Poster #21

# Predictable and systematic changes in ion distribution across the retina during induction of refractive error

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## Aim

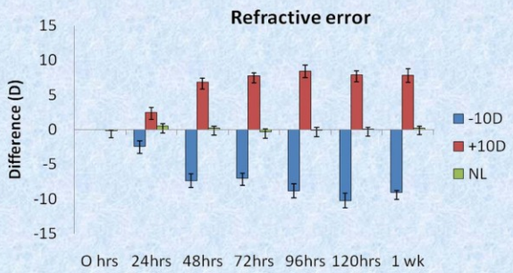
Form deprivation myopia (FDM) and refractive recovery has been shown to be associated with systematic changes in abundance of sodium (Na) Potassium (K) and chloride (Cl) ions across the retina reflecting a change in osmoregulation (Crewther et al., 2006).

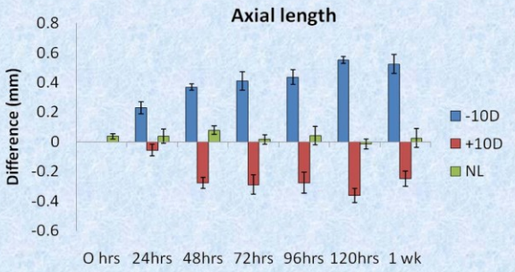
FDM and refractive recovery are also characterized by systematic ultrastructural changes indicative of variation in the rate of fluid movement across the retina and RPE to the choroid (Liang et al., 2004).

FDM and negative lens compensation also show similar retinal ultrastructure (Beresford et al., 2001) leading us to hypothesize:

(i) a similar pattern of ion changes to that seen in FD during induction of refractive compensation to negative lenses and (ii) that induction of refractive compensation to positive lenses would involve the same ions but in an opposing pattern.

## Methods

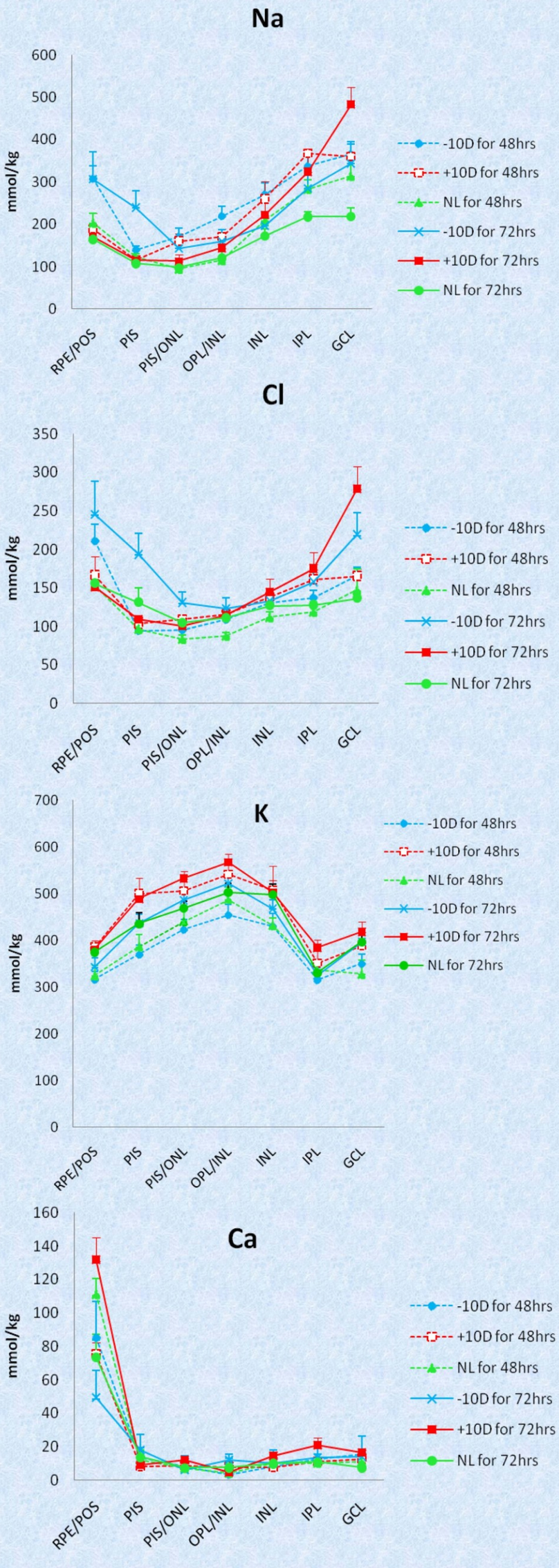



Figures 1a and 1b. Mean differences in Refractive State and Axial Length between the two eyes of experimental animals during the induction of myopia (blue) and hyperopia (red). Note that at 48 hrs ocular growth and refractive compensation is clearly migrating in a sign dependent manner and at 72 hours good and nearly complete refractive compensation is seen. Hence 48 and 72 hours were the time-points examined.

- 18 chicks raised from post-natal day 5 for either 48hrs or 72 hrs under standard light conditions in 3 lens groups (+1OD or -10D lenses or no lens control).
- Chicks were taken at 48 or 72 hours when refractive compensation to positive and negative lenses was beginning and also well underway but not completely compensated.
- Both eyes of chicks from 3 groups were prepared for elemental microanalysis using X-ray microanalysis (EDX).
- The posterior eyecup was frozen in liquid propane, and freeze dried for 72hrs. The dry tissue was then cracked transversely and platinum coated for analysis.
- Specimens were analysed on a scanning electron microscope using an x-ray detector with the electron beam at 15kv and at constant 20nA.
- Sampling was taken every 20um x 20um square in seven positions across the retina/RPE.

## Results




Figures 2a, 2b, 2c and 2d. Change in elemental abundance (mmol/kg) of Na (sodium) (2a), Cl (chloride) (2b), K (potassium) (2c) and Ca (Calcium) (2d) when measured at intervals (see Figure 3) across the retina for eyes wearing +10D, -10D or No Lenses for 48 or 72hrs. RPE: retinal pigment epithelium, PIS: photoreceptor inner segments, ONL: outer nuclear layer. OPL/INL: outer plexiform layer/inner nuclear layer, INL: inner nuclear layer IPL: inner plexiform layer and GLC: ganglion cell layer.

High Na/Cl was notable in the outer retina during myopic development . However, high Na/Cl in the inner retina was notable in both myopic and hyperopic development but more so for hyperopic development.

Potassium abundances were high during hyperopic development in both the inner and outer retina whilst K was low during myopic development.

Calcium levels were highest in abundance at the RPE in all groups. Slightly higher Ca abundances were seen during hyperopic development.

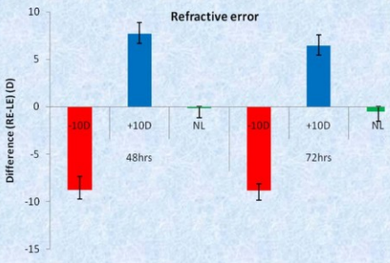



Figure 3. Refractive error status of x-ray tissue following optical defocus for 48hrs and 72hrs.

Note that refractive compensation is nearly fully compensated at 48hrs for negative lenses and was at 48hrs for positive lenses. At 72hrs, refractive compensation was similar to 48hrs suggesting that the eyes were maintaining the refractive status.



Figure 4. Sampling of the retina using x-ray microanalysis. Scanning was conducted in seven regions (blue boxes) across the retina: OS/RPE: outer segments/retinal pigment epithelium, IS: inner segments, IN: inner nuclear, IP/GC: inner plexiform/ganglion cell.

## Conclusion

Experimental induction of myopia (LIM) and hyperopia (LIH) by refractive compensation to negative and positive blur is accompanied by systematic predictable changes in distribution of Na, Cl, and K ions.

- Outer retina:
  - High Na and Cl with low K in the outer retina during LIM
  - High K and low Na and Cl in the outer retina during LIH
- Inner retina:
  - High Na and Cl in the inner retina during both LIM and LIH, however this was highest for LIH and corresponded with high K levels for LIH

Changes in ionic concentrations at the exit layers of the retina is indicative of a change in osmolarity that could be associated with volume change in response to blur and supports the ion-driven fluid movement hypothesis (Crewther, 2000).

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# ON/OFF Pathway Activation Ratio as a Predictor of Eye Growth and Refractive Compensation

**Poster #24**

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## Purpose

- Optimal retinal function is necessary for accurate identification and growth response to signed defocus.
- Conversely, Pharmacological or Environmental interference with the balance between the ON and OFF systems leads to differential, sign-dependant perturbation of compensation to imposed defocus.

- The following agents have been known to selectively affect activation of these pathways via a number of different mechanisms.
  - APB (2-amino-4-phosphonobutyrate) is a glutamatergic receptor agonist that selectively suppresses photoreceptor transmission to ON bipolar cells.
  - PDA (cis-2, 3-piperidicarboxylic acid) is an excitatory amino acid antagonist known to suppress communication to photoreceptors and OFF (an to some extent ON) bipolar cells.
  - The gliotoxins L or D α amino adipic acid (LAAA, DAAA) lead to selective suppression of the ON or OFF pathways at the photoreceptor/bipolar synapse.
  - Nitric Oxide (NO) also affects synaptic transmission of the ON and OFF systems
    - L-NAME has been shown to increase the OFF response, while L-Arg is reported to decrease this response.

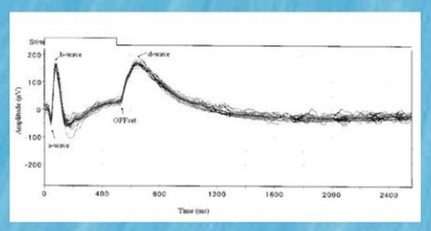
- We have now utilised these drugs to examine their effect on refractive compensation to optical blur and related the resultant change in eye growth to long term effects on the ON and OFF pathways, as shown by ERG.
  - Hence, the current study hypothesised that the effect of such drugs on the amplitude of the ON and OFF waves (ie the b/d ratio) would predict the direction of ocular growth.

## Method

- On day 4, 25 normally reared chicks were anaesthetised and injected intravitreally with physiological doses of either LAAA, DAAA, APB, PDA, L-Arg or L-NAME dissolved in PBS, or PBS alone. They were raised until day 9 and prepared for electrophysiological analysis.
- ERG waves were compared to the biometric data of chicks raised with +/-10D defocus in the same drug conditions.
- Electrode Placement
  - An intravitreal Ag/AgCl electrode was inserted via a catheter placement unit with a scleral reference.
- Stimulus Protocol
  - ON and OFF responses were assessed using a square wave 500ms onset/offset light pulse presented in a 150mm ganzfeld (peak luminance 50 cd/m$^2$ measured using a Tektronix J6523 narrow angle luminance probe).
  - Signals were recorded via a Powerlab amplifier and band-pass filtered (0.3 – 1000Hz).
  - 20 potentials were measured in each run, and 5 such runs were recorded for each eye.

*Data Analysis*

- Grand mean average waves from the ERG recordings were obtained by averaging across the multiple recordings from the chicks in each of the groups.
  - The main features of the ERGs analysed were the ampliitude and latency of the a-wave, b-wave peak, and d-wave peak.
  - The b/d amplitude ratio for each drug condition was calculated.




a wave = Photoreceptor response
b wave = Light ON response
d wave = Light OFF response

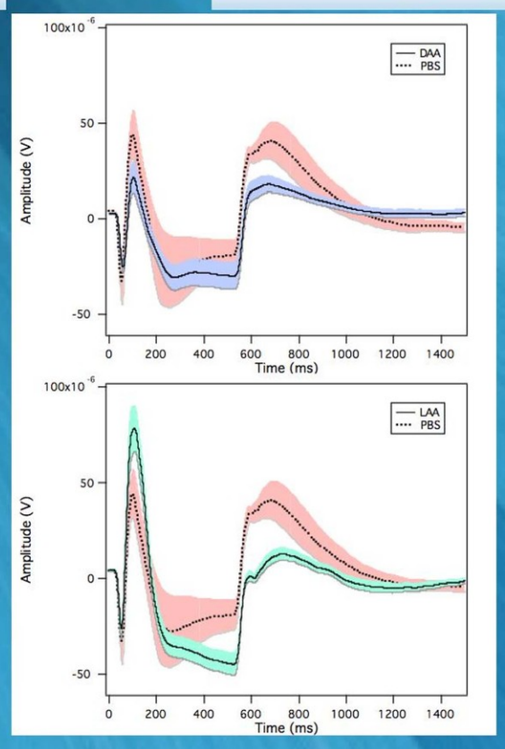



Figure 1.
Top - ERG of DAAA v PBS. Note the reduced amplitude of the d wave compared to that of PBS. (96hrs)

Bottom - LAAA v PBS. Note the increase in the b wave, and slightly diminished d wave in comparison to PBS. (96hrs)

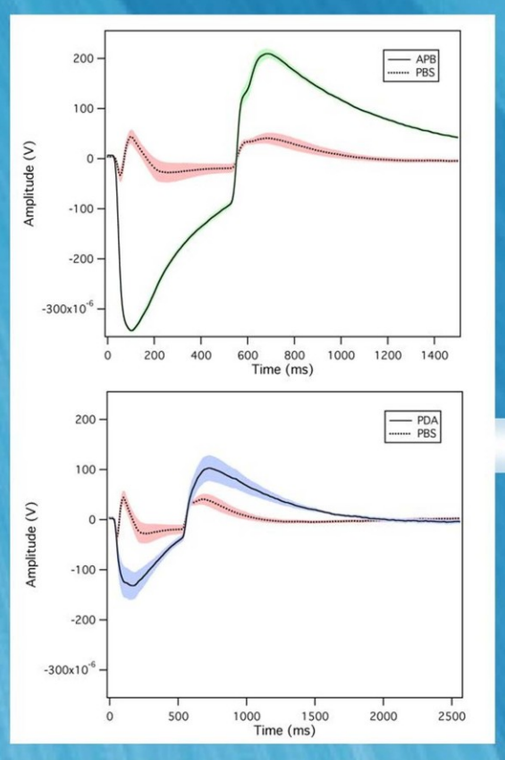



Figure 2.
Top - ERG of APB v PBS. Note the substantial negativity of the a wave amplitude lack of b wave positivity following the application of APB compared to PBS.

Bottom - PDA v PBS. Note the of lack of b wave positivity, and increased positivity of the d wave in comparison to PBS.

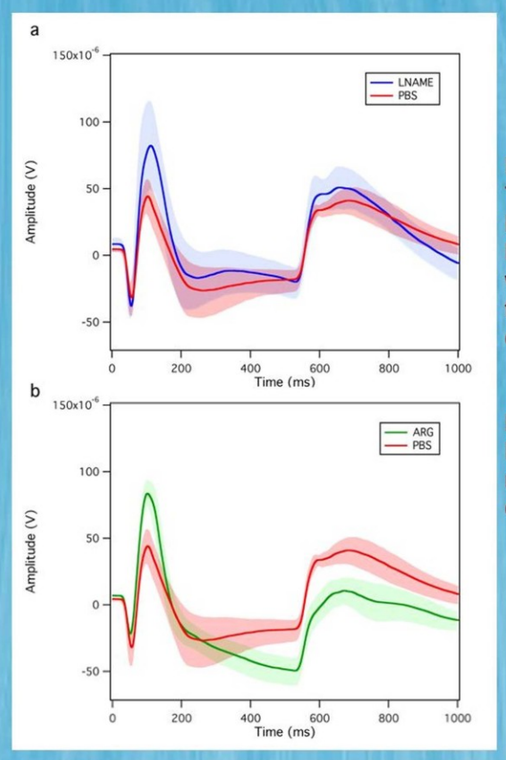



Figure 3.
Top - ERG of L-NAME v PBS.
Note the slight increase in b wave amplitude following the application of APB compared to PBS.

Bottom - L-Arg v PBS. Note the decreased magnitude in the d wave in comparison to PBS.

## Results

Table 1. Analysis of the b/d wave ratio for each agent tested

| Agent | Pathway Affected at Time of Injection | Refractive Compensation (+10/-10) | | b/d Wave Ratio |
|---|---|---|---|---|
| PBS | Control | 6.90 | -9.11 | 1.23 |
| LAAA | ON | 6.00 | -2.67 | 1.67 |
| DAAA | OFF | 2.91 | -6.61 | .9 |
| APB | ON | 7.53 | -9.60 | -1.1 |
| PDA | OFF | 7.23 | -7.61 | -.29 |
| L-Arg | Decrease OFF | 5.74 | -5.75 | 1.77 |
| L-NAME | Increase OFF | 6.82 | -5.78 | 1.68 |

- The magnitude of the ON and OFF response (b/d wave ratio) differed between drugs known to alter compensation to +10D or -10D lenses.
- A negative ratio (indicating total abolition of at least 1 wave) corresponds to no significant alteration of typical growth after 4 days injection.
- Where a change in growth is observed;
- a ratio greater than 1.23 leads to hyperopia,
- A ratio less than 1.23 leads to a myopic shift.

## Conclusion

- Results indicate that the direction of ocular growth may be predicted based on the b/d wave ratio.
- These results also suggest that any long term imbalance in the activation of the ON and OFF pathways must be seen as a long term shift in the ionic homeostasis of the micro-enviroment of the photoreceptors/bipolars of the outer retina.
- Such light induced ionic imbalances create changes in fluid dynamics, and thus ocular volume as predicted by Crewther (2000).

*References*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Poster #29

# Selective Activation of a Subpopulation of GABAergic Amacrine Cells by Focused and Defocused Images in Macaque Retina

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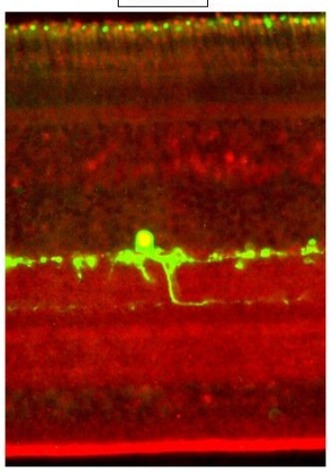
## INTRODUCTION

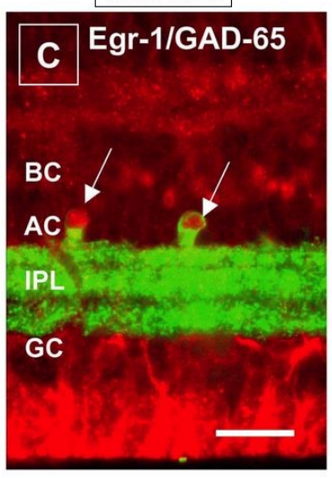
It is well known that the quality of visual images fine-tunes ocular elongation early in life. The purpose is to produce and maintain the ability to focus over a wide range of distances (emmetropia). Amacrine cells play key roles in this process.

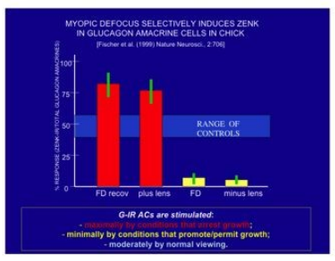
Previously, using the inducible factor Egr-1 to indicate focus-dependent cell activation, we have identified amacrines in chick, tree shrew, and macaque as possible mediators of growth- restraint due to plus-defocused images:

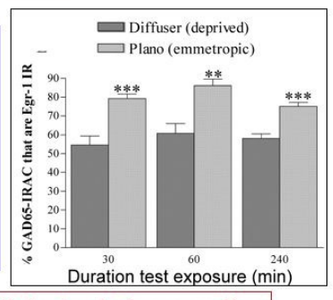
CHICK



MACAQUE




Results were consistent with tuning to focus and/or defocus, but state of focusing was not controlled.

Here we tested the hypothesis that GAD65-immunoreactive (IR) amacrines in macaque retina respond selectively to plus-defocused images.

## METHODS

Rhesus monkeys (*Macaca mulatta*, n=14, 20-30 days old) were divided into 3 groups: In-Focus (n=6), Plus-Defocus (n=6); and Binocular Open (n=2). Refraction under ketamine anesthesia and tropicamide cycloplegia revealed moderate hyperopia.

Three days later the animals were fitted with a spectacle-holder, and a diffuser was mounted over both eyes except in the Binocular Open group. The diffusers were worn overnight.

The next day, midway through the light period, the diffuser over the left eye was replaced by a fully correcting lens (In-Focus) or a lens overcorrecting by +1.50D (Plus-Defocus). Transmission by lenses and diffusers was equalized with N.D. (=0.1) filters.

For the next two hours, the animals viewed toys held and moved by a technician (to hold attention and fixation), 5 meters away across an otherwise featureless room. Horizontal fields of view were ~50 deg monocular, and ~108 deg binocular. Refraction while wearing lenses verified that the lenses had the desired effects, making the animals' viewing eye in focus, or myopically defocused by+1.5 D, respectively, at 5 meters.

After the two-hour viewing period, animals were anesthetized deeply, fixed by vascular perfusion with 4% PFA, cryoprotected, given a code number to hide the identity, and stored frozen.

Later, pieces of central (macular) retina, still attached to sclera, were cryosectioned at 12-15µm. Sections of viewing (treated) and fellow diffuser-covered (control) retinas were mounted on the same slide; double-labeled together with rabbit antibodies to Egr-1 + non-rabbit antibodies to GAD65, GABA, nNOS or VIP; labeled further with two different fluorophores; and viewed.

Labeled cells were counted visually in 15-29 fields (~350µm retina length) from 1-4 sections of each retina. The mean number per field of Egr-1-positive nuclei in the amacrine cell layer gave the number of "Egr-1 labeled cells. The fraction of a specific type of amacrine cell labeled having Egr-1-IR nuclei was taken to indicate the state of activation of that cell type.

## RESULTS

**Figure 1. Plus-Defocus and In-Focus Conditions ACTIVATE GAD65- IR Amacrine Cells.**

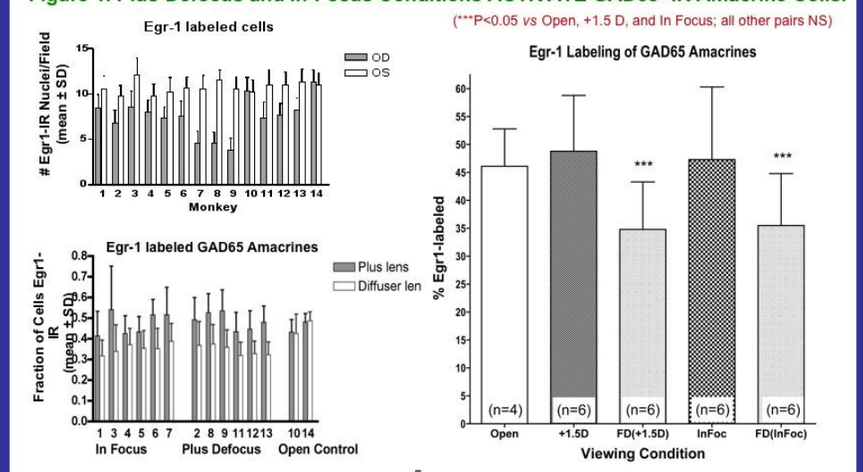



**Figure 2. Plus-Defocus and In-Focus Conditions ACTIVATE GABA- IR Amacrine Cells.**

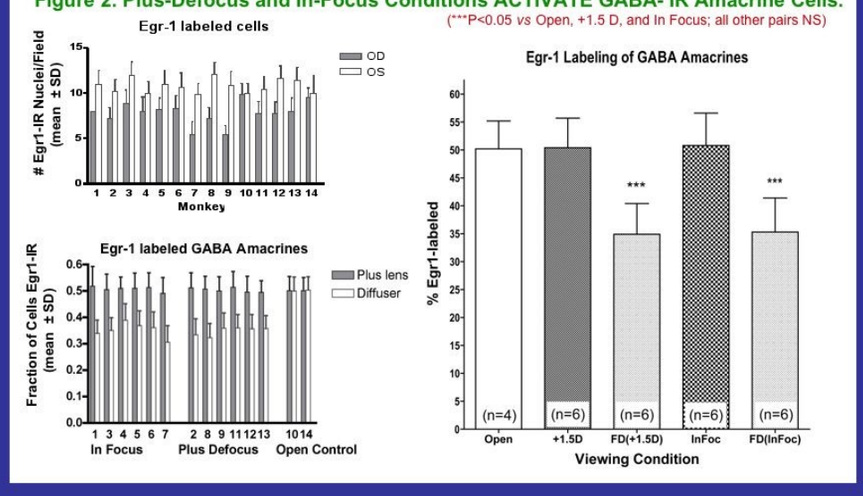



**Figure 3. Plus-Defocus and In-Focus DO NOT ACTIVATE nNOS-IR Amacrine Cells.**

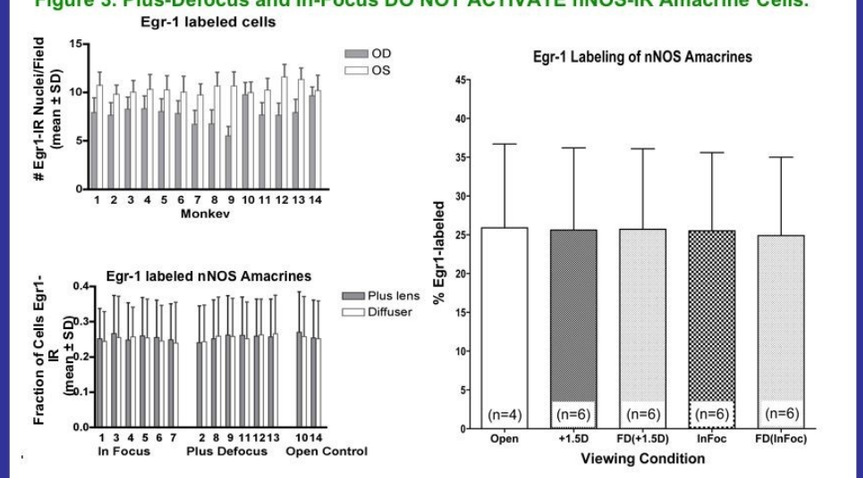
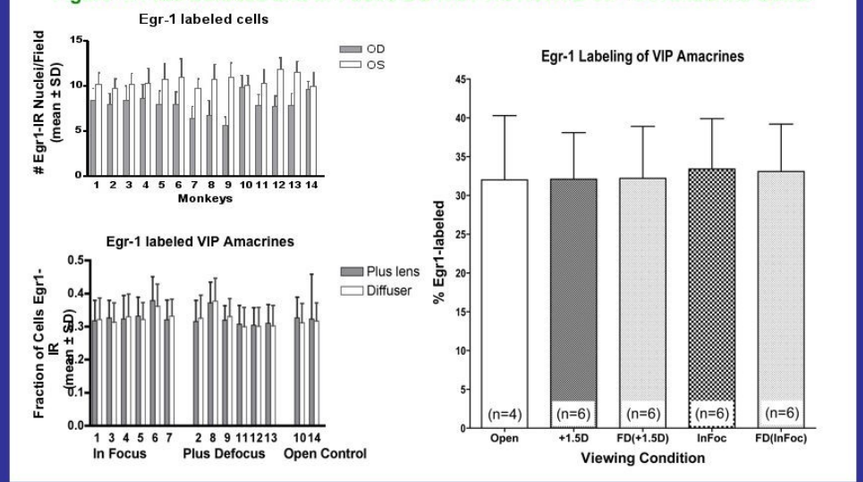



**Figure 4. Plus-Defocus and In-Focus DO NOT ACTIVATE VIP- IR Amacrine Cells.**




## SUMMARY

**Summary of Monkey Egr-1 Labeling Data**

| | GAD65 | | GABA | | VIP | | nNOS | |
|---|---|---|---|---|---|---|---|---|
| COND (n) | MEAN | ± SD | MEAN | ±SD | MEAN | ±SD | MEAN | ±SD |
| OPEN (4) | 46.1$^{+++}$ | 6.7 | 50.2$^{+++}$ | 5.0 | 32.0 | 8.3 | 25.9 | 10.8 |
| DEFOC (6) | 48.8*** | 10.0 | 50.4*** | 5.3 | 32.1 | 6.0 | 25.6 | 10.6 |
| FD (6) | 34.8 | 8.5 | 34.9 | 5.5 | 32.2 | 6.7 | 25.7 | 10.4 |
| INFOC (6) | 47.3*** | 13.0 | 50.8*** | 5.8 | 33.4 | 6.5 | 25.5 | 10.1 |
| FD (6) | 35.5 | 9.3 | 35.3 | 6.1 | 33.1 | 6.1 | 24.9 | 10.1 |

$^{+++}$ P<0.001 *vs* fellow eyes (FD) to Plus-Defocused (DEFOC) & In-Focus (INFOC) eyes
*** P<0.001 *vs* fellow eyes (FD) within own treatment group

1. In Open Control eyes (OPEN), which were not covered with any goggle overnight or while viewing the targets, substantial fractions of all 4 amacrine cell types were labeled for Egr-1. The frequency of Egr-1 labeling in all amacrine cells, of any type, was the same in both eyes.

2. The frequency of Egr-1 labeling of amacrine cells in eyes that experienced In-Focus or Plus-Defocused viewing for 2 hours, was similar to frequency of labeling in Open eyes. This was true for the four specific amacrine cell types tested as well as for amacrine cells in general.

3. The frequency of Egr-1 labeling of amacrine cells in eyes that were always covered by a diffuser (FD), and thus never experienced In-Focus or Plus-Defocused viewing, was significantly lower in GAD65- and GABA-labeled amacrine cells (and amacrine cells in general) than in eyes that experienced In-Focus or Plus-Defocused for 2 hours.

4. However, the frequency of Egr-1 labeling of VIP- and nNOS-labeled amacrine cells was not altered significantly by any treatment condition.

5. Therefore, the activity of GABAergic amacrine cells - likely a specific subpopulation of such cells - is increased by focus conditions (vision unrestricted, restricted to in-focus, and restricted to plus-defocus) that are known to restrain axial elongation and retard myopia development. This subpopulation of amacrine cells is a plausible candidate for a role in emmetropization and myopia-prevention.

6. Our data do not support such a role for VIP- or nNOS-labeled amacrine cells, however.

## ACKNOWLEDGMENTS

These studies were supported by grants from the Canadian Institutes for Health Research[1], the National Natural Science Foundation of China[2], and the National Eye Institute, National Institutes of Health, USPHS[3].

The authors thank Jin Tao for her technical assistance.

# CHANGES IN REFRACTIVE ERROR AND ITS OCULAR COMPONENTS OF KOREAN CHILDREN BETWEEN THE AGES OF 7 AND 11 YEARS: A 2-YEARS LONGITUDINAL STUDY

Poster #56

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## Aim

❖To obtain information which can be used for further myopia preventive research by analyzing the correlation between changes in refractive error and ocular components in Korean students as a function of age and gender.

## MATERIALS AND METHODS

❖This study investigated refractive error and ocular components changes in a group of 81 Korean schoolchildren from age 7 to 11 year over a two year period between 2004 and 2006.
❖Cycloplegic autorefraction, autokeratometry and A-scan ultrasonography (Sonomed A/B Scan 5500, USA) were performed three times at 12 month intervals.

Table 1. General characteristics of subjects in first, second and third measurements.

| Each year | Elementary schoolchildren (N) | Drop-out rates(%) |
|---|---|---|
| First year | 185 (F96/M89) | - |
| Second year | 152 (F72/M80) | 18 |
| Third Year | 81 (F47/M34) | 56 |

## RESULTS

Table 2. Mean values of refractive error(D) in first, second and third measurements.

| | Grade 1~2 | Grade 4 | Total (D) |
|---|---|---|---|
| 1st measurement | 0.086±1.793 | -1.590±2.196 | -0.617±2.133 |
| 3rd Measurement | -0.798±2.258 | -2.352 ±2.524 | -1.405±2.474 |
| Difference | -0.884±0.770 | -0.765±0.695 | -0.788±0.847 |
| Statistics(t) | 8.264 | 9.053 | 11.838 |
| Statistics(p) | 0.000 | 0.000 | 0.000 |

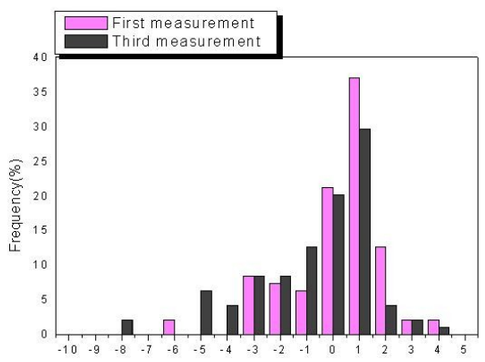


Fig 1. Distribution of refractive error (SE; D) in grade 1-2 students at first and third measurements.

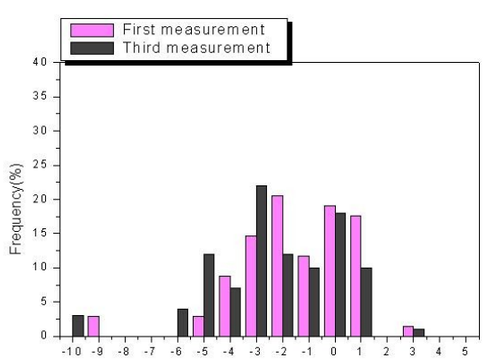


Fig 2. Distribution of refractive error (SE; D) in grade 4 students at first and third measurements.

Table 3. Change of kurtosis and skewness in grade 1-2 and 4 students during two years.

| Statistics | | Grade 1-2 | Grade 4 |
|---|---|---|---|
| Kurtosis | 1st | 1.719±0.493 | 2.302±0.574 |
| | 2nd | 1.285±0.493 | 1.199±0.574 |
| | 3rd | 1.310±0.493 | 0.842±0.574 |
| Skewness | 1st | -1.008±0.249 | -1.019±0.291 |
| | 2nd | -1.071±0.249 | -0.7271±0.291 |
| | 3rd | -1.079±0.249 | -0.610±0.291 |

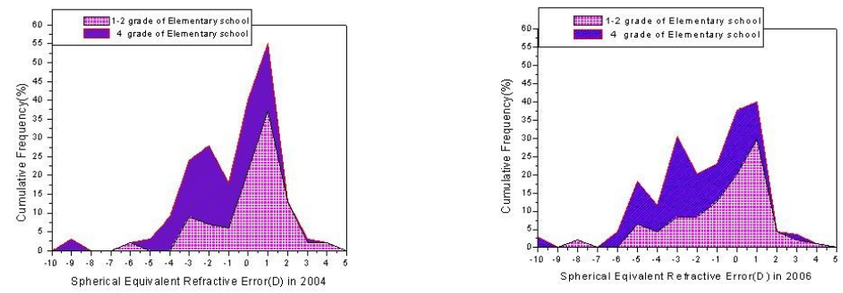


Fig 3. Cumulative distribution of spherical equivalent of refractive error in first and second measurements.

Table 4. Mean SER and ocular components of subjects in 2004 and 2006.

| | SE | CR | AL | AL/CR | ACD | Weight | Height |
|---|---|---|---|---|---|---|---|
| 1st measure ment | -0.617 ±2.133 | 7.844 ±0.172 | 23.247 ±1.301 | 2.965 ±0.165 | 3.772 ±0.195 | 44.213 ±13.90 | 133.66 ±9.602 |
| 3rd measure ment | -1.450 ±2.474 | 7.814 ±0.336 | 23.836 ±1.242 | 3.057 ±0.218 | 3.546 ±0.318 | 48.242 ±14.07 | 146.16 ±10.04 |
| Differ- ence | -0.788 ±0.847 | -0.033 ±0.290 | 0.589 ±0.642 | 0.022 ±0.195 | -0.225 ±0.293 | 4.029 ±3.377 | 12.501 ±3.121 |
| Statistics (t) | 11.838 | 1.317 | -11.68 | -5.969 | 9.766 | -28.98 | -50.98 |
| Statistics (p) | 0.000 | 0.189 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

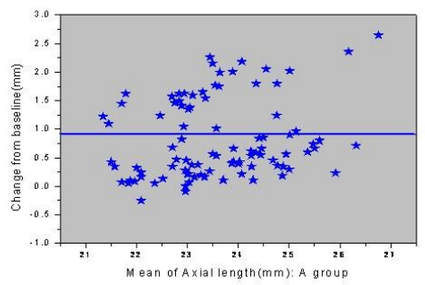


Fig 4. Scatter plot of Change in Axial length (A group: 1-2 grade of elementary school students)

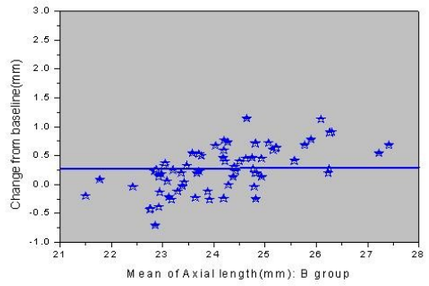


Fig 5. Scatter plot of Change in Axial length (B group: 4 grade of elementary school students)

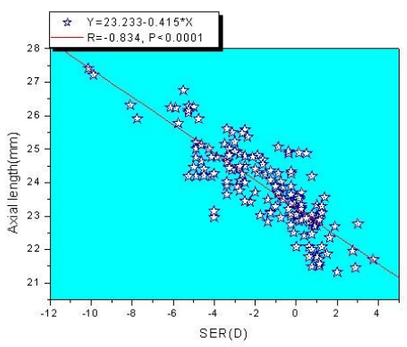


Fig 6. Correlation of refractive error and axial length in 3rd measurement

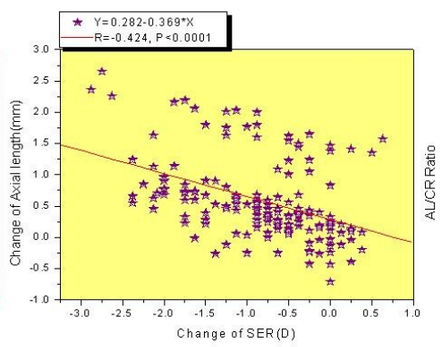


Fig 7. Correlation of change in refractive error and axial length in 3rd measurement

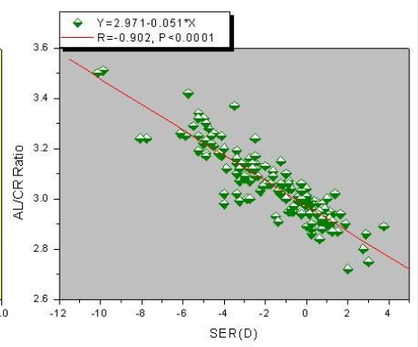


Fig 8. Correlation of refractive error and AL/CR ratio in 3rd measurement

## CONCLUSIONS

❖ At the end of the two year study, the mean spherical equivalent refraction(SER) was -1.405±2.474D and 53% of the schoolchildren were myopic.
❖ For lower grades of students, the skewness changed from -1.01±0.25 to -1.08± 0.25, kurtosis decreased from 1.72±0.49 to 1.31±0.49 and the refractive error changed from 0.09±1.79D to -0.72±2.21D.
❖ For higher grades of students, skewness changed from -1.02±0.29 to -0.61±0.29, kurtosis decreased from 2.30±0.57 to 0.84±0.57 and the refractive error changed from -1.59±2.20D to -2.35±2.52D.
❖The main factor in myopia progression for elementary school students is change of axial length and AL/CR ratio.
❖ Korean schoolchildren develop myopia as early as 7-8 years old.
❖ In order to investigate factor which are influential toward myopia progression, a prospective study with a longer period of observation and larger samples is important for future research.

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