvalbumin-positive (PV+) interneurons. We  
302 therefore investigated, whether ChR2 was expressed in PV+ neurons. In two cats, we  
303 stained histological slices with fluorescence-marked antibodies against parvalbumin  
304 (Fig. 2A-F). One cat had been injected with AAV9-CamKII $\alpha$ -ChR2-eYFP into  
305 area 21a, the other had been injected with AAV1-CamKII $\alpha$ -hChR2(H134R)-eYFP into  
306 area 17. Across several slices and imaging windows of area 21a, we identified 182  
307 unequivocally labeled neurons, which showed ChR2-eYFP expression or PV+ anti-  
308 body staining (Fig. 2A-C); of those, 73 were PV+ and 109 were ChR2-eYFP neurons,  
309 and there was zero overlap between these groups (Fig. 2G). Across several slices and  
310 imaging windows of area 17, we identified 282 unequivocally labeled neurons, which  
311 showed ChR2-eYFP expression or PV+ anti-body staining (Fig. 2D-F); of those, 154  
312 were PV+ and 128 were ChR2-eYFP neurons, and again there was zero overlap  
313 between these groups (Fig. 2G).

### 314 **Neuronal responses to optogenetic stimulation after transfection with AAV1,** 315 **AAV5 and AAV9**

316 Between 4 and 6 weeks after virus injection, we performed terminal experiments under  
317 general anesthesia. The injected part of cortex was illuminated with blue light while  
318 neuronal spike and LFP activity was recorded from the optogenetically stimulated  
319 region. As mentioned above, one injection used AAV5 in area 17 of one cat and failed  
320 to show transfected neurons in the later histology. Correspondingly, the recordings in  
321 this case also failed to show any neuronal response to light application (Fig. 3A, B;  
322 pulses of 18 mW strength and 2 ms duration, applied in a regular 40 Hz pulse train).  
323 Firing rates following light pulses (in a window from 2 to 10 ms after light onset) did  
324 not differ significantly from rates immediately preceding the pulses (-10 to 0 ms)  
325 (Wilcoxon rank-sum test = 2503,  $p = 0.88$ ,  $n = 5$  sites). This was in stark contrast to  
326 responses in a cat injected with AAV1 and AAV9. In one cat, AAV1 was injected into  
327 area 17 in the left hemisphere (Fig. 3C,D), and AAV9 was injected into area 17 in the  
328 right hemisphere (Fig. 3E,F). Both injections led to strong optogenetic responses.  
329 Pulse trains of 20 Hz resulted in strong firing rate enhancements with a clear 20 Hz  
330 modulation. The peri-stimulus time histograms showed response latencies after light  
331 pulses of 3.9 ms (AAV1, Fig. 3D) and 3.6 ms (AAV9, Fig. 3F).

## 332 **Constant optogenetic stimulation induces neuronal gamma-band** 333 **synchronization**

334 Visual cortex shows particularly strong gamma-band activity in response to visual  
335 stimulation that is sustained and devoid of temporal structure (Kruse and Eckhorn,  
336 1996). Thus, optogenetic stimulation of visual cortex might also be particularly suited  
337 to induce gamma if it is constant. Indeed, we have previously reported that constant  
338 optogenetic stimulation induces gamma-band activity in anesthetized cat visual cortex,  
339 in the context of an investigation of the gain-modulating effect of gamma (Ni et al.,  
340 2016). Here, we present a detailed analysis of gamma induced by constant  
341 optogenetic stimulation. Figure 4A shows an example LFP recording from area 21a of  
342 a cat transfected with AAV9, during one trial of optogenetic stimulation with 2 s of  
343 constant blue light. The raw LFP trace reveals strong optogenetically induced gamma,  
344 and the zoomed-in epoch illustrates that this emerged immediately after stimulation  
345 onset. Figure 4B shows the spike responses of this recording site for many interleaved  
346 trials of stimulation with blue or yellow light, confirming the selective optogenetic  
347 stimulation by blue light. Figure 4C shows the spike-triggered average of the LFP,  
348 demonstrating that spikes were locked to the LFP gamma-band component. The time-  
349 frequency analysis of both, LFP power (Fig. 4D, E) and MUA-LFP locking (Fig. 4F, G)  
350 showed a strong and sustained gamma-band peak for stimulation with blue light, that  
351 was absent for stimulation with yellow light.

352 This pattern was found very reliably across all recording sites. Stimulation with two  
353 seconds of constant blue light, as compared to yellow control light, induced strong  
354 enhancements in firing rate, which were sustained for the duration of stimulation  
355 (Fig. 5A,D; Wilcoxon rank-sum test = 39581,  $p < 0.0001$ ,  $n = 163$  sites). The ratio of LFP  
356 power during stimulation versus pre-stimulation baseline showed an optogenetically  
357 induced gamma-band peak around 70 Hz (Fig. 5B,E; Wilcoxon rank-sum test = 14751,  
358  $p < 0.0001$ ,  $n = 99$  sites). We note that the gamma-band peak frequency varies across  
359 animals and recording sessions, as previously shown (Ni et al., 2016). The LFP  
360 gamma-power changes reflected changes in neuronal synchronization, because  
361 optogenetic stimulation also induced strong MUA-LFP locking in the gamma band, as  
362 quantified by the MUA-LFP PPC (Fig. 5C,F; Wilcoxon rank-sum test = 9389,  
363  $p < 0.0001$ ,  $n = 84$  sites). In addition to the induction of gamma-band activity,  
364 optogenetic stimulation also caused a power reduction between 6 and 12 Hz (Fig. 5E  
365 left panel for lower frequencies, note the scale is much smaller than for the higher  
366 frequencies shown in the right panel; Fig.5B inset). At the same time, it caused a  
367 reduction in MUA-LFP locking between 10 and 12 Hz (Fig. 5F and Fig.5C inset).

## 368 **Neuronal responses to optogenetic pulse train stimulation at different** 369 **frequencies**

370 To characterize the temporal response properties of the network to optogenetic  
371 stimulation of the transfected neurons, we applied pulse trains of different frequencies.  
372 Pulses always had a duration of 2 ms, and were repeated at frequencies of 5, 10, 20,

373 40, and 80 Hz. Pulse intensity was adjusted per recording site (see Materials and  
374 Methods) and was kept constant for a given site across the different pulse train  
375 frequencies. The analysis was limited to spike trains and excluded LFP, to avoid LFP  
376 artifacts caused by light stimulation. Pulse trains of all employed frequencies resulted  
377 in clear increases in firing rate, with strong rhythmicity at the pulse train frequency  
378 (Fig. 6). We calculated spike density functions, subtracted the baseline values and  
379 averaged them across recordings sites. Figure 6A-C shows those average spike  
380 densities for 10 Hz, 40 Hz and 80 Hz. We quantified their rhythmicity by calculating  
381 the Fourier transforms at the pulse train frequency (F1, see Materials and Methods).  
382 Figure 6D shows F1 for the different pulse train frequencies. Different stimulation  
383 frequencies led to different F1 components (one-way ANOVA,  $p = 2.3E-9$ ,  $F_{(4,200)} =$   
384 12.9). F1 values showed a smooth dependence on pulse-train frequency, with a peak  
385 at 40 Hz. Auto-correlation histograms (ACHs) confirmed strong rhythmicity  
386 (Fig. 6E-G). F1 components of the ACHs also differed across frequencies (one-way  
387 ANOVA,  $p = 1.2E-6$ ,  $F_{(4,200)} = 8.91$ ) and increased with frequency. Note that the ACH  
388 for 80 Hz stimulation suggested a 40 Hz subharmonic response.

### 389 **Neuronal responses to optogenetic sine wave stimulation at different** 390 **frequencies**

391 The results so far suggest that the stimulated circuits resonate most strongly in the  
392 gamma-frequency band. Yet, for a fixed stimulation epoch, higher pulse train  
393 frequency imposed higher total light power onto the brain tissue. Therefore, we also  
394 employed optogenetic stimulation with sine waves of 5, 10, 20, 40 and 80 Hz, with  
395 total light power constant across frequencies. Results were similar to stimulation with  
396 pulse trains: The rhythmicity of the responses increased with stimulation frequency  
397 (Fig. 7A-H; one-way ANOVA: F1 of spike train,  $p = 3.6E-9$ ,  $F_{(4,295)} = 12.17$ ; F1 of ACH,  
398  $p = 2.2E-14$ ,  $F_{(4,295)} = 19.7$ ). Note that the ACH for 80 Hz stimulation showed a  
399 substantial 40 Hz subharmonic response.

### 400 **Neuronal response latencies to optogenetic stimulation with pulse trains and** 401 **sine waves of variable frequencies**

402 Next, we investigated the neuronal response latencies to optogenetic stimulation. This  
403 is highly relevant when optogenetic stimulation is used to produce temporal activation  
404 patterns at high frequencies. In addition, it provides a signature of true optogenetic  
405 stimulation. Optogenetic response latencies have typically been found on the order of  
406 3-8 ms. Spikes detected at shorter latencies are suspicious of reflecting photo-electric  
407 artifacts (Cardin et al., 2010). To investigate response latencies, we averaged MUA  
408 responses aligned to the light pulses (Fig. 8A) or to the peaks of the sine wave light  
409 stimuli (Fig. 8B). Light pulses caused a small enhancement of MUA starting within  
410 approximately 1 ms after light onset and lasting a fraction of a millisecond, which most  
411 likely reflects light artifacts. The main response to the pulses followed later, starting at  
412 latencies after pulse onset of approximately 3 ms and peaking at 5.4-6.9 ms (Fig. 8C).  
413 During sine wave stimulation, the light was modulated between the respective maximal

414 intensity and almost zero intensity. Thus, the light crosses the threshold for effective  
415 neuronal stimulation at an unknown intensity, and it is not possible to calculate  
416 response latencies in the same way as for the pulse trains. Therefore, we used a  
417 technique of latency estimation that has been developed in the study of synchronized  
418 oscillations, and that is based on the slope of the phase spectrum of the coherency  
419 between two signals (Schoffelen et al., 2005), in our case the light intensity and the  
420 MUA. Figure 8D shows this phase spectrum and reveals a strictly linear dependence  
421 of phase on frequency. The slope of this linear relationship allows to infer a latency of  
422 5.5 ms, in close agreement to the values obtained for the different pulse train  
423 frequencies.

#### 424 **Neuronal responses to optogenetic white-noise stimulation**

425 The described neuronal responses to pulse trains and sine waves of different  
426 frequencies suggest that the network responds more strongly to rhythmic stimulation  
427 with higher frequencies, potentially with a peak in the gamma-frequency range. It  
428 would be interesting to characterize the spectrum of neuronal response to a large  
429 number of driving frequencies. Yet, testing neuronal responses to optogenetic  
430 stimulation at a sufficiently large number of individual frequencies to fully characterize  
431 the spectrum would require excessively long recordings. We therefore employed  
432 optogenetic stimulation with light intensities following a Gaussian random process  
433 (sampled at  $\approx 1000$  Hz) with a flat power spectrum (Fig 9). This white-noise stimulus  
434 contains the same energy at all frequencies up to 500 Hz. Recordings obtained during  
435 white-noise stimulation allow the estimation of the transfer function, which specifies  
436 for each frequency the strength of the neuronal circuit's response given optogenetic  
437 stimulation at that frequency. Figure 9A shows an example LFP and MUA recording  
438 for an example trial of white-noise stimulation. The time-frequency analyses of the  
439 respective LFP power (Fig. 9B) and MUA-LFP locking (Fig. 9C), averaged over trials,  
440 showed sustained responses that peaked in the gamma-frequency range. The  
441 average transfer function between the white-noise stimuli and the example LFP  
442 recording site is shown in Figure 9D and reveals a dominant peak in the gamma band.  
443 Figure 9E shows the average transfer function across all recording sites stimulated  
444 with white noise (46 recording sites in area 17 of 3 cats), confirming a predominant  
445 peak in the gamma band.

#### 446 **Discussion**

447 We investigate the response of anesthetized cat areas 17 and 21a to optogenetic  
448 stimulation with constant light, rhythmically modulated light and white-noise modulated  
449 light. Neurons were transfected to express Channelrhodopsin-2 (ChR2) through the  
450 injection of different adeno-associated viral vectors. We found that AAV5 fails to  
451 transfect cells in cat cortex. By contrast, both AAV1 and AAV9 resulted in robust and  
452 widespread transfection. ChR2 expression was under the control of a CamKII $\alpha$   
453 promotor, which is expressed in excitatory neurons. In agreement with that,

454 immunohistochemical analysis in two cats found none of the transfected cells to  
455 express Parvalbumin, which suggests that optogenetic stimulation reached primarily  
456 or exclusively excitatory neurons. Optogenetic stimulation with constant light induced  
457 pronounced gamma-band activity, in agreement with several previous reports in other  
458 species and areas, as discussed in detail below; it also induced decreases in theta  
459 and alpha LFP power and alpha-band MUA-LFP locking. Rhythmic optogenetic  
460 stimulation with either pulse trains or with sinusoidal light modulation showed strongest  
461 resonance at 40 or 80 Hz. Stimulation with white-noise-modulated light revealed the  
462 full transfer function of the visual cortical circuit. This transfer function showed  
463 predominantly a peak in the gamma band, between 30 and 60 Hz.

464 The stimulation with light can cause artifacts in recordings with metal electrodes, as  
465 used here. We found artifacts in the LFP that were sizeable, yet constrained to the first  
466 few hundred milliseconds after light onset (data not shown). We also found artifacts in  
467 some of the MUA recordings, which were always constrained to the first 2 ms after  
468 light onset (Fig. 8A). The observation, that sustained optogenetic stimulation induces  
469 sustained gamma-band oscillations, is not due to artifacts, because gamma is  
470 sustained for the entire duration of optogenetic stimulation, long after the light-onset  
471 related artifact has ceased. The analysis of signals recorded with pulsed optical  
472 stimulation excluded LFPs, because under these conditions, our LFP recordings  
473 contained substantial artifact components, which were difficult to separate from  
474 neuronal components. By contrast, the analysis of MUA responses to pulsed light  
475 showed light-related artifacts that were small compared to the optogenetically driven  
476 neuronal responses (Fig. 8A). Finally, the analysis of signals recorded with white-noise  
477 optical stimulation most likely includes some artifacts in the LFP, yet these artifacts  
478 cannot explain the band-limited transfer function, because the power spectrum of light  
479 stimulation was by construction white.

480 Both, viral expression and light application was not homogeneous across cortical  
481 layers. Expression was stronger in superficial and deep layers than in middle layers,  
482 with a particular predominance in superficial layers. Optogenetic stimulation for all  
483 area 21a recordings and for most area 17 recordings was through surface illumination,  
484 leading to strongest light intensity in superficial layers. The combination of  
485 predominantly superficial expression with predominantly superficial light application  
486 likely led to a predominance of superficial neuronal activation. Superficial layers of  
487 macaque areas V1, V2 and V4, show substantially stronger visually induced gamma-  
488 band synchronization than infragranular layers (Buffalo et al., 2011; Xing et al., 2012).  
489 Thus, our finding of resonance in the gamma-frequency band might be partly due to a  
490 predominantly superficial localization of our optogenetic stimulation. Whether neuronal  
491 circuits in other layers resonate in different ways will require layer-specific expression  
492 and/or optical stimulation.

493 We found that visual cortical circuits resonate in the typical gamma-frequency band.  
494 Yet, across the different optogenetic stimulation protocols, we found variable peak  
495 frequencies. For constant optogenetic stimulation, the average gamma-band peak

496 extended from 50-100 Hz. For rhythmically pulsed stimulation, only a limited set of  
497 frequencies was tested and strongest resonance was found at 40 or 80 Hz. For white-  
498 noise stimulation, the average transfer function peaked between 30 and 60 Hz. This  
499 variability in gamma peak frequency is likely due to a combination of factors. First, the  
500 gamma peak frequency is partly genetically determined and thereby varies across  
501 individuals (van Pelt et al., 2012). The different stimulation protocols analyzed here  
502 were applied to different subsets of cats, such that inter-individual differences in  
503 gamma peak frequency could lead to apparent differences between stimulation  
504 protocols. Second, the gamma peak frequency is likely affected by state changes, that  
505 can occur during anesthesia and might resemble changes in attention, which have  
506 been shown to modulate gamma peak frequency (Bosman et al., 2012). Third, the  
507 peak frequency of visually induced gamma-band activity is strongly affected by visual  
508 stimulus parameters with stimuli of higher salience typically leading to higher gamma  
509 peak frequency (Fries, 2015). Gamma peak frequency is e.g. reduced for stimuli that  
510 are of low contrast or masked by noise (Ray and Maunsell, 2010; Jia et al., 2013;  
511 Roberts et al., 2013). It is conceivable that similar effects occurred for the optogenetic  
512 stimulation employed in the present study. For example, rhythmically pulsed  
513 stimulation might be highly salient, leading to gamma resonance at the upper end of  
514 the gamma-frequency range; by contrast, white-noise stimulation might be of lower  
515 salience and similar to noisy visual stimulation, leading to lower gamma peak  
516 frequencies.

517 The finding that constant optogenetic stimulation of cortex induces sustained gamma-  
518 band activity is consistent with previous reports. Several in-vitro studies applied slowly  
519 ramping optogenetic stimulation to slices of somatosensory cortex or hippocampus  
520 and found that this induces strong and narrowband gamma-band activity (Adesnik and  
521 Scanziani, 2010; Akam et al., 2012; Crandall et al., 2015). Several in-vivo studies  
522 found that slowly ramping and/or constant optogenetic stimulation induces strong  
523 gamma-band activity that is less narrowband than in-vitro. When awake mouse frontal  
524 cortex receives sustained activation by means of a step-function opsin, this enhances  
525 power in a 50-90 Hz band and reduces power at 4-25 Hz (Yizhar et al., 2011). When  
526 awake macaque motor cortex is transfected to express the C1V1 opsin and stimulated  
527 with constant or slowly ramping light, it shows gamma-band activity of constant or  
528 varying peak frequency (Lu et al., 2015). When anesthetized cat visual cortex is  
529 transfected to express ChR2 and stimulated by constant light, it generates sustained  
530 gamma-band activity (Ni et al., 2016). This latter study used partly data from the same  
531 animals as used here, yet from different recording sessions. In the present study, we  
532 confirm that constant optogenetic stimulation of cat visual cortex induces gamma-band  
533 synchronization, and we add that it also reduces LFP power in the theta and alpha  
534 bands and MUA-LFP locking in the alpha band (Fig. 5E, F). These reductions in low-  
535 frequency LFP power and MUA-LFP locking are very similar to effects of visual  
536 stimulation and selective attention in awake macaque area V4 (Fries et al., 2008).

537 The findings with pulsed stimulation differ from earlier reports. A previous study used  
538 mouse Cre-lines to express ChR2 selectively in either PV-positive fast-spiking (FS)  
539 neurons or CamKII $\alpha$ -positive regular-spiking (RS) cells (Cardin et al., 2009). Pulse-  
540 train stimulation of the PV-FS circuit led to LFP resonance in a 30-70 Hz frequency  
541 band. By contrast, pulse-train stimulation of the CamKII $\alpha$ -RS circuit led to resonance  
542 for low frequencies, up to 24 Hz. The cells expressing ChR2 in the CamKII $\alpha$ -Cre mice  
543 were 100% PV negative, consistent with exclusive expression in excitatory neurons.  
544 The present study in cat visual cortex cannot build on Cre lines to target expression to  
545 specific cell types. Any cell-type specificity of opsin expression is likely due to the  
546 employed CamKII $\alpha$  promoter. Promoters generally control cell-type specific  
547 expression less tightly than Cre-driver lines. Nevertheless, the immuno-histochemical  
548 analysis in both area 17 and area 21a found 100% of the ChR2-expressing neurons  
549 to be PV negative. This strong exclusion of PV-positive neurons by the CamKII $\alpha$   
550 promotor might be specific for cat (visual) cortex. Previous studies in monkeys and  
551 rodents, using the same promoter, though sometimes with other AAV serotypes, found  
552 at least small proportions of labeled GABAergic cells (Nathanson et al., 2009b;  
553 Nathanson et al., 2009a; Galvan et al., 2016). Pulse-train stimulation applied to those  
554 putative excitatory cells in cat visual cortex revealed resonance in the gamma-  
555 frequency band, which was further corroborated by the white-noise stimulation. This  
556 is different from the low-pass resonance found with pulse-train stimulation of excitatory  
557 neurons in barrel cortex. This difference is likely due to the different modalities,  
558 somatosensation versus vision, and the different species, mouse versus cat, or a  
559 combination of both. In cat visual cortex, specific cells with the intrinsic and/or network-  
560 borne property of rhythmic bursting at the gamma rhythm have been described (Gray  
561 and McCormick, 1996; Cardin et al., 2005).

562 The current results suggest that input to (superficial) visual cortical circuits will be most  
563 effective if it is gamma rhythmic. If the input is spectrally broad, then resonance will be  
564 strongest to the gamma-band components. Local gamma-band synchronization is  
565 particularly strong in superficial layers (Buffalo et al., 2011; Xing et al., 2012), and  
566 inter-areal Granger-causal influences in the gamma-band are predominant along  
567 feedforward type projections, originating from superficial layers (Bastos et al., 2015a;  
568 Bastos et al., 2015b; Michalareas et al., 2016). Thus, local and interareal  
569 supragranular circuits might be tuned to render both, their output and their sensitivity  
570 to input, selective to the gamma band.

## 571 **Figure legends**

572 **Figure 1. Transfection efficiency of different AAVs in cat visual cortex.** Each pair  
573 of images shows the fluorescence at low resolution on the left side and at higher  
574 resolution on the right (see scale bars at lower right of each image). (A) Fluorescence  
575 in area 17 after injection with AAV5. Only auto-fluorescence is present, but no labeled  
576 cells. (B) Same as (A), but for area 17 injected with AAV1. (C) Same as (A), but for  
577 area 17 injected with AAV9. (D) Same as (A), but for area 21a injected with AAV9.  
578 Injections with AAV1 and AAV9 resulted in clear labeling of cells, indicative of  
579 successful ChR2 expression.

580 **Figure 2. PV+ antibody staining.** (A-C) Slice from area 21a. (D-F) Slice from area 17.  
581 (A, D) Cells expressing the fusion protein ChR2-eYFP. (B, E) PV+ antibody labeled  
582 cells. (C, F) Merged images, testing for neuronal co-labeling with ChR2-eYFP and PV+  
583 antibody. No co-labeled neurons can be found. (G) Counts of PV+ labeled neurons,  
584 EYFP+ labeled neurons, and co-labeled neurons in area 21a (left side) and area 17  
585 (right side).

586 **Figure 3. Neuronal responses to light stimulation after injection of AAV5, AAV1  
587 or AAV9.** (A) MUA in AAV5-injected area 17 during 40 Hz pulse-train light stimulation.  
588 (B) Same MUA as shown in (A), averaged after aligning to the 2 ms light pulses. No  
589 optogenetic response was obtained after AAV5 injection. (C, E) MUA responses in  
590 area 17 after transfection with AAV1 (C) or AAV9 (E), and light stimulation with 20 Hz  
591 pulse trains. (D, F) Same data as (C, E), averaged after aligning to the 2 ms light  
592 pulses. MUA responses showed latencies of 3.9 ms and 3.6 ms (AAV1: 20 Hz, 10 -  
593 70 mW, N = 7 sites; AAV9: 20 Hz, 10 - 100 mW, N= 10 sites).

594 **Figure 4. The effect of constant light stimulation on an example MUA and LFP  
595 recording site.** (A) Constant-light induced gamma-band oscillation in the local field  
596 potential. (B) Constant-light induced MUA response. Blue: 473 nm wavelength light;  
597 Yellow: 594 nm wavelength light. The spike density was smoothed with a Gaussian  
598 function with  $\sigma = 12.5$  ms, truncated at  $\pm 2\sigma$ . Shaded area indicates  $\pm 1$  SEM.  
599 (C) Spike-triggered LFP. Shaded area indicates  $\pm 1$  SEM. (D) TFR of constant-light  
600 induced gamma power, for 473 nm light. (E) Same as (D), but for 594 nm light as  
601 control. (F) TFR of constant-light induced MUA-LFP coherence. (G) The same as in  
602 (F), but for 594 nm light as control.

603 **Figure 5. Group results for MUA rate, LFP power and MUA-LFP PPC induced by  
604 constant light.** (A) Scatter plot of MUA rate (between 0.3 to 2 s) induced by constant  
605 light for both blue and yellow light condition. The red cross indicates the median value.  
606 (B) Scatter plot of power ratio (50-90 Hz) between blue and yellow light condition. The  
607 inset shows the same for 4-14 Hz, motivated by the analysis shown in (E). (C) Scatter  
608 plot of MUA-LFP PPC (50-90 Hz) between blue and yellow light condition. The inset  
609 shows the same for 10-12 Hz, motivated by the analysis shown in (F). (D) Averaged  
610 MUA spike density. Smoothed by a Gaussian function with  $\sigma = 12.5$  ms and truncated



611 for  $\pm 2\sigma$ . (E) Averaged power spectrum. (F) Averaged MUA-LFP PPC spectrum.  
612 (D-F) Blue (yellow) lines show data obtained with 473 nm (594 nm) light stimulation.  
613 Shaded areas indicate  $\pm 1$  SEM, which is shown for illustration only. Black bars at the  
614 bottom indicate frequency ranges with statistically significant differences ( $p < 0.05$ ),  
615 based on a cluster-level permutation test. (E, F) use  $\pm 0.5$  s epochs for the analyses  
616 from 4 to 20 Hz, and  $\pm 0.25$  s long epochs for the analyses from 20-150 Hz.

617 **Figure 6. MUA resonance for optogenetic pulse-train stimulation.** (A-C) MUA  
618 spike density (Gaussian smoothing with  $\sigma = 1.25$  ms and truncated for  $\pm 2\sigma$ ) for 10 Hz  
619 (A), 40 Hz (B) and 80 Hz (C) optogenetic pulse train stimulation, respectively. Data  
620 were averaged over all MUA recording sites. Error regions show  $\pm 1$ SEM, but are  
621 barely visible. (D) The F1 component of MUA spike density as a function of optogenetic  
622 pulse train frequency. (E-G) MUA auto-correlogram for 10 Hz (E), 40 Hz (F) and 80 Hz  
623 (G) optogenetic pulse train stimulation, respectively. Data were averaged over all MUA  
624 recording sites. (H) The F1 component of the MUA auto-correlogram (maxlag =  
625 250 ms) as a function of the optogenetic pulse train frequency.

626 **Figure 7. MUA resonance for optogenetic sine wave stimulation.** (A-C) MUA spike  
627 density (Gaussian smoothing with  $\sigma = 1.25$  ms and truncated for  $\pm 2\sigma$ ) for 10 Hz (A),  
628 40 Hz (B) and 80 Hz (C) optogenetic sine wave stimulation, respectively. Data were  
629 averaged over all MUA recording sites. Error regions show  $\pm 1$ SEM, but are barely  
630 visible. (D) The F1 component of MUA spike density as a function of optogenetic sine  
631 wave frequency. (E-G) MUA auto-correlogram for 10 Hz (E), 40 Hz (F) and 80 Hz (G)  
632 optogenetic sine wave stimulation, respectively. Data were averaged over all MUA  
633 recording sites. (H) The F1 component of the MUA auto-correlogram (maxlag =  
634 250 ms) as a function of the optogenetic sine wave frequency.

635 **Figure 8. MUA response latencies to optogenetic pulse-train and sine-wave**  
636 **stimulation.** (A) MUA spike probability, averaged over recording sites, as function of  
637 time relative to optogenetic stimulus pulse onset. The optogenetic pulse is indicated  
638 by the blue bar. Note the very short latency, very short-lived MUA enhancement  
639 around 1 ms, most likely reflecting light-induced artifacts in some of the recordings.  
640 (B) MUA spike probability, averaged over recording sites, as function of the phase of  
641 the optogenetic sine wave stimulation. The optogenetic sine wave is indicated by the  
642 blue-shaded region. (A, B) Data from the five pulse-train or sine-wave frequencies are  
643 shown in separate panels. MUA responses were fitted with Gaussians, and the  
644 resulting peak latencies are indicated by dashed red lines. Peak latencies and their  
645 SEM (estimated through a jackknife procedure) are also indicated as text insets. For  
646 sine-wave stimulation, latencies are expressed relative to the time of peak light  
647 intensity. (C) MUA peak latencies from (A) as a function of the pulse-train frequency.  
648 (D) MUA peak latencies from (B) as a function of the sine-wave frequency.

649 **Figure 9. Cortical responses to white noise laser stimulation.** (A-C) Example  
650 single trial LFP and MUA response to a white noise light stimulus. (A) The blue trace  
651 in the bottom panel shows the white-noise time course of laser intensity. The sequence

652 of vertical lines above it indicates time points of MUA spike occurrence. The black  
653 continuous line shows the LFP. LFP and MUA for the epoch indicated by the dashed  
654 rectangle are shown at higher temporal resolution at the top. (B) LFP power as a  
655 function of time and frequency. (C) MUA-LFP PPC as a function of time and frequency.  
656 (D) The trial-averaged magnitude of the transfer function between white noise and the  
657 LFP shown in (A-C). (E) The magnitude of the transfer function, averaged over 46 LFP  
658 recording sites from 5 sessions in 3 cats. The blue region indicates  $\pm 1$  SEM.

## 659 **References**

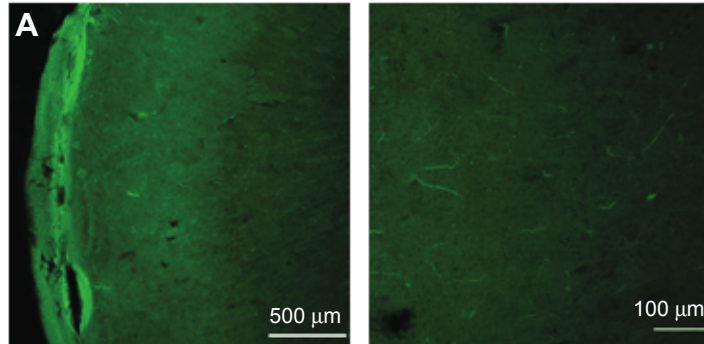
- 660 Adesnik H, Scanziani M (2010) Lateral competition for cortical space by layer-specific horizontal  
661 circuits. *Nature* 464:1155-1160.
- 662 Akam T, Oren I, Mantoan L, Ferenczi E, Kullmann DM (2012) Oscillatory dynamics in the hippocampus  
663 support dentate gyrus-CA3 coupling. *Nature neuroscience* 15:763-768.
- 664 Ball T, Demandt E, Mutschler I, Neitzel E, Mehring C, Vogt K, Aertsen A, Schulze-Bonhage A (2008)  
665 Movement related activity in the high gamma range of the human EEG. *NeuroImage* 41:302-  
666 310.
- 667 Bastos AM, Litvak V, Moran R, Bosman CA, Fries P, Friston KJ (2015a) A DCM study of spectral  
668 asymmetries in feedforward and feedback connections between visual areas V1 and V4 in the  
669 monkey. *NeuroImage* 108:460-475.
- 670 Bastos AM, Vezoli J, Bosman CA, Schoffelen JM, Oostenveld R, Dowdall JR, De Weerd P, Kennedy H,  
671 Fries P (2015b) Visual areas exert feedforward and feedback influences through distinct  
672 frequency channels. *Neuron* 85:390-401.
- 673 Bauer M, Oostenveld R, Peeters M, Fries P (2006) Tactile spatial attention enhances gamma-band  
674 activity in somatosensory cortex and reduces low-frequency activity in parieto-occipital areas.  
675 *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26:490-501.
- 676 Bosman CA, Schoffelen JM, Brunet N, Oostenveld R, Bastos AM, Womelsdorf T, Rubehn B, Stieglitz T,  
677 De Weerd P, Fries P (2012) Attentional stimulus selection through selective synchronization  
678 between monkey visual areas. *Neuron* 75:875-888.
- 679 Brainard DH (1997) The Psychophysics Toolbox. *Spat Vis* 10:433-436.
- 680 Brosch M, Budinger E, Scheich H (2002) Stimulus-related gamma oscillations in primate auditory  
681 cortex. *Journal of neurophysiology* 87:2715-2725.
- 682 Brown P, Salenius S, Rothwell JC, Hari R (1998) Cortical correlate of the Piper rhythm in humans.  
683 *Journal of neurophysiology* 80:2911-2917.
- 684 Buffalo EA, Fries P, Landman R, Buschman TJ, Desimone R (2011) Laminar differences in gamma and  
685 alpha coherence in the ventral stream. *Proceedings of the National Academy of Sciences of*  
686 *the United States of America* 108:11262-11267.
- 687 Buzsáki G, Wang XJ (2012) Mechanisms of gamma oscillations. *Annual review of neuroscience* 35:203-  
688 225.
- 689 Cardin JA, Palmer LA, Contreras D (2005) Stimulus-dependent gamma (30-50 Hz) oscillations in simple  
690 and complex fast rhythmic bursting cells in primary visual cortex. *J Neurosci* 25:5339-5350.
- 691 Cardin JA, Carlén M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai LH, Moore CI (2009) Driving  
692 fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* 459:663-  
693 667.
- 694 Cardin JA, Carlen M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai LH, Moore CI (2010) Targeted  
695 optogenetic stimulation and recording of neurons in vivo using cell-type-specific expression  
696 of Channelrhodopsin-2. *Nat Protoc* 5:247-254.
- 697 Colgin LL, Denninger T, Fyhn M, Hafting T, Bonnevie T, Jensen O, Moser MB, Moser EI (2009) Frequency  
698 of gamma oscillations routes flow of information in the hippocampus. *Nature* 462:353-357.
- 699 Crandall SR, Cruikshank SJ, Connors BW (2015) A corticothalamic switch: controlling the thalamus with  
700 dynamic synapses. *Neuron* 86:768-782.
- 701 Csicsvari J, Jamieson B, Wise KD, Buzsáki G (2003) Mechanisms of gamma oscillations in the  
702 hippocampus of the behaving rat. *Neuron* 37:311-322.

- 703 Diester I, Kaufman MT, Mogri M, Pashaie R, Goo W, Yizhar O, Ramakrishnan C, Deisseroth K, Shenoy  
704 KV (2011) An optogenetic toolbox designed for primates. *Nature neuroscience* 14:387-397.
- 705 Fries P (2015) Rhythms for Cognition: Communication through Coherence. *Neuron* 88:220-235.
- 706 Fries P, Womelsdorf T, Oostenveld R, Desimone R (2008) The effects of visual stimulation and selective  
707 visual attention on rhythmic neuronal synchronization in macaque area V4. *The Journal of*  
708 *neuroscience : the official journal of the Society for Neuroscience* 28:4823-4835.
- 709 Fries P, Schröder JH, Roelfsema PR, Singer W, Engel AK (2002) Oscillatory neuronal synchronization in  
710 primary visual cortex as a correlate of stimulus selection. *The Journal of neuroscience : the*  
711 *official journal of the Society for Neuroscience* 22:3739-3754.
- 712 Galvan A, Hu X, Smith Y, Wichmann T (2016) Effects of Optogenetic Activation of Corticothalamic  
713 Terminals in the Motor Thalamus of Awake Monkeys. *J Neurosci* 36:3519-3530.
- 714 Gerits A, Vancraeynest P, Vreysen S, Laramée ME, Michiels A, Gijssbers R, Van den Haute C, Moons L,  
715 Debysers Z, Baekelandt V, Arckens L, Vanduffel W (2015) Serotype-dependent transduction  
716 efficiencies of recombinant adeno-associated viral vectors in monkey neocortex.  
717 *Neurophotonics* 2:031209.
- 718 Gray CM, McCormick DA (1996) Chattering cells: superficial pyramidal neurons contributing to the  
719 generation of synchronous oscillations in the visual cortex. *Science* 274:109-113.
- 720 Gray CM, König P, Engel AK, Singer W (1989) Oscillatory responses in cat visual cortex exhibit inter-  
721 columnar synchronization which reflects global stimulus properties. *Nature* 338:334-337.
- 722 Gregoriou GG, Gotts SJ, Zhou H, Desimone R (2009) High-frequency, long-range coupling between  
723 prefrontal and visual cortex during attention. *Science* 324:1207-1210.
- 724 Grothe I, Neitzel SD, Mandon S, Kreiter AK (2012) Switching neuronal inputs by differential  
725 modulations of gamma-band phase-coherence. *The Journal of neuroscience : the official*  
726 *journal of the Society for Neuroscience* 32:16172-16180.
- 727 Hasenstaub A, Shu Y, Haider B, Kraushaar U, Duque A, McCormick DA (2005) Inhibitory postsynaptic  
728 potentials carry synchronized frequency information in active cortical networks. *Neuron*  
729 47:423-435.
- 730 Hoogenboom N, Schoffelen JM, Oostenveld R, Parkes LM, Fries P (2006) Localizing human visual  
731 gamma-band activity in frequency, time and space. *NeuroImage* 29:764-773.
- 732 Jia X, Xing D, Kohn A (2013) No consistent relationship between gamma power and peak frequency in  
733 macaque primary visual cortex. *The Journal of neuroscience : the official journal of the Society*  
734 *for Neuroscience* 33:17-25.
- 735 Kreiter AK, Singer W (1996) Stimulus-dependent synchronization of neuronal responses in the visual  
736 cortex of the awake macaque monkey. *The Journal of neuroscience : the official journal of the*  
737 *Society for Neuroscience* 16:2381-2396.
- 738 Kruse W, Eckhorn R (1996) Inhibition of sustained gamma oscillations (35-80 Hz) by fast transient  
739 responses in cat visual cortex. *Proceedings of the National Academy of Sciences of the United*  
740 *States of America* 93:6112-6117.
- 741 Lu Y, Truccolo W, Wagner FB, Vargas-Irwin CE, Ozden I, Zimmermann JB, May T, Agha NS, Wang J,  
742 Nurmikko AV (2015) Optogenetically induced spatiotemporal gamma oscillations and  
743 neuronal spiking activity in primate motor cortex. *Journal of neurophysiology* 113:3574-3587.
- 744 Lundqvist M, Rose J, Herman P, Brincat SL, Buschman TJ, Miller EK (2016) Gamma and Beta Bursts  
745 Underlie Working Memory. *Neuron* 90:152-164.
- 746 Maris E, Oostenveld R (2007) Nonparametric statistical testing of EEG- and MEG-data. *Journal of*  
747 *neuroscience methods* 164:177-190.

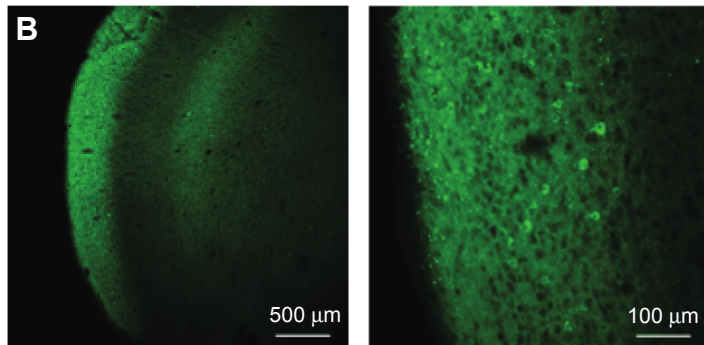
- 748 Michalareas G, Vezoli J, van Pelt S, Schoffelen JM, Kennedy H, Fries P (2016) Alpha-Beta and Gamma  
749 Rhythms Subserve Feedback and Feedforward Influences among Human Visual Cortical Areas.  
750 Neuron.
- 751 Nathanson JL, Yanagawa Y, Obata K, Callaway EM (2009a) Preferential labeling of inhibitory and  
752 excitatory cortical neurons by endogenous tropism of adeno-associated virus and lentivirus  
753 vectors. *Neuroscience* 161:441-450.
- 754 Nathanson JL, Jappelli R, Scheeff ED, Manning G, Obata K, Brenner S, Callaway EM (2009b) Short  
755 Promoters in Viral Vectors Drive Selective Expression in Mammalian Inhibitory Neurons, but  
756 do not Restrict Activity to Specific Inhibitory Cell-Types. *Front Neural Circuits* 3:19.
- 757 Ni J, Wunderle T, Lewis CM, Desimone R, Diester I, Fries P (2016) Gamma-Rhythmic Gain Modulation.  
758 *Neuron* 92:240-251.
- 759 Nichols TE, Holmes AP (2002) Nonparametric permutation tests for functional neuroimaging: a primer  
760 with examples. *Hum Brain Mapp* 15:1-25.
- 761 Oostenveld R, Fries P, Maris E, Schoffelen JM (2011) FieldTrip: Open source software for advanced  
762 analysis of MEG, EEG, and invasive electrophysiological data. *Computational intelligence and  
763 neuroscience* 2011:156869.
- 764 Payne BR (1993) Evidence for visual cortical area homologs in cat and macaque monkey. *Cereb Cortex*  
765 3:1-25.
- 766 Pesaran B, Pezaris JS, Sahani M, Mitra PP, Andersen RA (2002) Temporal structure in neuronal activity  
767 during working memory in macaque parietal cortex. *Nature neuroscience* 5:805-811.
- 768 Ray S, Maunsell JH (2010) Differences in gamma frequencies across visual cortex restrict their possible  
769 use in computation. *Neuron* 67:885-896.
- 770 Roberts MJ, Lowet E, Brunet NM, Ter Wal M, Tiesinga P, Fries P, De Weerd P (2013) Robust gamma  
771 coherence between macaque V1 and V2 by dynamic frequency matching. *Neuron* 78:523-536.
- 772 Salkoff DB, Zaghera E, Yüzgeç O, McCormick DA (2015) Synaptic Mechanisms of Tight Spike Synchrony  
773 at Gamma Frequency in Cerebral Cortex. *The Journal of neuroscience : the official journal of  
774 the Society for Neuroscience* 35:10236-10251.
- 775 Scheyltjens I, Laramée ME, Van den Haute C, Gijsbers R, Debyser Z, Baekelandt V, Vreysen S, Arckens  
776 L (2015) Evaluation of the expression pattern of rAAV2/1, 2/5, 2/7, 2/8, and 2/9 serotypes  
777 with different promoters in the mouse visual cortex. *J Comp Neurol* 523:2019-2042.
- 778 Schoffelen JM, Oostenveld R, Fries P (2005) Neuronal coherence as a mechanism of effective  
779 corticospinal interaction. *Science* 308:111-113.
- 780 Stark E, Eichler R, Roux L, Fujisawa S, Rotstein HG, Buzsáki G (2013) Inhibition-induced theta resonance  
781 in cortical circuits. *Neuron* 80:1263-1276.
- 782 van Pelt S, Boomsma DI, Fries P (2012) Magnetoencephalography in twins reveals a strong genetic  
783 determination of the peak frequency of visually induced gamma-band synchronization. *The  
784 Journal of neuroscience : the official journal of the Society for Neuroscience* 32:3388-3392.
- 785 Vasileva A, Jessberger R (2005) Precise hit: adeno-associated virus in gene targeting. *Nat Rev Microbiol*  
786 3:837-847.
- 787 Vinck M, van Wingerden M, Womelsdorf T, Fries P, Pennartz CM (2010) The pairwise phase  
788 consistency: A bias-free measure of rhythmic neuronal synchronization. *NeuroImage* 51:112-  
789 122.
- 790 Vinck M, Womelsdorf T, Buffalo EA, Desimone R, Fries P (2013) Attentional modulation of cell-class-  
791 specific gamma-band synchronization in awake monkey area V4. *Neuron* 80:1077-1089.

- 792 Vite CH, Passini MA, Haskins ME, Wolfe JH (2003) Adeno-associated virus vector-mediated  
793 transduction in the cat brain. *Gene Ther* 10:1874-1881.
- 794 Whittington MA, Traub RD, Kopell N, Ermentrout B, Buhl EH (2000) Inhibition-based rhythms:  
795 experimental and mathematical observations on network dynamics. *International journal of*  
796 *psychophysiology : official journal of the International Organization of Psychophysiology*  
797 38:315-336.
- 798 Womelsdorf T, Fries P, Mitra PP, Desimone R (2006) Gamma-band synchronization in visual cortex  
799 predicts speed of change detection. *Nature* 439:733-736.
- 800 Xing D, Yeh CI, Burns S, Shapley RM (2012) Laminar analysis of visually evoked activity in the primary  
801 visual cortex. *Proceedings of the National Academy of Sciences of the United States of America*  
802 109:13871-13876.
- 803 Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, Sohal VS, Goshen I, Finkelstein J,  
804 Paz JT, Stehfest K, Fudim R, Ramakrishnan C, Huguenard JR, Hegemann P, Deisseroth K (2011)  
805 Neocortical excitation/inhibition balance in information processing and social dysfunction.  
806 *Nature* 477:171-178.

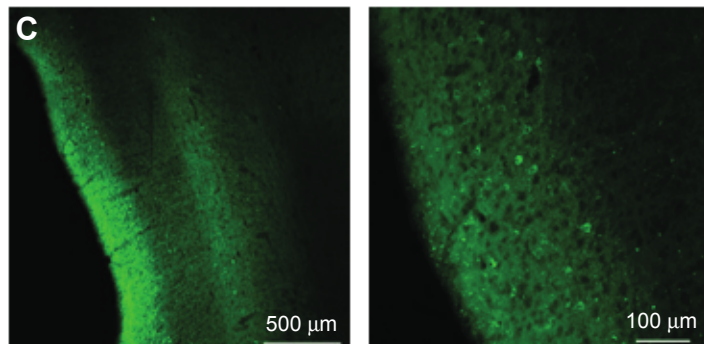
**Area 17, AAV5**



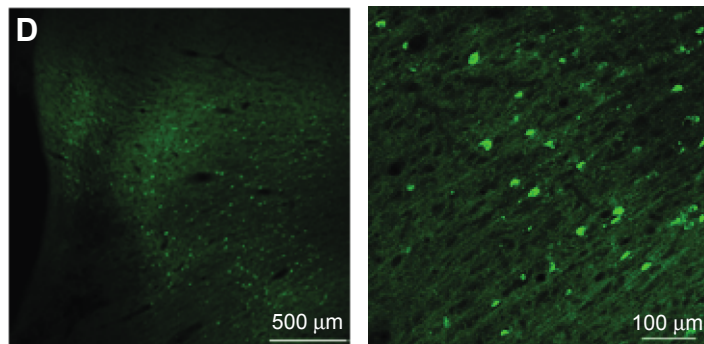
**Area 17, AAV1**

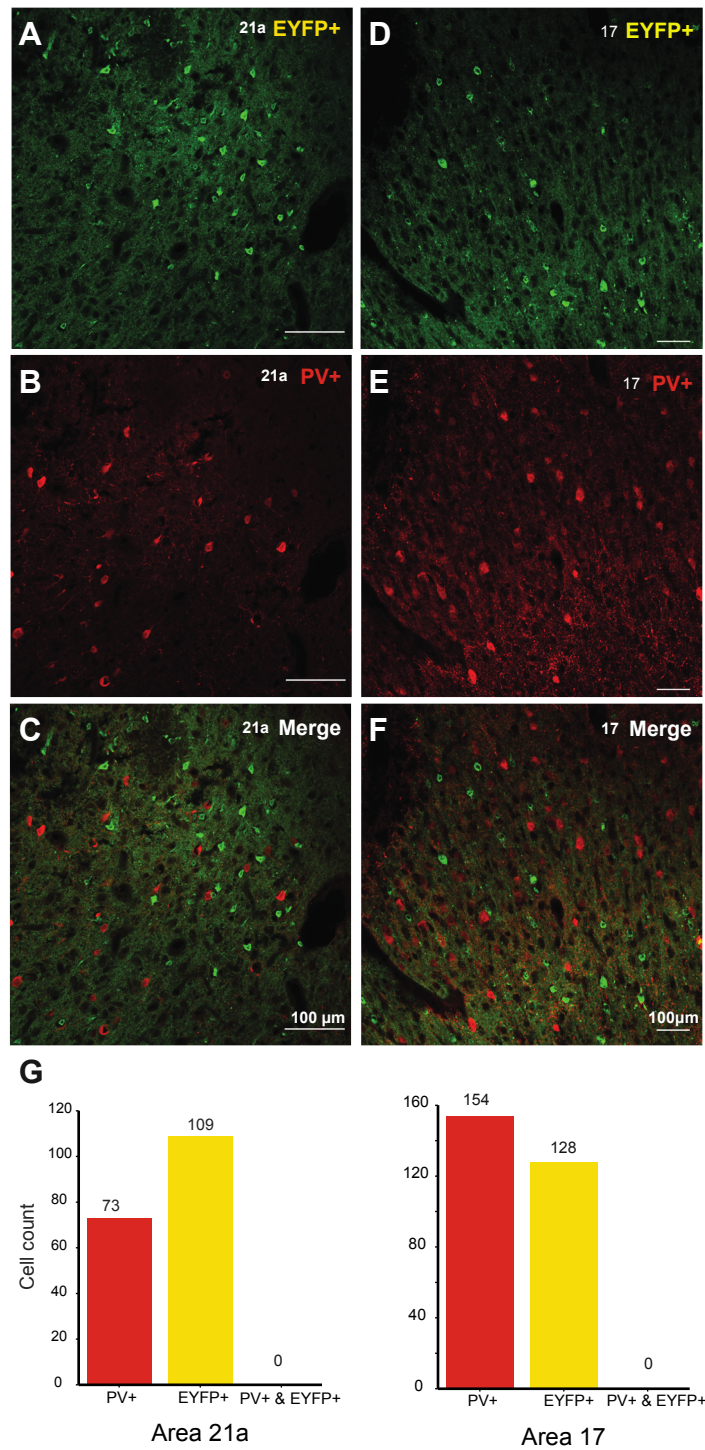


**Area 17, AAV9**



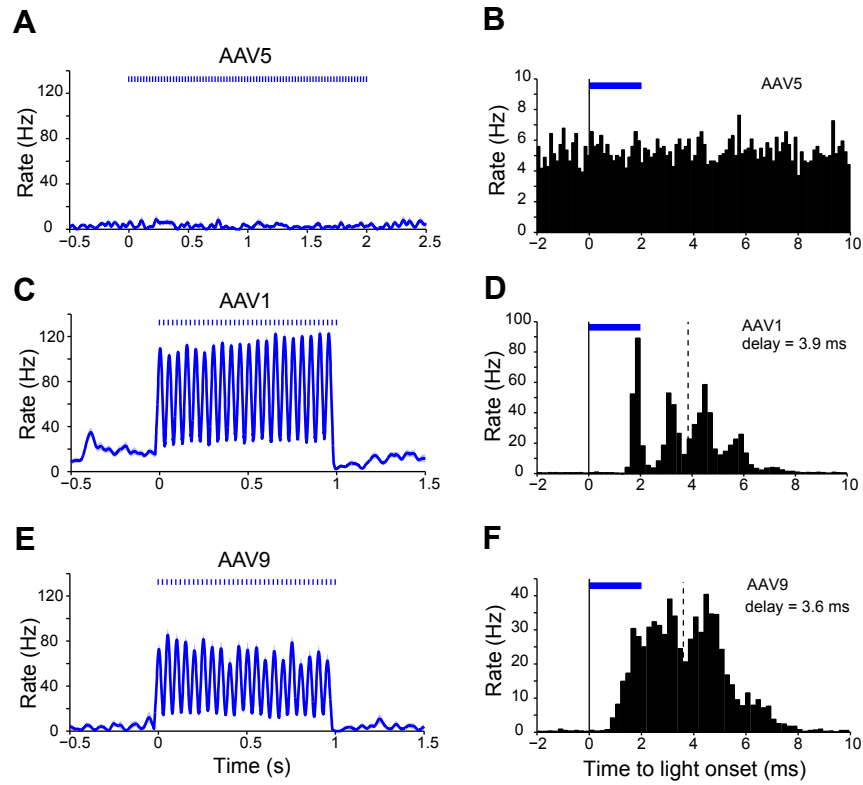
**Area 21a, AAV9**



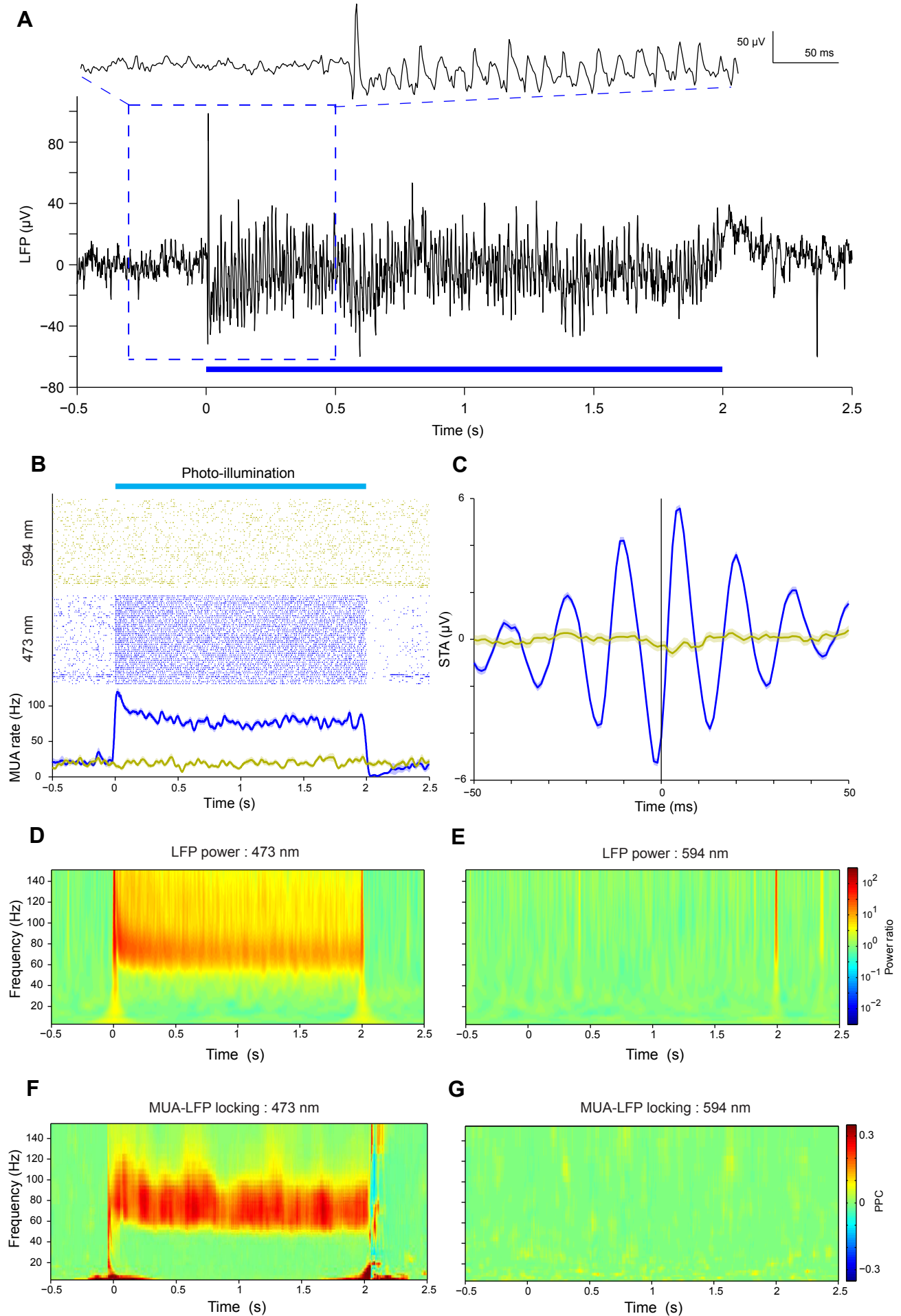


Ni et al., Fig. 2

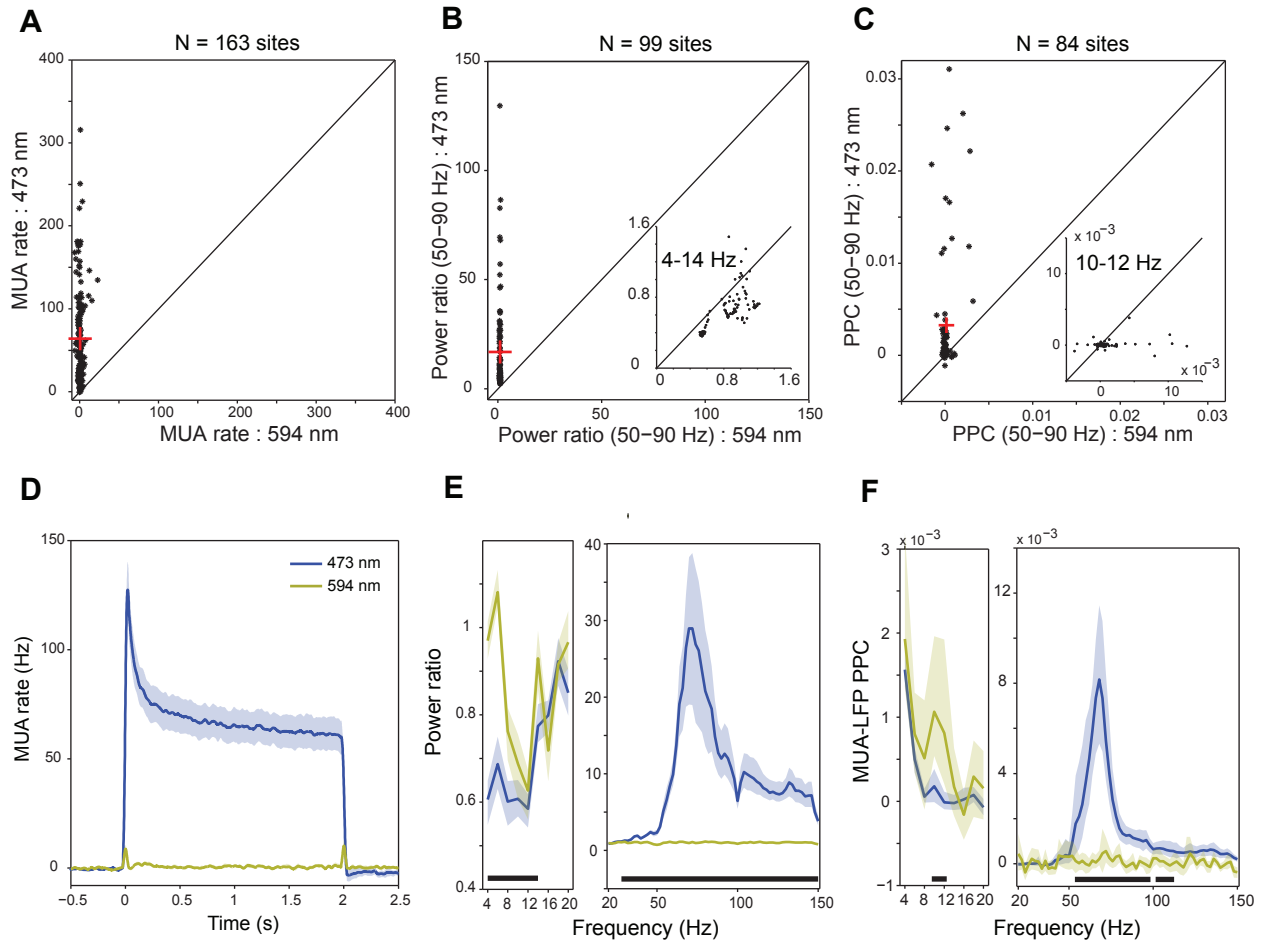




Ni et al., Fig. 3



Ni et al., Fig. 4



Ni et al., Fig. 5

