The Ferret as a Model for Visual System Development and Plasticity

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The exquisitely sculpted synaptic connections of the sensory pathway in mammalian brains are simultaneously fascinating in terms of their precision and complexity, and intensely challenging to understand even after decades of dedicated effort. The most extensively researched of these connections and pathways relate to the visual pathway, whose patterned connections are responsible for the finely tuned feature selectivity which underlies visual perception [1]. Over the years, the visual system of ferrets has become increasingly recognized as a model of choice for investigations concerning sensory systems, particularly for early developmental mechanisms. Examples include the form and function of retinal ganglion cell (RGC) topographies, eye-specific and on/off segregation of retinogeniculate pathways, patterning of geniculocortical connections, and the development of functional specialization and plasticity in neocortical circuits. The nervous system of ferrets provides several unique advantages because its sensory system is similar to that of the cat, but being born developmentally earlier like mice and rats, many events critical in shaping cell-circuit assemblies, synaptogenesis, laminar and sublaminar specialization, axonal targeting, and remodeling take place postnatally, thus enabling specific experimental manipulations *in vivo* [2–35]. By the time they open their eyes, at postnatal day 28, ferret pups are bigger and more robust than kittens at same stage [25,26,36]. This feature, combined with large litter sizes and the availability of timed-pregnant ferrets, makes them an attractive choice for investigations and manipulations in the early developmental period. Additionally, the mature ferret brain is semilissencephalic and much of its cortex, particularly the striate and extrastriate visual areas, are accessible for electrophysiological recording and application of modern imaging techniques of optical imaging and multiphoton microscopy [28,37–45].

We begin this chapter with an overview of the ferret visual system, focusing on the early visual pathway from retina to the striate cortex. We will then discuss the timeline of pre- and postnatal development with reference to major milestones during genesis and maturation of principal structures and their interconnectivity, before turning our attention to a myriad of maps in the ferret visual cortex and their structural and functional plasticity [25,37,38, 44,46–56]. Finally, we will describe the profound influence of patterned inputs in remodeling and respecifying sensory cortical networks revealed by the unique cross-modal paradigm involving rerouting of visual inputs to the auditory pathway and their functional and behavioral readouts [10,15,31,57–63].

GENERAL LAYOUT AND DEVELOPMENT OF THE FERRET VISUAL SYSTEM

Ferrets have binocular vision but their visual acuity and depth perception are quite poor compared with cats, likely due to smaller eyes and lower RGC density and central magnification [64,65]. However, like other open terrain vertebrates, laterally placed eyes aided by a prominent visual streak and horizontally split pupils endow them with better peripheral vision. Similar to cat, the ferret retina has morphologically and physiologically characterized

Biology and Diseases of the Ferret, Third Edition. Edited by James G. Fox and Robert P. Marini.

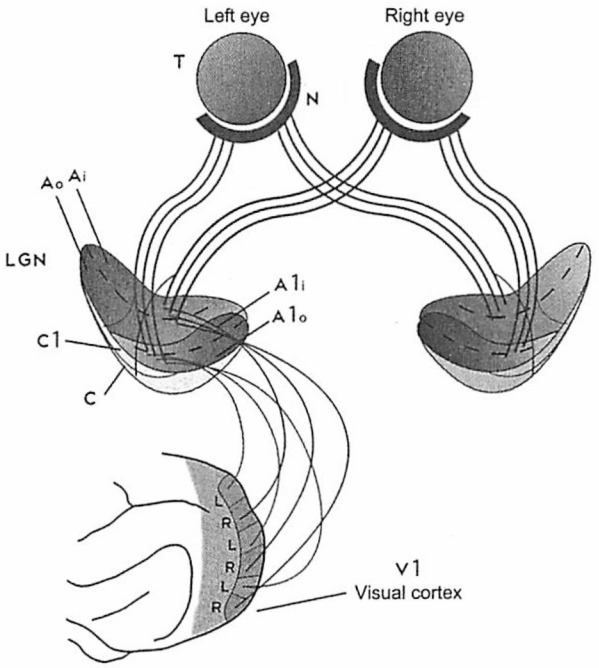


Fig. 30.1. The visual pathway in ferrets. Functional pathways arise from genetic programs of early development when intrinsic molecular cues induce regional and graded patterns of gene expression. These patterns translate into structurally parcellated and functionally differentiated brain regions. Axons of RGCs in the nasal (N) part of the two retinas cross to the opposite side and project to layers A and C of the contralateral lateral geniculate nucleus (LGN). Axons of cells in the temporal (T) retina remain ipsilateral and project to layers A1 and C1 of the LGN. The retinal axons from both eyes segregate to form eye-specific layers at the beginning of second postnatal week and by the end of fourth postnatal week into inner and outer sublayers that receive input from either ON- or OFF-center RGCs (shown here are subdivisions of the A1 layer into A1i and A1o, similar subdivision occurs in the A layer). In the mature visual pathway, spatial and temporal patterns of activity are conveyed from LGN to input layer IV of the visual cortex while preserving left and right eye preferences. The timeline of thalamocortical as well as corticothalamic and intracortical development is shown in Fig. 30.2 and Fig. 30.3. OD columns are regions within layer IV of the visual cortex that respond exclusively to inputs from the left (L) or right (R) eye, respectively, and are set up during very early development. While the role of activity in setting up OD columns remains debatable, there is convincing evidence that activity is required to maintain them. Courtesy of Samvaran Sharma.

X-, Y-, and W-cells as the principal cells (also see discussion later). These cells form three parallel and fairly independent neural pathways, dedicated to analyzing specific features of the visual scene [9,66,67]. The axons emanating from the nasal part of the retina cross the optic chiasm to innervate the lateral geniculate nucleus (LGN) and other subcortical structures in the opposite hemisphere [7]. Among the axons from the temporal part of the retina, the Y-cells entirely cross, but most X-cells and a subset of W-cells remain ipsilateral [3,7]. The information from the two eyes is mapped in separate layers dedicated to the inputs from each eye. All crossed axon terminals populate the A and C layers of the LGN and uncrossed ones home into A1 and C1 layers (Fig. 30.1; Refs. 3, 26, and 68). During development, further elaboration and refinement take place, when retinal axons of each class and subclass must find their appropriate hemispherical targets and follow correct retinotopic order. The next level of specialization occurs when eye-specific afferents functionally divide within layers A and A1 into inner and outer subdivisions. The inner sublaminae Ai and A1i of each layer receive afferent inputs from ON-center cells (these cells

respond when a spot of light is turned on in their receptive field) of both X and Y origins [13,30,69–71]. The outer sublaminae Ao and A1o receive inputs from OFF-center cells (activated by a spot turning off in their receptive field). The C layers also subdivide into C, C1, and C2 sublaminae. The W-cells project to both C1 and C2 while C lamina receives exclusively Y-cells. Besides the LGN and its substructures, the retina also projects to other loci in the brainstem, mainly the superior colliculus (SC), a midbrain structure that integrates visual, auditory, and somatosensory information in order to generate motor outputs such as saccadic eye movements in a "retinocentric" frame of reference. The axons emanating from LGN preserve retinotopic order in their projections to primary visual cortex (also called area 17 or V1). Other cortical areas, such as area 18 (V2) and area 19, also receive direct projections from the LGN; however, the largely serial arrangement of connections is limited to the first three stages, from retina to LGN to V1. In all, as many as 20 or more areas in ferret brain process visual information [40,72], where early serial connectivity gives way to a roughly distributed serial-cum-parallel arrangement. Thus, retrograde labeling of projection neuron classes combined with high-resolution functional imaging in ferrets reveals that the projection from V1 to higher areas, such as area PSS (in the posterior suprasylvian sulcus, analogous to primate area MT) and area 21 (analogous to primate area V4), arises from specific subsets of superficial layer neurons with subtle but clear differences in response properties [43]. These differences seem to be amplified in the target areas.

The eye-specific mapping from LGN continues to its main recipient region, the input layer V1. All three major classes of cells, X, Y, and W, project to V1. The area 18 or V2, on the other hand, appears to largely receive Y-cell inputs, and area 19 gets afferent inputs principally from W-cells [73]. V1 not only preserves the eye-specific information in maps of ocular dominance and retinotopic visual space but also creates new maps that encode specific features of the visual input such as orientation, direction, spatial frequency, etc. The supragranular layers (II/III) of V1 principally project to V2, while infragranular layers (V/VI) send axon terminals to midbrain and subcortical structures, and corticothalamic feedback to LGN and other nuclei, respectively [39,74,75]. In the cortical hierarchy, feedback pathways emanating from mainly infragranular layers provide information to lower areas [76–81]. The higher areas also tend to have larger receptive fields but do not follow strict retinotopy in their feedback to lower areas; however, they exert considerable influence on the functional properties of the lower area neurons [82–84].

During development, the crude and intermingled connectivity between neurons goes through a series of stages of refinement and rearrangement before giving rise to finely sculpted, feature-specific maps of the adult visual system. This process consists of weakening and elimination of incorrect and imprecise connections, and reordering and strengthening of correctly targeted ones [20,26, 85–89], much of which does not require external vision [3,90,91]. Some connections require vision to be maintained [36,51], while others remain agnostic to disruptions in visual experience [92–94]. Still others form only after eye opening and require normal visual experience in order to develop [55]. Despite the significant influence of intrinsic developmental mechanisms through genetic and molecular specification, the influence of extrinsic environmental factors such as patterned visual input has been shown both to influence profoundly the structure and function of pathways and regions, and also to reorganize and respecify entirely new substrates. This has been convincingly demonstrated in a cross-model plasticity paradigm, where early rerouting of retinal inputs into the ferret medial geniculate nucleus (MGN), by denervation of normal auditory inputs from the cochlea and inferior colliculus (IC), leads to a dramatic reorganization of the auditory cortex such that single neurons become visually selective and a topographic map of orientation selectivity develops [14,15,31,57,60,95–97]. The properties of underlying cells as well as their interconnectivity ultimately determine the quality of visual perception. A number of mechanisms, including innate axon-guidance cues provided by age-related "chronotopic" order, genes and molecules, spatial and temporally correlated waves of spontaneous neural activity that are self-generating, followed by patterned input from the environment, all seem to be necessary for the development of various receptive-field properties and visual feature maps [3,20,23,52,62,86,90,98–101].

DEVELOPMENTAL TIMELINE OF THE FERRET VISUAL SYSTEM

The time from conception to eye opening is roughly comparable in ferrets (72 days) and in cats (75 days), although ferrets are born earlier, after 41 days of gestation, roughly 3 weeks earlier than cats [3,6]. This means that a number of developmental events that take place *in utero* in carnivores and primates occur postnatally in ferrets, much like rodents, but at longer timescales, thereby allowing experimental manipulations in this comparatively larger brain at the same stage of development [5,102]. Figure 30.2 summarizes the broad timeline of visual cortex development and important milestones starting from conception

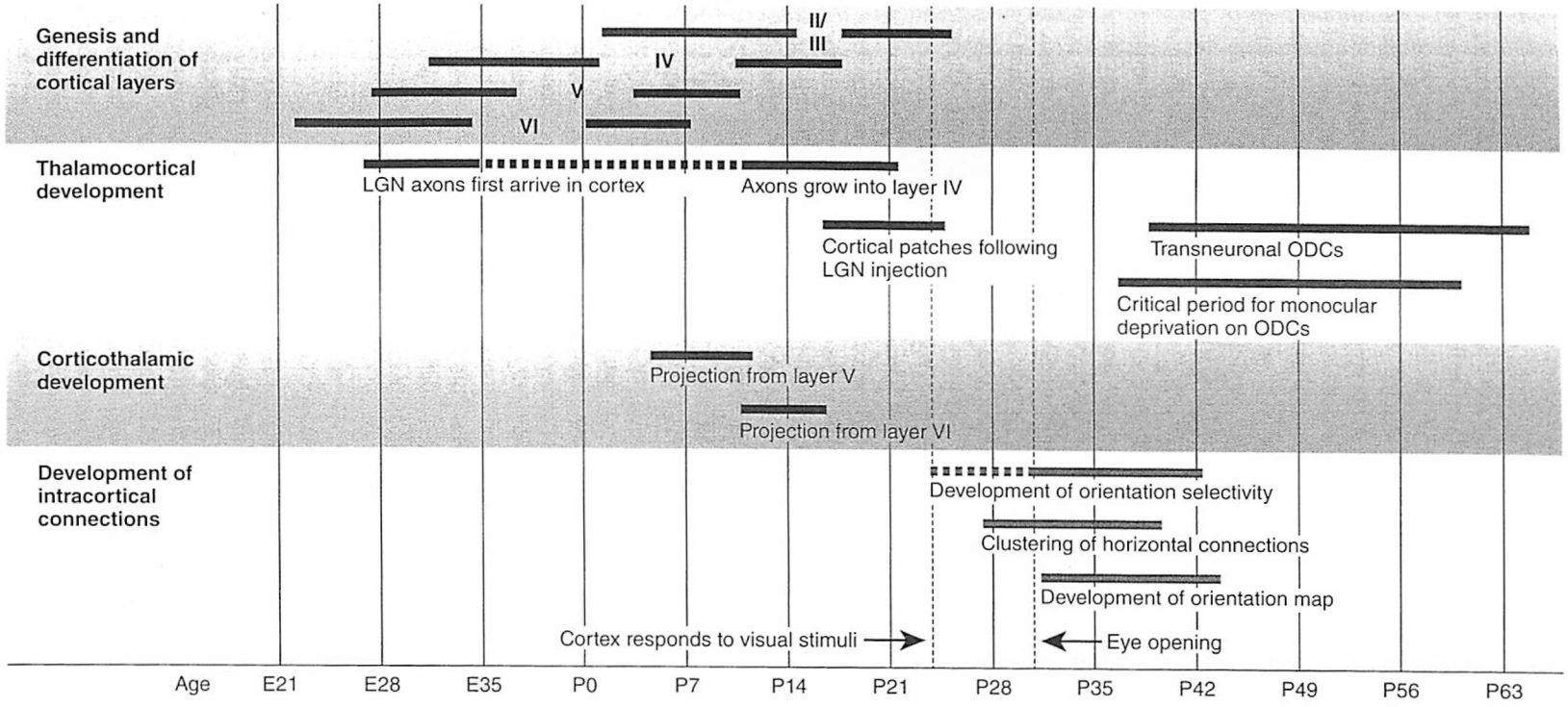


Fig. 30.2. Timeline of main events leading to visual cortical laminar differentiation, development of reciprocal connections between thalamus and visual cortex, and intracortical circuits in ferrets. The mature neocortex consists of six layers generated by precursor cells in ventricular and subventricular zones. The time of ventricular neurogenesis (shown in red) regulates cortical laminar differentiation (shown in blue) with earlier generated neurons forming deeper layers while later born cells progressively occupying superficial layers except in layer I (not shown), where neurogenesis occurs both pre- and postnatally [E20–E32; P1–P14]. The thalamocortical afferents (shown in green) seem to have major influence on specification of areal dimensions and identities of their cortical targets but not in setting up of broad parcellation of cortex, since they only arrive 3–4 days after the beginning of cortical neurogenesis (also see Fig. 30.3 for further details). The axons from dorsal LGN reach layer IV around P10, as soon as it differentiates from the CP and thalamocortical synapse formation, leading to eye-specific patch formation. The corticothalamic connections (shown in pink) are initiated by layer V neurons 3–4 days after birth, and layer VI follows a week later. The clustering of intracortical horizontal connections (in purple) that set up weak orientation selectivity and coarse orientation map formation takes place before eye opening but mature feature selective maps get established several weeks thereafter. Adapted from Reference 99.

to adulthood. The neurogenesis of RGCs, LGN, and posterior cortex takes place between E20 and E22. Immediately afterwards, the first signs of advancing axonal growth cones are discernible in each of these three regions (Fig. 30.2; Refs. 8 and 103–105). By E24, retinal axons begin crossing the optic chiasm [8,106], following a rough central-to-peripheral gradient [9,105]. The development of morphologically identifiable RGCs takes place from E22 to E32 [105]. The major thalamocortical axonal bundles reach posterior cortex between E25 and E27, and continue their growth in layer IV past the third postnatal week (P22). Within the cortex, genesis of layers and their differentiation, along with genesis of cells in each of these layers, occur at different times starting from E22 (also see Fig. 30.2 legend). Whereas the retinotopic and eye-specific maps are patterned roughly before birth and eye opening, the development of intracortical connections (shown in purple) responsible for the formation of functional maps probably starts a week before eye opening; a subset of neurons show orientation preference from the onset of visual responsiveness in the cortex, the orientation map is detectable only by the time of eye opening in ferrets [36,51,107], and the direction-selective map appears 1–2 weeks later [55]. Therefore, the formation and maturation of these maps likely depend critically on developmental processes coincident with patterned input into the cortex. The formation of orientation maps coincides with a period during which axonal connections in V1, especially long-range horizontal inhibitory projections in layer 2/3, proliferate [108]. The initial projection from the cortex to the thalamus, as shown by the *in vivo* transport of tracers, is formed by layer V neurons a few days after birth. The layer VI projections form about 1 week later [17].

THE FERRET RETINA

Early studies in ferrets were directed toward investigation of retinal growth and development of the area centralis [7,9,102]. Being born early, many events critical to shaping ganglionic form and function happen postnatally in ferrets, making it extremely valuable in studies of emergence of retinal topographic order, variation in synaptic density, and synaptogenesis [6,7,9,67,109]. The size of the ferret eyes is smaller than that of cats and is roughly comparable with that of rats and hamsters, but the eye has a prominent visual streak and area centralis. At birth, the ferret retina is about 12 mm^2 in area, and rapidly grows over next 2 weeks before tapering off, ultimately reaching the size of 64 mm^2 at full maturity [7,9]. In terms of RGCs, the mature ferret retina has about half the number (90,000) of that of a cat [9,88,110]. The ratio of cell density from center to the periphery is 10–12:1 in ferrets, compared with 30:1 in cats [9,111,112]. By postnatal day (P) 3, the number of RGCs peaks to reach around 150,000 before declining to around second/third of this number at P6. The ganglion cell density is also uniform across the retina at birth, but is followed by a cell degeneration cycle, depleting in a dorsal-to-ventral gradient, until P10. By day 24 postnatal, the area centralis and a visual streak emerge [8,9]. While most studies mention three major cell types based on combined morphological–physiological characteristics, recent studies have confirmed as many as eight to nine different ganglion cell types in the ferret retina, characterized mainly by their size and dendritic architecture as well as distance from the visual streak [7,67,113,114]. Of these, the prominent ones are X- and Y-cells whose dendrites mainly occupy inner plexiform layers, and consist of both ON- and OFF-center varieties [7,68,115]. However, these cell types exhibit no systematic difference in size of their dendritic fields as a function of their retinal location, nasal/temporal distribution, or age-related stratification [113,114,116].

WIRING OF RETINOTOPIC PATHWAYS

Several lines of evidence suggest that early retinotopic wiring follows genetic programs that initially establish structurally defined regions and later guide functional connectivity in a region-specific manner [98,117–120]. Signaling molecules during embryogenesis induce patterns that subsequently translate into structural and functional regions of brain, including the visual system [62,98,121–124]. Many of these insights on genetic programming and molecular signaling have come from mouse studies. In ferrets, there are two main targets of retinal axons: roughly one-third of the ganglion cell axons from the retina project to the LGN, specifically the dorsal and ventral subdivisions (LGNd, LGNv), while the remaining two-thirds target the SC in the brainstem [125,126]. Between E24 and E27, retinal axons begin crossing the optic chiasm, following a roughly central to peripheral gradient [8,105,106] and begin reaching their central targets, the LGN and SC around E28, reaching adult-like mapping before birth [68,121,127]. Early in development, the eye-specific axons reaching their LGN targets are intermingled [3] but subsequently segregate into precise eye-specific laminae, such that axons from the contralateral eye always occupy the inner part of the LGN and those from the ipsilateral eye reside in the outer portion of LGN [68,128]. In addition, the RGCs also follow retinotopic order (see discussion later). Numerous mechanisms have been proposed to explain this precise targeting of RGCs, including activity-dependent and activity-independent mechanisms which

include chronotopy [129,130], and genetic and molecular guidance cues (reviewed in Ref. 131). A number of genes are implicated in defining spatial and temporal orders of functionally specific identities of retinogeniculate connections and their region specificity within the target; however, it is not yet fully established how gradients of gene expression are translated into boundary-delimited regions [123,132] (reviewed in Ref. 133).

OPTIC TRACT AND OPTIC CHIASM

In the optic chiasm of binocular animals, the converging axons of RGCs from the two eyes segregate into crossed and uncrossed projections. From an initial intermingled arrangement of the crossed and uncrossed axons in their journey toward the chiasm [4,130], the ferret optic tract reflects a systematic rearrangement of fiber order with age such that older fibers which normally intermingle near the eye, occupy the perimeter of the optic nerve, and newer ones come to lie near the center. This chronotopic order is maintained right up to the optic chiasm [4,134]. The developmental processes producing the chiasmatic segregation into crossed and uncrossed components from the axons reaching the chiasm appear around E28–32 in ferrets [106]. During later developmental stages, there is a gradual increase in the number of axons from the temporal retina that cross at the chiasm [129,135]. Interestingly, in ferrets, the axons of the late-born alpha cells are all crossed, whereas in cats, it is the earlier born beta-cells that cross [7,136]. Such axonal crossing from the temporal aspect of the retina is not observed in primates [137,138]. Moreover in carnivores, a well-defined line of decussation forms early such that later arriving axons preferentially take a crossed course, indicating either the strengthening of nasal chiasmatic cues or weakening of temporal cues that direct axons into the uncrossed pathway as development proceeds [139]. An age-related change in fiber organization in their course from the retina to the optic chiasm across the optic tract indicates that some features of the local microenvironment in addition to possible change in the responsivity of axons may be instrumental in producing the reordering of the optic axons [134,140, 141]. Furthermore, the changing order of the fibers may also reflect a change in glial structure according to age [127,139,140,142,143]. In this regard, the distribution of cell adhesion molecules and extracellular matrix molecules, particularly chondroitin sulfate proteoglycans (CSPGs), the main inhibitory molecule whose density complements age-related segregation of the optic tract axons, may play an important role. The density of CSPGs is shown to be the highest in the deepest part of the tract, within the region of the oldest optic axons [134,144]. The temporal sequence of development of glial architecture and the CSPG distribution are coincident with the arrival of the first optic axons. Conversely, these glial and molecular features fail to form in the absence of optic axons, suggesting the importance of the radial gradient of CSPGs in the formation of chronotopic fiber rearrangement, possibly by providing a relatively unfavorable environment for subsequent axonal growth and by interfering with adhesion molecules on optic axons that normally promote elongation [134,145]. More recently, the presence of specific transcriptional factors, especially temporally precise regulation of Zic2, has been shown to regulate the proportion of ipsilaterally projecting RGCs at the optic chiasm of ferrets [146,147].

EYE-SPECIFIC LAYERS IN THE LGN

A fundamental feature of the visual pathway is the segregation of axons from the two eyes into eye-specific laminae or domains in the LGN. Such segregation may be necessary for emergence of depth perception from stereoscopic cues conferred by retinal disparity. In mammals such as ferrets, cats, and primates (including humans) with complex visual systems, the axons from the nasal retina of each eye project to the contralateral side, whereas the temporal retina projects ipsilaterally. In primates, the location of the eyes in front results in higher degree of binocular overlap with up to 40% uncrossed retinal axons. In ferrets and other animals having eyes further away to the side of the head, the binocular overlap is proportionally reduced, with only 15% uncrossed fibers in ferrets, for example [147]. The segregation into eye-specific domains may be influenced by positional cues in addition to activity-dependent processes. Developmental time course studies in the mouse show that early ipsilateral axons are diffusely targeted to the dorsal-medial portion of the LGN and progressively become more strictly confined to a central core [148]. Although activity-dependent processes contribute to the refinement of eye-specific layers in the LGN [149–151], the initial ingrowth of ipsilateral axons shows a bias toward the binocular region in the central part of the dorsal half of the LGN, and eye-specific guidance cues likely instruct this initial positioning [91]. A number of molecular mechanisms elucidating RGC guidance and mapping have been proposed, mainly in mice, but in ferrets and higher mammals, some of the same molecules that contribute to the topographic ordering of projections in retina and optic tract have been investigated in the LGN, primary visual cortex (V1), and the SC. The influential "chemoaffinity" model [152] proposes complimentary matching of molecular gradients

along the sheet of axons by its binding partner in the target. This model has received increasing validation with the discovery of a number of different receptor-ligand gradients expressed in projecting axons and target cells along the visual pathway (e.g., Leamey et al., [153]). The ephrin ligands and Eph family of tyrosine kinase receptors are the most comprehensively studied of these gradient mapping molecules. The role of ephrinA–EphA receptor interactions in topographic mapping was first shown in the optic tectum [154], where two complementary gradients, low-to-high ephrin-A2/A5 expression along the anterior–posterior axis and high-to-low EphA3 receptor gradient in terminals of the temporal–nasal axis of retina interact [155–157]. High lateral-to-medial gradients of ephrin-A2/A5 are also present in the ferret LGN, and direct topography of high levels of EphA5/A6 expression from the contralateral nasal projections and low levels in the ipsilateral temporal projections [158] (reviewed in Ref. 159). Loss and ectopic gain of ephrin-A2, A3, and A5 lead to disruptions of the topographic map in both the LGN and V1. While loss results in a medial shift in V1 and internal disorganization, the overexpression leads to a compression of V1, suggesting that EphA-expressing geniculocortical axons respond to a high medial to low lateral gradient of ephrinA [160].

ON–OFF SUBLAYERS IN THE LGN

Eye-specific segregation in ferrets starts soon after birth and is relatively complete by the first postnatal week [3]. Subsequently, on and off sublayers differentiate within the eye-specific A and A1 layers, starting in the third postnatal week. The formation of these sublayers also seems to be governed by a combination of activity-independent and activity-dependent cues. The stereotyped location and pattern of the sublayers indicate that molecular guidance mechanisms likely establish their initial parcellation. However, a variety of experiments demonstrate the crucial impact of electrical activity, and substrates that mediate the influence of activity, on sublayer patterning. Thus, blockade of retinal activity with tetrodotoxin (TTX) disrupts the sublayers [161], as does blockade of NMDA receptors in the LGN [12,13]. NMDA receptor blockade causes retinal afferents to be more widespread than normal rather than be restricted to sublayers, and also causes a rapid increase in dendritic spines on LGN neurons [70], consistent with a disruption in the coordinated regulation of pre- and postsynaptic connectivities that characterize pattern formation during development. A crucial substrate for sublayer patterning is the availability of nitric oxide (NO). NO synthase (NOS) is transiently expressed in the ferret LGN during sublayer formation [18], and inhibition of NOS during the third and fourth postnatal weeks prevents their segregation [19]. A target of NO is soluble guanylyl cyclase (sGC), which in turn produces cGMP, both of which are critically involved in the process of sublayer formation [33]. The cGMP expression is upregulated in both retinal terminals and postsynaptic LGN cells during sublamination, and this expression is controlled by the activity of both NMDA receptors and NOS. Furthermore, the infusion of specific inhibitors of sGC or protein kinase G (PKG), a target of cGMP, prevents sublamination. Thus sGC–cGMP–PKG pathway acts downstream of NMDA receptors and, together with NO, acts as an effector of the activity-dependent refinement of connections. Finally, calcineurin, a calcium/calmodulin-dependent serine threonine protein phosphatase known to mediate NMDA-receptor-dependent synaptic plasticity in the hippocampus, has a role in on/off patterning. The calcineurin expression is transiently upregulated in LGN cells and neuropil during the period of sublamination, and blocking the enzyme locally via intracranial infusion of FK506 disrupts the sublayers [162]. The formation of sublaminar connections is accompanied by a remarkable stability in the properties of NMDA and AMPA receptor-mediated spontaneous and miniature evoked excitatory postsynaptic currents (EPSCs) at retinogeniculate synapses [30]. Similarly, the anatomical AMPA/NMDA ratio, as assayed by quantifying the colocalized expression of AMPA and NMDA receptor subunits, is fixed throughout the period of sublayer formation [29]. This elucidation of the steps, intervening between electrical activity in retinal afferents, structural rearrangement of retinal axon arbors into LGN sublayers, and their synaptic connections with LGN target cells, remains one of the best characterized mechanisms of pattern formation in a developing mammalian system.

GENICULOCORTICAL PATHWAY AND OCULAR-DOMINANCE COLUMNS IN VISUAL CORTEX

The earliest axons from thalamus to cortex, thought to be of reticular/perireticular origin, extend from posterior thalamus all the way into the cortical subplate (SP). These nonganglionic cells are born early (E21–E24), and in ferrets, their major fiber bundles arrive in the SP of posterior cortex (future V1) between E25 and E27 [8,163]. Within the cortex, the cells localized in different laminae and those projecting to different spatial locations follow diverse temporal orders, the earliest being born within the marginal zone (MZ) and SP between E20 and E26 [99,103] (see Fig. 30.3). As in the case of the retina and the thalamus, this population of early transient cells is thought to play the role of "guide posts" [164] for later geniculocortical axons.

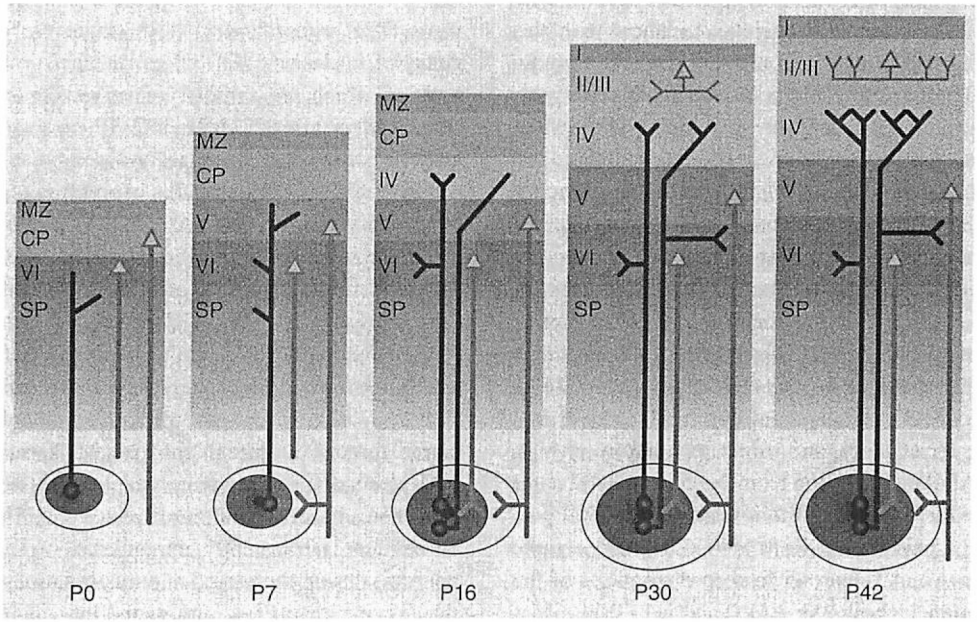


Fig. 30.3. Major milestones in development of visual cortex and its interconnections with the thalamus. At birth (P0), thalamocortical afferents (shown in red) have arrived at the base of the cortical plate (CP) which is undifferentiated with layer VI below. By postnatal day 7 (P7), layer V is visible below undifferentiated CP, and initial corticothalamic projection (shown in green) formed by collateral branches of layer V neurons that have grown past the thalamus. By postnatal day 16 (P16), layer IV has differentiated and thalamic axons have invaded to establish synaptic contacts that are segregated into patches reminiscent of OD columns. The corticothalamic projection from layer VI has also formed by this time. By postnatal day 30 (P30), differentiation of layer II/III has occurred and horizontal connections begin to form. These mature into clustered patterns by postnatal day 42 (P42). MZ, marginal zone; SP, subplate. Adapted from Reference 99.

Deletion of the SP in ferret cortex causes axons to bypass altogether their cortical targets [165]. In addition, there is some evidence that an early, coarse topographic pattern of connections in the cortical plate (CP) may simply be established based on temporal ordering alone. Much detailed analysis of chronological patterning, axon guidance, and targeting will be necessary to confirm this important developmental process. The synaptic profile and fine structure of thalamocortical connections in mature ferrets, after prenatal removal of all retinal afferents, before retinal fibers first invade LGN, show no significant compensation in growth of foreign afferents. The LGN itself is reduced in overall volume, but the geniculocortical and corticogeniculate interconnections show an essentially normal topography. However, in the presence of abnormal retinogeniculate inputs, the topographic pattern can be modified, indicating that separate mechanisms may be in play in formation of retinal maps within the geniculocortical pathways [166].

The spatial and temporal properties of visual inputs are conveyed to V1 in the form of distributed patterns of neural activity carried by axonal inputs arriving from the LGN. In the early 1960s, Hubel and Wiesel [167] first described that neurons in cat V1 showed remarkable preference for one or the other eye by which they were activated and that the neurons with similar eye preference grouped together into periodic stripes or columns of ocular dominance. It was initially argued that intrinsic mechanisms determined this stripe-like organization, whose structural basis was subsequently visualized with the use of a radioactively labeled, transneuronal tracer injected into one eye [168–170]. Not long after, they went on to show that closing one eye during first few months of life shifted the binocular equilibrium, such that the number of neurons responding to the open eye markedly increased, accompanied by a corresponding decrease in neurons activated by the closed eye [94,171]. This means that the activity-dependent processes involved in development of eye-specific lamination

in LGN carry their influence in guiding and in termination of axonal afferents in the input layers of V1. Furthermore, the effect of monocular deprivation (MD) was found to be most profound during a "critical period" of susceptibility, when alterations in the normal pattern of input activity dramatically alter the ocular-dominance (OD) pattern and neural responses, whereas similar manipulations done later in life produce minimal change [94,171]. The critical period for MD in ferret starts around P36, peaks roughly at P42, then tapers off to end approximately at P60 [47]. These early physiological and anatomical studies of OD development led to a set of conclusions that dominated the field: first, an initial intermingled pattern of terminals representing the two eyes segregates under the influence of spontaneously generated activity or visually driven activity; second, eye-specific afferents compete for synaptic space in the input layer of V1 determined by relative levels of input driven activity; third, balanced, binocular input during the critical period is necessary for synaptic refinement and modification, the absence of which leads to improper circuit development and generally irreversible visual deficits. The role of retinal inputs was further confirmed by studies done in ferrets and cats where repeated injections of binocular TTX silenced retinal activity and prevented OD column formation.

Since then, understanding the processes involved in formation of OD columns has become a major field of inquiry, particularly as a model for correlated development of structural and functional plasticities in visual cortical circuits. Later studies have challenged the earlier conclusions. An initial challenge to the role of activity in OD development was posed by Crawley and Katz [172], who injected the anterograde tracer biotinylated dextran amine directly in ferret LGN, and a retrograde label of fluorescent microspheres into V1. Both revealed patchy patterns of labeled neurons (see Fig. 30.4), having the same periodicity as normal controls as well as binocularly enucleated ferret kits that received lesions between P0 and P18, much before the onset of vision, suggesting that thalamocortical interactions were sufficient for segregation of OD patches in the primary visual cortex. In addition, when tracer injections were confined within individual layers of LGN, segregated patches of geniculocortical axons in layer IV of visual cortex were seen within a week after innervation around P16. Moreover, creating an imbalance in retinal inputs with MD before eye opening (P7–P14) did not significantly impact the periodicity of OD columns in V1, suggesting a nonessential role of retinal inputs in OD formation. There is general consensus now that the initial establishment of OD columns takes place well before the initiation of critical period, which only begins with the onset of visual experience, after P30 in ferrets.

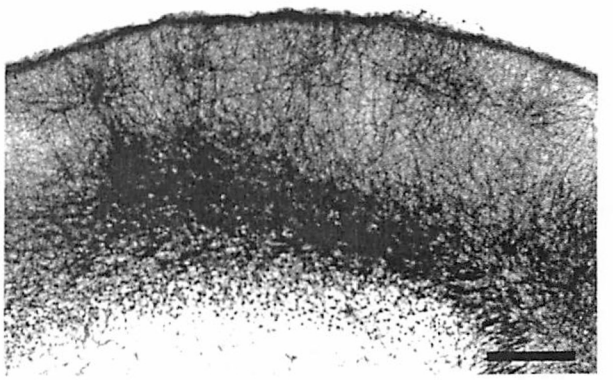


Fig. 30.4. Early OD column segregation in the ferret visual cortex. The formation of OD columns was believed to involve a process of prolonged, activity-dependent sorting from an intermixed early stage. However, as shown here in a coronal section of layer IV of visual cortex from a P21 ferret, labeled axons from the LGN clearly segregated into patches of ocular dominance, well before eye opening and the onset of the critical period for OD plasticity (pia mater is at the top; white matter is at the bottom of image). Scale bar: 500 μm. Adapted from Reference 50.

While it remains unclear what role activity plays in the initial establishment of the OD columns, it is now clear that activity is important for the development, maintenance, and plasticity in the segregation of the eye-specific layer in LGN and for plasticity of OD columns [23,36,173,174]. Activity has been shown to play an instructive role in OD column plasticity and a permissive role in the plasticity of retinal afferent segregation in the LGN. The exact patterning of activity needed to cause plasticity in the cortex and the LGN, as well as the role of activity in the initial development of both OD columns and LGN layers remains to be fully discovered. The possible involvement of activity-independent molecular cues in the initial establishment of OD columns and in the formation of layers in the LGN also remains an active area of research.

FORMATION AND MAINTENANCE OF FEATURE MAPS IN VISUAL CORTEX

Feature maps in the cortex constitute orderly neuronal representations of responses to particular stimulus features. In ferrets and other mammals, topographic feature

maps in the visual cortex constitute an emergent property of cortical circuitry. For example, single neurons in V1 are selective for orientation, spatial frequency, and direction and motion of visual stimuli. Of these, the selectivity for orientation is the most robust and extensively studied. The existence of periodic organization of orientation selectivity in the primary visual cortex was first described using extracellular recording by Hubel and Wiesel [175] and was later confirmed by the metabolic labeling technique of 2-deoxyglucose (2-DG) [176–180]. With the advent of optical imaging of intrinsic signals, the organization of cortical orientation responses to visual stimuli across a wide area provided unprecedented view of the functional organization of orientation selectivity at a much higher spatial resolution than was possible using electrophysiological recording [181–185]. Here again, the robustness of ferrets as experimental animals around the time of eye-opening (P32–P34) was especially useful, allowing chronic preparations where earliest emergence, maturation, and plasticity in organization of orientation preference could be studied by repeated imaging in the same animal over several days or weeks [36,41]. Based on these reports, the earliest clear orientation maps in ferret V1 can be discerned at P33, with cardinal orientations (horizontal and vertical) stronger than the oblique orientations [36]. The maps continue to become stronger over time, reaching adult-like clarity by P42. The single-orientation domains also appear to remain stable over time and with no change in their relative position, shape, or size. Orientation preference in mature ferret V1 (Fig. 30.5B) shows radial arrangement with prominent "pinwheel-like" structures interspersed by abrupt breaks in periodicity of otherwise smoothly mapped orientations, similar to that seen in cat and macaque V1 [37,186,187]. Interestingly, the density of pinwheels in the ferret V1 is twice as high as that in the cat V1 (5.5–2.4/mm^2), although the ferret map is less regular and more discontinuous than the orientation map in cat V1 (Fig. 30.5 for comparison). The average size of single-orientation domains in ferrets is 370 μm in diameter compared with 540 μm in cats [37]. Using combined tracer injections with optical imaging demonstrates that horizontal connections are patchy and target iso-orientation

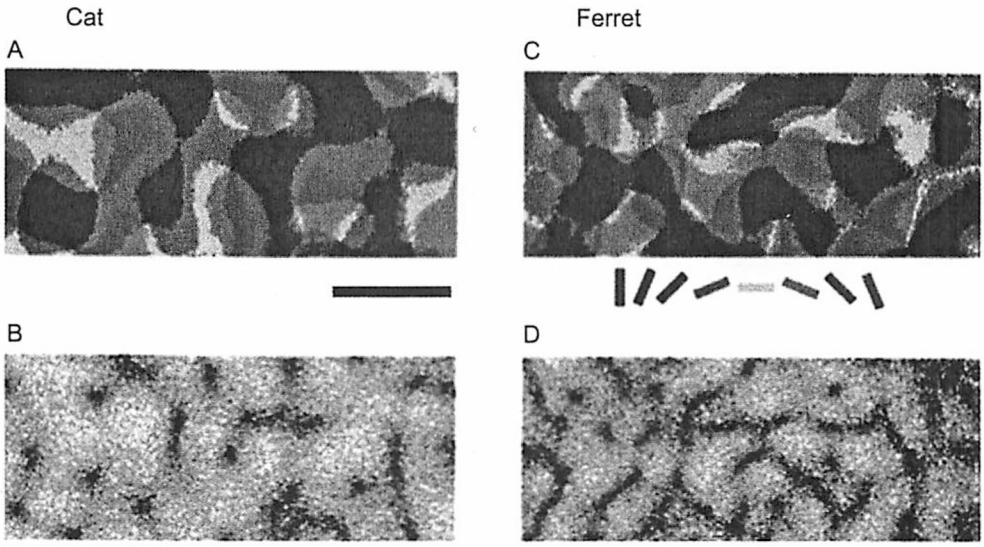


Fig. 30.5. Comparison of feature maps of orientation preference in primary visual cortex of cat (A, B) and ferret (C, D). Top panels (A, C) show that the composite maps of orientation preference in carnivores (cats and ferrets), as well as primates, show systematic organization of orientation. Each color in the map represents orientation preference of the underlying cortical neurons according to the color bar shown below C. Scale bar: 1 mm. Bottom panels (B, D) show orientation gradient maps representing rate of change of orientation preference obtained by applying a two-dimensional gradient operator to the maps in A and C. Dark areas represent high rates of change of orientation, which include pinwheel centers where multiple orientation domains converge. The lighter areas coincide with single-orientation domains where rate of change is slower. Adapted from Reference 37.

domains [188] with the mean interpatch distance (0.7 mm) resembling the mean spacing between isoorientation domains (0.72 mm). Similar focal injections in ferret V1 show a center-to-center spacing of 0.6–0.7 mm in the horizontal spread of label [74]. Interestingly, unlike V1 of cat and ferret, where horizontal connections link nearby iso-orientation domains regardless of eye specificity, horizontal connections in monkey V1 appear to link only iso-orientation domains related to the same eye, skipping intervening domains related to the other eye [189].

The cells in orientation maps are further organized into selectivity maps of their own. For example, stripes of multiple orientations converging around a pinwheel center on the cortical surface also represent graded regions of different orientation selectivities. Within these orientation-selective regions, directionally selective subregions are present. It is interesting that a number of topographic maps, each representing a distinct visual feature, can be mapped efficiently on the same two-dimensional sheet of cortex. An influential model that has received considerable experimental support proposes a layout that maximizes cortical coverage along with map continuity in which maps of multiple features are superimposed in systematic fashion, such that the regions of high gradient in each map are spatially segregated [44,190]. In this way, a particular feature is systematically mapped across the cortical surface but with interspersed low and high rates of change. A location with a slow rate of change in one feature has rapid change in other features [44]; this rule is necessary and sufficient for providing complete mapping of a single feature and complete coverage of all features, without "holes" or gaps in representations.

The mechanisms of formation of multiple feature maps in ferret primary visual cortex and other mammals are not well understood. There is general consensus that developmental processes dependent on intrinsic genetic programs coupled with activity-dependent patterned inputs both play important but differing roles [101]. Several lines of evidence point to distinct temporal windows during which activity-independent and activity-dependent processes exert permissive and/or instructive roles for development of functional maps in cortex. The maps of retinotopic space and OD patterns emerge before eye opening in ferrets and thus are activity independent, while the map of orientation in ferret V1 becomes detectable only by the time of eye opening and so requires activity [36,51,107]. The direction-selective map appears at least 1–2 weeks later [55]. The formation of feature maps coincides with a period during which axonal connections in V1, especially long-range horizontal inhibitory projections in layer 2/3, proliferate [108]. The feedforward patterns of inputs provided by thalamocortical connections provide initial tuning for orientation to V1 neurons [191] which are then shaped by recurrent excitation and balanced inhibition from intracortical connections [82,192,193]. The maturation of this supragranular inhibitory network may further contribute to the organization of orientation tuned cells into feature-selective domains.

Activity seems to play at least a permissive role in formation of maps as evidenced by a delay in the formation of the orientation map in ferret pups dark reared from birth [51]. Moreover, binocular lid suturing at birth leads to a near complete degradation of the orientation map [51], suggesting that low levels of nonpatterned activity have greater disruptive effect than the absence of any input. Therefore, intrinsic and yet unknown mechanisms instruct the formation of the maps of orientation and other feature preferences in the weeks after eye opening, but can induce the map even in the absence of patterned vision. In contrast to orientation maps, the direction-selective maps do not emerge for at least 2 weeks after eye opening, clearly showing the necessity of visual experience for their formation [55,101]. Thus, the direction-selective map may be induced by activity-dependent changes either in intracortical circuits or in feedforward inputs, as change in response latency and sharpening of retinotopic tuning of LGN cells have been shown to accompany the development of direction tuning [194]. Importantly, different feature maps in V1 appear to be guided by independent mechanisms. For instance, a recent study showed that disruption of the OD map following monocular enucleation does not interfere with the formation of the remaining feature maps in ferret V1 [38]. However, alterations in one map does impact the relative positioning of other feature maps in V1, as evidenced by monocular enucleation, which leads to the coordinated reorganization of the remaining map dimensions [38]. Taken together, while unique developmental mechanisms underlie the formation of each feature map, activity seems to play a key role in the fine-scale organization of each map and its structural and spatial coordination with other maps.

It is noteworthy that the unique developmental timeline of the ferret visual system has been invaluable in gaining important insights as detailed earlier, in formation and coordinated sculpting of feature maps in mammalian neocortex. Because of the independent origin of individual feature maps, the evolution of new maps may depend on unique events and developmental processes; however, the general properties inherent in neural circuits may allow for the introduction of a novel map. The examples of novel input leading to the introduction of a new map includes

the implantation of a third eye, leading to OD stripes in the tectum of the frog [195], and rewired retinal input to the MGN driving retinotopic maps to form in primary auditory cortex (A1) [10],

INTRINSIC MECHANISMS IN VISUAL SYSTEM DEVELOPMENT: SPONTANEOUS ACTIVITY AND RETINAL WAVES

Understanding mechanisms that guide early development has been an enduring theme and studies in ferrets have provided important insights. One of the important discoveries was demonstration of correlated spontaneous activity from fetuses of rat retina during embryonic days 17–21, which showed the key role of endogenous activity in early refinement of retinal projections [196]. Later studies from Wong [86], and Goodman and Shatz [197] in neonatal ferrets provided compelling evidence of the existence of waves of correlated activity that sweep across the retina before eye opening as the early initiators of activity-dependent mechanisms. *In vivo* recordings as early as postnatal day 1 (P1), made possible due to developmentally early-born ferrets, has provided crucial insights into this process. The spontaneous activity is present in RGCs prior to maturation of photoreceptors in neonatal ferrets, and bursts of action potentials of roughly 2–8 second duration, occurring every 30 seconds to a minute can be recorded [198,199]. These waves persist until a few days before eye opening, around P28–P30 in ferrets. The retinal waves are thought to be necessary for synaptic refinement and segregation in patterns of eye-specific connectivity. Indeed, correlated spatiotemporal patterns of activity within the cells of each retina have been shown to have clear nearest-neighbor bias, while they are distinctly uncorrelated in corresponding locations in the two retinas, thus seeming to occur independently [89]. It has therefore been suggested that within-eye correlated retinogeniculate activity, together with a lack of temporal correlation between the input of the two eyes, may drive lamination of the LGN [86,200] as a powerful demonstration of the Hebbian rule that cells that fire together, wire together [201].

A number of experiments, performed primarily in developing ferrets, have given credence to this theory. Multielectrode recordings and optical recordings using calcium indicator dyes that allowed visualization of fine grained RGC activity have been used to confirm the physiological presence of spontaneous bursts of activity [202,203]. Whole cell recording combined with calcium imaging in P1–P10 ferrets revealed that spontaneous waves are driven by acetylcholine acting via nicotinic receptors [204,205]. Furthermore, in cat LGN, blocking activity by intracranial injection of TTX disrupts eye-specific lamination, while silencing RGC activity in only one eye results in the shrinking of the deprived eye's territory in LGN [206]. Removal of activity could only prove a permissive role of retinal activity, however, but did not prove the necessity of waves in LGN lamina formation. Stellwagen and Shatz [34] induced an increase in retinal wave activity by elevated intracellular levels of cyclic adenosine monophosphate (cAMP) in ferret RGCs. When only one eye was injected, the ipsilateral projections of the injected eye expanded to nearly twice its normal size with only a modest increase in its contralateral projection. In contrast, when both eyes were injected, thereby increasing retinal wave activity binocularly, the two sets of afferents were indistinguishable from normal. This suggests that the amount of activity in the two eyes drives competition for territory, suggesting that activity plays an instructive, rather than simply a permissive role in the development of the LGN. The existence of patterned spontaneous activity preferentially initiated in the binocular retina and transmitted to subcortical and early visual cortex before the onset of vision has been recently shown in developing mice [201]. The wave activity ceases before eye opening, calling into question the instructiveness of this phenomenon in specification and maturation of LGN laminae. However, other evidence suggests this view to be insufficient for fully explaining the formation and segregation of eye-specific lamination in LGN [173,174]. In addition, the stereotypic termination of retinal axons in the same locations in LGN is difficult to explain by activity driven mechanisms based on spontaneous waves alone.

Synchronous bursts of spontaneous activity generated in the retina seem to travel through LGN to the visual cortex. Multielectrode recordings performed in visual cortex of awake ferrets before eye opening have been shown to have correlated waves of spontaneous activity [207]. A patchy organization of correlated activity reminiscent of the network of clustered horizontal connections appears to develop in the same temporal window. Moreover, this correlated activity is found to persist even in the absence of geniculate input [207]. Clearly, spontaneous activity in the visual cortex, not entirely dependent on geniculate input, may play a role in both the generation of orientation selectivity and the formation of OD columns. In the visual cortex of very young ferret kits, orientation-selective responses can be recorded through closed eyelids up to 2 weeks prior to natural eye opening [48] and is independent of the method of rearing [48,51]. These findings suggest that patterned spontaneous visual input prior to eye opening has a role in the development of orientation selectivity. There is also evidence that the activity in the LGN

caused by correlated waves of input from the retina is responsible for the early, experience-independent appearance of orientation selectivity in the visual cortex. In a series of studies, Weliky and Katz [25,208] showed that synchronous bursts of spontaneous activity occur in the LGN of ferrets before eye opening. The frequency of these bursts is similar to that of retinal waves, but significant correlation of activity between left-eye and right-eye layers is caused by cortical feedback. Disruption of the natural input with patterned electrical stimulation by placing wire cuffs around one of the optic nerves resulted in a degradation of cortical orientation selectivity. However, this manipulation did not disrupt the layout of orientation preference maps in the visual cortex. While this argues for an instructive role of correlated visual activity in generating orientation selectivity, the lack of change in orientation maps makes these results somewhat ambiguous, leaving open the issue of whether intrinsic cortical activity or residual correlated LGN activity, persisting despite experimental manipulations, is sufficient to impart topographic or feature-selective mapping to the visual cortex.

OCULAR-DOMINANCE PLASTICITY IN VISUAL CORTEX

Experience-dependent plasticity in the primary visual cortex (V1) has long been used for understanding how the quality and quantity of input activity influence functional and structural properties of cortical synapses and circuits. Blocking or reducing activity in one eye induces profound influence of deprivation in response properties during the critical period, with a smaller effect observed before or after this time. In particular, MD during the critical period of development in cats and ferrets leads to a physiological loss of response to stimulation of the deprived eye and an increase in response of the nondeprived eye [47,167,209]. Although traditionally, OD plasticity was studied by using long-term MD [47], a significant reduction in deprived-eye responses can be recorded even after a few hours of deprivation in kittens [210]. However, it is unclear whether the mechanisms underlying the effects of short- and long-term MDs are the same. Understanding such relationships requires examination of the OD map chronically in the same animal through these different manipulations. Again, the developmental timeline of ferrets, the ability to inject labels and viruses at a very early age allowing time for marker expression in advance of visual manipulations, and well-defined visual pathways and responses in ferret V1 have made ferrets an attractive model system for studying mechanisms of experience-dependent cortical plasticity. In particular, studies in ferrets have revealed important principles and mechanisms of rapid, short-term cortical plasticity during the critical period.

In a series of experiments in young ferrets, rapid physiological and anatomical changes associated with MD that occur within a few hours of deprivation at the level of dendritic spines were investigated [41]. These experiments were made possible by the advent of new, sophisticated imaging tools that allow dynamic high-resolution imaging of dendritic spines, which are motile structures whose dynamics likely have a role in their functional properties [211,212]. Brief periods of MD and recovery were used to study rapid alteration in spine structure and distribution by a combination of two-photon microscopy and intrinsic signal imaging in the same region of ferret V1 *in vivo* [41] The morphological arrangement of dendritic spines, together with the functional shift elicited by short-term MD, showed that structural plasticity of dendritic spines closely follows functional plasticity both in time course and underlying rule: local V1 regions experience rapid spine loss in proportion to deprived-eye drive. The structural and functional remodeling occurring on similar timescales and regulated by the same rules, occurring globally within the cortex, suggest that remodeling of connectivity by alterations at the level of dendritic spines is an important component of OD plasticity [41]. A previous study in a cat found little change in synapse number following short-term MD [213]. There are several plausible reasons for this discrepancy. It is possible that dynamic imaging of the same dendritic sections before and after MD allowed Yu et al [41]. to detect subtle changes that could be easily missed when comparing static images. The species difference could also be a factor. The ferret visual system exhibits some distinct features, for example, large contralateral eye regions within the central visual field representation of V1 which may induce rapid, correlated changes in functional visual drive and dendritic morphology. The molecular mechanisms underlying activity-dependent changes at the level of individual synapses *in vivo* were investigated in monocularly deprived ferrets by Mower and colleagues [42]. By using a genetically engineered Förster resonance energy transfer (FRET) probe for the detection of CaMKII activity, and two-photon imaging of single synapses within identified functional domains, they revealed unexpected and differential mechanisms in specific subsets of synapses. Specifically, they found that brief MD led to activation of CaMKII in most synapses of layer 2/3 pyramidal cells within deprived-eye domains, despite reduced visual drive, but not in nondeprived-eye domains. Synapses eliminated in deprived-eye domains had low basal CaMKII activity, implying a protective role for activated CaMKII against synapse elimination [42].

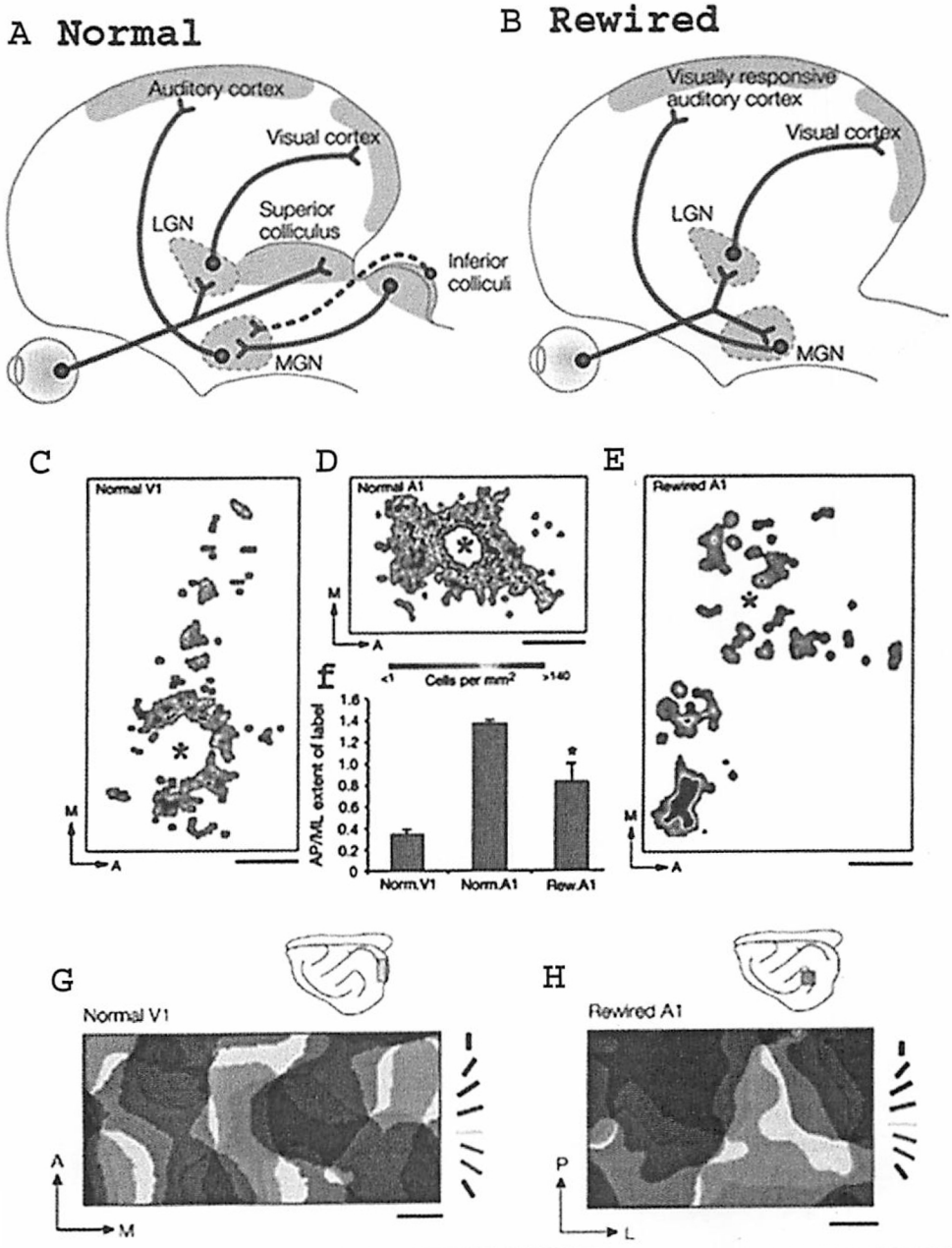
Rapid receptive-field and OD plasticity in the developing (and adult) visual cortex likely involve a reduction in drive of one set of connections coupled with a feedback or "homeostatic" enhancement in the strength of another set of connections to the same neurons. Thus, MD reduces feedforward drive to neurons in deprived-eye domains; the reduction is countered by an enhancement of lateral drive from neurons that receive input from the nondeprived eye, causing a change in neuronal ocular dominance. An overarching hypothesis underlying this proposal is "homeostatic regulation" of synaptic strength that allows neurons to preserve a certain level of total drive, and thus compensate for a reduction in one set of inputs by increasing the strength of another [193,214,215].

REWIRING CORTEX: INDUCTION OF VISUAL INPUTS TO THE AUDITORY PATHWAY

Paradigms using visual deprivation for investigation of functional plasticity induce overall loss of function from the deprived eye and demonstrate that visual experience is permissive for making appropriate cortical connections. On the other hand, a gain-of-function approach offers complementary insights into the role of activity in shaping cortical networks, by demonstrating that visual experience is instructive for making specific connections. For instance, kittens raised in an environment consisting of stripes of one orientation lead to an overrepresentation of that orientation in visual cortex [216]. A powerful way to probe the instructive role of patterned visual experience utilizes rerouting of visual inputs into the auditory pathway [217]. Following pioneering work in hamsters by Schneider [218] and Frost [219,220] that demonstrated redirection of retinal axons into auditory thalamus after deafferentation of its normal inputs, Sur and colleagues chose the ferret as a model of cross-modal plasticity. Besides having a well-developed visual pathway, similar to that of cats, as mentioned earlier, retinal axons in newborn ferrets are still developing projections to the thalamus and thalamocortical fibers have yet not innervated the CP [3,8]. In addition to investigation of the role of novel input in producing retinotopic and feature maps, the experimental "rewiring paradigm" can also provide insights into guidance cues involved in wiring sensory pathways.

The normal auditory pathway comprises cochlear afferents projecting to the IC, which sends fibers along the brachium of the IC (BIC) to the MGN in the thalamus, which then innervates the primary auditory cortex (A1). Similarly, the visual pathway begins with the retina projecting to its two major targets, LGN and SC. The LGN, in turn, sends projections to V1 (Fig. 30.6, top row).

Fig. 30.6. Anatomical and physiological substrates of visual and auditory pathways in normal ferrets, and consequences of rewiring on visual projections into the auditory pathway. (A) Normal visual pathway in ferrets (in blue). The retina projects to its major targets, lateral geniculate nucleus (LGN) and superior colliculus (SC). The LGN, in turn, projects to primary visual cortex. In the auditory pathway, the medial geniculate nucleus (MGN) receives its major inputs from the ipsilateral and contralateral inferior colliculi (IC) (red lines). The MGN projects to the auditory cortex. (B) Extensive deafferentation of the MGN in neonatal ferrets, by ablation of the BIC as well as SC at birth, induces retinal afferents to innervate the MGN. Besides deafferentation of inputs to the MGN, expression of membrane bound molecules such as ephrins in addition to possible attractant and diffusible molecules play influential roles in facilitating invasion of retinal axons into a novel target. The MGN projects normally to auditory cortex carrying novel two-dimensional patterned visual inputs (Adapted from Refs. 58 and 99). (C, D, E) Retrograde tracers (cholera-toxin, subunit B) reveal the pattern of horizontal connections in superficial layers of normal V1, normal A1, and rewired A1 of ferrets, respectively. Distribution of horizontal connections in rewired A1 shows patchy domains that closely resemble that of normal V1 compared with normal A1 and likely contribute to the refinement of orientation mapping in rewired A1. Scale bars: 500 μm. (F) Histogram of the ratio of anteroposterior extent of label to its mediolateral extent in V1, A1, and rewired A1 shows significantly lower ratio in rewired A1 compared with normal A1, indicating that the connectional field in rewired A1 is much more symmetric or isotropic than in normal A1. (G) Orientation map in normal V1 (inset: lateral view of the ferret brain showing imaged region in blue). Visual stimuli consisted of gratings of eight equally spaced orientations which elicited preferential responses from neurons that are clustered in orientation domains and organized periodically around singularities or pinwheel centers. (H) Orientation map in rewired auditory cortex (inset: orange patch in the lateral view of the brain). The map was obtained in a similar fashion as that in normal ferret visual cortex; it is similarly organized with single-orientation domains converging in quasiperiodic manner around singularities or pinwheel centers. Scale bar: 0.5 mm. Adapted from Reference 31.


A Normal
B Rewired
Auditory cortex
Visual cortex
LGN
Superior colliculus
Inferior colliculi
MGN
Visually responsive auditory cortex
Visual cortex
LGN
MGN
C
Normal V1
M
A
D
Normal A1
M
A
f
<1
Cells per mm²
>140
AP/ML extent of label
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Norm.V1
Norm.A1
Rew.A1
E
Rewired A1
M
A
G
Normal V1
A
M
H
Rewired A1
P
L

Removal of auditory inputs to the MGN at P0 by surgical ablation of the IC causes RGCs to find novel targets in the auditory thalamus, thereby inducing visual information into the auditory pathway (Fig. 30.6B). Subsequent to receiving RGC input, the MGN adopts some of the anatomic and physiologic features of the normal LGN while retaining many of its intrinsic features. Rewired MGN exhibit center-surround visual receptive fields [14,221], topographic ordering [57], and eye-specific segregation [24]. The potential to form ordered retinotopic and OD regions in MGN indicates that common patterning cues might exist between the LGN and MGN. For example, experiments in ephrin A2/A5 double knockout mice reveal that surgically induced rewiring is enhanced [222], with ipsilateral projections especially increased, as they originate from the temporal retina and express the highest levels of EphA receptor [223]. Furthermore, loss of innervation to the MGN may make this nucleus permissive to accepting growth of retinal axons. Further exploration using gene-expression analyses comparing the normal and rewired MGNs may facilitate the discovery of yet unknown tropic or repulsive agents regulating retinal axon affinity for different sensory nuclei of the thalamus [224].

Studies of axon morphology show that retinal axon terminations are elongated along the typical isofrequency axis, or lamellae, of the MGN as opposed to more focal, isotropic distributions in the LGN [225]. In addition, eye-specific clusters are smaller and cruder than the eye-specific layers of LGN [24] (Fig. 30.6C). In the cortex of rewired ferrets, cells in A1 respond to visual field stimulation and form a functional retinotopic map of visual space [57]. However, the thalamocortical axons transmitting this information retain their pattern of elongated projections along the anteroposterior axis of A1, which typically correspond to isofrequency bands [226]. In order to create the functional map of focal retinotopic representations, either a refinement of these elongated inputs by intracortical inhibitory network or a difference in drive along the projection itself is required [217]. Consistent with the first possibility, calbindin-immunoreactive GABAergic neurons of rewired A1 have more elongated axonal arbors [227]. Thus, despite persistent structural features of A1 and thalamocortical input that seem to resist change, functional retinotopy can be driven by novel patterns of activity.

Rewired A1 acquires novel maps of orientation selectivity with pinwheels and orientation domains (Fig. 30.6D) similar, in general, to maps in normal V1 [31,37]. In rewired A1, orientation maps are less organized, although intrinsic horizontal connections of superficial layer pyramidal neurons are clustered and bridge distantly located domains of the same orientation preference, as in V1 (Fig. 30.6E) [31]. This pattern of intracortical connectivity is in contrast to horizontal connections in normal A1, where horizontal connections are limited to isofrequency domains of the tonotopic map and stretch along these bands. Such reorganization of horizontal connections driven by visual activity is likely related to changes in the inhibitory circuits of rewired A1, and it suggests that coordinated activity-dependent changes in inhibitory and excitatory networks of at least the superficial cortical layers are a prominent feature of cortical map organization and plasticity.

Interestingly, the rewired auditory pathway is sufficient to instruct visually mediated behavior. Unilaterally rewired ferrets were initially trained to distinguish a visual hemifield stimulus presented in their left hemifield from an auditory stimulus. The same ferrets thereafter received LGN lesions on the rewired side (left LGN). When retested with visual stimulus presented in the right hemifield, animals were able to accurately perceive the stimulus [60]. The left LGN-lesioned ferrets also possess diminished yet intact spatial acuity in the right hemifield. Subsequent ablation of the rewired A1 completely abolished the animals' ability to distinguish a right hemifield stimulus presented as visual. This demonstrates that retinal inputs presented to the rewired A1 were necessary and, in fact, sufficient to detect a visual percept in the trained hemifield.

Taken together with other experiments, the findings in rewired A1 demonstrate that patterned activity has a role in at least maintaining thalamocortical, local intracortical, and long-range horizontal circuits. Axons that project from the MGN to A1 do not show obvious changes in their morphology and extent of arborization after rewiring, but the effectiveness of their synapses in creating a topographic map is shaped importantly by the pattern of activity that drives them. Rewiring and artificial stimulation of the optic nerve are seen to have complementary effects on cortical responses. Whereas artificial stimulation degrades orientation tuning but leaves the orientation map unaltered [25], rewiring leads to orientation tuning in rewired A1 that is highly comparable with V1 but an orientation map that is less organized. Together, these data demonstrate that the orientation tuning of cells is separable from their organization into an orientation map. Furthermore, the two kinds of experiment argue that the thalamocortical and local intracortical connections that generate orientation tuning are influenced by patterned activity. The orientation map reflects the nature of long-range horizontal connection, and difference in fine grain of the map points to difference

in connectivity patterns that underlie the differences in domain size and periodicity between V1 and rewired A1. Nor are the long-range connections a static feature of cortex, as they are altered in strabismic cats [228,229], and are shaped in rewired A1 so as to resemble somewhat the connections in V1. Finally, the corticocortical connections of rewired cortex appear not to be different from normal A1 [15], although more subtle changes in cortical projections that might underlie the ability of rewired cortex to mediate visual behavior cannot be ruled out.

CONCLUSIONS

The wide range of studies described here reveal the power of appropriately chosen model systems such as ferrets for revealing processes and mechanisms of brain development and function. The complexity of the mammalian brain demands a protracted developmental time course and complexly orchestrated developmental mechanisms. Ferrets provide a valuable model system for exploring such mechanisms—combining a longer timeline of development compared with rodents, short *in utero* development and early birth, and thus protracted *ex utero* development and access to a wide range of developmental stages. The ferret visual system, in particular, offers the additional advantage of sophisticated visual function and neuronal responses as appropriate models of human visual capacities and circuits. The description of the ferret genome, nearing completion at the time of this writing, will provide additional tools and approaches for studying mechanisms of brain development and plasticity. Indeed, transgenic ferrets [230], and rapidly evolving new technologies applied to ferrets, will undoubtedly bring new insights into the structure, function, development, and plasticity of the brain-wide networks of higher mammals.

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