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Fast-spiking interneurons have an initial orientation bias that is lost with vision

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We found that in mice, following eye opening, fast-spiking, parvalbumin-positive GABAergic interneurons had well-defined orientation tuning preferences and that subsequent visual experience broadened this tuning. Broad inhibitory tuning was not required for the developmental sharpening of excitatory tuning but did precede binocular matching of excitatory orientation tuning. We propose that experience-dependent broadening of inhibition is a candidate for initiating the critical period of excitatory binocular plasticity in developing visual cortex.

Broadly tuned inhibition, mediated by parvalbumin-positive GABAergic neurons[1], controls the timing and spatial spread of circuit activity in the cortex[2–4]. These interneurons are thought to regulate the onset of developmental critical periods of cortical plasticity[5], and deficits in their function are linked to autism spectrum disorders and schizophrenia[6,7]. Despite the centrality of these neurons to normal cortical development, it is not known how these neurons are recruited into cortical circuits during postnatal maturation.

To directly examine this, we recorded visually driven response properties of identified fast-spiking, parvalbumin-positive GABAergic neurons in cortical layer 2/3, 180–310 μm below the pia, of mouse primary visual cortex *in vivo* (**Fig. 1**). We recorded in the binocular zone of visual cortex just before the onset of the critical period for binocular plasticity (postnatal day 17–19 (P17–19), pre) and during the peak of this period (P23–30)[5,8]. We used intrinsic signal mapping to identify the position of binocular visual cortex (**Fig. 1a**) and, subsequently, *in vivo* two-photon imaging to target cell-attached recordings to parvalbumin-positive interneurons expressing a red fluorescent protein (RFP; **Fig. 1b**,**c**, also see the **Supplementary Methods**). The spike waveform of these cells was narrowly shaped, characterized by a rapid falling action potential and high-amplitude undershoot (**Fig. 1d**, see also ref. 1). In contrast, the waveform of neurons negative for RFP was broadly shaped, indicating that this population was highly biased to excitatory neurons[9]. Hereafter we refer to the RFP-negative neurons as excitatory neurons, although we do not rule out the possibility that up to 10% of the cells are inhibitory neurons. These spike waveform parameters clearly distinguished parvalbumin-positive interneuron recordings from excitatory neuron recordings under all of the experimental conditions that we examined (**Fig. 1d**,**e** and **Supplementary Fig. 1**). Our experiments were approved by the University of California Los Angeles Office for Protection of Research Subjects and the Chancellor's Animal Research Committee.

In the pre-critical period, we found that parvalbumin-positive interneurons had well-defined orientation tuning preferences. Orientation selectivity was measured by two independent metrics[10,11] that are commonly used to describe tuning response curves: the orientation selectivity index (OSI), which is a measure of global selectivity that takes into account both the preferred and nonpreferred components of the response, and bandwidth, which is specifically a measure of the tuned component around the preferred orientation. The mean OSI (defined as 1 minus the circular variance) of parvalbumin-positive interneurons was 0.18 ± 0.01 and the median bandwidth was 25.5 degrees (**Fig. 1f** and **Supplementary Fig. 2**).

In contrast, in critical-period mice, parvalbumin-positive interneurons did not have well-defined tuning preferences, as have been observed in adults[1,11–13]. The mean OSI at this age was 72% lower than what we observed in the pre-critical period (0.107 ± 0.01) and the median bandwidth was 2.2-fold larger (56.4 degrees; **Fig. 1f**). Taken together, the changes in these metrics indicate that the tuned component of parvalbumin-positive interneuron responses broadens during development (example tuning curves are shown in **Supplementary Fig. 3**).

To investigate whether visual experience is required for the developmental broadening of inhibitory tuning, we recorded responses from parvalbumin-positive interneurons in mice raised in the dark from P9 to the time of recording, between P23 and P30. We found that the orientation selectivity of parvalbumin-positive interneurons in dark-reared mice was indistinguishable from that of younger, pre-critical period mice but was significantly sharper than in age-matched controls ($P < 0.01$; **Figs. 1g** and **2a**,**b** and **Supplementary Fig. 3**). Thus, visual experience, and not developmental age, broadens the tuning of parvalbumin-positive interneurons *in vivo*.

There was a significant developmental increase in both the evoked and spontaneous firing rates of parvalbumin-positive interneurons that depended on vision (2.4-fold and 3.0–3.2-fold change, respectively, $P < 0.001$; **Fig. 1h**). Despite the fact that there may be a biological basis for these correlations (**Supplementary Fig. 4**), it is necessary to exclude the possibility that the appearance of tuning in these interneurons in pre-critical period and dark-reared mice is an artifact of higher noise levels, which can occur when evoked firing rates are low. We therefore compared the signal-to-noise ratio and response variability at the preferred orientation across all three rearing conditions. In addition, we estimated the contribution of spontaneous activity to the OSI and the reliability of our OSI measurements across stimulus trials. All of these measures were stable with age and experience (**Supplementary Figs. 5** and **6**), validating the conclusion that parvalbumin-positive interneurons are tuned in pre-critical period and dark-reared mice and lose this tuning with visual experience.

Does the developmental broadening of parvalbumin-positive interneuron tuning influence the development of excitatory receptive fields? To examine this, we measured the tuning of excitatory neurons under the same rearing conditions: pre-critical period, critical period

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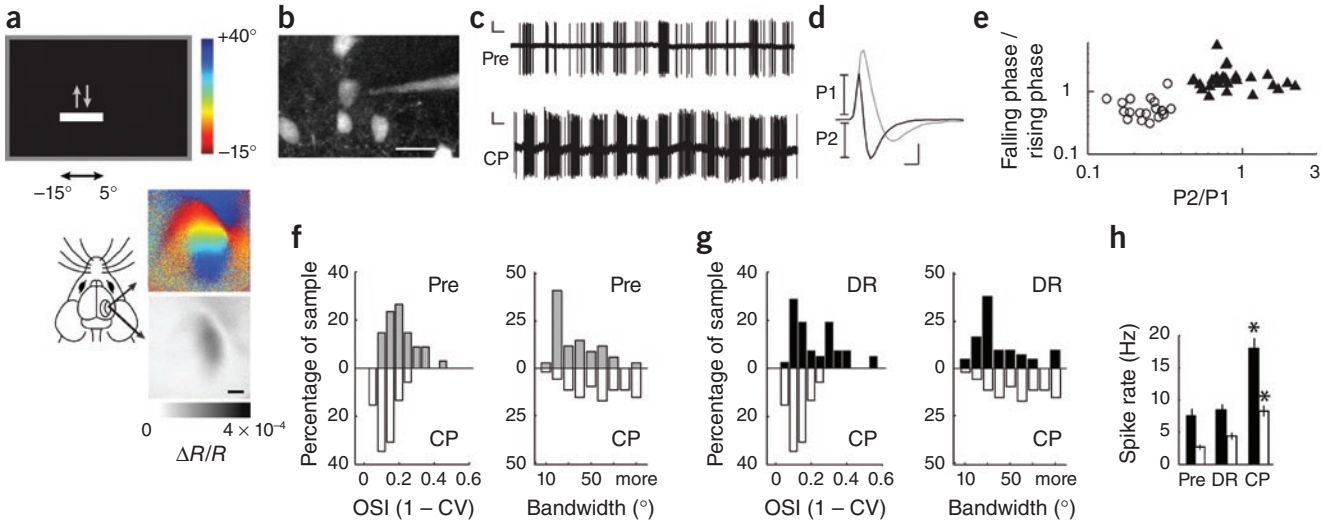

Figure 1 Broadening of parvalbumin-positive interneuron tuning requires visual experience. (**a**) Intrinsic signal optical imaging was used to identify the binocular zone. Scale bar represents 500 μm. (**b**) Two-photon image of recording pipette approaching a parvalbumin-positive interneuron. Scale bar represents 20 μm. (**c**) Example evoked spike responses to 12 orientations and inter-leaved gray-screen presentations. Scale bars represent 3 ms and 2.5 mV (top) and 3 ms and 1 mV (bottom). CP, critical period. (**d**) Average spike waveforms of a parvalbumin-positive interneuron (black) and an excitatory neuron (gray); P1 denotes the amplitude of the spike-wave peak and P2 denotes the nadir. Scale bars represent 1 ms and 0.5 mV. (**e**) Spike waveforms of inhibitory and excitatory neurons are distinct for all rearing conditions examined (see also **Supplementary Fig. 1**). (**f**,**g**) Histogram plots of interneuron OSI values (left) and bandwidth (right) (see **Fig. 2a**,**b** for statistics). The numbers of parvalbumin-positive cells recorded are $n = 17$ (9 mice) for pre, $n = 26$ (14 mice) for critical period and $n = 21$ (8 mice) for dark reared. Critical period values are plotted twice to aid comparison. CV, circular variance; DR, dark reared. (**h**) Baseline-subtracted evoked firing rates (filled bars) and spontaneous firing rates (open bars) (evoked: ANOVA, $P < 0.001$; pre versus dark reared, unadjusted $P = 0.66$; pre versus critical period, unadjusted $P < 0.001$; spontaneous: ANOVA, $P < 0.001$; pre versus dark reared, $P = 0.113$; pre versus critical period, unadjusted $P < 0.001$). $^*P < 0.05$. In all cases, we used the Holm-Sidak *post hoc* test to determine significance. Errors bars represent s.e.m.

and dark-reared. In contrast with parvalbumin-positive interneurons, the orientation selectivity of excitatory neurons sharpened significantly with age, increasing by 72% between the pre-critical period and the critical period (OSI, pre = 0.26 ± 0.03, critical period = 0.44 ± 0.04, $P < 0.01$; **Supplementary Fig. 7**). Thus, although the orientation selectivity of parvalbumin-positive interneurons and excitatory neurons were roughly equivalent during the pre-critical period, they diverged thereafter (**Fig. 2**). The median bandwidth of excitatory neurons, however, did not change with age.

When we examined the tuning of excitatory neurons in dark-reared mice, we found that tuning sharpened even in the absence of vision, although it did not reach the same level as in controls (**Fig. 2a** and **Supplementary Fig. 7d**). These findings are consistent with previous findings in carnivores[14]. Taken together, these results indicate that the maturation of excitatory tuning does not entirely depend on the broadening of fast-spiking inhibitory tuning (**Fig. 2**) and suggest that other mechanisms contribute to the sharpening of excitatory tuning.

Given the evidence suggesting that parvalbumin-positive interneurons regulate excitatory binocular plasticity, we sought to determine whether the broadening of their orientation tuning, a sign of their maturation, precedes or follows the binocular matching of orientation selectivity that is the most salient consequence of the critical period[15]. We found that, during the critical period, when these interneurons have attained mature tuning, binocular matching of excitatory

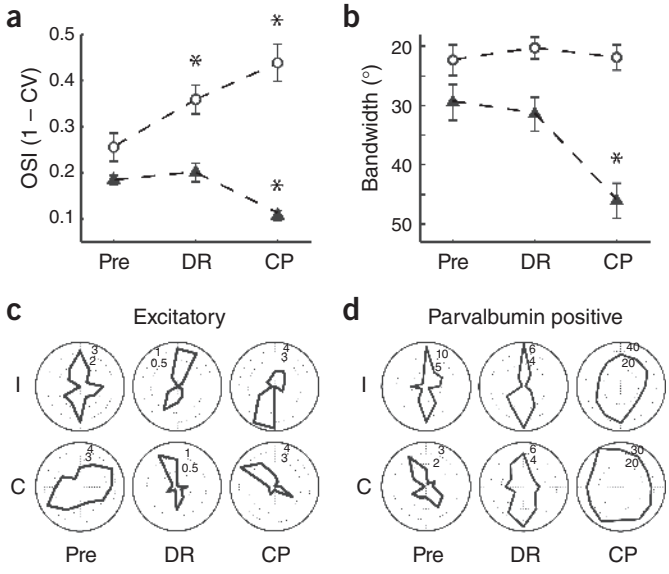

Figure 2 Inhibitory and excitatory tuning diverges with age and experience. (**a**) Plot of mean OSI values shown in **Figure 1f**,**g** and **Supplementary Figure 7c**,**d**. Open circles represent excitatory neurons and closed triangles represent interneurons. An ANOVA and subsequent Holm-Sidak tests were used to determine significance, $^*P < 0.05$ (excitatory neurons: ANOVA, $P < 0.01$; pre versus dark reared, unadjusted $P = 0.047$; pre versus critical period, unadjusted $P < 0.01$; interneurons: ANOVA, $P < 0.01$; pre versus dark reared, unadjusted $P = 0.45$; pre versus critical period, unadjusted $P < 0.01$). (**b**) Plot of mean bandwidth values shown in **Figure 1f**,**g** and **Supplementary Figures 7c**,**d**. Data are presented and statistics were calculated as in **a** (excitatory neuron: ANOVA, $P = 0.76$; interneuron: ANOVA, $P < 0.01$; pre versus dark reared, unadjusted $P = 0.65$; pre versus critical period, unadjusted $P < 0.01$). (**c**) Example polar plots of the spike response (Hz) from individual excitatory neurons for ipsilateral (I) and contralateral (C) eye stimulation. (**d**) Example polar plots of parvalbumin-positive interneurons. Tuning curves of the same cells depicting trial-to-trial variation are shown in **Supplementary Figure 3b**. Error bars represent s.e.m.

tuning remained undeveloped and was as poor as in dark-reared animals (pre, Δ43.89 ± 6.52°, n = 14; dark-reared, Δ29.30 ± 5.15°, n = 22; critical period, Δ34.22 ± 8.13°, n = 15; ANOVA, P = 0.28). Thus, the emergence of broad inhibitory tuning precedes binocular matching of orientation.

In summary, we found that vision preferentially regulates the maturation of parvalbumin-positive interneurons, increasing the strength of evoked responses and broadening orientation tuning. Previous investigations of parvalbumin-positive interneuron maturation have shown that vision increases the number of inhibitory synapses made onto excitatory neurons, concluding that this change is an important mediator of critical period plasticity[5]. Our findings that the experience-dependent strengthening and broadening of interneuron responses precede critical-period binocular matching identify these changes in interneuron responses as mediators of critical period plasticity in the cortex.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

S.J.K. and J.T.T. designed the experiments and wrote the manuscript. S.J.K. carried out the parvalbumin-positive experiments and analyzed the data, and S.J.K. and E.T. carried out the RFP-negative experiments and analyzed the data.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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