Original Article

Effect of Monocular Deprivation on Rabbit Neural Retinal Cell Densities

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Abstract

Effect of Monocular Deprivation on Rabbit Neural Retinal Cell Densities.

Purpose: To describe the effect of monoc[illegible]sities of neural retinal cells in rabbits.
Methods: Thirty rabbits, comprised of 1[illegible] animals, were included and monocular deprivation was achieved through unilat[illegible]bject animals. The rabbits were observed for three weeks. At the end of each wee[illegible]3 control animals were euthanized, their retinas was harvested and processed for [illegible]micrographs of the retina were taken and imported into FIJI software for analysis. [illegible]
Results: Neural retinal cell densities of[illegible]educed along with increasing period of deprivation. The percentage of reductions were 60.9% ($P < 0.001$), 41.6% ($P = 0.003$), and 18.9% ($P = 0.326$) for ganglion, inner nuclear, and outer nuclear cells, respectively. In non-deprived eyes, cell densities in contrast were increased by 116% ($P < 0.001$), 52% ($P < 0.001$) and 59.6% ($P < 0.001$) in ganglion, inner nuclear, and outer nuclear cells, respectively.
Conclusion: In this rabbit model, monocular deprivation resulted in activity-dependent changes in cell densities of the neural retina in favour of the non-deprived eye along with reduced cell densities in the deprived eye.

Keywords: Monocular; Deprivation; Neural Retinal Cells

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INTRODUCTION

The retina plays a critical role in visual perception as it contains the initial components of the visual pathway.[1] The retina develops from outpouchings of the neural tube known as optic vesicles[2] and is morphologically made up of the outer retinal pigment epithelium and inner neural retina. The neural retina is the photosensitive layer and contains several cell types including photoreceptors (rods and cones), conducting neurons (bipolar and retinal ganglion cells), interneurons (horizontal and amacrine cells) and glial cells.[3] These cells are arranged in three histologically distinct ''nuclear'' layers containing cell bodies but no synapses, separated by two ''plexiform'' layers having synapses but no cell bodies.[3] Axons of ganglion cells form the optic nerve which synapse with third order neurons at the lateral geniculate body of the thalamus.[4] The third order neurons mainly project to the primary visual cortex where processing of the visual information takes place.[3]

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Neuroplasticity is the ability of the nervous system to adapt its structural organization to new situations emerging from changes due to intrinsic or extrinsic inputs.[5,6] Monocular eyelid closure using sutures results in anatomical changes in the visual cortex in favor of the non-deprived eye.[7-9] This phenomenon is referred to as ocular dominance plasticity. The changes include synaptic modifications, changes in cell densities of the cortex,[5,6,10] as well as spatial changes in gene expression in the primary visual cortex.[11,12]

Structural changes occurring in the retina following monocular deprivation remain largely undescribed, despite the fact that the retina is considered as part of the nervous system based on its embryonic development from the diencephalon as well as its cellular content. The neural retina develops in an inside-to-outside manner; ganglion cells are formed first and photoreceptor cells are the last cells to become fully mature.[13] At birth, the retina and visual pathway are fully formed. Visual experience in the early postnatal period, as a critical period, is important for maturation of the visual system.[14] This period corresponds to the time in normal development during which geniculocortical axons attain their mature organization in the form of ocular dominance columns and is affected by monocular deprivation.[15] In rodents and cats, plasticity is low at eye opening, peaks around four weeks of age and declines over several weeks to months.[16] In human beings, the critical period appears to lie within the first 10 years of life.[17]

Rabbits offer an ideal model for vision research as they are readily available and easy to handle. Moreover, their visual capabilities and retinal cell types have been studied in detail and characterized in a fashion similar to those in humans.[18-23] The present study was aimed at describing the effect of monocular deprivation on densities of neural retinal cells in a rabbit model.

METHODS

Animals

Thirty Californian white rabbits (*oryctolagus cuniculus*) including 18 subject and 12 control animals were obtained from a local private commercial farm. Since the peak period for development of ocular dominance plasticity is between the 2nd and 4th postnatal week, the animals were recruited into the study on their 14th postnatal day. Rabbits with obvious congenital or acquired eye disorders were excluded. Approval to carry out the study was granted by the Biosafety, Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nairobi, Kenya.

Handling of Study Animals

The rabbits were kept in wire cages measuring 4 feet by 4 feet, floored with sawdust. Each cage housed one doe and its litter with a nest box for the litter and was cleaned daily. Since a nursing female and its litter require a minimum floor space of 7.5 square feet (for a doe with more than 5 kg body weight) and one doe would have 6-12 kits per litter (average 8), then a 16 square feet cage would be spacious enough for each doe and its kits. The rabbits were fed on commercial rabbit pellets, half a cup of pellets per 5 kg of body weight daily and were offered water *ad libitum* through sipper bottles with nozzles.

Monocular Deprivation

Eighteen subject rabbits were recruited on their 14th post natal day and were divided into two groups of nine animals. One group had their right eyelids sutured together while the other group had their left eyelids stitched up. These animals were restrained for body weight estimation and administration of medications using a restrain box, then anesthetized with intramuscular ketamine (50 mg/kg) and also given intramuscular analgesic (flunixin meglumine 1.1 mg/kg). Two drops of gentamycin eye drops were instilled into the eye to be deprived. The margins of the upper and lower lids of one eye were trimmed and sutured together using a single vertical mattress 5/0 nylon stitch under aseptic conditions.

Following tarsorrhaphy, the rabbits were returned to their home cages and observed daily for suture breakdown or infection. Post-operative pain was managed by intramuscular flunixin meglumine (1.1 mg/kg) every 24 hours for 4 days. In addition, the animals were clinically assessed for signs and symptoms of pain such as poor feeding, facing the back of the cage (hiding posture), vocalization by means of a piercing squeal, kicking and scratching, and teeth grinding. Rabbits, which continued to experience pain despite being on the regular analgesic, received a further dose of intramuscular butorphanol (0.5 mg/kg) 12 hourly until they were pain free.

Animals which developed suture dehiscence or infection were excluded from the study and treated accordingly. Those with suture infection received topical antibiotic eye drops (gentamycin) for five days while those with suture dehiscence were examined for any eye infection and treated with topical antibiotics.

Tissue Harvesting

Three control animals were sacrificed at the start of the study (14th postnatal day), the day on which the subject animals had also their eyelids sutured together. Thereafter nine rabbits including 3 controls and 6 experimental subjects were sacrificed each successive week, as shown in Table 1. Following weight determination, the rabbits were euthanized using intravenous Euthasol® (sodium pentobarbital 390 mg/ml + sodium phenytoin 50 mg/ml) at a dose of

Table 1. Study schedule

Study period	Control animals (*n*)	Experimental animals (*n*)	
		Right eye sutured	Left eye sutured
Week 0 (postnatal day 14)	3 rabbits (baseline)		
Week 1 (postnatal day 21)	3	3	3
Week 2 (postnatal day 28)	3	3	3
Week 3 (postnatal day 35)	3	3	3

1mL per 4.5 kg body weight (86.7 mg/kg pentobarbital and 11.1 mg/kg phenytoin). Once death was confirmed by loss of pupillary light reflex and corneal reflex, the thoracic cavity was opened then intracardiac prewash with saline was commenced, followed by perfusion with 4% paraformaldehyde solution as described by Gage et al[24] and Cunningham and Scouten.[25] Following perfusion, both eyes were enucleated then bisected along the vertical meridian. This was followed by removal of the vitreous humor from the eyecup to facilitate further penetration of the fixating medium (paraformaldehyde). The retina was stored in the fixative for 48 hours. The carcasses were incinerated after the tissues were harvested.

Histological Analysis

The eyes were dehydrated in a series of graded alcohols and embedded in paraffin wax. A microtome was used to produce 5-µm thick sections, obtained from cuts through the whole globe, oriented along the optic nerve. Sections within the central retina were used for analysis. For each eye, four sections obtained through systematic sampling technique were picked and stained with hematoxylin-eosin (H and E) stain.[26] Photomicrographs of the sections were taken using a Canon® digital camera (PowerShot A640 camera, 12 megapixels, Canon, Tokyo, Japan), then transferred to a computer installed with ImageJ-Fiji software[27] for morphometric and stereological analysis. Cell densities in the outer nuclear, inner nuclear and ganglion cell layers were determined by counting the number of cell bodies seen in the field and dividing this number with the field area. For each section, cell counting was done in four different areas and then averaged.

Statistical Analysis

Collected data was entered into the Statistical Package for Social Sciences (SPSS) software (Version 17.0, Chicago, Illinois, USA) for coding, tabulation and statistical analysis. After confirming that the data was normally distributed using histograms and box plots, parametric tests were used to compare the means of the variables measured. Analysis of variance (ANOVA) test was used to compare the means of each variable studied from baseline to the end of the third week of the study. Student's *t*-test was used to compare the differences in means between non-deprived and deprived eyes, non-deprived and control eyes, and deprived and control eyes. $P < 0.05$ were considered as statistically significant at 95% confidence interval.

RESULTS

Deprived Eyes

There was generalized reduction in retinal cell densities in deprived eyes along with increasing duration of monocular deprivation [Figure 1]. The percentages of reduction of cell densities from the baseline were 60.9%, 41.6%, and 18.9% for ganglion, inner nuclear and outer nuclear cells, respectively [Table 2]. Statistically significant reductions in cell densities were noted in the ganglion and inner nuclear cell layers (ANOVA test, $P < 0.05$).

Non-deprived Eyes

Cell densities in non-deprived eyes were increased along with increasing duration of monocular deprivation of the fellow eye [Figure 2]. The percentage of increase in ganglion, inner nuclear and outer nuclear layer cells were 116%, 52% and 59.6%, respectively [Table 2]. All mentioned differences were statistically significant (ANOVA, $P < 0.05$).

Control Eyes

The retinas of the control eyes did not display any statistically significant change in cell density during the period of the study [Table 2].

Deprived versus Non-deprived Eyes

Monocular deprivation resulted in an increase in cell populations of the non-deprived eyes while cell densities in the deprived eyes were reduced. The difference between the deprived and non-deprived eyes was more marked with increasing deprivation period [Figure 3 and Table 3].

Non-deprived versus Control Eyes

The non-deprived eyes had higher cell densities as compared to control eyes at all three study intervals [Figure 4] showing a statistically significant differences after two and three weeks of deprivation [Table 4]. At the end of the third week of deprivation, the non-deprived retinas, as compared to the control retinas, had 115.6%, 50.3%, and 56.6% increments in ganglion, inner nuclear, and outer nuclear cell densities, respectively. All these differences were statistically significant ($P < 0.05$).

Deprived versus Control Eyes

The deprived eyes had lower cell densities as compared to the control eyes, with differences being more marked while the period of deprivation increased [Figure 5]. There were more noticeable changes in the ganglion cell densities [Table 5]. As compared to the controls, cell densities of ganglion cells in the deprived eyes was reduced by 32%, 39%, and 54% after one, two and three weeks of monocular deprivation, respectively.

DISCUSSION

Neuroplasticity is the ability of the nervous system to adapt its structural organization to new situations emerging from changes due to intrinsic or extrinsic inputs.[5,6] The present study has revealed that monocular deprivation leads to significant reduction in neural retinal cell densities of deprived eyes with a compensatory increase in non-deprived eyes. This is in agreement with previous studies on tree shrews.[28] Similar findings have been reported in other stimulus deprived receptor organs such as the olfactory mucosa after unilateral naris occlusion,[29,30] and the organ of Corti after unilateral hearing loss.[31,32] These findings have been attributed to under-expression of pro-mitotic genes and increased expression of apoptotic genes on the deprived side leading to reduced cellular proliferation.[33,34] In the retina, growth factors such as brain derived neurotrophic factor (BDNF) have been shown to affect cellular proliferation.[35,36] In monocularly deprived eyes, BDNF expression is reduced in deprived eyes and increased in non-deprived eyes.[35] In the current study, the reduction in cell densities in deprived eyes could be as a result of reduced expression of promitotic factors such as BDNF or increased expression of apoptotic factors.

Although all cells demonstrated changes in their densities with monocular deprivation, the

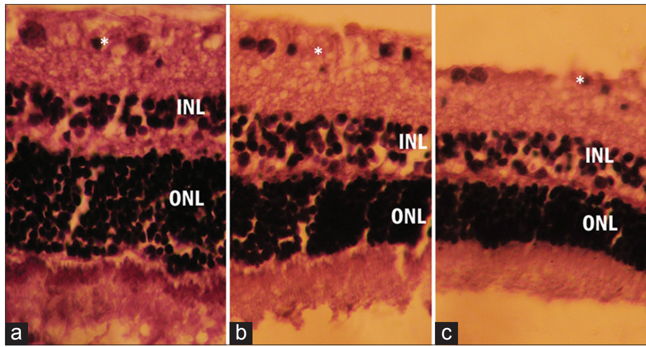

Figure 1. Photomicrograph of cell densities in deprived eyes. (a) After 2-week deprivation (b) after 3-week deprivation (c) after 4-week deprivation. Note that the ganglion cells (asterisk) are reduced in size and number along with increasing monocular deprivation period (Hematoxylin and eosin stain, ×92). INL, inner nuclear layer; ONL, outer nuclear layer.

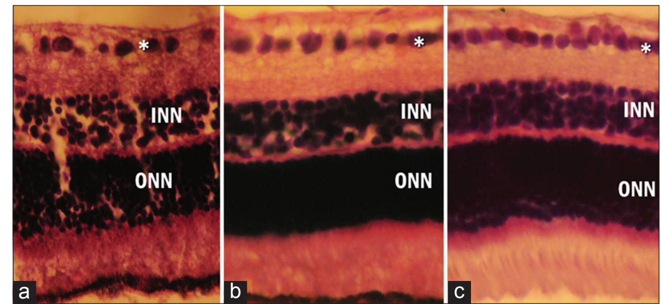

Figure 2. Photomicrograph of cell densities in non-deprived eyes; (a) after 2 weeks of monocular deprivation (b) after 3 weeks of monocular deprivation (c) after 4 weeks of monocular deprivation. Note that the cells become more densely packed with increasing monocular deprivation. This is clearly depicted in the ganglion cell layer (asterisk) (Hematoxylin and eosin stain, ×92). INL, inner nuclear layer; ONL, outer nuclear layer.

Table 2. Changes in the retinal cell densities

Retinal layer	Deprivation in weeks	Deprived		Nondeprived		Control	
		Mean±SD	*P*	Mean±SD	*P*	Mean±SD	*P*
Ganglion cell density (cells/mm²)	0	7775.0±1831.9	<0.001	7775.0±1831.9	<0.001	7775.0±1831.9	0.679
	1	4787.6±774.6		8456.0±3807.3		7049.8±2658.2	
	2	4135.9±1087.0		11,589.5±2401.2		6775.7±1787.7	
	3	3040.7±1086.9		16,803.2±5158.5		6575.3±821.1	
Inner nuclear cell density (cells/mm²)	0	38,488.8±1834.5	0.003	38,488.8±1834.5	<0.001	38,488.8±1834.5	0.995
	1	35,579.1±8322.8		37,218.6±8029.3		38,325.3±1490.6	
	2	34,147.7±5372.0		52,894.8±11,016.4		38,432.0±8879.4	
	3	22,490.8±8872.8		58,845.3±9177.6		39,163.4±4201.9	
Outer nuclear cell density (cells/mm²)	0	59,669.2±961.1	0.326	59,669.2±961.1	<0.001	59,669.2±961.1	0.996
	1	54,006.8±11,721.5		65,480.3±11,414.0		61,731.0±1281.0	
	2	53,105.8±9736.7		87,952.9±20,311.5		61,361.3±22,132.4	
	3	48,406.8±10,814.6		95,240.5±17,834.6		60,824.6±2982.3	

SD, standard deviation

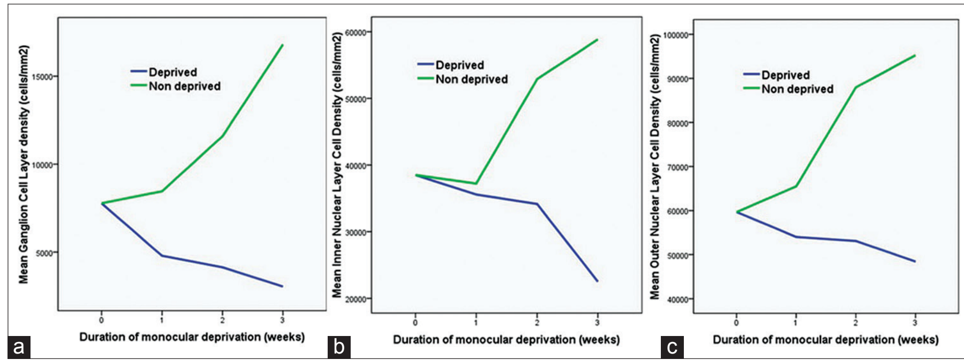

Figure 3. Mean plots for cell densities in non-deprived and deprived eyes; (a) ganglion cell density; (b) inner nuclear cell density; (c) outer nuclear cell density.

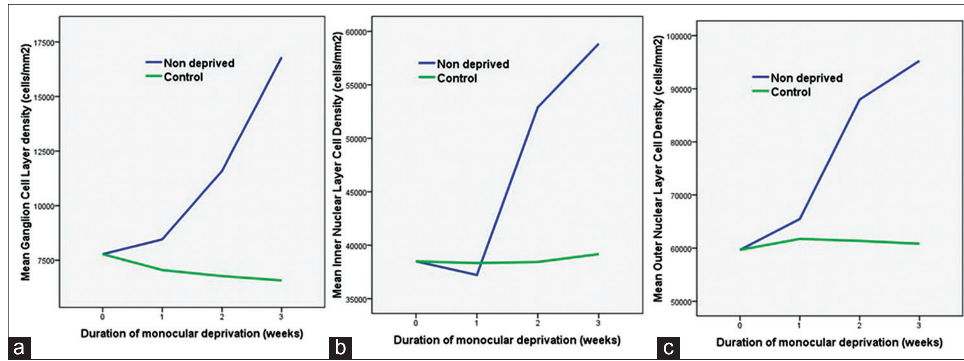

Figure 4. Mean plots for cell densities in the non-deprived and control eyes; (a) ganglion cell density; (b) inner nuclear cell density; (c) outer nuclear cell density.

Table 3. Mean cell densities in deprived and nondeprived eyes

Retinal layer	Eye	Week 1		Week 2		Week 3	
		Mean	*P*	Mean	*P*	Mean	*P*
Ganglion cell density (cells/mm²)	Deprived	4787.6	0.011	4135.9	<0.001	3040.7	<0.001
	Nondeprived	8456.0		11,589.5		16,803.2	
Inner nuclear cell density (cells/mm²)	Deprived	35,579.1	0.654	34,147.7	<0.001	22,490.8	<0.001
	Nondeprived	37,218.6		52,894.8		58,845.3	
Outer nuclear cell density (cells/mm²)	Deprived	54,006.8	0.039	53,105.8	<0.001	48,406.8	<0.001
	Nondeprived	65,480.3		87,952.9		95,240.5	

Table 4. Mean cell densities in nondeprived and control eyes

Retinal layer	Eye	Week 1		Week 2		Week 3	
		Mean	*P*	Mean	*P*	Mean	*P*
Ganglion cell density (cells/mm²)	Nondeprived	8456.0	0.509	11,589.5	<0.001	16,803.2	<0.001
	Control	7049.8		6775.7		6575.3	
Inner nuclear cell density (cells/mm²)	Nondeprived	37,218.6	0.793	52,894.8	0.005	58,845.3	<0.001
	Control	38,325.3		38,432.0		39,163.4	
Outer nuclear dell density (cells/mm²)	Nondeprived	65,480.3	0.837	87,952.9	0.015	95,240.5	0.001
	Control	66,731.0		61,361.3		60,824.6	

most marked changes were displayed in ganglion cells. Among the non-deprived eyes, ganglion cell density increased by 116% as compared to baseline ($P < 0.001$), while in deprived eyes, it was reduced by 60.9% ($P < 0.001$). Retinal ganglion cells are significant in the visual pathway as they are

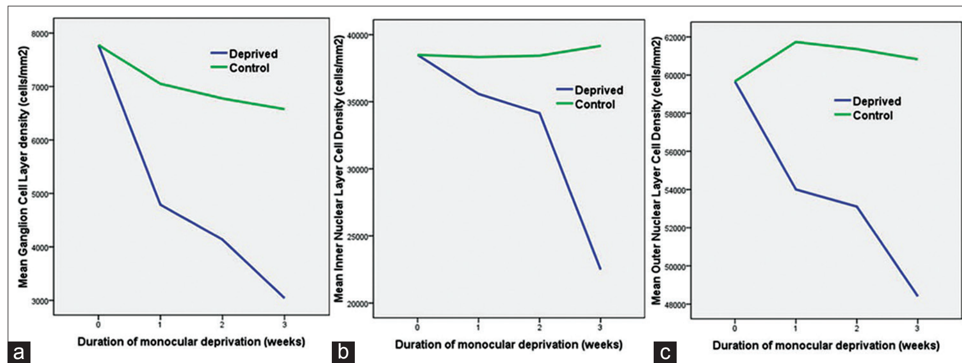

Figure 5. Mean plots for cell densities in the deprived and control eyes; (a) ganglion cell density; (b) inner nuclear cell density; (c) outer nuclear cell density.

Table 5. Mean cell densities in deprived and control eyes

Retinal layer	Eye	Week 1		Week 2		Week 3	
		Mean	*P*	Mean	*P*	Mean	*P*
Ganglion cell density (cells/mm^2)	Deprived	4787.6	0.032	4135.9	0.001	3040.7	<0.001
	Control	7049.8		6775.7		6575.3	
Inner nuclear cell density (cells/mm^2)	Deprived	35,579.1	0.535	34,147.7	0.199	22,490.8	0.001
	Control	38,325.3		38,432.0		39,163.4	
Outer nuclear cell density (cells/mm^2)	Deprived	54,006.8	0.228	53,105.8	0.303	48,406.8	0.036
	Control	61,731.0		61,361.3		60,824.6	

the output neurons from the retina.[37,1,3] Previous studies on the effects of monocular deprivation on retinal ganglion cell densities provide contradictory reports. Studies on the Rhesus monkeys[38] and rats[39] have reported a decrease in ganglion cell density in deprived eyes, while a study on three cats raised with monocular deprivation for 5.2-7.2 years did not reveal any differences in ganglion cell densities.[40] The findings by the latter study could be due to small sample size used, species of animal used or duration of deprivation.

In conclusion, the present study demonstrated that monocular deprivation results in activity-dependent changes in the neural retina and a reduction in all cell densities in deprived eyes with compensatory changes in the non-deprived eyes. These changes in the retina may contribute to changes seen in the visual cortex in monocularly deprived animals. However, further studies seem to be required to determine whether these changes in the retina are reversible, and if so, the maximum period of deprivation beyond which these changes cannot be reversed.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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