Responses of Neurons in Neonatal Cortex and Thalamus to Patterned Visual Stimulation Through the Naturally Closed Lids

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Krug, Kristine, Colin J. Akerman, and Ian D. Thompson. Responses of neurons in neonatal cortex and thalamus to patterned visual stimulation through the naturally closed lids. J Neurophysiol 85: 1436–1443, 2001. In studies of the developing mammalian visual system, it has been axiomatic that visual experience begins with eye-opening. Any role for neuronal activity earlier in development has been attributed to the patterned spontaneous activity found in retina and lateral geniculate nucleus (LGN). Here we show that, as early as 2 wk before eye-opening, visual stimuli presented through the closed eyelids can drive neuronal activity in LGN and striate cortex of the ferret. At this age, spontaneous activity in cortex is much lower than in LGN, and the visual responses of many cortical, but not geniculate, neurons depend on the orientation of a moving grating. Furthermore, the selectivity of cortical neurons to the orientation of gratings presented through the closed eyelids improves with age. Thus neuronal activity patterned by visual experience, rather than by spontaneous retinal activity, is present in visual cortex much earlier than previously thought. This could have important implications for the self-organization of visual cortex.

INTRODUCTION

One of the most intriguing issues in the development of the visual system is the balance between genetic programming and adaptation to early experience. Of particular interest has been the source of the information required to establish precise connections between neurons. In this context, one area of investigation has been the role of neuronal activity—patterned either by sensory experience or by intrinsic mechanisms. For instance, changes in visual experience after eye-opening can disrupt the framework of orientation columns and clustered horizontal connections in visual cortex, which is established in the period before and immediately after natural eye-opening (Chapman and Stryker 1993; Chapman et al. 1996; Durack and Katz 1996; Löwel and Singer 1992; Ruthazer and Stryker 1996; Sengpiel et al. 1999). Since orientation-tuned neurons can be found in visual cortex at eye-opening, or even earlier if the eyes are artificially opened (Blakemore and Van Sluyters 1975; Chapman and Stryker 1993; Hubel and Wiesel 1963), and orientation columns appear shortly afterward (Chapman et al. 1996; Gödecke and Bonhoeffer 1996; Thompson et al. 1983), it has been suggested that the basic architecture for orientation selectivity must develop independently of visual experience. However, experimental evidence does imply a role for neuronal activity prior to the time of eye-opening. Electrical stimulation of the optic nerve, cortical infusion of tetrodotoxin, or blockade of on-center retinal ganglion cell activity all disrupt the development of orientation tuning (Chapman and Gödecke 2000; Chapman and Stryker 1993; Weliky and Katz 1997).

One interpretation of these data is that the early manipulations are effective because they disrupt the intrinsic, spontaneous activity in the visual system. The nature of spontaneous activity in the neonatal ferret retina has been well characterized and displays two patterns (Wong and Oakley 1996; Wong et al. 1993; see Wong 1999). In very young neonates, waves of correlated activity spread slowly across regions of retina—a pattern that would be appropriate for refinement of retinotopic maps or segregation of inputs from the two eyes. In older ferrets, from about 2 wk before eye-opening, the correlations are much more localized spatially and are also ganglion cell class-specific—a pattern that may be more appropriate for the formation of cortical orientation tuning (see Miller et al. 1999). Indeed, this later pattern of retinal activity propagates into the lateral geniculate nucleus (LGN), where the statistics of firing resemble, but are not identical to, those in the retina (Weliky and Katz 1999). However, it is not known whether subcortical spontaneous activity can drive cortical neurons nor is it certain whether the patterning of neuronal activity in the visual system before eye-opening occurs only via intrinsic mechanisms.

Given indications that light passing through the lids may stimulate the visual system before eye-opening and after lid suture in kittens (Eysel et al. 1979; Huttenlocher 1967; Spear et al. 1978) and that ferret cortical neurons are responsive to visual stimuli if the eyelids are parted prematurely (Chapman and Stryker 1993), we examined whether geniculate and cortical neurons in neonatal ferrets respond to visual stimuli presented to the still closed eyes. And if so, how much information about the visual stimulus is encoded in the early spatiotemporal patterns of neuronal activity? We also directly compared spontaneous and visually driven neuronal activity in both cortex and LGN and examined the developmental changes in the stimulus selectivity of cortical neurons. Some of these results have been published in abstract form (Krug and Thompson 1997, 1998).

METHODS

Animals

Pigmented ferret kits from time-mated females (Marshall Farms, New Rose, NY; Glaxo, UK; University Laboratory of Physiology,
Oxford, UK) were studied between postnatal day 21 (P21) and P24 (n = 10), between P19 and P20 (n = 4), and between P29 and P32 (n = 3) for the cortical recordings. The geniculate data were collected from four ferret kits between P21 and P22. All ferret kits had firmly closed eyes at and during the time of recording.

**Surgery and anesthesia**

Anesthesia was induced with alphaxalone 0.9% and alphadalone acetate 0.3% (Saffan, Pitman-Moore, Uxbridge, UK; 1.5 ml/kg im). This was followed by 0.1 ml of atropine sulfate (BP 0.6 mg/ml; C-Vet, Leylands, UK) intraperitoneally or subcutaneously. During the surgery, anesthesia was maintained by intermittent intravenous administration of Saffan (diluted 1:2 in 0.9% saline). The trachea was usually intubated through the mouth; in some animals, however, a tracheotomy was performed, and the tube was inserted directly into the trachea. For cortical recordings, craniotomies were made roughly 1 mm rostral and 7 mm lateral of lambda in one or both hemispheres. For LGN recordings, larger craniotomies were made 8–9 mm rostral and 4–5 mm lateral of lambda. During recordings, anesthesia was maintained by a continuous infusion with a solution of medetomidine (Domitor, SmithKlineBeecham, Surrey, UK; 11–44 µg · kg⁻¹ · h⁻¹) and ketamine (KetaSet, Willows Francis Veterinary, UK; 2.5–10 mg · kg⁻¹ · h⁻¹) in 0.9% sterile saline, and the animal was paralyzed with gallamine tri-ethiodide (Sigma, 10 mg · kg⁻¹ · h⁻¹). To monitor anesthetic state, ECG and FCO₂ were continuously recorded during the experiment. In addition, control experiments performed without paralysis confirmed that the anesthetic regime was stable and adequate.

**Recordings**

Action potentials were recorded extracellularly with glass-coated tungsten microelectrodes with a 5-µm exposed tip. The majority of cortical units were recorded 350 µm or more beneath the pial surface—corresponding approximately to layers IV–VI. Visual stimuli were presented on a display screen that covered up to 49° of central visual space. Full-screen, high-contrast (90–100% Michelson contrast) squarewave gratings of low spatial frequency (0.01–0.03 cycles/°) and low temporal frequency (0.25 cycles/s) were displayed. For recordings in P19–P20 ferret kits, the Michelson contrast was high enough to force the animals to open their eyes. The trials were repeated until the eyelids were wide open. Visual stimuli were usually displayed to both eyes, but in a small number of experiments, monocular stimulation of the contralateral eye was used.

**Analysis**

The orientation selectivity index (OSI) assesses the shape of the orientation tuning curve according to the amplitude of the second harmonic (Chapman and Stryker 1993; Wörgötter and Eysel 1987). First, we subtracted the spontaneous rate from the average firing rate at each orientation. Then the amplitude of the second harmonic (A2) of the tuning curve was extracted by fast Fourier transform (Excel, Version 7.0a for Windows 95) and normalized by division through itself and the average firing rate (A0)

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\text{OSI} = \frac{(A2/(A0 + A2)) \times 100}{\text{minimum, 0 – 4 cdm}^2; \text{maximum, 77 – 80 cdm}^2} \text{was comparable to the background luminance measured in the animal house. In the animals' nest boxes, we measured 0.4 – 1.4 cdm}^2 \text{reflections of the walls, 24 cdm}^2 \text{toward the entrance hole, and 62 cdm}^2 \text{with the box lid open. In the cages, the reflectances of the walls were 2 – 48 cdm}^2, \text{and the front of the cage measured 4 – 89 cdm}^2 \text{(light meter horizontal or pointing down) and over 342 cdm}^2 \text{if angled upward.}

Gratings were displayed in four different orientations each moving in two directions. Additionally, a uniform display of zero contrast and identical mean luminance was used to record spontaneous activity. In a typical experiment, all nine stimuli were shown in a pseudo-randomized order, mostly each grating in blocks of five cycles (in few experiments blocks of 10 or 20 grating cycles were used). When each had been presented, the sequence was re-randomized, and the procedure was repeated between three and seven times. The stimuli were always displayed to the unopened eyelids. They were usually presented to both eyes, but in a small number of experiments, monocular stimulation of the contralateral eye was used.

**Results**

Ferret kits were studied between P21 and P24—their eyes normally open around P32. Single-unit recordings were made in primary visual cortex while the eyelids remained closed. Visually responsive neurons in cortex were first identified by their responses to an intense flashed light: a total of 86 single units were recorded in 10 ferrets. Qualitatively, their responses were sluggish with long latencies and, often, long intervals were required between stimulation to elicit reliable responses. All 86 units were subsequently tested with drifting squarewave gratings presented on a display screen in front of the animal. Fifty-eight units (67%) exhibited significant visual driving when presented with a particular oriented grating rather than a blank screen of matched mean luminance (Mann-Whitney U test, P < 0.05). These units that responded to patterned visual stimuli presented to the closed eyelids were then analyzed further.

Of the 58 neurons, a considerable number showed differential responses to moving gratings of varied orientations. The tuning curves of three neurons are illustrated in Fig. 1. The tuning curve in Fig. 1A is from a cell which responded to almost all orientations, but was biased to gratings at 45° and 225°. The unit in Fig. 1B showed a clear preference for horizontal gratings with other orientations not able to stimulate the neuron. The third neuron was strongly selective for both the orientation and the direction of the drifting grating (Fig. 1C). The corresponding spike rasters beneath each of the tuning curves reveal that in all three examples, despite varying firing and failure rates, the timing of the spikes was locked to particular phases of the grating. Overall, 86% of the neurons displayed clear temporal modulation of their responses. In 36% of these, two distinct clusters of spikes, roughly half a cycle apart, could be seen (as for example in Fig. 1B). The remaining 64% showed one peak only. The mean firing rate to the best stimulus was on average only 2.07 spikes per cycle (SE = 0.33, n = 58), but the mean spontaneous activity was also very low at 0.19 spikes per cycle (SE = 0.04, n = 58). This responsiveness of the visually driven neurons was much less than that in the adult (Baker et al. 1998). However, for the immature cortical neurons, mean firing rate may be misleading. Many presentations of the optimal stimulus did not lead to a response, but when neurons responded, they often did so strongly. For instance, a high failure rate (87% of the trials) is seen for the neuron illustrated in Fig. 1C. Across all the trials, this neuron had an average peak firing rate of 0.53 spikes per
cycle, but within a single trial it responded with up to 7 spikes per cycle.

Having established that visual responses in cortex could be elicited through unopened eyelids in P21–P24 ferrets, the extent of neuronal selectivity to drifting gratings of differing orientations was quantified in two ways. A Kruskal-Wallis test of the 58 neurons with significant visual responses revealed that 22 neurons (38%) showed a significant variation in their spike rate with stimulus orientation ($P < 0.05$; for example Fig. 1, B and C). However, these statistics reveal little about the shape of the tuning curve. Therefore the neurons were also ranked on the orientation selectivity index (OSI) (Chapman and Stryker 1993; Wörgötter and Eysel 1987) (see METHODS).

The unit in Fig. 1B, for instance, has a high OSI of 60, reflecting the fact that the tuning curve has two narrow peaks 180° out of phase. Using the OSI to visualize the distribution of tuning to oriented gratings in our sample of neurons, we find that the whole population covers a wide range of OSI values (Fig. 2), ranging from 4 (nonselective) to 65 (highly selective). The examples surrounding the central histogram in Fig. 2 show tuning curves from different parts of the distribution. Chapman and Stryker (1993) classify units with an OSI value of 25 and higher as orientation selective—79% of the units in Fig. 2 fall into this category. Taking the two measures together, of the 22 units passing the Kruskal-Wallis, all but four fulfill the OSI criterion for orientation selectivity. Different neurons were selective for the full range of orientations tested, suggesting that the tuning is not an artifact of optical filtering by the closed eyelids but is neuronal in origin.

**LGN responses through closed eyelids**

To further investigate the origin of the cortical neurons’ selectivity, we compared their responses to those of 46 geniculate neurons from animals of about the same age (P21–P22), again stimulating through the closed eyelids. Investigation of the responses to different oriented gratings revealed marked differences between geniculate and cortical responses. Figure 3 shows examples of orientation tuning curves, together with raster plots at the “best” orientation, for three different LGN neurons. Compared with the cortical data in Fig. 1, the most striking feature of the tuning curves is that the geniculate neurons respond reliably at all orientations. The cells in Fig. 3 are characteristic of the population in showing a stimulus-locked, monophasic response, although the width of the response window varied from cell to cell. Each cycle of the grating typically resulted in a burst of several action potentials lasting under 0.5 s. The geniculate neurons depicted in Fig. 3,
spikes per cycle (SE = 0.04, n = 58; Mann-Whitney U test P < 0.001): with a blank display, a geniculate action potential occurred on average once every 5 s, whereas a cortical action potential occurred only once every 20 s.

The lack of selectivity of geniculate cells to the orientation of the drifting grating illustrated in Fig. 3 was confirmed across the population. Our sample of 46 neurons had a mean OSI score of just 21 (SE = 1.43), only 15 neurons (33%) had an OSI of 25 or higher and none had a value above 50. Figure 4 shows the difference in OSI distributions between cortical and geniculate neurons at around 3 wk of age. As described in the preceding text, cortical neurons had a mean OSI of 38 (SE = 1.92, n = 58) with 79% of the population with an OSI of 25 or higher and 29% of 50 or higher. Thus cortical OSIs were clearly greater than OSIs obtained from LGN neurons in animals of similar ages (Mann-Whitney U test, P < 0.0005), strengthening our previous conclusion that the differential response of cortical neurons to the orientation of drifting gratings reflects a specialization of cortical circuitry.

Age-dependent changes in cortical selectivity

We next investigated whether there was any developmental change in the selectivity of cortical neurons for visual stimuli in the period before eye-opening. Recordings were made in animals younger than 3 wk of age, at P19–P20 (no visual responses could be elicited in LGN or optic nerve at P18) (C. J. Akerman, unpublished observations), and in older animals just before eye-opening, at P29–P32. The developmental trends are illustrated in Figs. 5 and 6.

At the earliest ages, it proved to be considerably more difficult to drive cortical neurons: in a total of four animals, only eight cortical cells could be shown to respond quantitatively to drifting gratings (Mann-Whitney U test, P < 0.05). The response rates of these neurons were low, failure rates were high, and phase-locking generally weak. The levels of spontaneous activity were also very low. Although the eight neurons responded to visual stimulation, their selectivity for the orientation of the gratings was limited. When ranked according to their OSI, there was very little difference between the 25th and 75th percentiles (see Fig. 5).

The selectivity of the cortical neurons quickly improves with age. Figure 5 includes additional examples from the P21–P24 cohort already described. The modulation of the response by this age was much tighter and failure rates for the optimal stimulus had fallen from a level of 66% (SE = 9.8, n = 6) at P19–P20 to 47% (SE = 3.1, n = 47) by P21–P24. Neurons recorded from animals just before eye-opening (P29–P32) showed the most robust response to drifting gratings. Response levels to the optimal stimulus were generally higher, and the failure rate had decreased to 32% (SE = 5.8, n = 13). The trend toward lower neuronal failure rates with age represented a significant change (Kruskal-Wallis, P < 0.01).

The neuronal selectivity to gratings of different orientations was analyzed quantitatively for the three age groups. The proportion of neurons showing a statistically significant variation in response with orientation (Kruskal-Wallis P < 0.05) was found to increase with age: from 0% at P19–P20 to 38% P21–P24 and 86% at P29–P32. The population distributions for different OSI groupings are shown in Fig. 6. Using the OSI criterion described in the preceding text, at P19–P20 only 38%
of the neurons were orientation selective (i.e., had an OSI \( \geq 25 \)) and none had an OSI \( \geq 50 \). By P21–P24, 79% of the neurons were orientation selective and 29% had a selectivity \( \geq 50 \). In the relatively small sample of cells at P29–P32, 36% had OSIs \( \geq 50 \) and 79% of the population were orientation selective. This distribution of cells among the three categories of orientation-selectivity represents a significant developmental trend (Kruskal-Wallis, \( P < 0.05 \)). It has already been demonstrated that a major improvement in orientation selectivity of cortical neurons takes place after eye-opening (Chapman and Stryker 1993). Our data imply that the emergence of a selective response to the orientation of drifting gratings before eye-opening is also a dynamic process.

**DISCUSSION**

The implication of our data is clear: visual experience can start before the eyes open. Almost 2 wk prior to eye-opening, the firing of neurons in the ferret visual system is modulated by drifting gratings presented to the closed lids. The important question is, what is the developmental significance of our observation: is it relevant to the generation of orientation tuning in cortical neurons and/or the organization of tuned neurons into orientation maps?

It might be argued that our stimuli, although conventional for the study of the neonatal visual system, are unnatural and fail to mimic the visual stimulation that ferret kits could experience at this stage. And this is the age when ferret kits begin to venture from the nest (Porter and Brown 1985). So what is the relation between our stimuli and “natural” visual stimuli? Although we have matched luminance to the ranges found in the animal house, the gratings are of high contrast and of low spatial and temporal frequency. The low spatial frequency is not a fundamental problem because square-wave stimuli contain multiple spatial frequencies and also because the statistics...
of natural scenes are dominated by low spatial frequencies (Field 1987). In fact, the spatiotemporal properties of our stimuli are not too different from those generated by the head movements of ferret kits. Videos of head movements reveal angular velocities in the range of 31–240°/s (C. J. Akerman, unpublished observations), and the velocity of the gratings was 8–25°/s. The concept of contrast in natural scenes is difficult and depends on the spatial scale at which it is measured (Tadmor and Tolhurst 1994). However, given the luminance range of the various surfaces in the animal house (see Methods), the head movements could generate temporal contrast ranging from 0 to 97%. For instance, a head movement across

![Comparison of selectivity of individual cortical neurons in P19–P20, P21–P24, and P29–P32 ferrets. Visually responsive neurons (all of which had passed the Mann-Whitney U test for visual stimulation at $P < 0.05$) were tested for orientation selectivity to stimuli presented through the closed eyelids at 3 different ages: P19–P20 ($n = 8$), P21–P24 ($n = 58$), and P29–P32 ($n = 14$). For each of the 3 ages, OSIs, tuning curves and raster plots are shown of the neurons at the 25th, 50th, and 75th percentile when ranked according to the OSI. The OSI scores and tuning curves reveal that cells with oriented responses were more common in older animals. Each percentile shows a trend of increasing OSI with age. For example, the OSI score for the 75th percentile increased with age from 26 first to 50 and then to 51 by P29–P32. The rasterplots demonstrate that failure rates decreased with increasing age before eye-opening.](http://jn.physiology.org/doi/10.1152/jn.00377.1616)
orientation selectivity (\(\Omega\)) to moving gratings (\(\text{ON} -\text{center vs. OFF} -\text{center}\)). Our results challenge the assumption that visual stimulation plays no role in patterning neuronal activity until the eyes have opened. We show that, in the 2 wk prior to eye-opening in the ferret, visual stimulation through the closed eyelids is able to pattern neuronal activity effectively in both LGN and cortex. At this age, spontaneous activity in the retina shows local correlations within and between different functional classes of neurons, but the spatially propagating waves of correlated spontaneous retinal activity have broken down (see Wong 1999).

Spontaneous retinal activity is relayed to the LGN and activates geniculate neurons (Mooney et al. 1996; Weliky and Katz 1999). There is also evidence for an intrinsic component to spontaneous activity in the LGN (McCormick et al. 1995). An in vivo study of spontaneous activity in the neonatal ferret LGN (Weliky and Katz 1999) showed that the statistics are broadly similar to those in the retina. Correlated activity between neurons of the same type (ON-center vs. OFF-center) is higher than between neurons of the opposite type, and correlations between neurons activated by the same eye are greater than that for neurons activated by different eyes. (Interestingly, inter-ocular correlations were only present when visual cortex was intact.) These multi-unit recordings showed spontaneous bursts of geniculate activity occurring about twice a minute, which is consistent with the level of spontaneous single-unit activity in our preparation. However, our finding is that visual stimulation induces a very different pattern of firing in the LGN. Not only is the mean level higher, but visually elicited bursts can occur much more frequently than twice a minute. The visually evoked activity is also temporally synchronised to the stimulus, and this should have significant implications for the patterns of correlated activity. We speculate that if convergent thalamic activity is necessary for cortical neurons to fire, local visual entrainment of geniculate neurons is the reason why stimulation through the eyelids appears much more effective at driving cortical cells than the spontaneous activity in the LGN.

Although we show that visual stimulation through the closed lids can pattern neuronal activity, we have not directly demonstrated whether this level of visual experience is important in normal development. However, the improvement in OSI with age seen in Figs. 5 and 6 indicates a possible role for through-the-lids experience. It will be interesting to see if dark rearing, prior to eye-opening, can affect the development of the visual system. Another important question pertaining to the emergence of orientation selectivity is to ask what circuitry underlies the earliest, relatively crude, orientation selectivity that is seen at P19–20: how different is receptive field structure at this age from that in more mature animals? Studies on the ferret should facilitate investigation of the relative contribution of spontaneous and of visually driven neuronal activity to the logical blockade of neuronal activity or electrical stimulation before eye-opening can disrupt the development of orientation tuning in the ferret (Chapman and Gödecke 2000; Weliky and Katz 1997). Normal patterned neuronal activity appears to be important for the establishment and improvement of orientation tuning both before and after eye-opening.
establishment and refinement of the circuits underlying orientation tuning in visual cortex.

Finally, the fact that closed eyelids are not necessarily a barrier to visual stimulation (a finding echoing earlier observations) (Eysel et al. 1979; Huttenlocher 1967; Spear et al. 1978) and the fact that orientation selectivity persists with the eyelids closed suggests a new interpretation of recent deprivation experiments. It is arguable that the failure of lid suture to disturb the early development of orientation maps (Craig et al. 1998; Gödecke and Bonhoeffer 1996) actually reflects the patterning of cortical activity that is possible with “closed eye” visual experience. The role of visual experience in the early development of visual cortex may have to be reconsidered.

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