



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Development of Direction Selectivity in Mouse Cortical Neurons

Citation for published version:

Rocheffort, NL, Narushima, M, Grienberger, C, Marandi, N, Hill, DN & Konnerth, A 2011, 'Development of Direction Selectivity in Mouse Cortical Neurons' *Neuron*, vol 71, no. 3, pp. 425-432. DOI: 10.1016/j.neuron.2011.06.013

Digital Object Identifier (DOI):

[10.1016/j.neuron.2011.06.013](https://doi.org/10.1016/j.neuron.2011.06.013)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Neuron

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Development of Direction Selectivity in Mouse Cortical Neurons

Nathalie L. Rochefort,^{1,2} Madoka Narushima,^{1,2,3} Christine Grienberger,¹ Nima Marandi,¹ Daniel N. Hill,¹ and Arthur Konnerth^{1,*}

¹Institute of Neuroscience and Center for Integrated Protein Science, Technical University Munich, Biedersteinerstrasse 29, 80802 Munich, Germany

²These authors contributed equally to this work

³Present address: Department of Physiology, Tokyo Women's Medical University, Kawada-cho 8-1, Shinjuku-ku, 162-8666 Tokyo, Japan

*Correspondence: arthur.konnerth@lrz.tum.de

DOI 10.1016/j.neuron.2011.06.013

SUMMARY

Previous studies of the ferret visual cortex indicate that the development of direction selectivity requires visual experience. Here, we used two-photon calcium imaging to study the development of direction selectivity in layer 2/3 neurons of the mouse visual cortex *in vivo*. Surprisingly, just after eye opening nearly all orientation-selective neurons were also direction selective. During later development, the number of neurons responding to drifting gratings increased in parallel with the fraction of neurons that were orientation, but not direction, selective. Our experiments demonstrate that direction selectivity develops normally in dark-reared mice, indicating that the early development of direction selectivity is independent of visual experience. Furthermore, remarkable functional similarities exist between the development of direction selectivity in cortical neurons and the previously reported development of direction selectivity in the mouse retina. Together, these findings provide strong evidence that the development of orientation and direction selectivity in the mouse brain is distinctly different from that in ferrets.

INTRODUCTION

Throughout the visual system of vertebrates, neurons are tuned to respond to different features of a visual scene, such as the position, orientation, or direction of motion of a given object. In the mammalian primary visual cortex, most neurons respond selectively to a preferred orientation of visual stimuli. Some of these neurons are also direction selective, in that they are significantly more activated by a preferred direction of stimulus motion than by any other direction (Hubel, 1959; Hubel and Wiesel, 1959). Since the first recordings of visual responses in the cat primary visual cortex (Hubel, 1959; Hubel and Wiesel, 1959), numerous studies have focused on the mechanisms underlying the development of selective properties of visual cortical neurons. These studies were mostly performed in carnivores, such as cats and ferrets, and in primates. In these species, orien-

tation and direction preferences are elaborated with a high degree of selectivity only at the cortical level along the retinogeniculo-cortical pathway (see review in White and Fitzpatrick, 2007) and neurons with a similar orientation or direction preference are clustered into radial columns (Hubel and Wiesel, 1962, 1968; Blasdel and Salama, 1986; Bonhoeffer and Grinvald, 1991; Bosking et al., 1997). It has been shown that orientation and direction selectivity are established by different mechanisms. While the initial establishment of orientation selectivity in cortical neurons is independent of visual experience (see review in White and Fitzpatrick, 2007), several lines of evidence indicate that the emergence of direction selectivity strictly requires visual experience. Thus, direction-preference maps are absent at eye opening and do not develop in ferrets that are reared in darkness (Li et al., 2006). Moreover, visual experience with moving stimuli just after eye opening drives the emergence of direction-selective responses in the ferret visual cortex (Li et al., 2008). However, the connectivity and the mechanisms that are necessary for the emergence of direction selectivity remain unclear.

In recent years, rodents and especially mice are becoming an attractive model for the investigation of such mechanisms *in vivo*. Various transgenic mice lines have been used to study visual system development (Fagiolini et al., 2003; Cang et al., 2005), plasticity (Fagiolini et al., 2004; Syken et al., 2006; Wang et al., 2010), and function of specific cell types in the visual cortex (Sohya et al., 2007; Kerlin et al., 2010; Runyan et al., 2010). It is important to remember that unlike in ferrets, cats, and primates, neurons in the primary visual cortex of rodents are not organized into orientation columns. Instead, orientation-selective neurons are distributed in a mixed “salt-and-pepper” manner throughout the primary visual cortex (Ohki et al., 2005; Van Hooser et al., 2005). Nevertheless, highly tuned orientation- and direction-selective neurons have been shown to be abundant in the mouse visual cortex (Dräger, 1975; Métin et al., 1988; Sohya et al., 2007; Niell and Stryker, 2008; Wang et al., 2010). While the emergence of orientation selectivity has been investigated in the rodent visual cortex (Fagiolini et al., 1994, 2003), the development of direction selectivity has so far received less attention, except in recent studies that investigated the emergence of direction selectivity at the level of the mouse retina.

In mice, retinal ganglion cells exhibit strong direction selectivity (Elstrott et al., 2008; Yonehara et al., 2009). Remarkably, this strong direction selectivity is already present at eye opening (Elstrott et al., 2008; Chen et al., 2009; Yonehara et al., 2009).

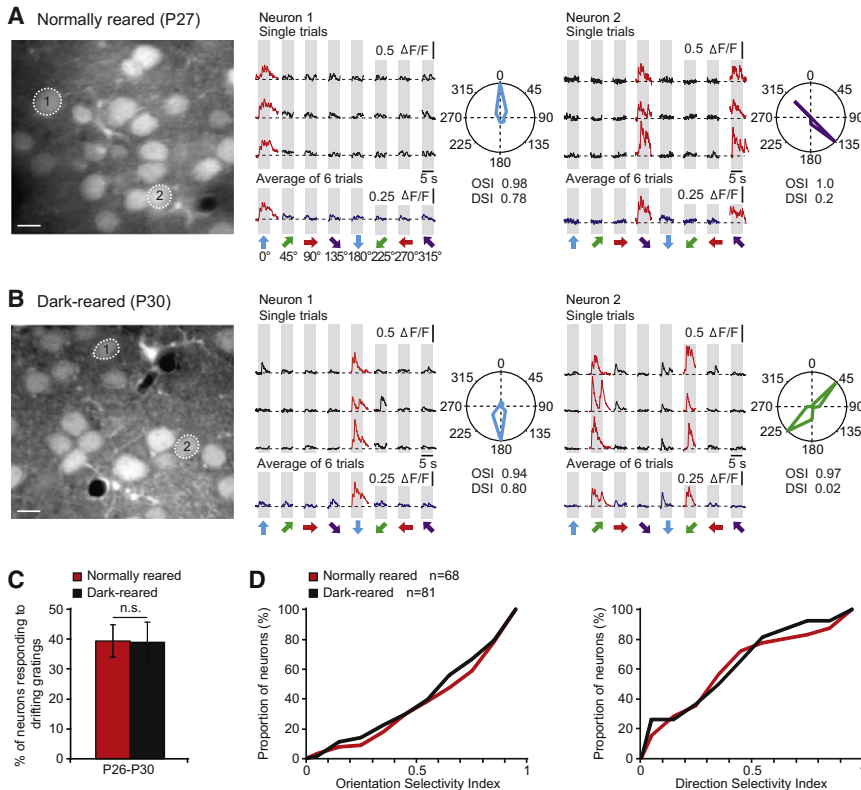


Figure 1. Orientation-Selective and Direction-Selective Neurons in Normally Reared and Dark-Reared Juvenile Mice

(A and B) In vivo two-photon images of layer 2/3 neurons in the visual cortex of normally reared (A) and dark-reared (B) juvenile mice (P27 and P30, respectively) are shown in the left panels. Calcium transients evoked by visual stimulation of orientation-selective neurons indicated in left panels are shown in the middle and right panels. Gray regions indicate periods of visual stimulation with drifting gratings (0.03 cpd, 1 Hz) schematized by oriented arrows on the bottom of each panel. Three single trials are represented with black lines and the average of six trials is shown in blue. Neuron 1 responded selectively to a horizontal drifting grating moving in the upward direction (red calcium transients). The right panels are polar plots showing the neuron's response function to oriented drifting gratings. The responses to each of the eight directions tested were normalized with respect to the maximal response. The response function was constructed by connecting lines between the eight values. OSI, orientation-selectivity index; DSI, direction-selectivity index. Scale bars = 10 μ m. (C) Percentage of neurons responding to drifting gratings (0.03 cpd) in normally reared (red) and dark-reared (black) juvenile mice (P26–P30) (n.s., not significantly different, Mann-Whitney test, $p = 0.767$; $n = 174$ and 220 neurons, respectively). Error bars indicate SEM. (D) Cumulative distributions of OSI and DSI from all responsive neurons of normally reared (red) and dark-reared (black) juvenile mice. No significant difference was found between the two groups (Mann-Whitney test, OSI, $p = 0.50$; DSI, $p = 0.15$).

Moreover, robust directional responses have been detected in dark-reared mice and in mice lacking cholinergic retinal waves (Elstrott et al., 2008; Chen et al., 2009), indicating that visual experience and patterned activity are not required for the development of direction selectivity in the mouse retina. At present, it is unknown how the early presence of direction selectivity in the retina is related to the motion sensitivity of cortical neurons and when and how direction selectivity emerges in the mouse visual cortex. In this study, we investigated the development of orientation and direction selectivity in the mouse primary visual cortex by using in vivo two-photon calcium imaging (Stosiek et al., 2003; Rochefort et al., 2009). We characterized the responses of layer 2/3 neurons to oriented drifting gratings with single-cell resolution at different developmental stages, from eye opening until adulthood, in normally reared mice as well as in mice reared in darkness for a month.

RESULTS

Orientation- and Direction-Selective Neurons in the Visual Cortex of Dark-Reared Mice

We first compared neuronal calcium signals evoked by drifting gratings in the monocular region of the primary visual cortex of normally reared and dark-reared juvenile mice (P26–P30). For this purpose, we presented drifting gratings to the contralat-

eral eye while performing two-photon calcium imaging recordings of layer 2/3 neurons stained with the fluorescent calcium indicator dye OGB1-AM (Rochefort et al., 2009). Figure 1A illustrates such recordings obtained in a normally reared juvenile mouse (P27). In the field of view (left panel of Figure 1A), neuron 1 displayed large calcium transients during the presentation of a specific orientation and direction of the drifting gratings (middle panel, direction of 0°, red calcium transients), but no significant responses for all the other directions. A quantitative analysis of the stimulus-evoked responses showed that the neuron had an orientation-selectivity index (OSI) of 0.98 and a direction-selectivity index (DSI) of 0.78. Thus, this neuron was defined as a highly tuned orientation- and direction-selective neuron (Niell and Stryker, 2008). In the same field of view, neuron 2 displayed large calcium transients for one orientation of the drifting gratings, but not for a specific direction of motion (right panel, responses for both directions of 135° and 315°). Thus, this neuron was identified as a highly tuned orientation-selective, but not direction-selective, neuron (OSI, 1.0; DSI, 0.2). Surprisingly, both orientation- and direction-selective neurons were also found in the visual cortex of dark-reared mice. Figure 1B illustrates an example of a direction-selective (middle panel) and of an orientation-selective neuron (right panel) in a juvenile dark-reared mouse (P30). An analysis of all recorded neurons in normally reared and dark-reared mice showed that the percentage of

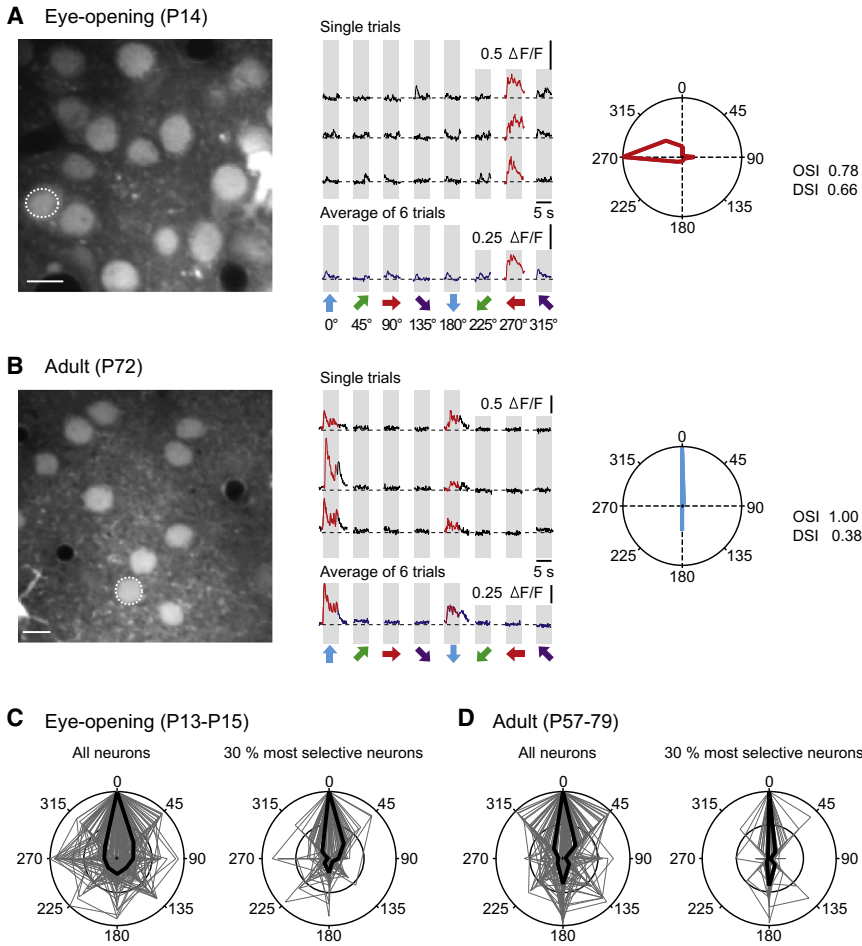


Figure 2. Direction-Selective Neurons Are Present Just after Eye Opening

(A and B) In vivo two-photon images of layer 2/3 neurons in the visual cortex of normally reared mice at eye opening (P14) and in adulthood (P72) are shown in the left panels. Calcium transients evoked by drifting gratings (0.03 cpd) in the neurons indicated in the left panels are shown in the middle panels. The same conventions are used as in Figure 1. The right panels are polar plots showing the neuron's response function to oriented drifting gratings. Scale bars = 10 μm . (C and D) Overlay of polar plots obtained from the visually evoked activity in all neurons (left panels, $n = 167$ and 81 neurons, respectively) and in the 30% most selective neurons (right panels) in normally reared mice just after eye opening (P13–P15) and in adulthood (P57–P79). Polar plots were normalized by the maximal response and rotated to place the preferred direction at 0°. Black lines indicate median tuning function.

either through closed eyelids or after the gentle opening of the eyelids with forceps (Figures S3A and S3B). Only strong luminance changes, consisting either of light flashes or of a sudden transition between a black and a gray screen (Figure S3C), could elicit a response from layer 2/3 neurons. It is important to note the remarkably dense response pattern, with more than 80% of neurons activated in the field of view (Figure S3C, left panel, red neurons; Figure S3D). A similarly dense activity pattern was also observed when

neurons responding to drifting gratings was not significantly different in the two groups (Figure 1C). Furthermore, the tuning level of the responsive neurons was also remarkably similar (Figure 1D and Figure S1, available online). The quantitative estimation of orientation and direction tuning showed no significant differences between the cumulative distributions of both OSIs and DSIs (Mann-Whitney test: OSI, $p = 0.50$; DSI, $p = 0.15$) (Figure 1D). In addition, we explored whether the visual stimulation itself modified the level of orientation and direction selectivity in dark-reared mice. We found that both OSIs and DSIs were not significantly different between the first and last trials of visual stimulation (Figure S2). Thus, we conclude that both orientation and direction selectivity develop without visual experience during the first postnatal month in the mouse visual cortex.

Orientation- and Direction-Selective Neurons at Eye Opening

It has been recently shown that the earliest light-evoked responses are detected in the mouse retina at P10 (Tian and Copenhagen, 2003; Chen et al., 2009), ~ 2 –3 days before the eyes open. We found that just before eye opening (P10–P12), neurons of the mouse visual cortex were spontaneously active, but no activity could be evoked by drifting gratings presented

the eyelids were gently opened with forceps in these mice (Figure S3C). These results indicate that, at early stages of development, activity patterns are dense not only spontaneously (Rocheffort et al., 2009), but also in response to a sensory stimulus. By contrast, in adult mice (P57–P79), the activity pattern becomes much sparser, with only 23% of the recorded neurons responding to such changes in luminance (Figure S3D).

The earliest responses evoked by drifting gratings were detected just after eye opening, i.e., on the day of eye opening or 1 day later (Figure 2A). Nearly half of these responsive neurons were orientation selective (OSI > 0.5) (Figures 2A, 2C, and 4D). We overlaid the polar plots obtained from all responsive neurons recorded just after eye opening (Figure 2C). The median tuning function (Figure 2C, black line) shows that the early responsive neurons were selective not only for the orientation of the drifting gratings but also for their direction of motion. Remarkably, nearly all (93%) of the orientation-selective neurons were also direction selective (OSI > 0.5 and DSI > 0.5) (Figures 2A, 2C, and 4D). Thus, in the mouse visual cortex, the earliest neurons tuned for the orientation of the stimulus appear just after eye opening and are strongly tuned for the direction of stimulus motion.

Interestingly, we noticed that at early stages after eye opening, most direction-selective neurons had a directional preference

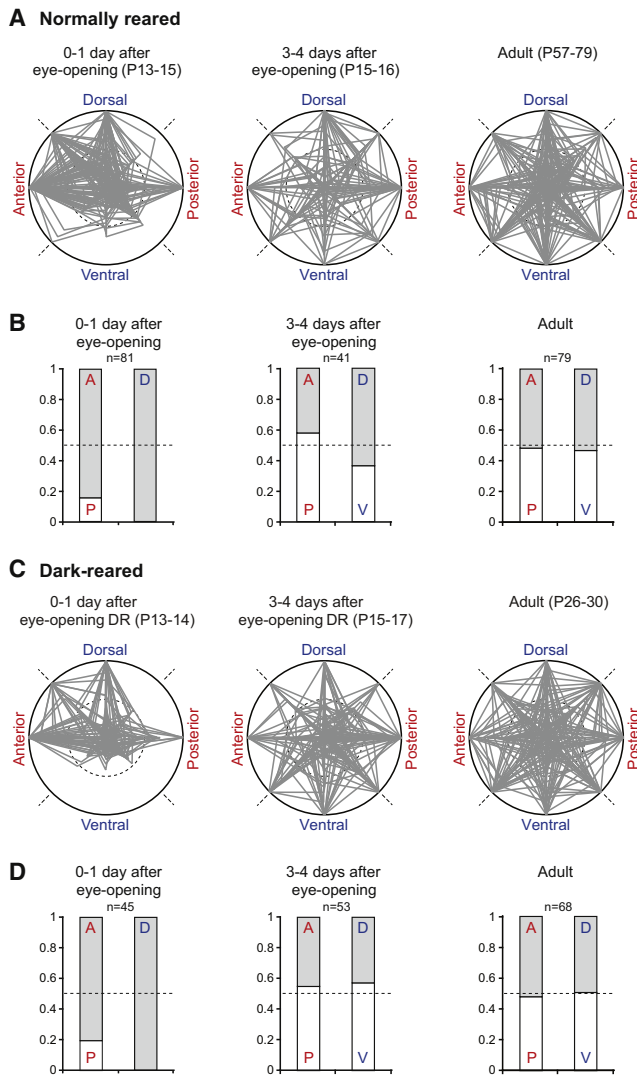


Figure 3. Preference for Anterodorsal Directions at Eye Opening
(A) Overlay of polar plots obtained from the responses evoked by drifting gratings (0.03 cpd) in all selective neurons (OSI > 0.5) of normally reared mice at different postnatal ages from eye opening (P13–P15) to adulthood (P57–P79). Polar plots were normalized by the maximal response. (B) The symmetry of preferred directions along a given axis (anterior-posterior or dorsal-ventral) was tested by calculating the fraction of neurons with a preference for a given direction (e.g., anterior [A]) along the axis versus the fraction of neurons with a preference for the opposite direction (e.g., posterior [P]). A perfectly symmetric axis would have 50% of neurons preferring either opposite direction (dashed lines). For the four postnatal ages, the light gray and white bars show for each axis the fraction of neurons with a preference for the anterior (A) versus posterior (P) direction and for the dorsal (D) versus ventral (V) direction, respectively. (C and D) Same conventions as in (A) and (B) for polar plots obtained for all selective neurons (OSI > 0.5) in dark-reared mice at three different postnatal ages from eye opening (P13–P15) to adulthood (P30).

for gratings moving toward either the anterior (270°, Figure 2A) or the dorsal direction (0°). In order to compare the distribution of preferred directions in young and adult mice, we overlapped the polar plots obtained from all orientation-selective neurons recorded at different ages: just after eye opening (0–1 day),

3–4 days after eye opening, and in 2-month-old adult mice (Figure 3A). Just after eye opening, the direction-selective neurons displayed a clear preference for the anterior direction along the anterior-posterior axis as well as a preference for the dorsal direction along the ventral-dorsal axis (Figures 3A and 3B). Not a single neuron was found with a preference for the ventral direction (Figure 3A). In order to quantify this bias, we counted the number of neurons with a preference for a given direction along each axis (anterior-posterior and ventral-dorsal). The representation of opposite directions was strongly biased along both axes, with anterior and dorsal directions being significantly overrepresented (Figure 3B). This distribution of direction preferences did not depend on the preferred spatial frequency of the drifting gratings (Figure S4). A few days later (3–4 days after eye opening), this asymmetric organization disappeared (Figures 3A and 3B). As in 2-month-old adult mice, the distributions along the anterior-posterior and ventral-dorsal axes were roughly symmetric in that the number of neurons that prefer a given direction of motion was roughly the same along both axes (Figures 3A and 3B). It is noteworthy that the oblique orientations were strongly underrepresented just after eye opening (especially 45°, 135°, and 225°) and this bias disappeared 3–4 days after eye opening.

We found that the bias in the distribution of direction preferences was similarly present in the visual cortex of dark-reared animals just after eye opening. As in normally reared animals, this bias disappeared 3–4 days after eye opening (Figures 3C and 3D). At the three ages tested (0–1 day, 3–4 days after eye opening, and young adult, P26–P30), no differences were noticed in the direction preference of visual cortical neurons of normally reared and dark-reared mice. These results indicate that the developmental change of the direction-preference distribution is a highly robust intrinsic process that does not depend on visual experience.

Developmental Changes in Orientation and Direction Selectivity

The above-mentioned developmental increase in the responsiveness to all directions of stimulus motion was paralleled by a steep increase in the overall number of motion-sensing visual cortex neurons. Thus, the proportion of neurons responding to drifting gratings (0.03 cpd) increased significantly between the day of eye opening and just 2–3 days later (from 12% to 33%, $n = 1216$ neurons in 20 mice and $n = 201$ neurons in 7 mice, respectively, Mann-Whitney test, $p < 0.001$) (Figure 4A). This proportion increased further during the next 2 months, eventually reaching a value of 42.5% ($n = 174$ neurons in 7 mice). This proportion of neurons responding to drifting gratings in layer 2/3 of the visual cortex of adult mice is similar to what has been described in other studies by using two-photon imaging in mouse (Zariwala et al., 2011) and rat visual cortex (Ohki et al., 2005). In addition, we tested five other spatial frequencies of drifting gratings (0.01, 0.015, 0.02, 0.04, and 0.08 cpd) in two age groups (0–1 day after eye opening and at 2 months old). Taking into account the responses to all spatial frequencies tested, we found an increase of 12% in the proportion of neurons responding to drifting gratings in both age groups (Figure S5A). We thus reached a value of 55% of neurons responding to drifting gratings in adult mice, which is very close to what was found in a previous study testing a larger set of spatial frequencies (Kerlin et al., 2010).

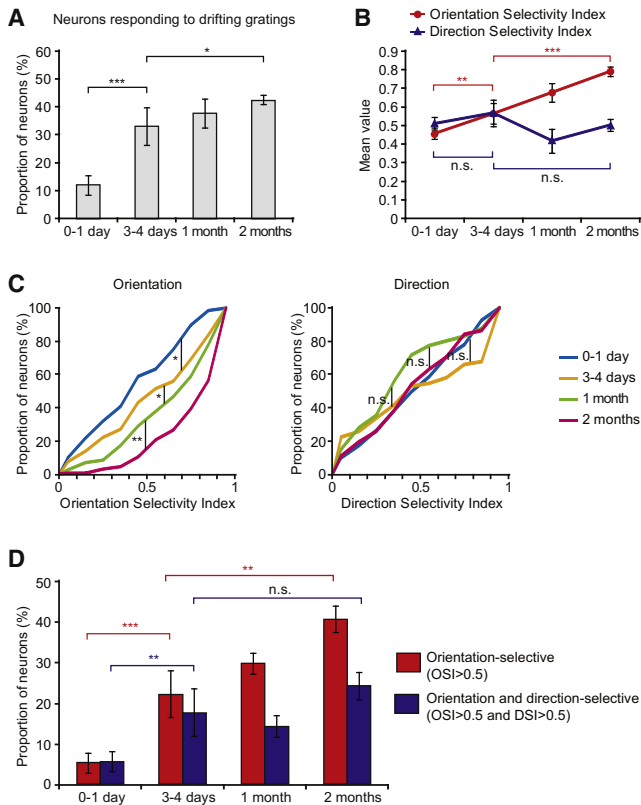


Figure 4. Proportion of Responsive Neurons and Population Tuning Develops during the First Two Postnatal Months

(A) Percentage of neurons responding to drifting gratings (0.03 cpd) in normally reared mice at four different ages from eye opening (0–1 day after eye opening, P13–P15) to adulthood (P57–P79) (Mann-Whitney test, $*p < 0.05$, $***p < 0.001$, $n = 1216, 201, 174$, and 174 neurons from $20, 7, 10$, and 7 mice, respectively). Error bars indicate SEM. (B) Mean OSI (red) and DSI (blue) in all neurons responding to drifting gratings (0.03 cpd) in normally reared mice. The results showed a significant increase of the mean OSI during the first 2 postnatal months (Mann-Whitney test, OSI, $**p < 0.01$, $***p < 0.001$). In contrast, no significant difference (n.s., Mann-Whitney test, $p = 0.62$, 0–1 and 3–4 days after eye opening; $p = 0.56$, 3–4 days and 2 months) was found between mean DSIs. Error bars indicate SEM ($n = 167, 62, 68$, and 81 neurons, respectively). (C) Cumulative distributions of OSI and DSI from all neurons responding to drifting gratings (0.03 cpd) recorded in normally reared mice of four different ages. The results show a significant increase of OSI during the first 2 postnatal months (Mann-Whitney test, OSI, $*p < 0.05$, $**p < 0.01$). In contrast, no significant difference was found between DSI at different ages ($n = 167, 62, 68$, and 81 neurons, respectively). (D) Percentage of orientation-selective neurons (OSI > 0.5 , red) and of direction-selective neurons (OSI > 0.5 and DSI > 0.5 , blue) among all neurons recorded in normally reared mice at four different ages, during the presentation of drifting gratings (0.03 cpd) (Mann-Whitney test, $**p < 0.01$, $***p < 0.001$; n.s., $p = 0.14$). Error bars indicate SEM.

During the first 2 postnatal months, not only did the proportion of neurons responding to drifting gratings increase, but also the proportion of orientation-selective neurons increased among the responsive neurons. Figure 4B compares the development of orientation and direction selectivity during this period. The mean OSI values indicate a significant increase of the orientation tuning between the day of eye opening, 3–4 days after eye opening, and 2 months later (Mann-Whitney test, $p < 0.05$

(Figure 4B; see also Figure 2). In addition, the tuning width of the orientation-selective responses decreases slightly during development from a mean value of 32° at eye opening to 27° in 2-month-old adults (Figure S6). The values found in adult mice (mean, 27° ; median, 26°) are similar to those previously described for orientation-selective neurons in the adult mouse visual cortex (Niell and Stryker, 2008; Wang et al., 2010). Notably, already in the youngest age group (0–1 day after eye opening), a significant proportion (35%) of the orientation-selective neurons had a narrow tuning width ($<30^\circ$) (Figure S6A). Whereas orientation tuning increased during development, the mean DSI values (Figure 4B and Figure S7) showed no significant change in the direction tuning between the day of eye opening, 3–4 days later, and in adults. In line with these results, the cumulative distributions of OSIs and DSIs clearly showed a significant increase of orientation but not of direction selectivity during the first 2 postnatal months (Figure 4C). These tuning properties did not depend on the preferred spatial frequency of the drifting gratings (Figure S5B).

Thus, just after eye opening, among orientation-selective neurons (5% of all recorded neurons with gratings of 0.03 cpd) nearly all were highly tuned for the direction of stimulus motion (Figure 4D and Figure S8). At 3–4 days after eye opening, the proportion of neurons responding to drifting gratings increased and the vast majority of the orientation-selective neurons were still strongly direction selective (17.5% of all cortical neurons with gratings of 0.03 cpd, Figure 4D and Figure S8). At this early stage, most of the orientation-selective neurons did not respond at all to the opposite direction of movement of the preferred orientation (Figure 2A and Figures S7A and S7B) and only 4% of all cortical neurons were strictly orientation selective (responding to both directions of movement). This was followed by a 4-fold increase in the proportion of strictly orientation-selective neurons, whereas the proportion of direction-selective ones remained stable (Figure 4D). As a consequence, at the age of 2 postnatal months, only half of the orientation-tuned neurons were also direction selective (Figure 4D and Figure S8). The tuning properties of these neurons were largely similar to those reported by previous studies in normally reared adult mice (Niell and Stryker, 2008; Wang et al., 2010). Altogether, these results establish that the early development of direction selectivity is distinctly different from that of orientation selectivity in the mouse visual cortex.

DISCUSSION

In this study, we obtained unexpected insights into the development of direction selectivity in neurons of the mouse visual cortex. Neurons selective for the orientation of drifting gratings were detected just after eye opening and nearly all were also highly tuned for the direction of stimulus motion. Furthermore, we found a marked preference of these cortical neurons for anterodorsal directions. During later development, the number of neurons responding to drifting gratings increased in parallel with the fraction of neurons that were orientation selective but not direction selective. This developmental increase was similar in normally reared and dark-reared mice. Together, these findings indicate that the early development of orientation and direction selectivity depends on intrinsic factors of mouse visual cortical neurons, without a detectable contribution from visual experience.

Different Development of Orientation Selectivity and Direction Selectivity in Mice and Ferrets

Before eye opening, cortical neurons can respond to visual stimuli through closed eyelids. For example, in ferrets, the firing of visual cortex neurons is modulated by drifting gratings presented through closed eyelids (Krug et al., 2001). These results, however, contrast with those obtained in the present study in mice, where drifting grating stimuli were ineffective before eye opening. In our hands, only strong luminance changes could evoke cortical activity before eye opening and this activity was characterized by simultaneous calcium transients in the majority of layer 2/3 neurons. This dense activity is reminiscent of the spontaneous activity pattern recorded before eye opening (Rocheffort et al., 2009). An important feature of the spontaneous activity is that it undergoes a transition from dense to sparse just after eye opening (Rocheffort et al., 2009). Our present results indicate that such a transition from a dense activity to a stimulus-specific one also occurs around eye opening for stimulus-evoked neuronal responses. Interestingly, a recent study provides additional support for major functional changes in the rat visual cortex during the period just preceding eye opening (Colonnese et al., 2010). It has been suggested that this switch prepares the developing cortex for patterned vision (Colonnese et al., 2010).

Neurons responding to drifting gratings were first observed in the mouse visual cortex soon after eye opening. At this early stage (0–1 day and 3–4 days after eye opening), nearly all orientation-selective neurons are also direction selective. Then the fraction of neurons that are orientation, but not direction, selective gradually increases during the first 2 postnatal months. These results are in contrast to those obtained in the ferret visual cortex, where the developmental sequence is characterized by the presence of orientation-selective neurons at eye opening that subsequently acquire direction selectivity and achieve functional maturity around 2 weeks after eye opening (Li et al., 2006; White and Fitzpatrick, 2007). Thus, from different states at eye opening, the mouse and ferret visual systems undergo converging developmental processes, such that in adults of both species, nearly half of the orientation-selective neurons are also direction selective. The origin of the orientation-selective neurons that are lacking direction selectivity in the mouse visual cortex is unknown. This fraction of neurons appears around 3–4 days after eye opening and increases during the following 2 months (Figure 4D; red area in Figure S8). Future studies need to establish whether these purely orientation-selective neurons evolve from direction-selective ones or whether they constitute a separate class that emerges *de novo* at about 3–4 days after eye opening.

Importantly, in ferrets, dark rearing prevents the formation of direction-selective maps. This indicates a crucial role of visual experience for this developmental process (Li et al., 2006). In the mouse visual cortex, our data show that dark rearing has no detectable influence on the development of direction selectivity (Figure 1 and Figure S9). It should be noted that we focused our study primarily on the early development of orientation selectivity and direction selectivity and not on the effect of long-term visual deprivation. It has previously been shown that in the absence of visual input, orientation selectivity normally appears during the first postnatal month (Iwai et al., 2003; Wang et al., 2010), but then degrades after prolonged lack of visual experience in rodents

(Benevento et al., 1992; Fagiolini et al., 1994, 2003; Iwai et al., 2003) and cats (Frégnac and Imbert, 1978; Crair et al., 1998).

Distinctive Features of the Mouse Visual System

In mice, direction selectivity is already present at the level of the retina (Elstrott and Feller, 2009). On-Off direction-selective ganglion cells have been detected in mouse retina at the time of eye opening (P14) (Elstrott et al., 2008; Chen et al., 2009). It was shown that at this developmental stage these direction-selective ganglion cells exhibit a strong preference for motion toward either the temporal or the ventral pole of the retina, which in visual coordinates corresponds to anterior and dorsal motion direction (Elstrott et al., 2008). Similar results were obtained in the retina of dark-reared mice of the same age (Elstrott et al., 2008). In adult retinas, the anteroposterior asymmetry disappears and the ventro-dorsal one is strongly reduced (Elstrott et al., 2008). Interestingly, our present results demonstrate a strikingly similar developmental pattern of direction selectivity in the upper layer visual cortical neurons. Thus, as in the retina, direction selectivity was detected at eye opening and emerges independently of visual experience. Furthermore, direction-selective neurons recorded just after eye opening in both the cortex and the retina have a similar preference for the dorsal and anterior directions of motion. This preference disappeared in the cortical neurons of adult mice.

One possible conclusion from these results is that in the mouse visual system direction selectivity emerges in the retina and is relayed to the visual cortex. This notion finds support in the previous observations that On-Off direction-selective retinal ganglion cells project both to the LGN and to the superior colliculus in specific laminae (Huberman et al., 2009). In line with this anatomical evidence, direction-selective neurons were recorded in the rat superior colliculus around eye opening (P13) and, as in the mouse visual cortex, the proportion of direction-selective neurons was found to remain stable from P15 to adulthood (Fortin et al., 1999). By contrast, the relay of direction-selective information through the rodent LGN is less clear. While the receptive fields of neurons in the mouse LGN were described as center-surround with exclusively ON-center or OFF-center responses (Grubb and Thompson, 2003), direction-selective cells in mouse or rat LGN are not yet described. However, it remains unclear whether LGN neurons that receive direct projections from direction-selective retinal ganglion cells were ever studied specifically. Another possibility is that LGN-receptive fields are more broadly tuned and that direction selectivity is generated again at the cortical level. It is noteworthy that the directional tuning of the cortical neurons recorded in this study is more narrow than the directional tuning of the mouse retinal ganglion cells (Elstrott et al., 2008). This result indicates that in mice the direction selectivity is refined along the retinogeniculo-cortical pathway. It is unclear whether such a possible refinement is found only in mice. Interestingly, there is some evidence for direction bias in the retinal ganglion cells of cats (Levick and Thibos, 1980; Shou et al., 1995) as well as in the cat and primate LGN (Vidyasagar and Urbas, 1982; Thompson et al., 1994; Xu et al., 2002). However, detailed studies in the retina and LGN of these species are needed for solving this issue.

Taken together, there is accumulating evidence that the anatomical difference between the primary visual cortices of higher mammals (ferrets, cats, or primates) and rodents, i.e., columnar organization versus salt-and-pepper structure, is paralleled by functional differences during development. These differences include the amount of visual experience before eye opening (through eyelids), the developmental time course of the maturation of orientation selectivity and direction selectivity, and the relative contributions of visual experience versus intrinsic factors. However, despite these differences during development, the mature visual cortex of mice preserves many fundamental properties of visual circuit function (Ohki et al., 2005; Niell and Stryker, 2008). A detailed comparison and evaluation of these differences may be critical for a better understanding of visual information processing in the mammalian visual system.

EXPERIMENTAL PROCEDURES

Animals and Surgery

All experimental procedures were performed in accordance with institutional animal welfare guidelines and were approved by the government of Bavaria, Germany. C57BL/6 mice were either reared in 12 hr/12 hr light/dark cycles (P10–P12, n = 5; P13–P15, n = 20; P15–P16, n = 7; P26–P30, n = 10; P57–P79, n = 7) or born and reared in complete darkness (P13–P15, n = 12; P15–P17, n = 10; P26–P30, n = 9). The day of birth (P0) was accurately ascertained as was the day of eye opening. For this, the eyes were checked four times per day (at 8 am, 1 pm, 6 pm, and 8 pm) beginning at the age of P10 and the eyes were considered opened as soon as we observed the initial break in the membrane sealing the eyelids. Strips of Ilford-FP4 plus 125 film were attached to the wall of the dark-rearing room and then developed to confirm that the films (and the mice) had not been exposed to light.

Animals were prepared for *in vivo* two-photon calcium imaging as described previously (Stosiek et al., 2003; see Supplemental Information). Ophthalmic ointment (Bepanthen, Bayer) was applied to both eyes to prevent dehydration during surgery. After surgery, the level of anesthetic was decreased to 0.8% isoflurane for recordings (breathing rate: 110–130 breaths/min). For dark-reared animals, the surgery was done under red light and the eyes were covered with an opaque eye cream and a black cone. The cone and the cream were removed just before (around 2–3 min) starting the recordings.

High-Speed Two-Photon Ca²⁺ Imaging

In vivo calcium imaging was performed by using a custom-built two-photon microscope based on a Ti:Sapphire pulsing laser (model: Chameleon; repetition rate: 80 MHz; pulse width: 140 fs; Coherent) and resonant galvo/mirror (8 kHz; GSI Group Inc.) system (Sanderson and Parker, 2003). The scanner was mounted on an upright microscope (BX51WI, Olympus, Tokyo, Japan) equipped with a water-immersion objective (60×, 1.0 NA, Nikon, Japan or 40×/0.8, Nikon, Japan). Emitted photons were detected by photomultiplier tubes (H7422-40; Hamamatsu). Full-frame images at 480 × 400 pixels resolution were acquired at 30 Hz by custom-programmed software written in LabVIEW™ (version 8.2; National Instruments). At each focal plane, we imaged spontaneous activity for at least 4 min and visually evoked activity for 6 to 10 trials.

Visual Stimulation

Visual stimuli were generated in Matlab™ (release 2007b; Mathworks Inc.) by using the Psychophysics Toolbox (<http://psychtoolbox.org/wikka.php?wakka=HomePage>). Visual stimuli were projected onto a screen placed 30 cm from the contralateral eye, covering 80° × 67° of the visual field. Each trial of visual stimulation started with a gray screen (mean luminance) for 5 s, followed by a stationary square-wave grating for 5 s and the corresponding drifting grating for 5 s (0.03 cpd, 1 Hz, 8 directions, contrast 98%, mean luminance 19.1 cd/m²). At each focal plane, evoked activity was imaged during 6–10 trials. See Supplemental Information for more details.

Data Analysis

Image analysis was performed offline in two steps. First, the software ImageJ (<http://rsb.info.nih.gov/ij/>) was used to draw regions of interest (ROIs) around cell bodies and around a large area of cell-free neuropil. In the next step, custom-made routines written in Igor Pro (Wavemetrics, Lake Oswego, OR) were used for the detection of wave-associated calcium transients in individual neurons. Calcium signals were expressed as relative fluorescence changes ($\Delta f/f$) corresponding to the mean fluorescence from all pixels within specified ROIs. For each ROI, a transient was accepted as a signal when its amplitude was greater than three times the standard deviation of the noise band. After the automatic analysis, all traces were carefully inspected. Neurons were defined as responsive to moving gratings when their activity during the presentation of at least one of the eight directions was significantly higher than their activity during the interstimuli period (ANOVA test). The activity was evaluated by the integral of the calcium transients. An OSI (e.g., Niell and Stryker, 2008) was calculated in order to quantify the tuning level of the neurons with regard to the orientation of the drifting grating. The OSI was defined as $(R_{\text{pref}} - R_{\text{ortho}}) / (R_{\text{pref}} + R_{\text{ortho}})$, where R_{pref} , the response in the preferred orientation, was the response with the largest magnitude. R_{pref} was determined as the mean of the integrals of the calcium transients for the two corresponding opposite directions. R_{ortho} was similarly calculated as the response evoked by the orthogonal orientation. With this index, perfect orientation selectivity would give OSI = 1, an equal response to all orientations would have OSI = 0, and 3:1 selectivity corresponds to OSI = 0.5. Highly and poorly tuned neurons were defined as neurons with an OSI > 0.5 and OSI < 0.5, respectively. Similarly, a DSI was defined as $(R_{\text{pref}} - R_{\text{opp}}) / (R_{\text{pref}} + R_{\text{opp}})$, where R_{opp} is the response in the direction opposite to the preferred direction.

Statistical Analysis

The following values were compared between normally reared and dark-reared mice and between different age groups, by using a Mann-Whitney test with a two-tailed level of significance set at $\alpha = 0.05$ (SPSS 16.0 software): percentage of neurons responding to drifting gratings, cumulative distributions of OSI and DSI, OSI and DSI mean values.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and nine figures and can be found with this article online at doi:10.1016/j.neuron.2011.06.013.

ACKNOWLEDGMENTS

We thank Jia Lou for excellent technical assistance. This study was supported by grants of the Deutsche Forschungsgemeinschaft to A.K. and by the Friedrich Schiedel Foundation. A.K. is a Carl von Linde Senior Fellow of the Institute for Advanced Study of the Technische Universität München. N.L.R. was supported by the DFG (IRTG 1373). M.N. was supported by the Japan Society for the Promotion of Science Postdoctoral Fellowships for Research Abroad.

Accepted: June 8, 2011

Published: August 10, 2011

REFERENCES

- Benevento, L.A., Bakkum, B.W., Port, J.D., and Cohen, R.S. (1992). The effects of dark-rearing on the electrophysiology of the rat visual cortex. *Brain Res.* 572, 198–207.
- Blasdel, G.G., and Salama, G. (1986). Voltage-sensitive dyes reveal a modular organization in monkey striate cortex. *Nature* 321, 579–585.
- Bonhoeffer, T., and Grinvald, A. (1991). Iso-orientation domains in cat visual cortex are arranged in pinwheel-like patterns. *Nature* 353, 429–431.
- Bosking, W.H., Zhang, Y., Schofield, B., and Fitzpatrick, D. (1997). Orientation selectivity and the arrangement of horizontal connections in tree shrew striate cortex. *J. Neurosci.* 17, 2112–2127.

- Cang, J., Rentería, R.C., Kaneko, M., Liu, X., Copenhagen, D.R., and Stryker, M.P. (2005). Development of precise maps in visual cortex requires patterned spontaneous activity in the retina. *Neuron* 48, 797–809.
- Chen, M., Weng, S., Deng, Q., Xu, Z., and He, S. (2009). Physiological properties of direction-selective ganglion cells in early postnatal and adult mouse retina. *J. Physiol.* 587, 819–828.
- Colonnese, M.T., Kaminska, A., Minlebaev, M., Milh, M., Bloem, B., Lescure, S., Moriette, G., Chiron, C., Ben-Ari, Y., and Khazipov, R. (2010). A conserved switch in sensory processing prepares developing neocortex for vision. *Neuron* 67, 480–498.
- Crair, M.C., Gillespie, D.C., and Stryker, M.P. (1998). The role of visual experience in the development of columns in cat visual cortex. *Science* 279, 566–570.
- Dräger, U.C. (1975). Receptive fields of single cells and topography in mouse visual cortex. *J. Comp. Neurol.* 160, 269–290.
- Elstrott, J., and Feller, M.B. (2009). Vision and the establishment of direction-selectivity: a tale of two circuits. *Curr. Opin. Neurobiol.* 19, 293–297.
- Elstrott, J., Anishchenko, A., Greschner, M., Sher, A., Litke, A.M., Chichilnisky, E.J., and Feller, M.B. (2008). Direction selectivity in the retina is established independent of visual experience and cholinergic retinal waves. *Neuron* 58, 499–506.
- Fagiolini, M., Pizzorusso, T., Berardi, N., Domenici, L., and Maffei, L. (1994). Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark rearing and monocular deprivation. *Vision Res.* 34, 709–720.
- Fagiolini, M., Katagiri, H., Miyamoto, H., Mori, H., Grant, S.G., Mishina, M., and Hensch, T.K. (2003). Separable features of visual cortical plasticity revealed by N-methyl-D-aspartate receptor 2A signaling. *Proc. Natl. Acad. Sci. USA* 100, 2854–2859.
- Fagiolini, M., Fritschy, J.M., Löw, K., Möhler, H., Rudolph, U., and Hensch, T.K. (2004). Specific GABA circuits for visual cortical plasticity. *Science* 303, 1681–1683.
- Fortin, S., Chabli, A., Dumont, I., Shumikhina, S., Itaya, S.K., and Molotchnikoff, S. (1999). Maturation of visual receptive field properties in the rat superior colliculus. *Brain Res. Dev. Brain Res.* 112, 55–64.
- Frégnac, Y., and Imbert, M. (1978). Early development of visual cortical cells in normal and dark-reared kittens: relationship between orientation selectivity and ocular dominance. *J. Physiol.* 278, 27–44.
- Grubb, M.S., and Thompson, I.D. (2003). Quantitative characterization of visual response properties in the mouse dorsal lateral geniculate nucleus. *J. Neurophysiol.* 90, 3594–3607.
- Hubel, D.H. (1959). Single unit activity in striate cortex of unrestrained cats. *J. Physiol.* 147, 226–238.
- Hubel, D.H., and Wiesel, T.N. (1959). Receptive fields of single neurones in the cat's striate cortex. *J. Physiol.* 148, 574–591.
- Hubel, D.H., and Wiesel, T.N. (1962). Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *J. Physiol.* 160, 106–154.
- Hubel, D.H., and Wiesel, T.N. (1968). Receptive fields and functional architecture of monkey striate cortex. *J. Physiol.* 195, 215–243.
- Huberman, A.D., Wei, W., Elstrott, J., Stafford, B.K., Feller, M.B., and Barres, B.A. (2009). Genetic identification of an On-Off direction-selective retinal ganglion cell subtype reveals a layer-specific subcortical map of posterior motion. *Neuron* 62, 327–334.
- Iwai, Y., Fagiolini, M., Obata, K., and Hensch, T.K. (2003). Rapid critical period induction by tonic inhibition in visual cortex. *J. Neurosci.* 23, 6695–6702.
- Kerlin, A.M., Andermann, M.L., Berezovskii, V.K., and Reid, R.C. (2010). Broadly tuned response properties of diverse inhibitory neuron subtypes in mouse visual cortex. *Neuron* 67, 858–871.
- Krug, K., Akerman, C.J., and Thompson, I.D. (2001). Responses of neurons in neonatal cortex and thalamus to patterned visual stimulation through the naturally closed lids. *J. Neurophysiol.* 85, 1436–1443.
- Levick, W.R., and Thibos, L.N. (1980). Orientation bias of cat retinal ganglion cells. *Nature* 286, 389–390.
- Li, Y., Fitzpatrick, D., and White, L.E. (2006). The development of direction selectivity in ferret visual cortex requires early visual experience. *Nat. Neurosci.* 9, 676–681.
- Li, Y., Van Hooser, S.D., Mazurek, M., White, L.E., and Fitzpatrick, D. (2008). Experience with moving visual stimuli drives the early development of cortical direction selectivity. *Nature* 456, 952–956.
- Métin, C., Godement, P., and Imbert, M. (1988). The primary visual cortex in the mouse: receptive field properties and functional organization. *Exp. Brain Res.* 69, 594–612.
- Niell, C.M., and Stryker, M.P. (2008). Highly selective receptive fields in mouse visual cortex. *J. Neurosci.* 28, 7520–7536.
- Ohki, K., Chung, S., Ch'ng, Y.H., Kara, P., and Reid, R.C. (2005). Functional imaging with cellular resolution reveals precise micro-architecture in visual cortex. *Nature* 433, 597–603.
- Rochefort, N.L., Garaschuk, O., Milos, R.I., Narushima, M., Marandi, N., Pichler, B., Kovalchuk, Y., and Konnerth, A. (2009). Sparsification of neuronal activity in the visual cortex at eye-opening. *Proc. Natl. Acad. Sci. USA* 106, 15049–15054.
- Runyan, C.A., Schummers, J., Van Wart, A., Kuhlman, S.J., Wilson, N.R., Huang, Z.J., and Sur, M. (2010). Response features of parvalbumin-expressing interneurons suggest precise roles for subtypes of inhibition in visual cortex. *Neuron* 67, 847–857.
- Sanderson, M.J., and Parker, I. (2003). Video-rate confocal microscopy. *Methods Enzymol.* 360, 447–481.
- Shou, T., Leventhal, A.G., Thompson, K.G., and Zhou, Y. (1995). Direction biases of X and Y type retinal ganglion cells in the cat. *J. Neurophysiol.* 73, 1414–1421.
- Sohya, K., Kameyama, K., Yanagawa, Y., Obata, K., and Tsumoto, T. (2007). GABAergic neurons are less selective to stimulus orientation than excitatory neurons in layer II/III of visual cortex, as revealed by in vivo functional Ca²⁺ imaging in transgenic mice. *J. Neurosci.* 27, 2145–2149.
- Stosiek, C., Garaschuk, O., Holthoff, K., and Konnerth, A. (2003). In vivo two-photon calcium imaging of neuronal networks. *Proc. Natl. Acad. Sci. USA* 100, 7319–7324.
- Syken, J., Grandpre, T., Kanold, P.O., and Shatz, C.J. (2006). PirB restricts ocular-dominance plasticity in visual cortex. *Science* 313, 1795–1800.
- Thompson, K.G., Zhou, Y., and Leventhal, A.G. (1994). Direction-sensitive X and Y cells within the A laminae of the cat's LGNd. *Vis. Neurosci.* 11, 927–938.
- Tian, N., and Copenhagen, D.R. (2003). Visual stimulation is required for refinement of ON and OFF pathways in postnatal retina. *Neuron* 39, 85–96.
- Van Hooser, S.D., Heimel, J.A., Chung, S., Nelson, S.B., and Toth, L.J. (2005). Orientation selectivity without orientation maps in visual cortex of a highly visual mammal. *J. Neurosci.* 25, 19–28.
- Vidyasagar, T.R., and Urbas, J.V. (1982). Orientation sensitivity of cat LGN neurones with and without inputs from visual cortical areas 17 and 18. *Exp. Brain Res.* 46, 157–169.
- Wang, B.S., Sarnaik, R., and Cang, J. (2010). Critical period plasticity matches binocular orientation preference in the visual cortex. *Neuron* 65, 246–256.
- White, L.E., and Fitzpatrick, D. (2007). Vision and cortical map development. *Neuron* 56, 327–338.
- Xu, X., Ichida, J., Shostak, Y., Bonds, A.B., and Casagrande, V.A. (2002). Are primate lateral geniculate nucleus (LGN) cells really sensitive to orientation or direction? *Vis. Neurosci.* 19, 97–108.
- Yonehara, K., Ishikane, H., Sakuta, H., Shintani, T., Nakamura-Yonehara, K., Kamiji, N.L., Usui, S., and Noda, M. (2009). Identification of retinal ganglion cells and their projections involved in central transmission of information about upward and downward image motion. *PLoS ONE* 4, e4320.
- Zariwala, H.A., Madisen, L., Ahrens, K.F., Bernard, A., Lein, E.S., Jones, A.R., and Zeng, H. (2011). Visual tuning properties of genetically identified layer 2/3 neuronal types in the primary visual cortex of cre-transgenic mice. *Front Syst Neurosci* 4, 162.