Critical Period for the Monocular Deprivation Effect in Rats: Assessment With Sweep Visually Evoked Potentials

ERIC S. GUIRE, MARVIN E. LICKEY, AND BARBARA GORDON
Institute of Neuroscience, 1254 University of Oregon, Eugene, Oregon 97403

Guire, Eric S., Marvin E. Lickey, and Barbara Gordon. Critical period for the monocular deprivation effect in rats: assessment with sweep visually evoked potentials. J. Neurophysiol. 81: 121–128, 1999. Rats and mice are the species most frequently used for cellular and biochemical studies of plasticity, but only a few studies have examined developmentally regulated visual plasticity in these species. Here we report a study of the critical period for monocular deprivation in Long-Evans rats in which visual pattern sweep evoked potentials (sweep VEP) was used. Successful recording of sweep VEPs depended on establishing a stable light plane of anesthesia. We found a mixture of halothane and NO₂ to be suitable. During a single trial lasting 10 s, anesthetized rats (n = 28) viewed a sinusoidal contrast grating (spatial frequency of 0.13 cycles/deg) that reversed phase at 3 Hz. During the trial, the grating contrast increased logarithmically from 1 to 70%. Extracellular recording pipettes were placed bilaterally in layers II/III of the binocular regions of primary visual cortex. Stimulating the right and left eye on alternate trials, sweep VEP amplitudes were collected for 30 trials from each eye. In monocularly deprived animals, the right eyelid had been sutured for 5 days before recording. Age at suture varied from P19 to P86. In 12 of 13 rats sutured between P19 and P50, the crossed response from the deprived eye was smaller than the crossed response from the nondeprived eye. The same relation prevailed for the uncrossed responses in 11 of 13 animals. There was no significant monocular deprivation effect in animals sutured between P55 and P86 (n = 9). Dark rearing until approximately P90 followed by 5 days of eyelid suture resulted in a strong monocular deprivation effect in both crossed and uncrossed pathways (n = 3). There was little effect of dark rearing alone on the size the sweep VEPs (n = 3). The critical period reported here lasts at least 2 wk longer than reported for rats by Fagioliini et al. and for mice by Gordon and Stryker. Both previous studies used single unit recording rather than the sweep VEP method.

INTRODUCTION

The monocular deprivation effect pioneered by Wiesel and Hubel (1963) is the classic model system for studies of experience dependent remodeling in the cerebral cortex. Wiesel and Hubel found that if one eye is deprived of visual experience during infancy, most cells in the visual cortex fail to respond to that eye. Most studies of monocular deprivation have been performed in cats and monkeys because these higher mammals are thought to have visual capacities similar to humans. For studies of cortical development at the cellular level, however, rodents are superior to cats and monkeys. We know more about the biochemistry, genetics, neural development, and synaptic physiology of the rat brain than we know about the cat and monkey brain. Rodents are also less expensive and have a shorter generation time. Therefore research on the cellular mechanisms of experience dependent plasticity in visual cortex will benefit from precise information about the extent and timing of visual plasticity in rodents. We chose to use rats rather than mice because of their larger size. We chose pigmented Long Evans rats because of their superior vision relative to albino rats.

There was one recent study of the critical period for monocular deprivation in rats. Fagioliini et al. (1994) recorded from single units in the primary visual cortex and assessed the effect of monocular deprivation on the development of visual receptive fields. They found a monocular deprivation effect following 10 days of eyelid closure from P23 to P33. In a study on mice, Gordon and Stryker (1996) observed a monocular deprivation effect with 4 days of lid closure from P29 to P32, and a smaller effect with closure from P23 to P28; they observed no monocular deprivation effect with closure from P32 to P36 or later. By measuring the visual response with the visual pattern sweep evoked potentials (sweep VEP), we observed robust visual plasticity at later ages than those examined in previous studies. Furthermore, we were unable to detect a decrease in the monocular deprivation effect in animals deprived prior to P28. Because Gordon and Stryker studied mice, not rats, their results may not be comparable with ours or with those of Fagioliini et al.

We recorded the cortical field potential evoked by a pattern stimulus. Recording electrodes were placed in the binocular portion of the primary visual cortex (V1) bilaterally, where they picked up the summed currents from a population of cortical neurons that receive competing inputs from both eyes. With this technique one can efficiently evaluate the monocular deprivation effect in a single animal in a few hours. A special advantage is that this technique is used extensively in humans, and it is known that the contrast threshold as measured with sweep VEPs is quite similar to the psychophysical threshold (Allen et al. 1986).

METHODS

Animals

We used 22 normally reared and 6 dark reared Long-Evans rats. All procedures were approved by the University of Oregon Institutional Animal Care and Use Committee. Normally reared rats were on a L:D 12:12 h light cycle (L:D). The LD rats were given monocular deprivation by eyelid suture for 5 days before recording. The sutures were performed at various ages between P19 and P86. The age of LD animals is reported as postnatal days at the time of suture. We obtained recordings from six more rats after they were reared in continuous darkness (DD) from birth to about 90 days. The mothers of dark reared rats were transferred to continuous darkness 2 days before delivery. Photographic films (both T-MAX 400 and TMZ p3200) were left in the dark room...
FIG. 1. Relation between EEG and evoked response on 2 individual trials. Left: EEG tracings obtained concurrently with binocular presentation of the reversing grating. Right: analyses of the tracings. Graphs: solid line = signal (amplitude of 6 Hz component); disconnected circles = noise, (the average amplitude of the 4 and 8 Hz components); dashed line = average noise pooling all values of contrast; function at the bottom of each graph is the phase difference between the 6 Hz EEG component and the grating reversal. A physiological response to the grating is recognized when the signal rises consistently above the noise and the phase difference becomes constant. When the concurrent EEG included slow waves and lacked components above 10 Hz, the response was weak or absent, as in Trial 68. Horizontal scale bar = 1 s. Vertical scale bar = 250 mV.

Eye suture

Under isoflurane anesthesia, the left eye was protected from desiccation with Tear-Gel (Ciba Vision), and the area around the right eye was cleaned with isopropyl alcohol and Betadine. Using a surgical microscope and sterile technique, the lids margins were trimmed and closed with three or four horizontal mattress sutures, using 6/0 or 7/0 surgical silk. A final layer of tissue adhesive (Vetbond) strengthened the lid closure. Ophthalmic antibiotic ointment was applied. During surgery, rectal temperature was monitored and maintained at 37–38°C by a heating pad under the animal. Dark reared rats were anesthetized for eye suturing before light exposure. Rats were inspected daily for general good health, signs of discomfort, and suture integrity.

Preparation for recording

We induced anesthesia with 3% halothane carried in 65% NO2–35% O2. We switched to 1.5% halothane administered through a nose cone, performed a tracheotomy, and inserted an endotracheal tube. Thereafter respiration was controlled by a pump operating at 70–160 breaths/min and providing a gas flow of 200–1,000 ml/min. The pumping rate and stroke volume were adjusted to match the respiratory movements of the anesthetized rat. Small rats required much less gas than large rats. The corneas were protected with Tear-Gel (applied every 2 h), and 27 gauge needles were inserted subcutaneously into the right rear and left front legs to continuously monitor the EKG on an oscilloscope and loudspeaker. The rat rested on a water circulating heating pad adjusted to maintain rectal temperature at 37.5 ± 0.5°C. The rats were slightly hyperventilated, and CO2 was added to the inspired gas to produce 4.5% CO2 in the expired gas. About once per hour we injected saline subcutaneously, not exceeding 7 ml kg⁻¹ h⁻¹.

The rats were held in a stereotaxic instrument modified to permit full field vision. Using a surgical microscope, we made craniotomies ~1.5 mm diam over the binocular portion of the primary visual cortex of both hemispheres using a Dremel tool. Care was taken to avoid touching the brain or causing it to bleed. Continuous superfusion with saline prevented the cortex from drying. After removal of the dura, electrodes were positioned 300–400 μm anterior to the lambda suture and 4.2–4.8 mm lateral to the midline, depending on the size of the skull; the smaller skulls of younger rats were accommodated by proportional adjustments of coordinates. Injection of dexamethasone (0.125 mg · kg⁻¹ · h⁻¹) controlled edema.

We opened the sutured eye, rinsed it with ophthalmic saline, and treated it with Tear-Gel. To keep the eye lids open and the eyes in their forward position, two Minuten pins (Carolina Biological) were inserted through the conjunctiva at the point where it joins the sclera. One pin was positioned above the pupil and the other below so that the pupils were bilaterally symmetric and not dilated. Both pupils were visible from in front of the rat and were not obviously misaligned. Finally, the cortex was penetrated with the recording pipette, taking care to avoid rupture of visible blood vessels.

Recording

We used Ag-AgCl2 electrodes in glass pipettes containing 0.9% NaCl. The electrodes had a tip diameter of ~8 μm and an impedance
FIG. 2. Contrasts sensitivity of the 4 pathways of a single P37 rat averaged more than 30 trials for each eye. Each pathway shows the 30-trial average signal (●) or noise (■) at each value of contrast. Crossed pathways produce greater signal relative to the noise than the uncrossed pathways. Nondeprived pathways produced a greater signal to noise ratio than did the corresponding deprived pathways.

Visual stimulus

The stimulus was a sinusoidal contrast grating of constant luminance displayed on a 19 in. video monitor, 30 cm in front of the eyes. Exactly 10 cycles of the grating appeared on the screen yielding a spatial frequency of 0.13 cycles per degree. The grating reversed phase 6 times per second (3 Hz). The stimulus was presented in a series of 10 s trials; in each trial, the grating contrast started at 1% and increased logarithmically to 70%. Sixty trials were run, switching eyes after each trial. Twenty seconds elapsed between trials. The interleaving of right and left eye stimulation controlled for minor, unnoticed variations of anesthesia state. The experiment was temporarily halted if the state of anesthesia noticeably deteriorated. Deterioration was indicated by a decrease in the high-frequency component of the cortical EEG or an increase in high-amplitude, low frequency components.

Data collection

Responses were recorded simultaneously from both hemispheres using two symmetrically placed pipettes. Prior to each experiment, electrode placement in the binocular portion of primary visual cortex was verified by recording the sweep VEP of the uncrossed pathway of the nondeprived eye (left cortex, left eye). If a response was not obtained, the electrodes were repositioned. Relocation of the recording pipette was always matched stereotaxically by a mirror symmetric relocation on the opposite side. In dark reared animals both eyes were deprived, and this preliminary test could not be applied. To ensure that the ocular occluders were effective, several rats were given a series of trials with both eyes occluded; no responses were observed.

When data collection was complete, the fluid in the recording pipettes was replaced with a solution of 2% pontamine blue in 0.5 M acetate buffer (pH 5.6). Then current (2–10 μA, electrode negative) was passed until a blue spot appeared in the cortex at the pipette tip. The rat was killed with 5% halothane and perfused transcardially with phosphate buffer saline (PBS) followed by 4% paraformaldehyde or 10% formalin. The brain was removed and stored in 4% paraformaldehyde or 10% formalin until sectioned at 30 μm on a cryostat. Sections containing pontamine blue marks were photographed and compared with Paxinos and Watson’s (1986) rat atlas for verification of recording sites.

Data analysis

The display and analysis were controlled by an Apple II computer and custom hardware described previously in Norcia et al.
FIG. 3. Response amplitude as a function of contrast. Data are grouped by age, pathway, and deprivation condition. Age is postnatal days at the time of monocular eye suture. All rats were sutured for 5 days prior to recording. ○: signal (6 Hz component). ▲: noise (average of 4 and 8 Hz component). Error bars are ± SE.

(1989). The amplitude and phase of the evoked response were calculated with a discrete Fourier transform. The amplitude of the 6 Hz component was defined as the “signal” because this was the rate of phase reversal of the stimulus. The average amplitude of the field potentials at 4 and 8 Hz was defined as “noise.” The data consisted of a signal value and a noise value for each value of contrast.

Each animal yielded four data sets of 30 trials each: 1) right eye right cortex (deprived uncrossed), 2) right eye left cortex (deprived crossed), 3) left eye left cortex (nondeprived uncrossed), and 4) left eye right cortex (nondeprived crossed). To describe the average response as a function of contrast for each animal, we averaged all 30 trials for each data set. For statistical analysis, the mean (30 trials) signal to noise ratio (S/N) in the crossed pathway and in the uncrossed pathway of each eye was used as the index of response strength. Changes in the signal to noise ratio were mainly due to changes in signal; the noise varied little as a function of age, pathway, or stimulus contrast.

An index, D, of the monocular deprivation effect was constructed by dividing the S/N of the deprived eye (Sd/Nd) by the S/N of the nondeprived eye (Sn/Nn) and then taking the common logarithm of the ratio, that is D = \log \left( \frac{S_d/N_d}{S_n/N_n} \right). The purpose of taking the logarithm is to cast the deprivation scores into an approximately normal distribution for running parametric tests of statistical significance. A deprivation effect is indicated when D < 0. D was calculated separately for the crossed and uncrossed pathways of each rat. For the purpose of statistical analysis with analysis of variance (ANOVA), the D scores were categorized into four 20–day age bins based on age at suture: P15–P34, P35–P54, P55–P74, and P75–P94. As seen in Fig. 4, the youngest animal successfully tested was P19 at the time of suture, and animals were not evenly distributed within the age categories.

RESULTS

Pattern-evoked potential

Responses to the counterphase (reversing) grating occurred only if the concurrent EEG lacked obvious low frequencies and had strong signals above 10 Hz. Figure 1 illustrates this point with results from two single trials a few minutes apart, each having a duration of 10 s. These data were collected with both eyes open after the formal experiment on this animal had been completed. On trial 65, the EEG showed robust high frequencies and lacked slow waves; concomitantly there was a clear response to the counterphase grating. As contrast increased from 1 to 70%, the 6 Hz component of the EEG rose above the noise, and the phase of the response became locked to the stimulus. The growth of the 6 Hz component can actually be seen in the raw EEG record. On trial 68 the high-frequency components of the EEG were weaker and slow waves were more prominent. On this trial there was little, if any, response.

The optimal state of the EEG was more easily produced with a mixture of halothane and nitrous oxide than with other anesthetic agents we tested. In pilot experiments we tried and rejected urethane, chloral hydrate, ketamine/xylazine, fentanyl/fluanisone, sufentanil/fluanisone, pento-barbital, and methohexital. Only halothane and nitrous oxide permitted minute to minute adjustments of anesthesia level. The proper EEG occurred at a halothane dose of 0.9–1.2% carried in a 65:35% mixture of NO2- O2. In animals younger than P24 at the time of recording (P19 suture),
threefold greater than the amplitudes of the uncrossed response (Fig. 2).

The contrast threshold and response amplitude can be estimated from the averaged data for each animal (e.g., Fig. 2). In this study we did not analyze the threshold data, instead concentrating on the amplitude. We neglected the threshold for two reasons. First, it was not our aim to measure the contrast sensitivity in the rat. Therefore we did not repeat the measurements at values of spatial frequency other than 0.13 cycles/deg. Second, at 0.13 cycles/deg the contrast threshold does not appear to be very sensitive to the effects of monocular deprivation. Indeed, the contrast threshold was typically about 7% independent of age, pathway, or deprivation. The exception was when there was little or no response to the grating and therefore no threshold at all. This type of exception was frequently seen in young monocularly deprived animals.

Monocular deprivation effect

Average response amplitude was strongly influenced by 5 days of monocular deprivation during the sensitive period. We measured the monocular deprivation effect separately for the crossed and uncrossed pathways. As seen in the example of Fig. 2, the signal from the nondeprived eye is greater than the signal from the deprived eye in both crossed and uncrossed pathways; the noise is similar in both pathways. Figure 3 shows the 30 trial means and standard errors for each age group. Figure 4 shows the index of the monocular deprivation effect, $D = \log (S_d/N_d / S_{nd}/N_{nd})$. In the crossed pathway, $D$ was $<0$ in 12 of 13 rats in the two youngest age groups. In the uncrossed pathway, $D$ was $<0$ in 11 of 13 animals. The individual scores in Fig. 4 indicate that the monocular deprivation effect occurred in animals as young as P19 and in animals as old as P50. There was no consistent monocular deprivation effect in the two older age groups ($n = 9$), in which the youngest animals were sutured at P55. Recording locations were successfully marked with pontamine blue for 14 of 22 rats summarized in Figs. 3 and 4, and in all cases the pipette tip was within layer II/III of the binocular area of V1 (Fig. 5).

We tested the statistical significance of the effect of monocular deprivation using ANOVA. The dependent variable was the index of the monocular deprivation effect, $D$. The two independent variables were age and pathway. Pathway was categorized as either crossed or uncrossed. Age was categorized into four 20-day bins starting at age P15. The data and its categorization are shown in Fig. 4. The effect of age on the monocular deprivation effect was significant ($P = 0.006$). The effect of the pathway was also significant ($P = 0.03$), i.e., the monocular deprivation effect was larger in the uncrossed pathway. The age by pathway interaction was not significant ($P = 0.9$). Bonferroni paired comparisons were performed between the groups deprived between P75 and P94 and each of the three younger age groups. The difference was significant for the P15 to P34 group ($P = 0.007$) and P35 to P54 group ($P = 0.009$), but not significant for the P55 to P74 group ($P = 0.8$).

**Crossed and uncrossed responses**

In the formal experiment, the stimulus was always presented monocularly and responses were recorded simultaneously in both hemispheres. Crossed responses were responses recorded in the cortex contralateral to the stimulated eye, and uncrossed responses were recorded in the cortex ipsilateral to the stimulated eye. Perhaps because there are more crossed than uncrossed fibers in the rat visual pathway, amplitudes of the crossed responses could be as much as
Dark rearing

The amplitudes of sweep VEPs from rats reared in total darkness (DD) for about 90 days (Fig. 6) were similar to the amplitudes from nondeprived eyes in monocularly sutured rats (Fig. 3). Thus, lack of visual experience, by itself, did not diminish the amplitude of the sweep VEPs. The monocular deprivation effect reflects the unequal use of the two eyes.

Monocular deprivation following dark rearing

Dark rearing had a marked impact on plasticity. Monocular eyelid suture following 90 days of dark rearing (DDMD) produced a clear monocular deprivation effect in both crossed and uncrossed pathways (Fig. 7). This contrasts markedly with the absence of a monocular deprivation effect in LD rats of similar age (Figs. 3 and 4). The amplitudes of the sweep VEPs in the deprived pathways of the DDMD rats were reduced to a level well below the amplitudes following dark rearing alone (Fig. 7 cf. Fig. 6). Comparing the DDMD rats with the DD rats, the deprivation index was significantly more negative in the DDMD animals than in the DD animals (Fig. 8; t-test, 1-tail, $P < 0.003$, $n = 3$, crossed and uncrossed averaged). In this test the right eye was the “nominal” deprived eye in the DD rats and the “actual” deprived eye in the DDMD rats. The recording locations of all six dark reared rats were successfully marked,
and all were within the binocular portion of the primary visual cortex.

DISCUSSION

Summary of results

Our aim was to assess in the rat the susceptible period for the monocular deprivation effect and the effect of dark rearing on this period. Eyelid suture for 5 days beginning between P19 and P50 produced a monocular deprivation effect; that is, lid suture reduced the amplitude of the sweep VEP in the binocular visual cortex in response to stimulation of the deprived eye. Lid suture beginning at P55 or thereafter did not produce a consistent monocular deprivation effect. Rats reared in continuous darkness from birth to about P90 showed a robust monocular deprivation effect. Dark rearing by itself did not diminish the amplitude of sweep VEPs at the spatial frequency tested.

Our experiments did not establish an age of onset for the critical period. Following eyelid suture from P19 to P24 we observed a monocular deprivation effect, but we were unable to record reliable responses in rats younger than P24. This failure may derive from difficulty in establishing the proper level of anesthesia in young animals, or it may derive from some type of immaturity of cortical connections that prevents vigorous responses to a counterphase grating. Intracortical connections are probably required for normal receptive fields in the primary visual cortex (Allison et al. 1995; Gilbert and Wiesel 1989; Weliky et al. 1995). Immaturity of receptive field organization, presumably because of the immaturity of intracortical connections, was observed in both rats and mice that are younger than their 5th postnatal week (Fagiolini et al. 1994; Gordon and Stryker 1996).

Comparison with previous results

Previous investigations of the monocular deprivation effect in rats and mice have reported a critical period that is shorter than observed here. Fagiolini et al. (1994) found that 10-day deprivations beginning in the 4th postnatal week (at P23) produced a deprivation effect, but that deprivations beginning in the 5th week (at P33) no longer did so. In mice, Gordon and Stryker (1996) found that lid suture beginning during the 4th week produced an effect, but that sutures in the 6th week (P36) did not. In our experiments, deprivations initiated throughout the 6th and 7th weeks (P37–P50), including four after P41, consistently showed a monocular deprivation effect (Fig. 4).

Several differences between our methods and those of previous investigators can easily account for the differences in the observed length of the critical period. First are the differences in anesthesia. Fagiolini et al. (1994) used urethan, Gordon and Stryker (1996) used barbiturate with chlorprothixene, and we used halothane with nitrous oxide. Because we found it difficult to obtain desynchronized EEGs while using urethan or barbiturates, we think it unlikely that
our results could be obtained with these anesthetics. Second, our electrodes were probably always located in layers II/III, whereas the previous studies sampled cells from all cortical layers. Several investigators believe that neurons of layers II/III retain plasticity longer than do cells of layer IV (Daw et al. 1992; LeVay et al. 1980). In addition, long-term potentiation can be produced at later ages in layers II/III than in deeper cortical layers (Kirkwood and Bear 1994). Third, we measured the amplitude of sweep evoked potentials rather than the ocular dominance of single units. The sweep VEP method stimulates the retina with a reversing grating and simultaneously records the summed current from a large population of visual neurons. The population responses are generated by postsynaptic potentials as well as action potentials. In contrast, the single unit method uses oriented edge stimuli and gives equal weight to cells that spike vigorously and those that spike weakly; it completely ignores cells that do not spike. Finally, the results of Gordon and Stryker are on mice, not rats. Whereas mice and rats are both rodents, they are different species, and there is no reason that their critical periods should be identical.

The two methods both yield important information, but the information they yield is not quite identical. Our data show that a monocular deprivation effect exists in the 6th and 7th postnatal weeks. An advantage of the sweep VEP method is that it permits rapid assessment of the monocular deprivation effect in individual animals. In addition, there is good correspondence between contrast thresholds determined with sweep VEPs and contrast thresholds determined psychophysically in conscious humans. Our observation that the sweep pattern evoked potential requires a desynchronized state of the EEG validates the opinion that it is closely linked to functional vision.

**Multiple critical periods**

Prior studies have shown that there are many plastic periods. For example, in the cat the plastic period for morphological segregation of thalamocortical afferents occurs earlier than the plastic period for ocular dominance in the extragranular layers (Daw et al. 1992; LeVay et al. 1980). The plastic period for specification of ocular dominance appears to be later than the plastic period for specification of directional selectivity (Berman and Daw 1977). In the rodent somatosensory cortex, the plastic period for specification of the whisker barrels in layer IV is earlier than the plastic period for specification of receptive fields of the overlying neurons in layer II/III (Fox 1992; Woolsey and Wann 1976). To effectively search for the cellular, molecular, and biochemical bases of visual plasticity, it is necessary to have a detailed description of the various critical periods. Our study provides such a description for one of these critical periods.

Electronic equipment and consultation for the sweep VEP technique was generously provided by A. Norcia, Smith-Kettlewell Eye Research Institute, San Francisco, CA, 94115-1821. We thank D. Tullar and D. Gokcay for technical assistance.

This work was supported by Grant R01 04050 from the National Eye Institute to B. Gordon.

Address reprint requests to B. Gordon.

Received 22 December 1997; accepted in final form 14 September 1998.

**REFERENCES**


