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The contribution of sensory experience to the maturation of orientation selectivity in ferret visual cortex

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Sensory experience begins when neural circuits in the cerebral cortex are still immature; however, the contribution of experience to cortical maturation remains unclear. In the visual cortex, the selectivity of neurons for oriented stimuli at the time of eye opening is poor[1–5] and increases dramatically after the onset of visual experience[3–8]. Here we investigate whether visual experience has a significant role in the maturation of orientation selectivity and underlying cortical circuits[9–12] using two forms of deprivation: dark rearing, which completely eliminates experience, and binocular lid suture, which alters the pattern of sensory driven activity[13]. Orientation maps were present in dark-reared ferrets, but fully mature levels of tuning were never attained. In contrast, only rudimentary levels of orientation selectivity were observed in lid-sutured ferrets. Despite these differences, horizontal connections in both groups were less extensive and less clustered than normal, suggesting that long-range cortical processing is not essential for the expression of orientation selectivity, but may be needed for the full maturation of tuning. Thus, experience is beneficial or highly detrimental to cortical maturation, depending on the pattern of sensory driven activity.

The interactions between visually driven activity and endogenous mechanisms of development that shape the maturation of orientation selectivity remain unclear. Some studies suggest that visual experience is not required for the full maturation of selectivity[6,7,14,15], whereas others propose a more constructive influence[3,8,16]. To clarify these interactions, we first asked whether the rapid maturation of orientation selectivity that follows eye opening could be attributed solely to endogenous developmental mechanisms. We analysed the development of orientation selectivity in 16 ferrets that were dark reared from about two weeks before eye opening and brought into the light only after the induction of anaesthesia for the terminal experiment. Representative results are shown in Fig. 1. About one week after eye opening, this dark-reared ferret showed considerable maturation of orientation selectivity, despite the complete absence of previous visual experience (see Fig. 1b). Qualitatively, the map of orientation preference was very similar to normal in its areal extent and internal structure: orientation preference was organized in a continuous fashion except for point discontinuities around which preference was mapped in pinwheel-like arrangements (see insets in Fig. 1). However, careful examination of the difference images indicated that the responses were less selective than normal, as judged by the reduced contrast in the optical maps compared with normal (compare Fig. 1a with b).

This impression was substantiated by a quantitative analysis of cardinal difference images (that is, horizontal versus vertical) in a large series of normal and deprived ferrets that sampled the period of orientation map development. In normal animals, differential optical responses to orthogonal angles were first seen at or just before the time of natural eye opening (postnatal days 30–35), as reported previously[4,5] (Fig. 2). The selectivity of these responses increased greatly during the next two weeks and achieved a plateau by the eighth or ninth week (Fig. 2b). Ferrets that were raised in complete darkness showed evidence of a similar progression from before the time of eye opening through to the sixth postnatal week; thereafter, the degree of selectivity was significantly less than normal. Although cortical responses can be evoked through naturally closed eyelids in very young ferrets (postnatal weeks 4–5)[17], our results indicate that the contribution of visual experience to the maturation of orientation selectivity is confined to the developmental period that follows eye opening.

These data show that experience-independent mechanisms have the capacity to establish the map of orientation preference and promote its maturation. However, they also demonstrate that this capacity is limited: fully mature levels of selectivity were achieved only with the benefit of normal experience. This may suggest that orientation-selective responses are determined primarily by endogenous factors and that the influence of visually driven activity is limited to sharpening orientation tuning. On the other hand, it is possible that visually driven activity has a more important role, one that cannot be inferred from experiments in which its influence has been completely eliminated. To address this possibility, we analysed the development of orientation selectivity in ferrets that were subjected to binocular lid suture, which does not prevent light activation of the retina but does alter experience by passing only very low spatial and temporal frequencies[13].

In contrast to the more modest effects of dark rearing, lid suture had devastating effects on the maturation of orientation selectivity. In the representative case shown in Fig. 1c, there was very little evidence of differential cortical response to orthogonal orientations. Notably, the cortex had not simply become unresponsive; single-condition optical imaging (that is, stimulus versus blank) showed robust but much less modular cortical activation to each stimulus. The group analysis indicated that unlike the dark-reared animals, lid-sutured ferrets were similar to normal only at the time of natural eye opening (see Fig. 2 and Supplementary Information). Thereafter, lid-sutured ferrets failed to show any improvement in selectivity; indeed, this group of ferrets showed a steady decline with ongoing deprivation. To be sure that the effects of lid suture were related to altered patterns of neural activity rather than some non-specific consequence of lid suture, one ferret was lid-sutured and reared in total darkness. The map of orientation preference developed in this animal to the same degree as an unsutured littermate that was imaged on the same day (see Supplementary Information).

The maturation of the map of orientation preference proceeded to a limited extent in the absence of vision, but not with abnormal experience engendered by vision through sutured eyelids. This effect of lid suture differs from reports in kittens in which binocular lid suture did not prevent the maturation of the map, only the long-term maintenance of adult levels of selectivity[6,7]. These discrepancies may be explained by species differences in the timing of susceptibility to the influence of experience or, more simply, by differences in the amount of light transmitted through sutured eyelids.

We next sought to confirm our results with electrophysiological recordings in a subset of the same animals that were used for imaging. The electrophysiological assessments of orientation selectivity were consistent with the results obtained by optical imaging: dark-reared ferrets showed an intermediate level of tuning that was sharper than the lid-sutured group but not as selective as that seen in normal ferrets (see Supplementary Information). The mean values of an orientation selectivity index for electrophysiological data (OSI_{EP}) ($\pm$ standard error) for each of three groups were: normal = 66.2 $\pm$ 2.7; dark-reared = 44.0 $\pm$ 3.8; and lid-sutured = 36.4 $\pm$ 4.3 ($F_{2,76} = 21.3$, $P < 0.001$).

Finally, we asked whether the rapid maturation of orientation

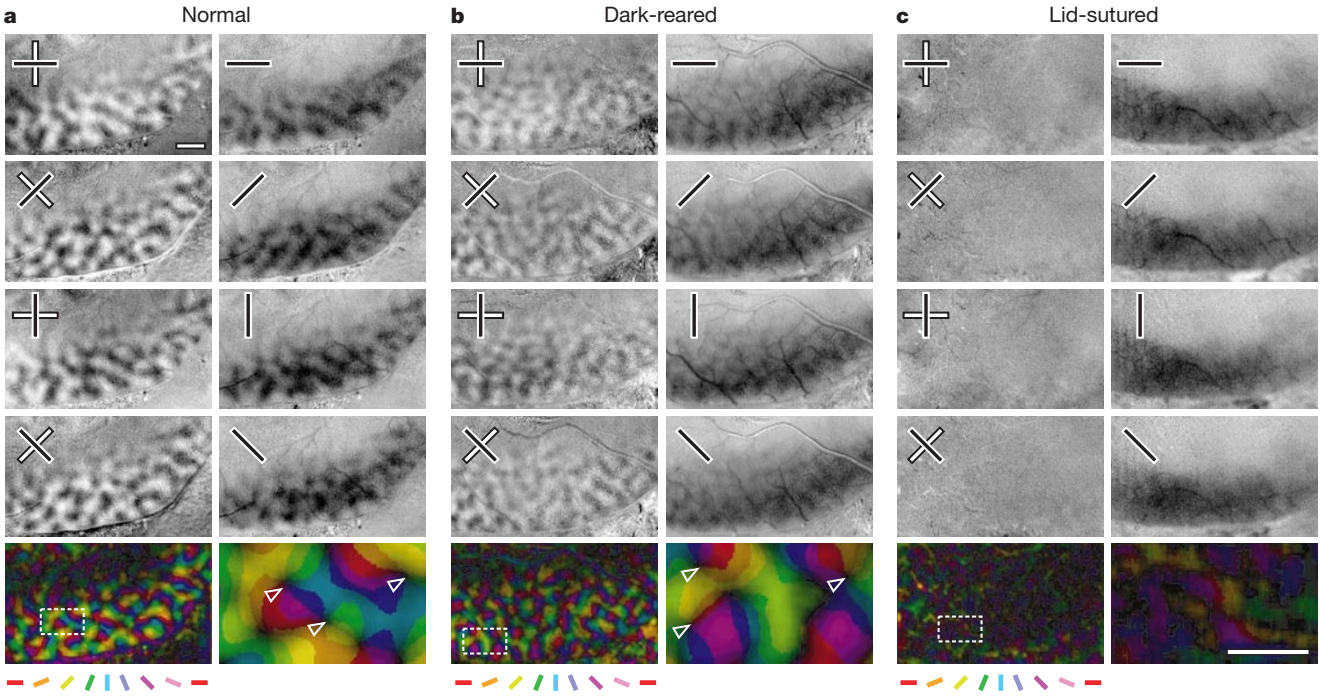

Figure 1 Maps of orientation preference in normal and visually deprived ferrets. **a**, Difference images (left column) show selective responses to orthogonal gratings in a 39-day-old ferret reared normally. The same patterns are seen in the adjacent single-condition images. The overall organization is shown in the polar-magnitude map where colour represents preference and brightness represents selectivity. Pinwheel centres (white arrowheads) are shown at higher magnification to the right. **b**, Images from a 37-day-old, dark-reared ferret. **c**, Images from a 43-day-old, lid-sutured ferret. Note the absence of selectivity despite the strong visual response. Scale bars: 1 mm.

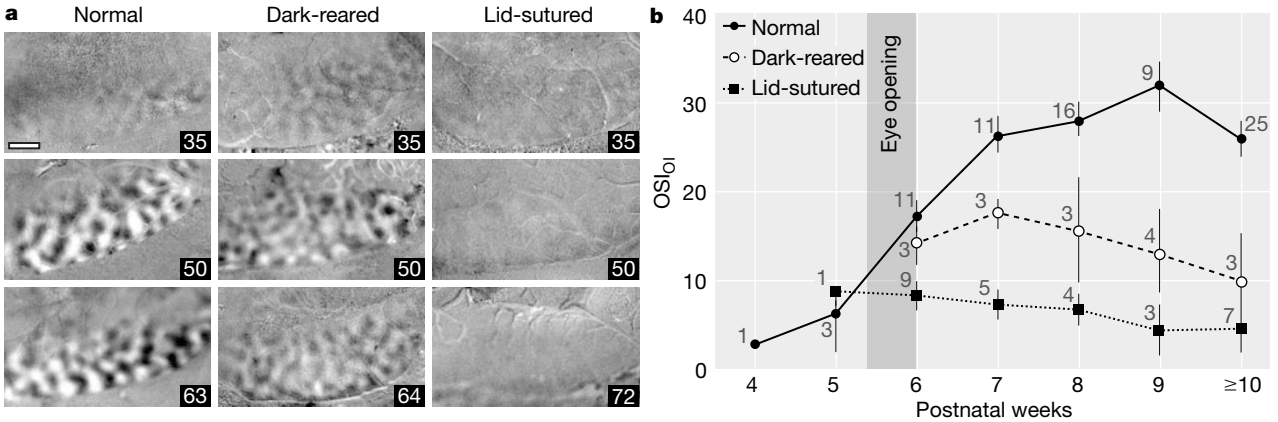

Figure 2 Quantitative assessment of orientation preference in normal and visually deprived ferrets. **a**, Cardinal difference images show the increase in selectivity seen in normal ferrets after eye opening, the substantial development of the map in dark-reared ferrets, and the devastating effects of lid suture (numbers indicate postnatal age; scale bar: 1 mm). **b**, Plot of OSI_{OI} (mean $\pm$ standard error) versus postnatal age for each treatment group (numbers next to symbols indicate sample size). After week six, rearing condition was significant ($F_{2,99} = 3.3$, $P < 0.05$), as was the condition/age interaction ($F_{8,99} = 3.6$, $P < 0.001$) with significant differences among all conditions ($P < 0.01$, *post hoc* Tukey–Kramer Honestly Significant Difference).

selectivity that follows eye opening and its disruption by visual deprivation could be understood in terms of the development of the horizontal network in layer 2/3. This system of long-range, clustered connections that link together cortical columns that share similar orientation preferences[9–12] has been proposed to be involved in the establishment and maturation of the map of orientation preference[10,18]. Moreover, previous studies in cats demonstrated that binocular lid suture prevents the expression of the mature pattern of horizontal connectivity[19,20]. Our results confirm this conclusion: axonal distributions in lid-sutured ferrets gave rise to fewer terminal clusters and covered less cortical area, in comparison with normal (Fig. 3). However, our data show that the devastating effects of lid suture on orientation selectivity cannot be attributed solely to alterations in the horizontal network, as these connections are also severely affected in dark-reared animals. This effect is different from the results of an earlier study in which horizontal connections appeared normal in two dark-reared kittens[21]. This inconsistency may be due to methodological differences (tracer substance, injection size, sample size) and/or differences in the timing of horizontal connection outgrowth relative to the onset of visual experience. Our results suggest that the restricted distribution of horizontal connections underlies the effects of dark rearing on orientation preference, whereas the more devastating effects of lid suture are explained by alterations in the functional organization of other cortical circuits.

The role of experience in the development of orientation selectivity has been the subject of numerous studies with results and interpretations that often conflict[13,22,23]. However, most recent accounts distinguish two phases of maturation: an initial period of experience-independent development in which the map of orientation preference emerges and selectivity reaches maturity, followed by a second experience-dependent phase in which experience is required to consolidate and maintain orientation-selective responses[6,7,14,15,24]. Our results showing that experience exerts a powerful influence during the maturational phase of orientation development are difficult to reconcile with this view. In the ferret, adult levels of orientation tuning are achieved only with the benefit of visual experience. Our results emphasize that the pattern of visually driven activity, not merely its presence, is a critical factor in promoting the complete maturation of orientation selectivity. Indeed, abnormal patterns of visually driven activity are far more disruptive to the maturation of orientation-selective responses than the total absence of visually driven activity. Additional evidence to support the disruptive influence of altered patterns of retinal activity has come from studies using electrical or pharmacological manipulations[25,26]. Although it is unclear whether these interventions altered developmental events during the initial establishment of orientation selectivity or during the later phase of maturation (or both), our results demonstrate that disruptions in the pattern of visually driven activity during the maturational phase alone would be sufficient to explain these observations.

But how might neural activity engendered by normal and

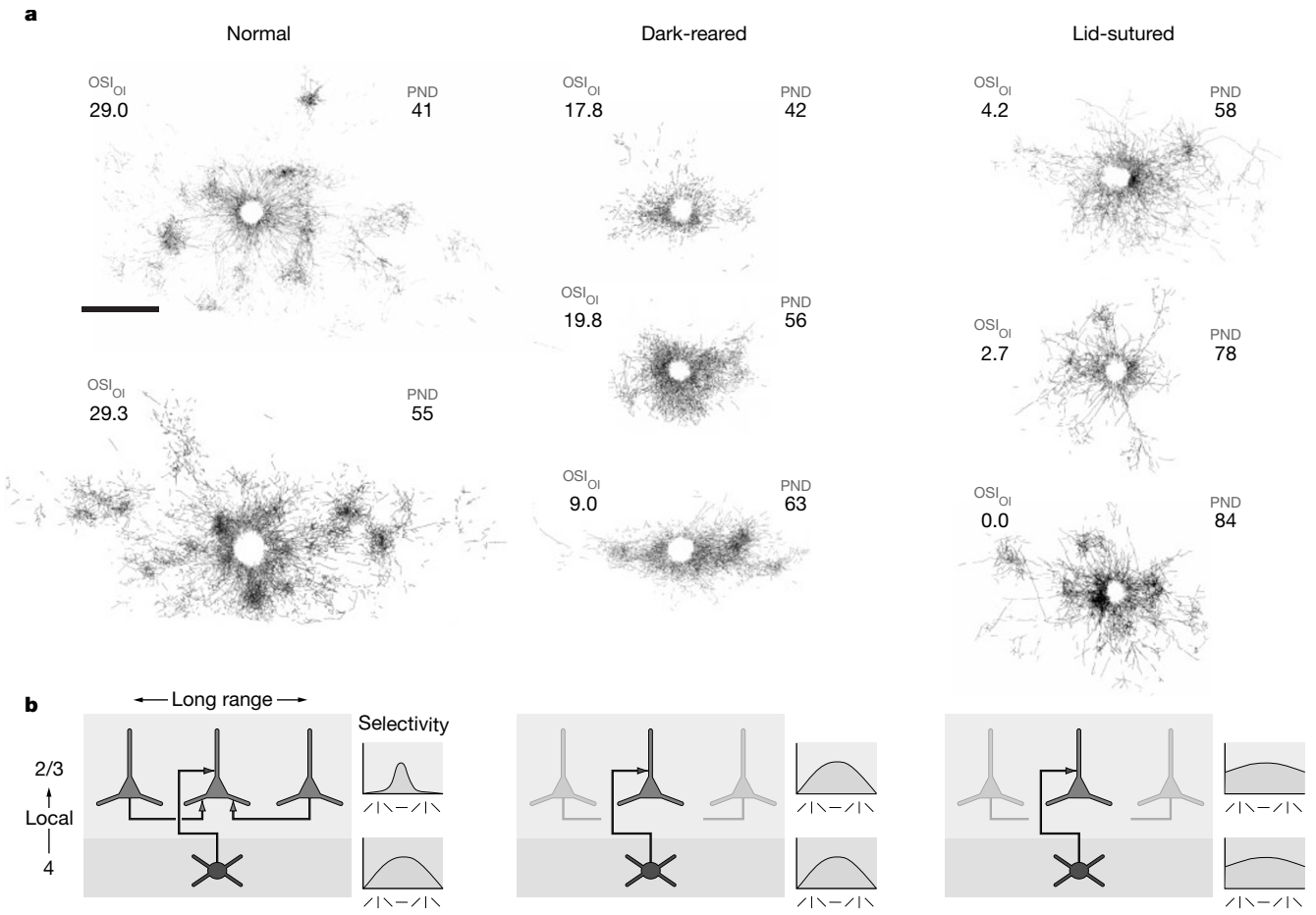

Figure 3 Horizontal connections in layer 2/3 of normal and visually deprived ferrets. **a**, Representative cases: the vacant area in the centre of each tracing contains the injection site (scale bar: 1 mm). PND, postnatal day. **b**, Models for the effects of experience on columnar and long-range processing. Normal experience sharpens orientation tuning within layer 2/3 (see ref. 4). Dark rearing prevents the full elaboration of horizontal connections and the increased selectivity that requires long-range computations. Horizontal connections are similarly impaired with lid suture and altered patterns of visually driven activity interfere with the establishment of orientation selectivity in layer 4.

abnormal visual experience interact with endogenous factors to produce sharpening or broadening, respectively, of orientation selectivity? Current models propose that intrinsic patterns of activity, in particular the local correlation structure of converging on-centre and off-centre inputs from the lateral geniculate nucleus, are sufficient to establish the orientation-selective responses of neurons in cortical layer 4 (reviewed in ref. 14). We suggest that the endogenous activities of on-centre and off-centre inputs[27] shape local columnar circuitry and account for the establishment and partial maturation of orientation selectivity in the total absence of visual experience, without the benefit of long-range, clustered horizontal connections (see Fig. 3b). However, lighted rearing conditions allow for interactions between endogenous factors and sensory driven activity. Given the prevalence of long contours in the visual environment, normal experience would be expected to act synergistically with intrinsic activity and other endogenous factors to increase the temporal and spatial precision of orientation-biased correlations, thereby sharpening cortical orientation circuits by a hebbian process. This should occur both within local columnar circuits and across the horizontal network in layer 2/3; indeed, our anatomical data show that the full elaboration of this network requires the influence of normal visual experience. In contrast, abnormal experience through light-scattering eyelids should abrogate this synergy by co-activating inputs over a broader spatial scale, thus increasing the correlation of inputs with different centre types that would otherwise tend to segregate. Consequently, both intra- and inter-columnar stages of orientation processing would be impaired, and the rudimentary map of orientation preference present at the time of eye opening fails to mature and progressively weakens. In conclusion, the pattern of activity evoked by visual experience affects the architecture of intracortical circuits and is a critical factor in the maturation of orientation selectivity in the developing visual cortex. □

Methods

All experimental procedures were approved by the Duke University Institutional Animal Care and Use Committee and were performed in compliance with guidelines published by the National Institutes of Health (USA).

Rearing conditions

Normal sable ferrets (*Mustela putorius furo*) of both sexes ($n = 76$) were reared in a 12 h light/dark cycle. Other ferrets ($n = 16$) were subjected to continual, absolute dark rearing beginning on postnatal day 21 or 23 (with day of birth being postnatal day 0) to maximize exposure of the mother and kits to normal circadian cycles, while ensuring the onset of deprivation before the development of reliable responses in cortical neurons[4,17]. Between postnatal days 21–27, 29 ferrets were briefly anaesthetized and subjected to binocular lid suture.

Optical imaging

Optical imaging of intrinsic signals was performed with an enhanced video acquisition system (Optical Imaging), as described previously[12,28]. The stimuli were moving, high-contrast rectangular wave gratings (0.1 cycle per degree) oriented at 0, 45, 90 or 135°. Difference images were generated by subtracting the optical responses to presentation of each member of a pair of orthogonal gratings. To compare different animals, an index of orientation selectivity for optical images (OSI_{OI}) was computed from the cardinal difference images by first clipping at ± 3 s.d. from the median and then calculating the s.d. of the distribution of greylevels within V1 and V2. Mean OSI_{OI} values binned by postnatal week were analysed using two-way analysis of variance, with rearing condition and age as the main effects (JMP Statistical Software). To assess cortical responsiveness, single condition images were generated by subtracting the response to a blank screen from the response to a single grating.

Electrophysiology

In a subset of cases (38 sites from 28 normal ferrets, 25 sites from 6 dark-reared ferrets, and 16 sites from 5 lid-sutured ferrets), a tungsten microelectrode (impedance = 8–14 MΩ) was inserted into selected cortical sites. Multi-unit activity was recorded from cortical layer 3 and the signals discriminated and tabulated using Spike2 software (Cambridge Electronic Design). For each recording site, the optimum orientation preference of the multi-unit activity was determined by panning an orientated bar (40°×0.4°) across the receptive field of the site in one of 9 or 18 orientations. The average spike counts from five replications were used to construct histograms, which were then fit by a gaussian distribution using a modified downhill simplex method[29]. We then calculated an orientation selectivity index for electrophysiological data (OSI_{EP}):

$$OSI_{EP} = [P2/(P1 + P2)] \times [1 - (P4/\text{number of stimuli})] \times 100$$

where $P1$ is baseline, $P2$ is peak height minus baseline, and $P4$ is 0.83 times half-width at half-height.

Anatomy

After optical imaging, 21 normal ferrets, five dark-reared ferrets and five hemispheres from three lid-sutured ferrets each received a small iontophoretic injection of biocytin into the visual cortex, as previously described[12]. Labelled axons in every tangential section through layers 2/3 were traced, and the areal extent of the composite distribution and the number of terminal clusters were measured using NIH Image software (developed at the US National Institutes of Health; available at http:/rsb.info.nih.gov/nih-image/).

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