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

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Abstract

We find that following eye opening fast-spiking parvalbumin-positive GABAergic interneurons in mice have well-defined orientation tuning preferences and that subsequent visual experience broadens this tuning. Broad inhibitory tuning is not required for the developmental sharpening of excitatory tuning, but does precede binocular matching of orientation tuning. We propose that the experience-dependent broadening of inhibition is a novel candidate for opening the critical period.

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 microns below the pia, of mouse primary visual cortex *in vivo*. We recorded in the binocular zone of visual cortex just prior to the onset of the critical period for binocular plasticity (postnatal days 17–19 (Pre)), and during the peak of this period (postnatal days 23–30)[5,8]. We used intrinsic signal mapping to identify the position of binocular visual cortex (Fig. 1a), and, subsequently, *in vivo* 2-photon imaging to target cell-attached recordings to parvalbumin-positive interneurons expressing a red fluorescent protein (Fig. 1b,c). The spike waveform of these cells was narrowly-shaped, characterized by a rapid falling action potential and high-amplitude undershoot (Fig. 1d, see also[1]). In contrast, the waveform of neurons negative for red fluorescent protein (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, though we do not rule out the possibility that up to 10% of the cells were inhibitory. These spike waveform parameters clearly distinguished parvalbumin-positive interneuron recordings from excitatory neuron recordings under all experimental conditions that we examined (Fig. 1d,e and Supplementary Fig. 1). Our experiments were approved by the

Author contributions

SJK and JTT designed the experiments and wrote the manuscript. SJK carried out the parvalbumin-positive experiments and analyzed the data, SJK and ET carried out the RFP negative experiments and analyzed the data.

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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] 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 non-preferred components of the response, and bandwidth, a measure specifically of the tuned component around the preferred orientation. The mean OSI (defined as 1 – circular variance) of parvalbumin-positive interneurons was 0.18±0.01, and the median bandwidth was 25.5 degrees (Fig. 1f).

In contrast, in critical period mice, parvalbumin-positive interneurons did not have well-defined tuning preferences, as others have found 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 median bandwidth was 2.2-fold larger (56.4 degrees; Fig. 1f). Taken together, the changes in these metrics show that the tuned component of parvalbumin-positive interneuron responses broadens during development. Example tuning curves are shown in Supplementary Fig. 2.

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 postnatal day 9 to the time of recording, between postnatal days 23 and 30. We found that orientation selectivity of parvalbumin-positive interneurons in dark-reared mice was indistinguishable from that of younger, pre-critical period mice, but significantly sharper than in age-matched controls (Fig. 1g and 2a,b and Supplementary Fig. 2). Thus, visual experience, not developmental age, broadens the tuning of parvalbumin-positive interneurons *in vivo*.

There was a significant developmental increase in both evoked and spontaneous firing rates of parvalbumin-positive interneurons that depended on vision (2.4-fold and 3.0–3.2-fold change, respectively, Fig. 1h). Despite the fact that there may be a biological basis for these correlations (Supplementary Fig. 3), 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 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 also estimated the reliability of our OSI measurements across stimulus trials. All of these measures were stable with age and experience (Supplementary Figs. 4 and 5), 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, and dark-reared. In contrast to 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. 6). Thus, while the orientation selectivity of parvalbumin-positive interneurons and excitatory neurons are roughly equivalent during the pre-critical period, they diverge 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, though it did not reach the same level as in controls (Fig. 2a and Supplementary Fig. 6d). These findings are in agreement with previous findings in carnivores[14]. Taken together, these results show 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 additionally contribute to the sharpening of excitatory tuning.

Given the evidence 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 tuning remains undeveloped, and 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 show 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 showed that vision increases the number of inhibitory synapses made onto excitatory neurons, concluding that this change is a key mediator of critical period plasticity[5]. Our results showing that the experience-dependent strengthening and broadening of interneuron responses precede critical period binocular matching identify these changes in interneuron responses as novel mediators of critical period plasticity in the cortex.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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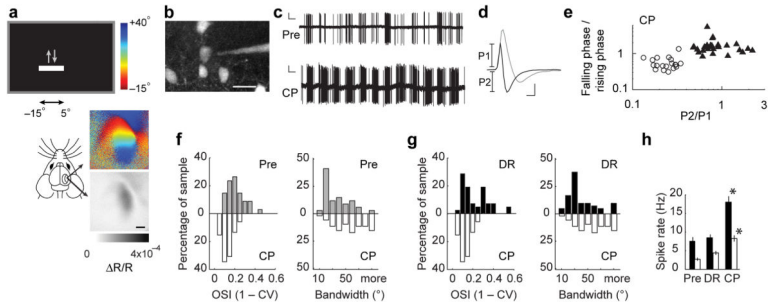

Figure 1. Broadening of parvalbumin-positive interneuron tuning requires visual experience

(**a**) Intrinsic signal optical imaging was used to identify the binocular zone. Scale bar in 500 μm. (**b**) Two-photon image of recording pipette approaching a parvalbumin-positive interneuron. Scale bar is 20 μm. (**c**) Example evoked spike responses to 12 orientations and inter-leaved gray-screen presentations. Scale bars: top, 3 ms, 2.5 mV; bottom, 3ms, 1 mV. (**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 bar: 1 ms, 0.5 mV. (**e**) Spike waveform 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. Number of parvalbumin-positive cells recorded: Pre: n=17 (9 animals); critical period: n= 26 (14 animals); dark reared: n= 21 (8 animals). Critical period values are plotted twice to aid comparison. (**h**) Baseline-subtracted evoked firing rate (filled bars) and spontaneous firing rate (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-values <0.05 are indicated by an asterisk. Abbreviations: Pre: pre-critical period; CP: critial period; DR: dark-reared. Errors bars report s.e.m.

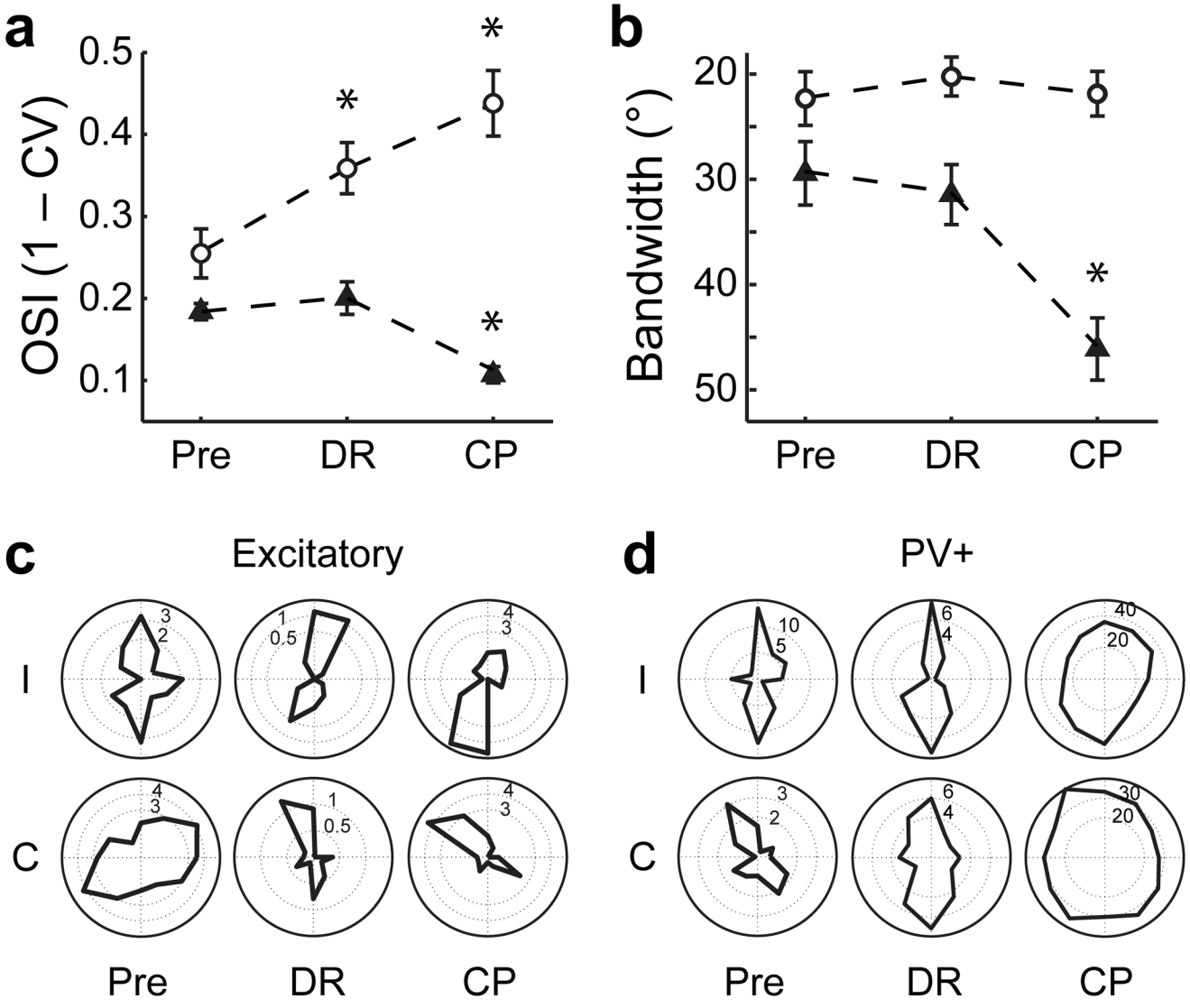

Figure 2. Inhibitory and excitatory tuning diverges with age and experience

(**a**) Plot of mean OSI values shown in Fig. 1f,g and Supplementary Fig. 6 c,d. Excitatory neurons, open circles; interneurons, closed triangles. An ANOVA and subsequent Holm-Sidak tests were used to determine significance, P-values <0.05 are indicated by an asterisk. Excitatory neuron ANOVA results: P<0.01. Pre versus dark-reared: unadjusted P= 0.047; Pre versus critical period: unadjusted P<0.01. Interneuron ANOVA results: 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 Fig. 1f,g and Supplementary Fig. 6c,d. Symbols as in 'a'. Statistics calculated as above. Excitatory neuron ANOVA results: P=0.76. Interneuron ANOVA results: 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 (PV+) interneurons. Tuning curves of the same

cells depicting trial-to-trial variation are shown in Supplementary Fig. 2b. Error bars report s.e.m.