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Balanced Enhancements of Synaptic Excitation and Inhibition Underlie Developmental Maturation of Receptive Fields in the Mouse Visual Cortex

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1 **Balanced Enhancements of Synaptic Excitation and Inhibition Underlie**
2 **Developmental Maturation of Receptive Fields in the Mouse Visual Cortex**

3

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14

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18

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26

27 **Abstract**

28 Neurons in the developing visual cortex undergo progressive functional maturation as
29 indicated by the refinement of their visual feature selectivity. However, changes of the
30 synaptic architecture underlying the maturation of spatial visual receptive fields (RFs) *per*
31 *se* remain largely unclear. Here, loose-patch as well as single-unit recordings in layer 4 of
32 mouse primary visual cortex (V1) of both sexes revealed that RF development in a post-
33 eye-opening period is marked by an increased proportion of cortical neurons with spatially
34 defined RFs, together with the increased signal-to-noise ratio (SNR) of spiking responses.
35 By exploring excitatory and inhibitory synaptic RFs with whole-cell voltage clamp
36 recordings, we observed a balanced enhancement of both synaptic excitation and inhibition,
37 and that while the excitatory subfield size remains relatively constant during development,
38 the inhibitory subfield is broadened. This balanced developmental strengthening of
39 excitatory and inhibitory synaptic inputs results in enhanced visual responses and together
40 with a reduction of spontaneous firing rate contributes to the maturation of visual cortical
41 RFs. Visual deprivation by dark rearing impedes the normal strengthening of excitatory
42 inputs but leaves the apparently normal enhancement of inhibition while preventing the
43 broadening of the inhibitory subfield, leading to weakened RF responses and a reduced
44 fraction of neurons exhibiting a clear RF, as compared to normally reared animals. Our
45 data demonstrate that an experience-dependent and coordinated maturation of excitatory
46 and inhibitory circuits underlie the functional development of visual cortical RFs.

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49 **Significance Statement**

50 The organization of synaptic receptive fields (RFs) is a fundamental determinant of feature
51 selectivity functions in the cortex. However, how changes of excitatory and inhibitory synaptic
52 inputs lead to the functional maturation of visual RFs during cortical development remains not
53 well-understood. In layer 4 of mouse primary visual cortex (V1), we show that a coordinated,
54 balanced enhancement of synaptic excitation and inhibition contributes to the developmental
55 maturation of spatially defined visual RFs. Visual deprivation by dark rearing partially interferes
56 this process, resulting in a relatively more dominant inhibitory tone and a reduced fraction of
57 neurons exhibiting clear RFs at the spike level. These data provide an unprecedented
58 understanding of the functional development of visual cortical RFs at the synaptic level.

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62 **Introduction**

63 High-quality visual function is progressively established during development, which depends on
64 the concurrence of correct initial neural wiring and appropriately patterned sensory inputs (Cang
65 and Feldheim, 2013; Espinosa and Stryker, 2012; Hooks and Chen, 2020; White and Fitzpatrick,
66 2007). This experience-dependent visual development is most prominent at the cortical level and
67 peaks at the critical period (Berardi et al., 2000; Hensch, 2005; Trachtenberg, 2015), during
68 which various important visual functionalities, including ocular dominance (Fagiolini et al., 1994;
69 Hofer et al., 2006; Shatz and Stryker, 1978; Stephany et al., 2018), binocularity (Jenks and
70 Shepherd, 2020; Tian et al., 2020), orientation selectivity (Chapman and Stryker, 1993; Hoy and
71 Niell, 2015; Li et al., 2012; Moore and Freeman, 2012; White et al., 2001), direction selectivity
72 (Clemens et al., 2012; Hoy and Niell, 2015; Li et al., 2006, 2008; Rochefort et al., 2011; Roy et

al., 2020; Smith et al., 2015) and binocular matching (Chang et al., 2020; Wang et al., 2010; Wang et al., 2015), are established, or refined and strengthened. Sensory deprivation such as dark rearing (DR) can affect the critical period and normal development of visual functionalities (Fagiolini et al., 1994; Gianfranceschi et al., 2003; Hubel and Wiesel, 1970; Jenks and Shepherd, 2020; Kang et al., 2013; Ko et al., 2014; Li et al., 2012; Ma et al., 2013; Morales et al., 2002; Sarnaik et al., 2014; Shatz and Stryker, 1978; Wang et al., 2010). Of all these visual functionalities, the spatial arrangement of ON/OFF receptive field (RF) is a major fundamental determinant (Hirsch and Martinez, 2006; Niell, 2015; Priebe and Ferster, 2012), as integration of individual sensory inputs from various visual field locations determines the response to the overall visual stimulation pattern. Importantly, the RF structure *per se* may undergo developmental changes, which in turn can instruct the refinement of other visual functions. However, research on the developmental evolution of RF structure in the visual cortex has remained limited (Braastad and Heggelund, 1985; Hoy and Niell, 2015; Ko et al., 2013; Roy et al., 2020; Sarnaik et al., 2014; Smith et al., 2017). While a recent study in the developing mouse primary visual cortex (V1) has reported a slight shift towards more RF subunits and more elongated RFs over development (Hoy and Niell, 2015), how the synaptic input architecture underlying the spatial RF evolves during normal development and whether this process can be affected by sensory deprivation remains elusive.

To address these questions, in this study, we systematically examined both the spiking and synaptic RFs in layer 4 excitatory neurons of the mouse V1 during development with a repertoire of electrophysiological and modeling approaches. We found that significant maturation of spatially defined RFs occurred within one week after the onset of visual experience, which was accompanied by decreased spontaneous firing rates, increased evoked firing rates, and an enhancement of the signal-to-noise ratio (SNR) of visual responses. At the synaptic level,

excitatory and inhibitory inputs were strengthened by a similar proportion, and the inhibitory subfield was enlarged in size over the same developmental period. In addition, we found that sensory deprivation by DR caused an apparently normal developmental strengthening of inhibition but an impedance of the broadening of the inhibitory subfield, while excitation paradoxically remained premature. The resulting abnormally dominant inhibition over excitation led to reduced response levels and smaller spike subfield sizes. Together, our data suggest that the balanced strengthening of excitation and inhibition contributes to the functional maturation of visual RFs during cortical development, which can be interfered at least partially by sensory deprivation.

Materials and Methods

Animal preparation

All experimental procedures used in this study were approved by the Animal Care and Use Committee of the University of Southern California. C57BL/6J mice of both sexes aged P14–P17 (ST1) and P21–P28 (ST2) were used. For anesthetized preparations, animals were sedated with an intramuscular injection of chlorprothixene (5 mg/kg) and then anesthetized with urethane (1.2 g/kg, i.p., at 20% w/v in saline), as previously described (Li et al., 2012, 2018). The level of anesthesia was confirmed by the lack of toe pinch reflex and additional urethane (0.2 g/kg) was administered as needed. The animal's body temperature was maintained at $\sim 37.5^\circ$ by a thermostatic heating pad (Harvard Apparatus). A tracheotomy was performed, and a small glass capillary tube was inserted to maintain a clear airway. A small craniotomy ($\sim 1 \times 1$ mm) was performed to expose the underlying V1, and the dura mater was partially resected. Warm artificial cerebrospinal fluid (ACSF) [containing the following (in mM): 140 NaCl, 2.5 KCl, 2.5 CaCl_2 , 1.3 MgSO_4 , 1.0 NaH_2PO_4 , 20 HEPES, 11 glucose, pH 7.4] was applied to the exposed

121 cortical surface when necessary. The eyes were covered with ophthalmic lubricant ointment until
 122 recording. During recording sessions, the eyes were rinsed with saline, and a thin layer of
 123 silicone oil (30,000 centistokes) was applied to prevent drying while allowing clear optical
 124 transmission. The eye movement and receptive field drift of single units were negligible within
 125 the recording time windows (Liu et al., 2010; Sarnaik et al., 2014).

126 For awake head-fixed preparations, a screw was glued to the skull surface of the mouse with
 127 acrylic dental cement under the anesthesia with 1.5% isoflurane (v/v). The screw was clamped
 128 tightly with a metal head post on the recording setup to achieve head fixation. After recovery
 129 from anesthesia, the mouse was trained to get accustomed to the head fixation and to run freely
 130 on a rotatable plate. On the day of recording, the mouse was again anesthetized with isoflurane
 131 and a craniotomy window was made over V1. A durotomy was further performed to allow the
 132 insertion of silicon probes. After the surgery, the exposed cortex was covered with a silicon
 133 elastomer (Kwik-CAST, WPI). The mouse was fully recovered from anesthesia before recording
 134 sessions.

135 ***In vivo* electrophysiology**

136 Whole-cell voltage-clamp recordings were performed with an Axopatch 200B (Molecular
 137 Devices) following our previous studies (Li et al., 2012, 2018). The patch pipette had a tip
 138 opening of $\sim 2 \mu\text{m}$ (4–6 M Ω). For whole-cell voltage-clamp recordings, we used a Cs⁺-based
 139 intrapipette solution containing the following (in mM): 125 Cs-gluconate, 5 TEA-Cl, 4 MgATP,
 140 0.3 GTP, 8 phosphocreatine, 10 HEPES, 10 EGTA, 2 CsCl, 1 QX-314, 0.75 MK-801, pH 7.25.
 141 The pipette and whole-cell capacitance were compensated completely, and series resistance (25–
 142 50 M Ω) was compensated by 50–60% (at 100 μs lag). An 11-mV junction potential was
 143 corrected. Data acquisition scripts was custom developed with LabVIEW (National Instruments).
 144 Signals were filtered at 2 kHz for voltage-clamp recording and sampled at 10 kHz. The evoked

145 excitatory and inhibitory currents were resolved by clamping the cell at -70 and 0 mV,
 146 respectively (Li et al., 2012; Liu et al., 2010). As discussed before, our blind whole-cell
 147 recording method highly biases sampling toward pyramidal neurons (Liu et al., 2010, 2009). For
 148 cell-attached loose-patch recordings, glass electrodes containing the ACSF were used. A 100–
 149 250 M Ω seal was formed on the targeted neuron. The pipette capacitance was completely
 150 compensated. Spikes were recorded under voltage-clamp mode, with a command potential
 151 applied to achieve a zero-baseline current. The spike signal was filtered at 10 kHz and sampled
 152 at 20 kHz. All neurons recorded in this study were located at a depth of 350–500 μ m below the
 153 pia according to the microdrive reading, corresponding to layer 4, which was determined based
 154 on the distribution of genetically labeled layer 4 cells in the Scnn1a-Cre mouse (Li et al., 2012;
 155 Petrus et al., 2015).

156 For awake recordings, we used a 64-channel silicone probe (NeuroNexus), coated with DiI
 157 (Invitrogen) to allow *post hoc* track recovery. The electrode was placed at an angle of 45°
 158 relative to the cortical surface and inserted to an appropriate cortical depth (~ 500 μ m). After
 159 insertion, agarose was added to stabilize the electrode, and it was allowed to settle for 45 min.
 160 Signals were recorded by an Open-Ephys system (Open Ephys) at a 30-kHz sampling rate. Raw
 161 unfiltered traces were saved for offline spike sorting and analysis.

162 **Visual stimulation**

163 Visual stimulation was generated with MATLAB (MathWorks) and presented on a 34.5×25.9
 164 cm monitor (refresh rate 120 Hz, mean luminance ~ 41.1 cd/m 2) placed 0.25 m away from the
 165 right eye. A distance of 0.25 m from the mouse eye is equivalent to infinity (Liu et al., 2010).
 166 The center of monitor was placed at 45° azimuth and 0° elevation, covering $\pm 35^\circ$ horizontally
 167 and $\pm 27^\circ$ vertically of the mouse visual field. Recordings were made in the monocular zone of
 168 the V1, contralateral to the stimulated eye. For forward mapping of RFs in anaesthetized

conditions, flashing bright (57.5 cd/m^2) and dark (24.7 cd/m^2) squares ($5^\circ \times 5^\circ$ in size) were individually presented on a gray background (41.1 cd/m^2) at different positions on a 11×11 grid according to a predetermined pseudorandom sequence. RFs were mapped for 5–10 repetitions. The stimulus duration was 200 ms and interstimulus interval was 240 ms. Spontaneous activity was determined by averaging spike rates during the interstimulus intervals when gray background was displayed.

In awake recordings, for forward RF mapping, small ($5^\circ \times 5^\circ$) or large ($10^\circ \times 10^\circ$) bright and dark squares were presented individually at different positions on the screen (stimulus duration = 200 ms, interstimulus interval = 240 ms, 5–10 repetitions). To map fine-scale RFs, sparse and dense noise stimuli, composed of static bright and dark squares ($4^\circ \times 4^\circ$) were used for STA (Hoy and Niell, 2015). Each stimulus pattern was presented for 32 ms (i.e., updated every other frame) without an inter-stimulus interval between consecutive patterns. As shown in Fig. 3A, RFs were consistently revealed by different stimulation methods.

182 **Data analysis**

Spikes were sorted offline. Spikes evoked by flashing stimuli were counted within a 70–270 ms time window after the stimulus onset. Stimulus-evoked spike rate (after subtracting the average spontaneous firing rate) was considered as significant if it exceeded the average spontaneous firing rate by three SDs of baseline fluctuations. RF was identified as a spatially contiguous area within which multiple flashing squares evoked significant responses. The cells analyzed in this study had one ON (where bright squares evoked responses) and/or one OFF subfield (where black squares evoked responses). The signal-to-noise ratio was calculated as the ratio of the mean evoked firing rate within the dominant subfield over the average spontaneous firing rate.

To quantify the spatial properties of the two-dimensional ON and OFF subfields, we fit the hard boundary of the subfield with an ellipse (Fang et al., 2020; Li et al., 2018; Liu et al., 2010),

193 and quantified the goodness of fitting by calculating the adjusted r^2 . Only subfields that were
 194 well fitted ($r^2 > 0.9$) were considered to have a clear boundary. The radius of a circle with the
 195 same area of the fitted ellipse was determined as the size of the subfield. We measured the size
 196 for the dominant subfield (ON or OFF sign). Overlap index (OI) was calculated between the
 197 excitatory and inhibitory RFs of the same cell and defined as follows (Liu et al., 2010):

$$OI = \frac{W1 + W2 - d}{W1 + W2 + d}$$

198 d is the distance between the peaks of two subfields, and $W1$ and $W2$ are the half widths on the
 199 inner side of the two subfields, respectively.

200 For voltage-clamp recording data, average excitatory and inhibitory response traces to
 201 flashing stimuli were first smoothed by averaging within a sliding 40-ms window. Excitatory and
 202 inhibitory synaptic conductance were derived according to the following equation (Li et al., 2012;
 203 Liu et al., 2010):

$$204 \quad I(t) = G_r \times (V_m(t) - E_r) + G_e(t) \times (V_m(t) - E_e) + G_i(t) \times (V_m(t) - E_i).$$

205 $I(t)$ is the amplitude of current at any time point, G_r and E_r are the resting leak conductance and
 206 resting membrane potential, respectively, G_e and G_i are the excitatory and inhibitory synaptic
 207 conductance, respectively, $V_m(t)$ is the membrane voltage, and E_e (0 mV) and E_i (-70 mV) are
 208 the reversal potentials. $V_m(t)$ is corrected by $V_m(t) = V_h - R_s \times I(t)$, where R_s was the
 209 effective series resistance, and V_h is the applied holding voltage. Measurement of currents at two
 210 different voltages yielded a system of two equations that could be solved for G_e and G_i at any
 211 particular t . The peak synaptic conductance (subtracted by the baseline activity) was considered
 212 as the stimulus evoked synaptic response. Similar as those in the loose-patch recording, synaptic
 213 ON and OFF subfields were defined as spatially continuous positions on which bright and dark

214 squares evoked significant synaptic responses (i.e., averaged stimulus evoked synaptic
215 conductance above the baseline by three SDs of baseline fluctuation).

216 The awake recording data were processed as previously described (Chou et al., 2020; Fang et
217 al., 2020). Recording sites in layer 4 were selected. For single-unit activity, the raw signals were
218 filtered through a 300–6000 Hz band-pass filter. The spatially varying motion artifacts were
219 removed by applying a common average referencing. The nearby four channels of the silicon
220 probe were grouped as tetrodes, and semi-automatic spike detection and sorting was performed
221 using the Plexon offline sorter (Dallas, Texas). Clusters with isolation distance > 20 was
222 considered as separate clusters. Spike clusters were classified as single units only if the
223 waveform SNR exceeded 4 (12 dB) and the inter-spike interval was longer than 1.2 ms for more
224 than 99.5% of the spikes.

225 To determine the spatiotemporal RF, the spike train evoked by the sparse or dark noise
226 stimuli was reversely correlated with the stimulus sequence to derive the spike-triggered average
227 (STA) of the stimuli (Jones and Palmer, 1987; Fang et al., 2020). A 2D standard deviation was
228 calculated at each time lag from 0–200 ms as the strength of RFs. The RF showing the strongest
229 response was used to determine the size of RF. The background response level was determined
230 by averaging the spike numbers at the 4 borders of RF and subtracted from RF. The RF map was
231 further divided by its 2D standard deviation to obtain Z scores. Pixel values with a Z score < 4
232 were set to 0.

233 **Neuron modeling**

234 A conductance-based single-compartment integrate-and-fire Neuron model (Li et al., 2012; Liu
235 et al., 2010) was built with a neuron receiving excitatory and inhibitory synaptic inputs evoked
236 by flash stimuli to simulate the membrane potential response, as follows:

$$V_m(t + dt) = -\frac{dt}{C} [G_e(t) \times (V_m(t) - E_e) + G_i(t) \times (V_m(t) - E_i) + G_r(t) \times (V_m(t) - E_r)] + V_m(t)$$

237 where $V_m(t)$ is the membrane potential at time t , C is the whole-cell capacitance, G_r is the
 238 resting leak conductance, and E_r is the resting membrane potential, set as -65 mV). E_e and E_i
 239 were set at 0 and -75 mV, respectively. C was set as 50 pF. G_r was calculated based on the
 240 equation $G_r = C \times G_m / C_m$, where G_m , the specific membrane conductance, is 10^{-5} S/cm², and
 241 C_m , the specific membrane capacitance, is 10^{-6} F/cm². Since previous studies in cortical
 242 pyramidal cells did not find drastic changes in intrinsic membrane properties including spike
 243 threshold after eye opening (Zhang, 2004), we assumed that the parameters defining membrane
 244 properties were constant between ST1 and ST2. The peak potential amplitudes above the resting
 245 membrane potential were quantified as postsynaptic potentials (PSPs).

246 The temporal profile of the evoked synaptic conductance was approximated by fitting the
 247 average waveform of synaptic response with an alpha function (Liu et al., 2010; Li et al., 2015,
 248 2018), as follows:

$$249 \quad G = G_{max} \times (t - onset) / \tau \times e^{-(t - onset - \tau) / \tau}, \text{ for } t > onset,$$

250 with $\tau = 63$ ms for the excitatory response and 83 ms for the inhibitory response. The onset of
 251 the inhibitory current was set at 5 ms after that of the excitatory current. G_{max} , the peak
 252 amplitudes of the excitatory or inhibitory tuning curves, was determined from experimental data
 253 of the average peak synaptic conductance at ST1 and ST2. To simulate the enhancement of
 254 synaptic strength during development, we increased G_{max} from 1 ns to 7 ns for excitation and
 255 determined the G_{max} for inhibition according to a constant E/I at 0.58. To simulate the effect of
 256 broadening of inhibitory subfield during development, we systematically set the inhibitory
 257 subfield size to be the same or 5°, 10°, or 15° larger than that of the excitatory subfield, while

258 kept the E/I at 0.58. To simulate the effect of dark rearing in preventing the developmental
 259 strengthening of excitation, we kept the inhibitory synaptic strength at ST2 and determined the
 260 excitation strength based on E/I ratio values ranging from 0.4 to 0.7. The peak amplitudes of the
 261 evoked synaptic conductances across spatial locations were derived from the spatial tuning
 262 curves approximated by a Gaussian function.

263 The peak firing rates were simulated by using a power law function (Carandini and Ferster,
 264 2000; Li et al., 2015): $R(V_m) = k[V_m - V_r]_+^p$. R is the predicted firing rate, V_r is the resting
 265 membrane potential (-65 mV), k is the gain factor (6.2×10^{-7}), p is the exponent, and “+”
 266 indicates rectification. V_r , k , and p were assumed to be constant during development. Because R
 267 is sensitive to variations of p , we chose a p value (5.3) to generate firing rates comparable to
 268 those experimentally observed. The predicted spiking subfield size was determined by the spatial
 269 range of PSPs above a 20-mV spike threshold. For the DR condition, we observed a significant
 270 reduction of spontaneous firing rates compared to NR mice (Fig. 6A). While it was not trivial to
 271 incorporate random spontaneous synaptic events in the Neuron model, we simulated the effect of
 272 their reduction by an equivalent lowering of the spike threshold (to 17 mV). It should be noted
 273 that any differences in values from empirically observed data could be due to some unknown
 274 changes of parameters. For example, the physical spike threshold could be lowered after DR,
 275 which we could not examine during our experiments due to the Cs+-based internal solution used
 276 for voltage-clamp recording.

277 To model the dependence of detectability of RFs on spontaneous firing rate (Fig. 6J), we
 278 used the mean spontaneous firing rate ($\pm$ SD) from ST1 and ST2 data, respectively. We then
 279 generated a large pool of evoked firing rates for each testing SNR: the mean of the generated
 280 evoked firing rates within the pool was equal to the mean spontaneous firing rate times SNR, and
 281 the standard deviation of the pool was selected based on experimental observations. We

282 randomly sampled from the pool and the sampled evoked firing rate was compared to the
283 spontaneous firing rate. It was scored as “detected” if it was larger than the spontaneous firing
284 rate by $> 3SD$. For each SNR level, we performed 1000 trials and calculated a detection rate.

285 **Statistics**

286 The Shapiro-Wilk test was first performed to test the normality of the data set. If the data were
287 normally distributed, a parametric two-sample t -test was applied. Otherwise, a nonparametric
288 Wilcoxon rank-sum test was used. Fisher’s exact test and χ^2 test were used to compare the ratios.
289 Multiple comparisons were based on the one-way analysis of variance (ANOVA) with *post hoc*
290 tests with Bonferroni correction. The significance level of the tests was set as 0.05. Data were
291 reported as mean $\pm$ SD, unless mentioned in the text. Statistical analysis was performed with
292 MATLAB (MathWorks) and Prism (GraphPad).

293 Results

294 Maturation of spatial RFs in layer 4 of mouse V1

295 To understand developmental changes of visual RFs, we performed *in vivo* cell-attached loose-
 296 patch recordings in layer 4 of the mouse monocular V1 at different developmental stages (see
 297 Materials and Methods). Our recording paradigm has been proved to preferentially sample from
 298 excitatory pyramidal neurons (Liu et al., 2010; Li et al., 2012). The cells we recorded from were
 299 segregated into two groups according to the age of animals: within three days after eye opening
 300 (stage 1, ST1, P14–P17, pre critical period) and during the critical period for ocular dominance
 301 plasticity (stage 2, ST2, P21–P28). The reason for us to choose these two stages is that several
 302 monocular visual selectivity features (e.g. orientation selectivity and linearity) mature
 303 significantly over one week after eye opening, in particular in layer 2–4 (Hoy and Niell, 2015;
 304 Jenks and Shepherd, 2020; Ko et al., 2013; Li et al., 2012; Rochefort et al., 2011; Wang et al.,
 305 2010). To map the ON and OFF subfields of the RF, we presented bright and dark squares ($5^\circ \times$
 306 5°), respectively, flashing individually on the screen (Liu et al., 2009; 2010; Li et al., 2015). As
 307 shown by the post-stimulus spike-time histograms (PSTHs) of an example neuron at ST1 (Fig.
 308 1A), the response areas to the bright (ON) and dark (OFF) squares were not clearly
 309 distinguishable from the spontaneous activity and therefore neither a ON nor OFF subfield with a
 310 definite boundary could be defined. Indeed, the RFs of recorded cells at ST1 were mostly
 311 obscure or “sluggish” (33 out of 41 cells, Fig. 1B,C), and only a small portion of the cells
 312 showed a spatially defined or “clear” RF (8 out of 41 cells, see an example in Fig. 1D). In stark
 313 contrast, a typical neuron at ST2 exhibited a brisk burst of firing to the square stimulation and a
 314 clear RF boundary could be delineated (Fig. 1E, green ellipse). Such spatially defined RFs were
 315 observed in the majority of cells recorded at ST2, with diversified spatial arrangements of
 316 ON/OFF subfields (Fig. 1F–H). This striking difference between the premature sluggish RFs at

ST1 versus the clear RFs at ST2 (and at adult ages, as shown in our previous study, Liu et al., 2009) prompted us to study the functional maturation of visual cortical RFs during this critical developmental period when basic monocular response properties are refined (Hoy and Niell, 2015).

To better understand the nature of the RF development, we quantified RF properties of the cells at ST1 and ST2. We found that distributions of spontaneous firing rates of the cells at ST1 versus ST2 were greatly diverged, as the median value was 1.4 Hz at ST1 versus 0.41 Hz at ST2 (Fig. 2A, left). Accordingly, the mean spontaneous firing rate was significantly decreased at ST2 (Fig. 2A, right, $p < 0.001$, t test). On the contrary, the evoked firing rate (measured as the maximum within the RF) was significantly increased at ST2 (Fig. 2B, $p < 0.001$, t test). Because of these opposite changes of spontaneous and evoked firing rates, the SNR values of cells at ST2 were greatly enhanced compared to those at ST1 (Fig. 2C, $p < 0.001$, t test). The reduced spontaneous firing rate and enhanced SNR would allow neurons to better encode visual information. Indeed, while the cells at ST1 predominantly showed sluggish RFs, about 90% of ST2 cells (19 out of 21) exhibited spatially well-defined RFs (Fig. 2D). Out of cells showing clear RFs, 50% (4/8), 25% (2/8) and 25% (2/8) of ST1 cells had ON-OFF, ON-only and OFF-only RF types, respectively, while the fractions at ST2 were 37% (7/19), 26% (5/19) and 37% (7/19), respectively ($p = 0.79$, χ^2 test). All these RF types have been observed in layer 4 of the adult cortex (Liu et al., 2009). Interestingly, the size of the dominant subfield (ON or OFF sign) of those apparently “precocious” ST1 cells with clear RFs was similar to their ST2 counterpart (Fig. 2E, $p = 0.46$, t test). Overall, cells with clear RFs exhibited lower spontaneous firing rates than those with sluggish RFs (Fig. 2F, left, $p < 0.001$, t test). Even for the precocious ST1 cells, the rare cells with clear RFs tended to have lower spontaneous firing rates than those with

340 sluggish RFs (Fig. 2*F*, right, $p = 0.094$, t test). These data indicate that the RF maturation is
 341 accompanied by a reduction of the spontaneous firing rate and enhancement of SNR.

342 It is possible that anesthesia has differential effects on circuits at different ages, contributing
 343 to the observed firing rate differences between ST1 and ST2. To avoid this confound, we further
 344 carried out extracellular multichannel recordings (with single-unit sorting) in awake mice at ST1
 345 and ST2 (see Materials and Methods). We used different methods to map RFs: forward mapping
 346 with singly flashed 5° and 10° squares, as well as sparse-noise and dense-noise mapping with
 347 spike-triggered average (STA) (see Materials and Methods). As shown by three example cells
 348 (Fig. 3*A*), the different methods either consistently failed to reveal a spatially organized RF, or
 349 revealed a RF at a similar location. The proportion of neurons exhibiting a clear RF greatly
 350 increased from ST1 to ST2 (Fig. 3*B*), and at ST2 it nearly reached the adult level (Fig. 3*B*, black
 351 column, data from our previous study Fang et al., 2020). Also consistent with data from
 352 anaesthetized mice, there was a significant decrease in the spontaneous firing rate as well as an
 353 increase in the evoked firing rate and SNR (Fig. 3*C-E*). The SNR at ST2 was comparable to the
 354 adult level (4.83 ± 4.36 , $n = 27$ cells, $p = 0.56$, t test, data from our previous study Fang et al.,
 355 2020). Consistent with data from anaesthetized animals, the size of the dominant subfield
 356 (measured with 5° squares) was not significantly changed from ST1 to ST2 (Fig. 3*F*). Together,
 357 these awake recording data further support the notion that maturation of monocular RFs mostly
 358 occurs between ST1 and ST2 and is characteristic of an enhancement of SNR.

359 **Synaptic inputs underlying the developmental maturation of RFs**

360 The reduction of spontaneous firing rates and popping-out of clear RFs at ST2 are reminiscent of
 361 a general increase of inhibitory tone in the visual cortex during development, as reported in
 362 previous studies (Chattopadhyaya, 2004; Li et al., 2012; Morales et al., 2002). Thus, we sought

363 to compare excitatory and inhibitory synaptic inputs underlying the spatial RF between ST1 and
 364 ST2. Since dense-noise mapping with STA tends to generate segregated ON/OFF subfields
 365 although the underlying ON and OFF inputs could be overlapped (Fig. 3A, bottom panel), we
 366 chose to use forward mapping with sparse stimuli as to better understand the spatial distribution
 367 of synaptic inputs. We performed whole-cell voltage-clamp recordings from layer 4 neurons and
 368 recorded excitatory and inhibitory synaptic responses to flashing bright and dark squares ($5^\circ \times 5^\circ$)
 369 by holding the membrane potential at -70 mV and 0 mV, respectively (see Materials and
 370 Methods). No significant difference in input resistance was found between the recorded ST1 and
 371 ST2 cells ($324 \pm 78 \text{ M}\Omega$ vs. $292 \pm 57 \text{ M}\Omega$, $p = 0.34$, t test, $n = 10$ and 12 cells, respectively). We
 372 observed that both excitatory and inhibitory synaptic responses were evoked by both ON and
 373 OFF stimuli in every cell recorded, even at ST1. As shown by an example neuron at ST1 (Fig.
 374 4A), excitatory and inhibitory responses to ON and OFF stimuli (upper panels) were spatially
 375 organized into largely overlapping excitatory and inhibitory ON/OFF subfields (outlined by blue
 376 and red ovals, respectively). To quantify the strength of synaptic responses, we deduced the
 377 synaptic conductance and measured the peak conductance after smoothing the conductance-time
 378 curve with a 40-ms sliding window (see Materials and Methods). The peak conductances evoked
 379 by ON and OFF stimuli at various locations were color coded to display the synaptic subfield
 380 maps (Fig. 4A, bottom panels). Similar to the example neuron in Fig. 4A, in most of neurons
 381 recorded at ST1 (8 out of 10), we observed clear ON and OFF subfields for both excitatory and
 382 inhibitory inputs (see Fig. 4B for an additional example). This is evidently different from the
 383 spiking response data at ST1, where in most of neurons no spatially clustered evoked responses
 384 were observed. At ST2, clear ON and OFF subfields could be identified for both excitatory and
 385 inhibitory inputs in every cell recorded (12 out of 12) (Fig. 4C-D). The arrangement of ON and
 386 OFF subfields at this stage appeared similar to ST1.

387 To quantitatively understand the progression of synaptic RF development, we compared two
 388 parameters, i.e. synaptic strength (the maximum peak conductance within the RF) and subfield
 389 size (size of the subfield of the dominant sign), between ST1 and ST2. We found that strengths
 390 of both excitation ($p = 0.0097$, t test) and inhibition ($p = 0.0072$, t test) increased from ST1 to
 391 ST2 (Fig. 5A-B). The excitation-to-inhibition (E/I) ratio however remained the same (Fig. 5C, p
 392 $= 0.49$, t test), indicating that excitation and inhibition were enhanced by a similar proportion.
 393 Moreover, while the excitatory subfield size remained the same (Fig. 5D, $p = 0.31$, t test), the
 394 inhibitory subfield size significantly increased from ST1 to ST2 (Fig. 5E, $p = 0.019$, t test). Thus,
 395 the ratio of excitatory to inhibitory subfield size was significantly reduced (ST1: 1.01 ± 0.13 ,
 396 ST2: 0.87 ± 0.11 , $p = 0.012$, two-sample t -test, $n = 8$ and 12 cells). In addition, the spatial
 397 arrangement of largely overlapping excitatory and inhibitory subfields appeared similar between
 398 ST1 and ST2, as shown by the quantification of overlap indices (OI, with 1 indicating complete
 399 overlap) (Fig. 5F). Together, the changes of these synaptic RF parameters suggest that excitatory
 400 and inhibitory synaptic inputs are substantially strengthened during development, presumably
 401 contributing to the maturation of spike RFs. The spatially enlarged inhibitory RF may further
 402 help to sharpen and delineate the boundary of spike RF at ST2, similar to the function of
 403 broadened inhibitory orientation tuning in the sharpening of orientation selectivity at the spiking
 404 response level (Li et al., 2012).

405 **The balanced enhancement of synaptic inputs leads to increased detection of RFs**

406 To further understand how the balanced enhancement of excitatory and inhibitory synaptic inputs
 407 during development contributes to the observed changes in spike RFs, we used a single-
 408 compartment Neuron model (Liu et al., 2011; Li et al., 2015) to simulate the membrane potential
 409 response resulting from the integration of synaptic excitation and inhibition evoked by each

410 visual stimulus (see Materials and Methods). The temporal dynamics of the synaptic
 411 conductances was based on our experimental data (Fig. 6A, upper panel). We kept the E/I ratio at
 412 0.58 (the average E/I ratio of the recorded neurons across ST1 and ST2, see Fig. 5C) and
 413 systematically changed the strength of excitation from 1 nS to 7 nS (the range of excitatory input
 414 strengths across ST1 and ST2, see Fig. 5A). That is, the strength of inhibition was also increased
 415 proportionally from 1.7 nS to 12.1 nS. As expected from our experimental data, we found that
 416 the peak amplitude of the simulated postsynaptic potential (PSP) response (Fig. 6A, lower panel)
 417 monotonically increased as the strengths of synaptic inputs were proportionally enhanced (Fig.
 418 6B and Fig. 6C, along the red dash line).

419 To understand the direct effect of synaptic strengthening on RF development, we simulated
 420 one-dimensional (1D) subfields (of the same sign) of excitation and inhibition (Fig. 6D, inset),
 421 based on the average synaptic strengths observed at ST1 and ST2. By integrating the excitation
 422 and inhibition evoked at each spatial location, we then obtained PSPs at different locations and
 423 thus the subfield of PSP at ST1 and ST2 (Fig. 6D), assuming that there were no changes in the
 424 synaptic subfield sizes. We found that the enhanced synaptic inputs at ST2 produced stronger
 425 PSPs than those at ST1, resulting in a pronounced spike subfield above the presumed spike
 426 threshold (20 mV, see Materials and Methods). This indicates that the balanced enhancement of
 427 excitation and inhibition can directly contribute to the functional maturation of spatially defined
 428 spike RFs. Next, we systematically increased the maximum excitatory conductance within the
 429 RF from 1 nS to 7 nS while keeping the E/I ratio at 0.58, and predicted evoked firing rates based
 430 on the derived PSPs by using a power law function (Carandini and Ferster, 2000; Priebe and
 431 Ferster, 2008; Liu et al., 2011). As expected from PSPs, only above a certain threshold
 432 conductance value (~3 nS) did the spike subfield appear and after that the peak firing rate and the
 433 spike subfield size increased with increasing synaptic strengths (Fig. 6E-F). When we further

enlarged the inhibitory subfield as observed for ST2, the increase in spike subfield size was then largely limited (Fig. 6F, red).

Next, using a similar modeling approach, we predicted the peak PSP within the RF for each recorded neuron by integrating the experimentally obtained excitatory and inhibitory conductances (Fig. 6G, top inset). We found that neurons at ST2 tended to have larger PSPs than those at ST1 (21.0 ± 3.8 mV vs. 18.0 ± 5.5 mV) (Fig. 6G). Among the ST1 neurons, only 38% of them (3/8 cells) had a peak PSP that surpassed the presumed spiking threshold of 20 mV, whereas 67% of ST2 neurons (8/12 cells) did so (Fig. 6H). Moreover, the predicted firing rate tended to be lower at ST1 than at ST2 (Fig. 6I), consistent with the experimental observation of higher evoked firing rates at ST2. Therefore, the observed balanced enhancement of excitatory and inhibitory synaptic inputs can directly contribute to the increased responses and RF detectability of layer 4 neurons during development.

The reduction of spontaneous activity contributes to the increased RF detection

In addition to the synaptic input changes, the observed reduction of spontaneous firing rates could also influence how well a spatially organized RF can be detected. As shown in a schematic illustration (Fig. 6J, top panel), a reduction of the spontaneous firing rate alone can lower the detection threshold for evoked responses, equivalent to lowering the effective spike threshold, and therefore enhances the detection of a spatially organized RF. By random sampling from a large pool of evoked firing rates consistent with experimental observations (see Materials and Methods), we examined how the detection probability depended on the spontaneous firing rate. Detection rate was increased with increasing SNR, and the spontaneous firing rate at the ST2 level shifted the curve leftward relative to that at the ST1 level (Fig. 6J, bottom panel). Therefore, with the reduced spontaneous firing rate and enhanced SNR at ST2, the detection probability

could be greatly enhanced. This result demonstrates that the reduction of spontaneous activity per se is an important factor contributing to the maturation of RFs.

Effects of dark rearing on the development of visual RFs

Previous studies have demonstrated that in excitatory neurons of mouse visual cortex orientation selectivity develops more-or-less normally when visual experience is deprived by dark rearing (DR) (Jenks and Shepherd, 2020; Ko et al., 2014; Kuhlman et al., 2011; Li et al., 2012; Rochefort et al., 2011; Sarnaik et al., 2014; Wang et al., 2010). To examine whether visual experience is similarly dispensable for the development of RFs, we reared animals in total darkness from P9 and examined spiking and synaptic responses to bright and dark squares at P21–P28 (after DR, comparable to ST2). We found a similar trend of response level changes to the normal development: compared with those at ST1, spontaneous firing rate was significantly reduced after DR (Fig. 7A, $p < 0.001$, one-way ANOVA and *post hoc* test), while evoked firing rate was noticeably increased (Fig. 7B, $p = 0.014$, one-way ANOVA and *post hoc* test), and thus SNR was enhanced (Fig. 7C, $p < 0.001$, one-way ANOVA and *post hoc* test). In addition, the proportion of cells showing a clear RF was significantly higher than that at ST1 (Fig. 7D, DR vs. ST1: 73% vs. 20%, $p < 0.001$, Fisher's exact test). These data demonstrate that despite the deprivation of vision the development of RFs proceeds relatively well, suggesting that a large component of RF development is experience independent. However, comparing to normally reared (NR) animals at ST2, we did find a significant reduction in both the spontaneous (Fig. 7A, $p = 0.024$, one-way ANOVA and *post hoc* test) and evoked firing rates (Fig. 7B, $p = 0.0024$, one-way ANOVA and *post hoc* test) as well as in the proportion of cells showing a clear RF (Fig. 7D, DR vs. ST2: 73% vs. 90%, $p < 0.001$, Fisher's exact test). For these cells, 41% (9/22) had ON-OFF RFs, 9% (2/22) had an ON-only and 50% (11/22) had an OFF-only RF (DR vs. ST2, $p =$

0.33, χ^2 test). In addition, the dominant subfield size was reduced after DR compared to both ST1 and ST2 of normal development (Fig. 7E, ST1 vs. DR: $p = 0.046$, ST2 vs. DR: $p = 0.0078$, one-way ANOVA and *post hoc* test). These results suggest that DR does interfere some aspects of RF development, possibly through altering the excitation/inhibition balance.

We next sought to investigate synaptic mechanisms underlying the reduction of response level and subfield shrinkage after DR, using whole-cell voltage clamp recording. The input resistance of cells recorded in DR mice was not different from ST2 cells in NR animals ($315 \pm 78 \text{ M}\Omega$ vs. $292 \pm 57 \text{ M}\Omega$, $p = 0.51$, t test, $n = 13$ and 12 cells, respectively). We found that excitatory and inhibitory synaptic subfields remained well spatially defined (13 out of 13 cells) and were seemingly normal in shape after DR (Fig. 7F, gray ovals). However, a closer examination revealed an impedance of subfield development caused by DR: the excitatory input was under-developed and remained as weak as that at ST1 (Fig. 7G, ST1 vs. DR: $p = 0.27$, ST2 vs. DR: $p = 0.015$, one-way ANOVA and *post hoc* test), whereas the inhibitory input appeared normally developed in strength (Fig. 7H, ST1 vs. DR: $p < 0.001$, ST2 vs. DR: $p = 0.35$, one-way ANOVA and *post hoc* test). This unbalanced development led to a significant reduction of E/I ratio (Fig. 7I, ST1 vs. DR: $p = 0.015$, ST2 vs. DR: $p < 0.001$, one-way ANOVA and *post hoc* test), i.e. relatively more dominance of inhibition over excitation after DR, which may explain the suppression of both spontaneous and evoked firing rates. Additionally, while the excitatory subfield size was not affected (Fig. 7J, ST2 vs. DR: $p = 0.43$, one-way ANOVA and *post hoc* test), the developmental broadening of the inhibitory subfield was prevented by DR (Fig. 7K, ST2 vs. DR: $p = 0.015$, one-way ANOVA and *post hoc* test). Thus, the ratio of excitatory over inhibitory subfield size was substantially increased compared to ST2 but remained the same as ST1 (Fig. 7L, ST1 vs. DR: $p = 0.40$, ST2 vs. DR: $p < 0.001$, one-way ANOVA and *post hoc* test). On the other hand, the spatial overlap between excitatory and inhibitory subfields (of the same

sign) was not affected by DR (OI after DR: 0.78 ± 0.096 , DR vs. ST2, $p = 0.47$, t test). Together, these data indicate that DR results in abnormal under-development of excitation and paradoxically “normal” development of inhibition, leading to abnormal dominance of inhibition over excitation.

Discussion

A proper spatial organization of RFs is fundamental for normal visual function (Niell, 2015; Priebe and Ferster, 2012). However, how the RF structure matures during development and what are underlying changes of synaptic inputs remain not well-understood. In the present study, we found that premature V1 layer 4 neurons around eye-opening rarely exhibited spatially organized visual RFs at the spike level, whereas clear RFs were observed in most of neurons one to two weeks after. In accompany with this RF maturation, spontaneous firing rates were reduced while evoked firing rates were increased, leading to a robust enhancement of SNR of visual responses within the RF.

The suppression of high spontaneous activity is consistent with the notion of an increasing inhibitory tone during the post-eye-opening development (Chattopadhyaya, 2004; Morales et al., 2002). The developmental trends of decreases in spontaneous firing rates and increases in visually evoked responses generally agree with the results of several previous studies (Hoy and Niell, 2015; Li et al., 2012; Roy et al., 2020), although they have not reached statistical significance in layer 4 in one study (Hoy and Niell, 2015). As shown in Fig. 6J, the decrease in spontaneous firing rate and the increase in SNR together contribute importantly to the increased detectability of RFs. These changes may similarly contribute to the developmental increase in the fraction of visually responsive neurons reported previously (Hoy and Niell, 2015; Jenks and Shepherd, 2020). Therefore, it is possible that SNR could be a critical indicator of maturation

528 status of cortical neurons. It should be noted that early neurons without exhibiting a clear RF
 529 may still be responsive to other stimulation patterns such as full-field moving gratings, leading to
 530 an apparently higher fraction of overall visually responsive neurons (Hoy and Niell, 2015) than
 531 the fraction reported here.

532 Different from the spike RF, we found that synaptic RFs (both excitatory and inhibitory)
 533 were mostly clear-cut at the earliest post eye-opening stage. The largely overlapping patterns of
 534 excitatory and inhibitory subfields are similar to what have been found in the adult cortex (Liu et
 535 al., 2010). This indicates that spatial RFs of thalamic relay neurons are already formed (Davis et
 536 al., 2015; Tschetter et al., 2018) and that the basic thalamocortical wiring pattern is already
 537 established around eye opening, likely under the influence of cortical/subcortical activity driven
 538 by spontaneous retinal waves (Blankenship and Feller, 2010; Katz and Shatz, 1996). This notion
 539 is also supported by observations that several visual functions such as orientation and direction
 540 selectivity are already present at the time of eye opening (Ko et al., 2014; Kuhlman et al., 2011;
 541 Li et al., 2012; Rochefort et al., 2011; Wang et al., 2010). Nevertheless, both excitatory and
 542 inhibitory inputs undergo robust developmental changes after the onset of visual experience (Fig.
 543 8A). On a global scale, excitation and inhibition were scaled up in a balanced manner, as the E/I
 544 ratio of visually evoked synaptic conductances was constant during development. Such balanced
 545 enhancement of excitatory and inhibitory synaptic strengths can help to increase evoked firing
 546 rates while preventing response saturation (Liu et al., 2011; Pouille et al., 2009). However,
 547 simply upregulating excitation and inhibition would generally lead to a reduction of tuning
 548 strength (Liu et al., 2011; Li et al., 2012), specifically in this study to enlarged spike subfields.
 549 Fortunately, inhibitory subfield size was increased during development. Such broadening of
 550 inhibitory spatial tuning, together with the enhancement of its strength, can effectively refine the
 551 spike subfield (Fig. 6F) by suppressing membrane potential (and spiking) responses at RF

552 surrounding regions, creating a clear-cut boundary. Remarkably, only moderately broader than
 553 the excitatory subfield, inhibition can already effectively sharpen the spike subfield (Fig. 8B).
 554 These modeling results are reminiscent of previous demonstrations that inhibition more broadly
 555 tuned than excitation can sharpen orientation selectivity in the face of weakly tuned excitation
 556 (Liu et al., 2011; Somers et al., 1995) and that broadening of inhibitory orientation tuning can
 557 lead to developmental sharpening of orientation selectivity (Li et al., 2012).

558 Different from the inhibitory subfield, the excitatory subfield remained unchanged in size. A
 559 previous study in the mouse dorsal lateral geniculate nucleus (dLGN) has shown that the spatial
 560 RF of thalamic relay neurons is reduced in size by half during development (Tschetter et al.,
 561 2018), which is attributable to reduced convergence (i.e. pruning) of retinogeniculate
 562 connections (Litvina and Chen, 2017; Tschetter et al., 2018). The unchanged excitatory subfield
 563 size in layer 4 thus suggests that the number of dLGN neurons connecting to a layer 4 neuron
 564 might be increased after eye opening, which can also contribute to the increased excitatory (and
 565 inhibitory) input strength. In this sense, retinogeniculate and geniculocortical connectivity might
 566 be developmentally regulated in an opposite manner. In addition, as layer 4 is the major thalamo-
 567 recipient layer of V1, changes of both feedforward thalamocortical and recurrent intracortical
 568 connectivity (Ko et al., 2013) can contribute to the developmental change of excitatory input. It
 569 will be of great interest to know how each of these components can account for the
 570 developmental maturation of the RF structure (Sun et al., 2019).

571 In addition to the synaptic input changes, any possible changes of intrinsic membrane
 572 properties of neurons can influence the detectability of spike RFs. A previous slice recording
 573 study of pyramidal neurons in the rat prefrontal cortex has reported only minor or negligible
 574 changes in input resistance, membrane time constant and spike threshold after eye opening
 575 (Zhang, 2004). Consistently, we did not find a significant difference in input resistance between

576 ST1 and ST2 cells. Nevertheless, we do not exclude the possibility that changes of other
 577 membrane properties, e.g. lowering of spike threshold (Roy et al., 2020), can further contribute
 578 to the increased salience of visually evoked activity and detectability of RFs.

579 Sensory experience is required for various aspects of normal visual functional development
 580 in the cortex during the critical period (Fagiolini et al., 1994; Kuhlman et al., 2011; Li et al.,
 581 2012; Sarnaik et al., 2014; Wang et al., 2010; White et al., 2001). Indeed, in this study, our data
 582 demonstrate that both the spontaneous and evoked firing rates as well as spike subfield sizes
 583 were diminished after DR, suggesting an overall reduction of responsiveness, in agreement with
 584 a number of previous studies (Braastad and Heggelund, 1985; Fagiolini et al., 1994; Tropea et al.,
 585 2010; Tsumoto and Freeman, 1987). A more recent study, however, reports no significant
 586 change in the overall RF size (i.e. considering both On and Off subfields) in slightly older (P31-
 587 P35) DR mice compared to NR control (Sarnaik et al., 2014). On the one hand, DR effects could
 588 be dependent on when and for how long it is applied (Benevento et al., 1992). On the other hand,
 589 the above observation may be in fact in line with our result of unaffected excitatory subfield
 590 sizes, considering that On/Off synaptic subfields are largely overlapping. Despite the largely
 591 normal development of monocular RFs, the development of spatial acuity as measured
 592 behaviorally is significantly delayed by DR (Kang et al., 2013), possibly because the acuity
 593 examined under binocular conditions is impacted by binocular matching of monocular RF
 594 properties, which is affected by DR (Sarnaik et al., 2014).

595 The change in spike subfield sizes can be explained by the imbalanced changes of synaptic
 596 strengths: excitation remained weak and not strengthened, while inhibition underwent apparently
 597 normal developmental strengthening, creating an abnormal dominance of inhibition over
 598 excitation and resulting in reduced subfield sizes (Fig. 8C). The underdevelopment of excitation
 599 is likely a result of combined effects: delayed pruning of retinogeniculate synapses (Hooks and

600 Chen, 2008) and a delayed increase in the convergence of thalamocortical synapses. The
601 relatively enhanced inhibition is consistent with previous *in vitro* slice recording results showing
602 that inhibitory synaptic transmission in layer 4 is potentiated after sensory deprivation (Maffei et
603 al., 2006; Nahmani and Turrigiano, 2014). This paradoxical enhancement of inhibition may be
604 attributed to a compensatory recruitment of more inhibitory inputs and/or strengthening of pre-
605 and postsynaptic functions of inhibitory synapses during DR (Maffei et al., 2006; Miao et al.,
606 2016; Nahmani and Turrigiano, 2014). Combined thalamocortical and intracortical effects may
607 lead to the failed enlargement of inhibitory subfields after DR. It is worth noting that some of DR
608 effects on inhibition might be masked since it has been shown previously in layer 2/3 pyramidal
609 neurons that restoring vision after DR only for a short period of time (2 hr, comparable to our
610 time window for visual stimulation) can partially reverse some DR effects on miniature
611 inhibitory postsynaptic currents (Gao et al., 2014). Finally, recent studies have suggested that the
612 impact of parvalbumin (PV) and somatostatin (SOM) inhibitory neurons on pyramidal cells is
613 increased and decreased, respectively, during a similar developmental window to this study
614 (Miao et al., 2016; Yaeger et al., 2019) and that the development of PV-neuron function is
615 regulated by visual experience (Kuhlman et al., 2011; Li et al., 2012). How the inhibitory-neuron
616 subtypes contribute distinctly to the experience-dependent RF maturation awaits further
617 investigations.

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796 **Figure legends**

797 **Figure 1.** Developmental maturation of spatial receptive fields (RFs) in mouse primary visual
 798 cortex. **A**, One example layer 4 (L4) pyramidal neuron at stage 1 (ST1). Upper, plots of post-
 799 stimulus spike time histograms (PSTHs) of its responses to bright (ON, left) and dark (OFF, right)
 800 squares ($5^\circ \times 5^\circ$) presented at various locations. Scale bar: 5 Hz and 50 ms. Lower, ON (left),
 801 OFF (right), and merged ON+OFF (middle) subfield maps of the neuron. The response strength
 802 is color coded. Scale bar: 5 Hz and 300 ms. Scale bar: 8° . **B-D**, Three additional L4 pyramidal
 803 neurons at ST1. Green and red ellipses mark the boundary of defined ON and OFF subfields,
 804 respectively. **E**, One example L4 pyramidal neuron at stage 2 (ST2). Data display is similar to **A**.
 805 Upper, PSTHs to bright and dark squares. Scale bar: 10 Hz and 50 ms. Lower, ON, OFF, and
 806 merged subfield maps. Scale bar: 8° . **F-H**, Three additional L4 pyramidal neurons at ST2.

807
 808 **Figure 2.** Summary of spike RF properties during development. **A**, Left, cumulative distribution
 809 of spontaneous firing rates of neurons at ST1 (light orange) and ST2 (dark orange), respectively.
 810 Right, comparison of spontaneous rates at two stages. ST1: 1.9 ± 1.6 Hz (mean $\pm$ SD), ST2: 0.6
 811 ± 0.5 Hz, *** $p < 0.001$, two-sample t -test, $n = 41$ and 21 cells, respectively. **B**, Comparison of
 812 evoked firing rates of neurons at ST1 versus ST2. ST1: 2.0 ± 4.9 Hz, ST2: 7.9 ± 4.8 Hz, *** $p <$
 813 0.001 , two-sample t -test, $n = 41$ and 21 cells, respectively. **C**, Comparison of the signal-to-noise
 814 ratios (SNR) of neuronal responses. ST1: 2.7 ± 8.0 , ST2: 19.1 ± 12.5 , *** $p < 0.001$, two-sample
 815 t -test, $n = 41$ and 21 cells, respectively. **D**, Fractions of clear versus sluggish RFs at ST1 and
 816 ST2. *** $p < 0.001$, Fisher's exact test, $n = 41$ and 21 cells, respectively. **E**, Comparison of
 817 dominant subfield sizes. ST1: $13.8^\circ \pm 5.9^\circ$, ST2: $13.6^\circ \pm 5.8^\circ$, ns: non-significant, $p = 0.46$, two-
 818 sample t -test, $n = 8$ and 19 cells, respectively. **F**, Spontaneous firing rates of neurons with clear

819 versus sluggish RFs. Left, all neurons at ST1 and ST2 are counted. Clear RF: 0.8 ± 0.9 Hz,
 820 Sluggish RF: 2.0 ± 1.6 Hz, *** $p < 0.001$, two-sample *t*-test, $n = 27$ and 35 cells, respectively.
 821 Right, only neurons at ST1 are counted. Clear RF: 1.3 ± 1.4 Hz, Sluggish RF: 2.1 ± 1.6 Hz, $p =$
 822 0.094 , two-sample *t*-test, $n = 8$ and 33 cells, respectively. Error bar = SEM (for all panels).
 823
 824 **Figure 3.** Development of spike RFs in awake mice. **A**, Top row, an example neuron without a
 825 clear RF when mapped with large (10° in size) or small (5° in size) flashing squares, or sparse or
 826 dense noise. Middle row, an example neuron with a clear and consistent RF of a single sign (On),
 827 mapped with different sets of visual stimuli. The red dashed line marks the boundary of the RF.
 828 Bottom row, an example neuron with a RF with both On and Off signs. Scale bar = 4° . **B**,
 829 Summary of the percentage of neurons with a clear RF across developmental stages. *** $p <$
 830 0.001 , Fisher's exact test, $n = 85$ (ST1) and 75 (ST2) cells with 10° squares, 91 and 76 cells with
 831 5° squares, 93 and 62 cells with dense noise, and 87 , 62 and 51 (adult) cells with sparse noise. **C**,
 832 Left, comparison of the spontaneous rates of neurons at ST1 versus ST2. ST1: 4.1 ± 2.8 Hz, ST2:
 833 2.3 ± 1.5 Hz, *** $p < 0.001$, two-sample *t*-test, $n = 91$ and 76 cells. Right, spontaneous rates of
 834 neurons with and without a clear RF at ST1 only (2.8 ± 1.9 Hz and 4.3 ± 2.8 Hz, respectively, p
 835 $= 0.13$, two-sample *t*-test, $n = 82$ and 9 cells). **D**, Comparison of evoked firing rates of neurons.
 836 ST1: 0.3 ± 0.6 Hz, ST2: 6.2 ± 6.6 Hz, *** $p < 0.001$, two-sample *t*-test, $n = 91$ and 76 cells. **E**,
 837 Comparison of SNR values. ST1: 0.04 ± 0.14 , ST2: 4.00 ± 5.75 , *** $p < 0.001$, two-sample *t*-test,
 838 $n = 91$ and 76 cells. **F**, Comparison of subfield sizes (mapped with 5° squares). ST1: $14.7^\circ \pm 4.3^\circ$,
 839 ST2: $15.2^\circ \pm 3.8^\circ$, ns: not significant, $p = 0.68$, two-sample *t*-test, $n = 14$ and 49 cells.
 840 **Figure 4.** Synaptic inputs underlying developing RFs of L4 excitatory neurons. **A**, Upper,
 841 averaged excitatory (Exc) and inhibitory (Inh) responses to bright (ON) and dark (OFF) squares

842 ($5^\circ \times 5^\circ$) for an example neuron at ST1. Blue and red ellipses mark the boundaries of the
 843 excitatory and inhibitory subfields, respectively. Scale bar: 250 pA, 300 ms. Lower, color maps
 844 of peak response amplitudes for excitatory and inhibitory ON (left), OFF (right) and merged
 845 (middle) subfields. Red and green ellipses delineate the boundaries of ON and OFF synaptic
 846 subfields, respectively. **B**, An additional example neuron at ST1. **C**, Excitatory and inhibitory
 847 subfields for an example L4 neuron at ST2. Scale bar: 300 pA, 300 ms. **D**, An additional
 848 example neuron at ST2. Scale bar: 8° (for all panels).

849
 850 **Figure 5.** Changes of synaptic response properties during development. **A**, Comparison of the
 851 maximum peak conductance of excitatory responses (averaged) to a flashing square within the
 852 RF between two stages. ST1: 2.5 ± 1.0 nS, ST2: 3.8 ± 1.3 nS, $**p = 0.0097$, two-sample *t*-test, *n*
 853 = 10 and 14 cells, respectively. **B**, Comparison of the maximum peak conductance of inhibitory
 854 responses within the RF. ST1: 4.3 ± 1.3 nS, ST2: 6.8 ± 2.7 nS, $**p = 0.0072$, two-sample *t*-test, *n*
 855 = 10 and 14 cells, respectively. **C**, Excitation-to-Inhibition (E/I) ratio. ST1: 0.58 ± 0.19 , ST2:
 856 0.58 ± 0.13 , $p = 0.49$, two-sample *t*-test, *n* = 10 and 14 cells, respectively. **D**, Comparison of
 857 dominant excitatory subfield sizes. ST1: $20.5^\circ \pm 4.4^\circ$, ST2: $21.6^\circ \pm 5.1^\circ$, $p = 0.31$, two-sample *t*-
 858 test, *n* = 8 and 12 cells, respectively. **E**, Comparison of dominant inhibitory subfield sizes. ST1:
 859 $20.3^\circ \pm 3.8^\circ$, ST2: $24.8^\circ \pm 4.9^\circ$, $*p = 0.019$, two-sample *t*-test, *n* = 8 and 12 cells, respectively. **F**,
 860 Overlap index calculated between the excitatory and inhibitory subfields of the same sign. ST1:
 861 0.79 ± 0.10 , ST2: 0.78 ± 0.11 , $p = 0.39$, two-sample *t*-test, *n* = 8 and 12 cells, respectively. Error
 862 bar = SEM (for all panels).

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Figure 6. Neuron modeling to simulate membrane potential and subfield size changes during development. **A**, Top, simulated square stimulus-evoked excitatory (Ge) and inhibitory (Gi) synaptic conductances of the neuron (upper) and the derived membrane potential response using the single-compartment Neuron model (lower). **B**, Change of the peak postsynaptic potential (PSP) response with increasing excitatory conductances (E) under a constant E/I ratio of 0.58, the average value observed experimentally at both ST1 and ST2. **C**, Color-coded amplitudes of PSPs at various combinations of E-I strengths. The maximum conductance is 12.1 nS. Red dashed line marks the trajectory of E/I ratio = 0.58. **D**, Spatial tuning of PSP responses at two different stages. Dash line marks the presumed 20mV spike threshold. Inset, schematic spatial tuning of excitation (blue) and inhibition (red) and sample synaptic conductances at the corresponding locations. In this stimulation, the spatial tuning of synaptic conductances was kept the same between ST1 and ST2, while the response amplitude (for both excitation and inhibition) was increased from ST1 to ST2, keeping the E/I ratio the same. **E**, Predicted evoked firing rate with increasing excitation amplitudes under a constant E/I ratio of 0.58. **F**, Predicted spike subfield size with increasing excitation amplitudes under a constant E/I ratio of 0.58. Black, excitatory and inhibitory subfield sizes are equal. Red, inhibitory subfield is 5° larger than the excitatory subfield as observed experimentally at ST2. **G**, Peak PSP amplitudes derived by integrating experimentally obtained excitatory and inhibitory inputs with the Neuron model. ST1: 18.0 ± 5.5 mV, ST2: 21.0 ± 3.8 mV, $p = 0.11$, two-sample t -test, $n = 8$ and 12 cells, respectively. Top inset, example experimentally obtained excitatory and inhibitory conductances in a cell responding to the same flash square (left) and the derived PSP response (Vm, right). **H**, Percentage of neurons with PSPs surpassing a spike threshold of 20 mV at two stages. **I**, Predicted evoked firing rates of neurons at ST1 versus ST2 based on a power-law function. ST1:

887 3.9 ± 5.8 Hz, ST2: 7.4 ± 6.4 Hz, $p = 0.14$, two-sample t -test, $n = 8$ and 12 cells, respectively. The
 888 firing rates of neurons with PSPs lower than 20 mV were set as 0. Error bar = SEM. **J**, Top,
 889 illustration of the influence of spontaneous firing rate on detectability of RFs. The black line and
 890 gray shaded area represent the mean and SD of spontaneous firing rate, respectively, with the
 891 blue dash line labeling spike rate = 0. The green bar represents the spike rate to a square stimulus
 892 within the subthreshold RF (represented by the light green eclipse). Red dash line marks the
 893 detection threshold (mean + 3SD). Solid green squares mark visual stimuli that result in the
 894 detection of a significant evoked response. Bottom, detection probability (see Material and
 895 Methods) plotted against SNR. The two curves are generated by using spontaneous firing rates at
 896 ST1 and ST2 level, respectively. Colored bars depict the range of SNR at ST1 and ST2,
 897 respectively (note that the ST2 range has been cut off at 5 since the mean value is much higher
 898 than 5).

899
 900 **Figure 7.** Effects of dark rearing on the development of spiking and synaptic RFs. **A-C**,
 901 Spontaneous firing rates (**A**), evoked firing rates (**B**), and SNR (**C**) of neurons at ST1 ($n = 41$
 902 cells) or ST2 ($n = 21$ cells) during normal development versus after dark rearing (DR, $n = 30$
 903 cells). Statistics: spontaneous firing rates (DR: 0.3 ± 0.3 Hz; ST1 vs. DR: $p < 0.001$; ST2 vs. DR:
 904 $p = 0.024$), evoked firing rates (DR: 4.3 ± 3.5 Hz; ST1 vs. DR: $p = 0.014$; ST2 vs. DR: $p =$
 905 0.0024), and SNR (DR: 20.5 ± 20.2 ; ST1 vs. DR: $p < 0.001$; ST2 vs. DR: $p = 0.38$); one-way
 906 ANOVA and *post hoc* test. **D**, Fractions of clear (C) versus sluggish (S) RFs at ST1, ST2, and
 907 after DR, respectively. *** $p < 0.001$, Fisher's exact test, $n = 41$, 21, and 30 cells, respectively. **E**,
 908 Dominant subfield sizes of cells at ST1 ($n = 8$ cells) or ST2 ($n = 19$ cells) versus after DR ($n =$
 909 22 cells). DR: $9.6^\circ \pm 3.6^\circ$; ST1 vs. DR: $p = 0.046$; ST2 vs. DR: $p = 0.0078$; one-way ANOVA

910 and *post hoc* test. **F**, Excitatory and inhibitory On/Off subfields of an example L4 neuron after
 911 DR. Gray ellipses delineate the subfield boundary. Scale bar: 300 pA and 300 ms. **G-I**, Peak
 912 evoked excitatory (**G**) and inhibitory (**H**) conductances and E/I ratio (**I**) of the cells at ST1 (n =
 913 10 cells) or ST2 (n = 12 cells) versus after DR (n = 13 cells). Statistics: excitatory conductance
 914 (DR: 2.8 ± 0.9 nS; ST1 vs. DR: $p = 0.27$; ST2 vs. DR: $p = 0.015$), inhibitory conductance (DR:
 915 7.1 ± 1.7 nS; ST1 vs. DR: $p < 0.001$; ST2 vs. DR: $p = 0.35$), and E/I ratio (DR: 0.40 ± 0.11 ; ST1
 916 vs. DR: $p = 0.015$; ST2 vs. DR: $p < 0.001$); one-way ANOVA and *post hoc* test. **J-L**, Excitatory
 917 subfield size (**J**), inhibitory subfield size (**K**), and the ratio of excitatory over inhibitory subfield
 918 sizes (**L**) of the cells at ST1 (n = 8 cells) or ST2 (n = 12 cells) versus after DR (n = 13 cells).
 919 Statistics: excitatory subfield size (DR: $20.1^\circ \pm 4.6^\circ$; ST1 vs. DR: $p = 0.43$; ST2 vs. DR: $p =$
 920 0.24), inhibitory subfield size (DR: $19.9^\circ \pm 5.3^\circ$; ST1 vs. DR: $p = 0.41$; ST2 vs. DR: $p = 0.015$),
 921 and excitatory-to-inhibitory subfield size ratio (DR: 1.03 ± 0.09 ; ST1 vs. DR: $p = 0.40$; ST2 vs.
 922 DR: $p < 0.001$); *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$, one-way ANOVA and *post hoc* test. Error
 923 bar = SEM.

924

925 **Figure 8.** A working model for the maturation of RF during development. **A**, Blue, red and
 926 green curves represent the spatial tuning (or RF subfield) of excitation, inhibition and membrane
 927 potential (Vm) responses, respectively. Gray dash line represents the spike threshold. At ST1, the
 928 premature excitation and inhibition result in relatively weak Vm responses (pale green) and only
 929 suprathreshold Vm responses (or spike responses that are above the detection threshold) in a
 930 small fraction of neurons. Under normal development at ST2, the amplitudes of excitation and
 931 inhibition are enhanced in proportion (vertical arrows) and the inhibitory subfield is broadened
 932 (horizontal arrows), resulting in stronger suprathreshold Vm responses and emergence of a

933 distinct spike subfield (bright green). Note that without the developmental broadening of the
934 inhibitory RF, the resultant spike subfield would be broadened (compare dashed green and solid
935 green). Under DR, the maturation of excitation is arrested, while inhibition enhances its strength
936 to the mature state (red vertical arrow) without broadening its spatial tuning. This un-balanced
937 change of inhibition results in reduced Vm responses (bright green, compared to pale green).
938 Note that spontaneous firing rate is reduced after DR, equivalent to lowering the detection
939 threshold. Together, these effects lead to a smaller spike subfield. **B**, Simulation of the effect of
940 the developmental broadening of inhibitory subfield on the spike subfield size (see Materials and
941 Methods). Δ Size is the difference between inhibitory and excitatory subfield sizes, while the
942 peak amplitudes of excitation and inhibition (E/I ratio = 0.58) as well as the excitatory subfield
943 size are kept the same. The maximum conductances of excitation and inhibition are 4.3 and 6.8
944 nS, respectively. Note that the mean experimentally observed Δ Size at ST2 is 5°. **C**, Simulation
945 of the effect of impaired maturation of excitation and reduced spontaneous firing rates on the
946 spike subfield size after DR (see Materials and Methods). The peak amplitude of inhibition (6.8
947 nS) as well as the inhibitory and excitatory subfield sizes are kept the same, whereas the
948 amplitude of excitation (therefore, E/I ratio) is varied.

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