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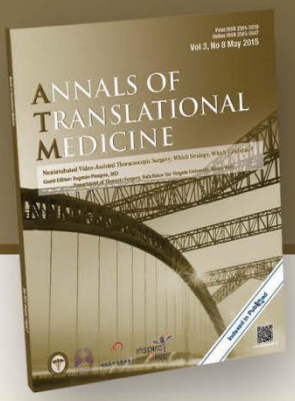

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Peer Review File

Article information: http://dx.doi.org/10.21037/atm-20-5433

Reviewer A

Comment 1:

For transmission to the authors:

1) Overview: The aim of this study is praiseworthy: to test the hypothesis that form-deprivation causes not only myopia, but also amblyopia, in a novel animal model – the guinea pig. It succeeds in this aim, perhaps – though the incomplete description of sweep-VEP recording (viz., whether the refractive error of the form-deprived (FD) eye was corrected during stimulation and recording) weakens the significance of the low VEP-acuity. The findings do not, I believe, differentiate reliably between effects of FD at retinal vs cortical level; and therefore, the references to "neuroplasticity of visual cortex" are not provided with a strong foundation. It would be wise to focus the discussion regarding the relationship between the time-course of FDM and 'amblyopia', and 'critical period' and 'plasticity', with a brief, clear, well-documented summary of what is known about the time-course of visual development in pigmented guinea pigs. The writing is not bad, but it would benefit from editing by a professional English-language science writer/editor. Examples, and further comments, follow.

Reply 1: We thank the reviewer for the helpful comments. We have revised them as your suggestion. Manuscript had been touched up and each change was highlighted in yellow color in the revised manuscript (details below).

Comment 2:

Detailed Questions, Comments, and Suggestions [Manuscript page, and line number, in brackets]:

Examples of how language will benefit from further editing:

[p. 24]: Start the abstract with 1-2 sentences to say what myopia is.

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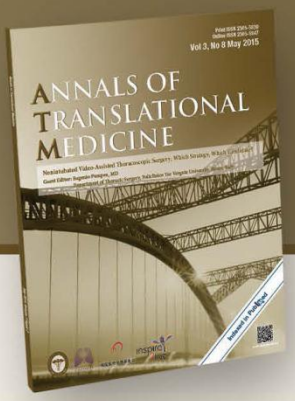

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Reply: We thank the reviewer for the suggestion. This has been added to the text in Abstract: "Form deprivation myopia is one of the ametropia in humans with identifiable causes and has been induced in many animals" (see Page 3, Line46-47)

[27, 29]: 'whether' is better than 'if'

Reply: We thank the reviewer raises a thoughtful consideration. We have revised 'if 'to 'whether' (see Page 3, Line50,53)

[29]: Place comma after 'deprivation'

Reply: We thank the reviewer raises a thoughtful consideration. We have revised it according to your suggestion (see Page 3, Line52).

[31]: 'pigmented' guinea pigs

Reply: We thank the reviewer for the suggestion. We have revised it according to your suggestion (see Page 3, Line55)

[34]: 'time points' is common, but overused, in my opinion; try using 'duration of FD', 'times', or 'intervals' here, instead

Reply: We thank the reviewer raises a thoughtful consideration. We have revised it according to your suggestion (see Page 3, Line58).

[35]: Indicate the type of retinoscopy, and the A-scan oscillation frequency

Reply: We thank the reviewer for the suggestion. This has been added to the text in Abstract: "using cycloplegic streak retinoscopy, A-scan ultrasonography (with an oscillation frequency of 10 MHz)" (see Page3, Line59-60).

[40-41], 'The acuity of form deprived eyes was unchanged': Compared to what? (initial, pretreatment status)

Reply: We thank the reviewer for the suggestion. We have revised it as "The acuity of form-deprived eyes was unchanged compared to that at the pretreatment time point". (see Page4, Line 67-68).

[42-44], "There was a significant difference ...': I find this sentence confusing; I suggest adding some details to make it clearer.

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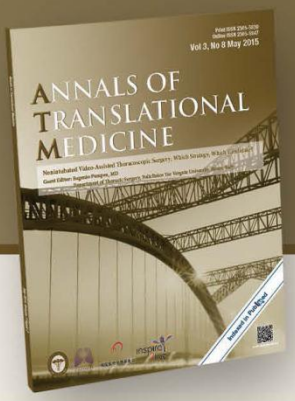

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Reply: We thank the reviewer for the suggestion. We have revised it as “there were significant differences between deprived eyes and the other two open eyes from 1 to 8 weeks, showing that amblyopia was possibly established during 1 week of form deprivation”. (see Page4, Line69-71).

[44-45], “Although not statistically significant ...’: Since it's not significantly different, you can't know whether VA increased, or not; so, delete this sentence.

Reply: We thank the reviewer for the helpful comments. We have deleted this sentence.

[68]: Start the Introduction with a brief statement, something like, what is myopia, and what/why are animal models used for studying it?

Reply: We thank the reviewer for the suggestion. This has been added to the text in Introduction: “Form deprivation myopia is one of the ametropia in humans with identifiable causes such as congenital cataract, ptosis, and corneal opacity. In order to investigate the mechanisms of myopia in humans, form deprivation myopia has been induced in many animals” (see Page5, Line 90-93).

[70+], references on animal models: These references are mostly outdated and do not contain typical results. And anyway, what is the point? All you really need to do here is summarize what is currently known about FDM in guinea pigs, to lay the foundations for understanding what you 2 are doing and why you are doing it. If there are things of importance in other animal models, the Discussion is the place to introduce them and discuss what's important. Here are some useful recent reviews:

1) Troilo D, et al. IMI – Report on Experimental Models of Emmetropization and Myopia. IOVS. 2019; 60:M31–M88. https:// doi.org/10.1167/iovs.18-25967

2) Schaeffel F, Feldkaemper M. Animal models in myopia research. Clin Exp Optom 2015; 98:507–17.

Chakraborty et al, 2020, Understanding Myopia: Pathogenesis and Mechanisms in Updates on Myopia. https://doi.org/10.1007/978-981-13-8491-2_4

[105+]: Same comment as above. Get on with what you are going to do, and why. (It's good to introduce amblyopia as a consequence of FD, but make it brief and to the point in this Intro.)

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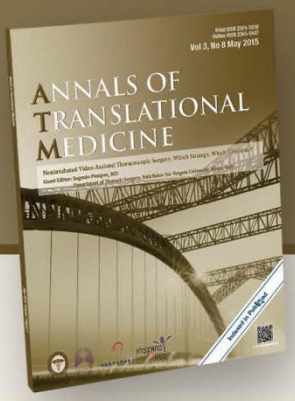

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Reply: We thank the reviewer for the helpful comments and suggested references. We have added suggested references to our manuscript (see Reference 6,7,8).
We have made Introduction brief and revised it as " Animal experimental studies have established the importance of visual feedback in eye and refractive state development, and have demonstrated that form deprivation myopia is a graded phenomenon; increases in the degree of image degradation have been positively correlated with the severity of the induced axial myopia, the uncoordinated ocular growth has been found to be due to reduced retinal image contrast, and the absence of visual feedback has been reported to be related to the effective refractive state of the eye (6-8). Many experiments regarding normal visual development in these animals have shown that the age of onset of form deprivation myopia in these animals coincided with the period of visual development and that larger refractive errors were present in younger animals and after a longer duration of form deprivation (9-13). For example, monocular form deprivation was performed in monkeys at the age of 2 weeks and led to the development of myopia of -13.5 diopters (D) after 18 months; performing monocular form deprivation at the age of one year led to the development of only -4.5 D myopia after 26 months. Eye development in the guinea pigs was basically completed by 3 weeks, and considerable myopia of approximately -3.4, -5.8, and -5.7 D was present after 6, 11, 16 days, respectively, of form deprivation performed at the age of 5 days. Similarly, any abnormal visual experience (e.g., monocular form deprivation, strabismus, or anisometropia) imposed during the period of visual development could result in abnormalities of the visual system (e.g., amblyopia); however, as this was initiated at progressively older ages, the resulting abnormality was smaller or even without visual impairment at all (14). For example, in rhesus monkeys, monocular form deprivation initiated during early life produced a severe degree of amblyopia, and the effect on spatial vision decreased systematically as the age of onset was delayed (15,16)" (see Page 5-6, Line 93-116).
We have also added a table to summarize the onset time, duration, final induced diopter, and course of time from birth for emmetropization in each animal model of form deprivation myopia, as well as the relationship between the deprivation onset time and the amblyopia severity in rhesus monkeys. (see Table1)

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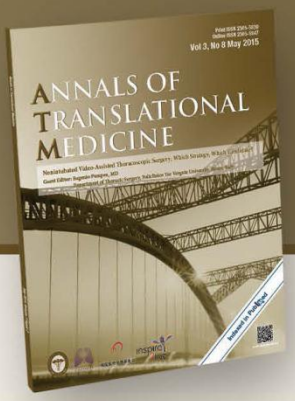

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[121-122], 'we first developed a monocular form deprivation model of guinea pig': You make it seem as if you are the first ones ever to do this, but that of course is far from the truth. I suggest instead something like this: 'we established and verified FDM in our own animals, with our own procedures'.
Reply: We thank the reviewer for the suggestion. We have revised it as "we established a monocular form deprivation model in guinea pig with our own procedures". (see Page 6, Line 126-127).
[125], 'similar to sweep VEPs recorded in': It's simpler, and clearer, to replace this with 'as in'.
Reply: We thank the reviewer for the suggestion. We have revised it according to your suggestion. (see Page 6, Line129).
[134]: Change 'Every five', to 'Groups of five' ...
Reply: We thank the reviewer for the suggestion. We have revised it according to your suggestion. (see Page 7, Line139)
[136]: Light in holding area: give details of source (type, spectrum, frequency, etc.)
Reply: We thank the reviewer for the suggestion. This has been added to the text in Methods: "with light provided by white LEDs (spectrum: broad-band, illuminance: 500 lux, color temperature: 4500 K, frequency: 50-60 Hz)" (see Page 7, Line 140-142).
[145], 'biometric measurements': Insert, '(see details below)'
Reply: We thank the reviewer for the suggestion. We have revised it according to your suggestion. (see Page 7, Line152).
[152], 'Guinea pigs were anesthetized': How? With what agent(s)?
Reply: We thank the reviewer for the suggestion. This has been added to the text in Methods: "Guinea pigs were anesthetized by inhaling 2% isoflurane" (see Page 8, Line 159).
[158-159]: 'Reti-Port System ... was connected ...'
Reply: We thank the reviewer raises a thoughtful consideration. We have revised it according to your suggestion (see Page 8, Line 168).
[160-161], 'The sweep VEPs stimulus was a horizontally ...': Another way to say this would be, 'The sweep VEP stimulus, a horizontally ...'

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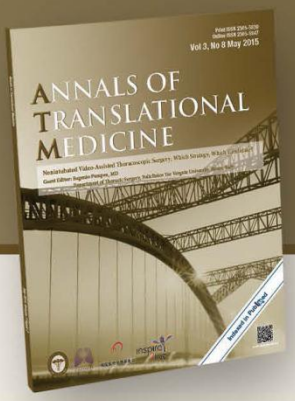

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Reply: We thank the reviewer raises a thoughtful consideration. We have revised it according to your suggestion (see Page 8, Line 169).

[161]: What kind of display screen? Emission spectrum? Refresh rate? Etc.

Reply: We thank the reviewer raises a thoughtful consideration. This has been added to the text in Methods: “The stimuli were presented using a 21-inch CRT monitor. The stimulus contrast was 80% and the temporal reversal rate (grating wave) was 3 Hz (about 6 reversals/s). The mean luminance of screen was 100 cd/m^2. The stimulus screen was viewed monocularly at 20cm (100°wide ×82°high)” (see Page8-9, Line171-177).

[162-165]: For me, this description is a bit difficult to follow. Think about providing a sample stimulus trace, and using it to show how response sampling was related to stimulus.

Reply: We thank the reviewer raises a thoughtful consideration. We have added a sample stimulus trace (see Figure2) and explained it in Figure Legends: “Figure 2: A diagram regarding the course of time and the eleven stimuli trace. Each spatial frequency was presented for 11 s and there was 1 s of adaptation before data collection. The stimulus contrast was 80% and the temporal reversal rate (grating wave) was 3 Hz (about 6 reversals/s)” (see Page26, Line552-555).

[167], Figure 2: The x-axis parameter is not "acuity", but spatial frequency of the stimulus.

Reply: We thank the reviewer for the suggestion. We have revised it as “spatial frequency (cpd)”. (see Figure3)

[174]: ‘previously’ **** At this point I will refrain from making further comments that concern mainly correctness of writing; but it would be useful to have the manuscript edited (in the way that I have done so far) by an English-language scientific writer/editor.

Reply: We thank the reviewer raises a thoughtful consideration. Manuscript had been touched up and each change was highlighted in yellow color in the revised manuscript. We had tried our best to improve the manuscript and made some changes which were related to grammatical errors in the manuscript. These changes will not influence the content and framework of the paper.

Comment 3:

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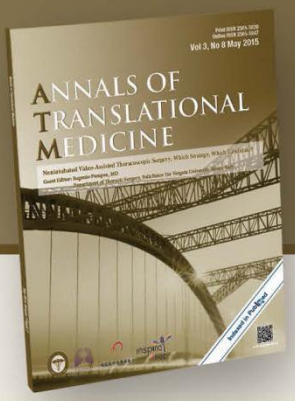

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Substantive issues:
[36], sweep VEP: It's critical to indicate, here – whether this was done without, or with, correction of the induced myopic RE.
Reply: We thank the reviewer for the helpful comments. Sweep VEP was done with correction of the induced myopic RE. This has been added to the text in Abstract and Methods: “Sweep VEPs were done with correction of the induced myopic refractive error” (see Page 3, Line61-62), (see Page 7, Line150-151).
[51-53]: You can't say this; you have a correlation, but no evidence that either myopia or amblyopia causes the other one. Also, I find that it is stretching even the evidence that you do have, to suggest (a) that the deficiency of visual acuity is due to “neuroplasticity”, and (b) that amblyopia affects FDM or other refractive development – although I admit, the Kiorpes paper that you’ve cited (ref. 29) does suggest so. You might find it useful to read and reference the recent review by Kiorpes: “Understanding the development of amblyopia using macaque monkey models” (PNAS 2019); www.pnas.org/cgi/doi/10.1073/pnas.1902285116 3
Reply: We thank the reviewer for the helpful comments and suggested reference. We have deleted this sentence and added suggested references to our manuscript (see Reference 14).
[155], ‘the right eye was stimulated’: Was it still under the latex diffuser? Or was the face-mask removed, and then was the FDM eye viewing with no corrective lens? Or was a corrective lens employed, to compensate for the induced myopia? THESE ARE CRITICAL ISSUES.
Reply: We thank the reviewer raises a thoughtful consideration. We have revised it as “When the right eye was stimulated, the face-mask was removed, and the stimulated eye viewing with a corrective lens was employed to compensate for the induced myopia” (see Page8, Line163-165).
[171]: As shown in Fig. 2, it's obvious only that "acuity" is ≤0.4 cpd, and it appears that the fitting of a line to just 2 points is of doubtful validity as a precise measure. I realize that this method may be common practice, but at the very least you should think about it. 'Linear regression' denotes a specific statistical procedure, doesn’t it? – but here it seems that you have

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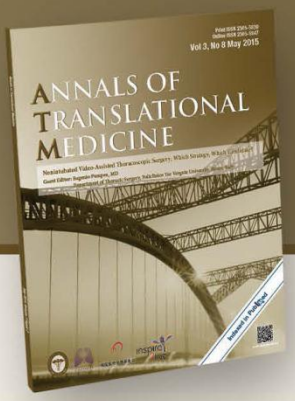

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simply drawn a line through two points, and measured its intersection with amplitude = 0. Also one could easily argue, regarding the data in this figure, that the highest amplitude is an outlier – in which case, by your own definition, the 'acuity' figure would be ~0.3 cpd.

Reply: We thank the reviewer raises a thoughtful consideration. We have added reference "20. Ridder WH, 3rd. Methods of visual acuity determination with the spatial frequency sweep visual evoked potential. Doc Ophthalmol 2004;109:239-47"and detailed explanation for the method of acuity extrapolation: "The data were determined to be noise if the signal to noise ratio (SNR, open/filled circles) was less than 2. As seen in Figure 3a, the SNR did not exceed 2 for the 0.39, 0.50, 0.65, or 0.80 cpd data. Acuity was determined by fitting a line between the high spatial frequency data that were above noise (0.3 cpd) and the first spatial frequency that entered the noise (0.39 cpd) (solid arrows). The linear fit was extrapolated to the X-axis (zero amplitude) for visual acuity (dashed arrows)" (see Page 9, Line 181-187).

We have also revised the Figure (see Figure3).

[178-179], 'completely dilated pupil' ... and complete cycloplegia, yes?

Reply: We thank the reviewer raises a thoughtful consideration. We have revised it as "complete cycloplegia" (see Page 9, Line 193).

[180] Was the experimenter blinded as to treatment?

Reply: We thank the reviewer for the suggestion. This has been added to the text in Methods: "The experimenter was blinded to the treatment performed." (see Page 9, Line 194).

[182]: Please define 'axial length'

Reply: We thank the reviewer for the suggestion. This has been added to the text in Methods: "The axial length was the distance from the cornea to the vitreous-retina interface" (see Page10, Line 205-206). This has been also added to the text in Figure Legends: "The double-headed arrow between C and R represents axial length" (see Page 27, Line 572-573).

[183-184]: 'conduction velocities ... were assumed to be' [insert; and cite a reference from which you got those values]

Reply: We thank the reviewer for the suggestion. We have added the reference to the text in Methods: "The conducting velocity of the anterior chamber and the vitreous chamber was 1540

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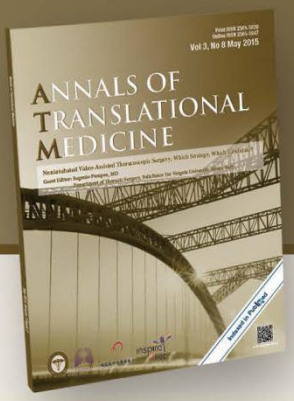

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m/s, which has been previously used for measurements in guinea pigs (19)" (see Page 9-10, Line 198-200).

[194], ANOVA: Did you verify that the data were normally distributed?

Reply: We thank the reviewer for the suggestion. We have verified that the data were normally distributed by using Shapiro-Wilk test. This has been added to added to the text in Methods: "Data were presented as continuous variables and evaluated for normality using the Shapiro-Wilk test" (see Page 10, Line 209-210).

[200], Table 1: I think it would be helpful to keep the Table, but also to display the data as graphs.

Reply: We thank the reviewer for the suggestion. We have also displayed the data as graphs (see Figure 5)

[202-203], 'deprivation period': Better than 'time point'! ('duration', or 'duration of FD(M)', also would be appropriate.)

Reply: We thank the reviewer for the helpful comments

[204+]: I think that it's unnecessary to include all these details in the text, since they are available already in the graph(s) and Table. It's better just to say what the overall findings are, and leave the numerical details to the Table and figures - IMO. J

Reply: We thank the reviewer for the helpful comments. We have deleted the numerical details in Results of the text.

[208], Figure format: Symbols in 4a,b,c are small and difficult to read. Perhaps make the symbols for normal controls larger, and empty circles (though I concede that the differences are fairly obvious); just extend the lines a little bit longer, in the key on the figure, so you can see which line is dashed and which one is dot-dash. Also, it's inappropriate to display the time intervals as you have done here (as neither linear nor power/exponential functions) in a line-graph. Either space them differently (linear or logarithmic scale); or keep the time scale as-is, but replace the line-graph with bar graph.

Reply: We thank the reviewer for the helpful comments. We have revised it as your suggestion. (see Figure 5).

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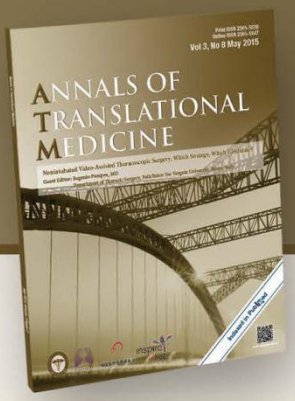

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[226]: [insert after 'acuity of']: 'vision via the'. (You don't know whether the defect in acuity is based in the eye, or in the brain.)
Reply: We thank the reviewer for the helpful comments. This has been added to the text in Results "there was a highly significant difference in the acuity of vision via the eyes from 1 to 8 weeks of form deprivation" (see Page 11, Line 230).
[238-239; 243], 'decrease ... between any two time-points': It doesn't appear that there is any difference between values in FD eyes at 0 vs. 1 week, nor in open eyes from weeks 1 to 4 to 8; I think you must mean something else.
Reply: We thank the reviewer raises an important consideration. This paragraph described the change trend of diopter with time in each group, there were differences between values in FD eyes at 0 vs. 1 week, and in open eyes from weeks 1 to 4. (see Table2, a indicates significant difference compare with 0-week time-point; b indicates significant difference compare with 1-week time-point). We added further details in Results of the text: "From week 0 to 1, the diopter of both deprived eyes and open eyes decreased significantly, indicating that the guinea pig eyes have still been in the stage of visual development" (see Page 11-12, Line 240-242).
[253], 'followed by a slow increase ($p>0.05$)': You can't claim that there is an increase, if the difference or change over time is not statistically significant.
Reply: We thank the reviewer for the helpful comments. We have deleted this sentence.
[263]: The reader might remain unconvinced (as I am) that you have used the method 'successfully'; I recommend that you delete that word.
Reply: We thank the reviewer for the helpful comments. We have deleted this word.
[266]: Again: Use more recent references - maybe a recent review of animal models for myopia.
Reply: We thank the reviewer for the helpful comments. We have added it to our manuscript. "7. Schaeffel F, Feldkaemper M. Animal models in myopia research. Clin Exp Optom 2015; 98:507–517." (see Page 12, Line 259)

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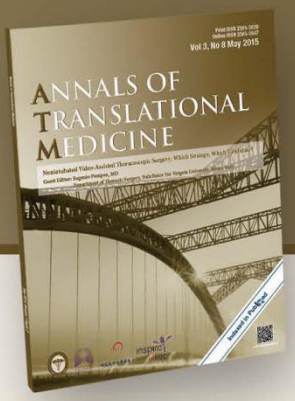

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[270-271]: Yes, the retina of rhesus monkey has a fovea, and probably an area centralis or macula; but it would be more useful to say something like, 'a central cone-rich region and rodrich periphery' 4

Reply: We thank the reviewer for the suggestion. We have revised it as "the retina of monkey has a central cone-rich region and rod-rich periphery, which enables better vision". (see Page 12, Line 262-263)

[274]: A particular strength of mouse as experimental model is the availability of many useful transgenic strains.

Reply: We thank the reviewer for the suggestion. We have revised it as "Mice constitute the most widely used animal model in biomedical research, with the availability of many useful transgenic strains and complete genome data" (see Page 13, Line 265-266).

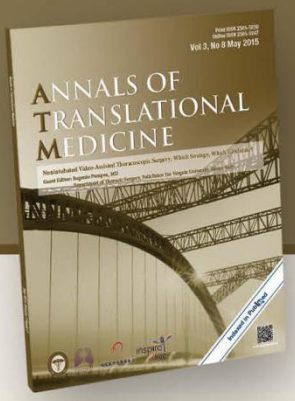
[276]: Emphasize how small the change in axial length is, by adding 'only', before '5.4-6.5 μm'.

Reply: We thank the reviewer for the suggestion. We have revised it as "a calculated axial eye elongation of only 5.4-6.5 μm was sufficient to make the schematic eye more myopic by 1 D". (see Page 13, Line 269)

[300]: You can't say that amblyopia developed 'after' 1 week of FD, because you didn't measure earlier; it might have developed at any time during the first week. (Wouldn't it be interesting to do further measurements, at shorter intervals!) [301] Similarly, you can say only that FDM developed between 1 and 4 weeks of FD.

Reply: We thank the reviewer for the helpful comments. We have revised it as "form deprivation amblyopia was developed during1 week of form deprivation, while form deprivation myopia was established between 1 and 4 weeks of form deprivation". (see Page 14, Line 294-295).

There are some limitations to our study, this has been added to the text in Discussion: "Fourth, we only used 3 timepoints to investigate changes with form deprivation. It is unclear whether the sVEP changes of the deprived eyes occurred in a shorter deprivation time than 1 week, and it is also difficult to know if the myopia development was happening at 1.5 or 2 weeks after form



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deprivation, a further study is needed to determine the measurement changes at shorter intervals" (see Page 17, Line 357-362).

[301], 'acuity decline at the last two time points': Really? It doesn't appear to me that there is any decline at all; and you have declared repeatedly that acuity remained unchanged in the FD eyes.

Reply: We thank the reviewer for the helpful comments. We have deleted this sentence.

[307]: Here is an even more relevant and important paper to discuss: Prusky et al., J. Neurosci., (2006) 26(45):11554 –11561. "Enhancement of Vision by Monocular Deprivation in Adult Mice"; and another study from the same group: Douglas et al., Visual Neuroscience (2005), 22, 677–684; "optokinetic acuities [in rats & mice] were ... consistently lower than acuity estimates from the Visual Water Task"

Reply: We thank the reviewer for the helpful comments and suggested references. We have added suggested references to our manuscript (see Reference29,30). We have added them to the text in Discussion: "even in adult mice, over 5 days of monocular deprivation could lead to an enhancement of the optokinetic response selectively through the nondeprived eye(30,31)" (see Page 14, Line 301-303) and "There are some limitations to our study. First, electrode placement would affect visual recording; implanted electrode or virtual-reality optokinetic system would provide stronger reliability for repeated measurement of visual acuity(31)" (see Page 17, Line 351-353).

[341-344]: This strikes me as mere speculation; both the basis in evidence, and the line of reasoning, are unclear.

Reply: We thank the reviewer for the suggestion. We have deleted this sentence and revised this paragraph as "Based on the results and discussion above, we speculate that early brief monocular form deprivation, setting up competition between the eyes' inputs to the cortex, leads to a dramatic shift in the ocular dominance distribution of primary visual cortex units in favor of the nondeprived eye and residual suppressive binocular interactions, resulting in amblyopia of the deprived eye(14), while after the retina receives the blurred visual stimulation input of long-term duration of form deprivation, the morphological structure of the retina and the content

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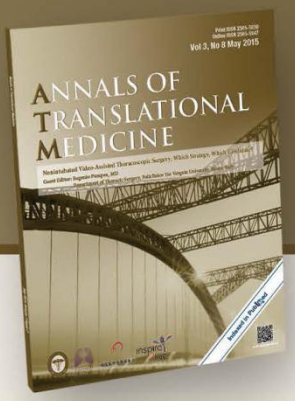

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of a variety of bioactive substances are changed, leading to sclera remodeling, ocular elongation, and eventually the development of myopia." (see Page 15-16, Line327-335).

[353], 'relatively large crytstal size' à 'relatively thick crystalline lens'

Reply: We thank the reviewer for the suggestion. We have revised it as "relatively thick crystalline lens" (see Page 16, Line344).

[370-372]: A short literature review on what is known about structural and functional maturation of the GP retina and cortex would be helpful – more helpful, perhaps, than all the cross-species comparisons that have taken up a lot of space in this MS.

Reply: We thank the reviewer for the helpful comments. We have added several references to our manuscript (see Reference48-50). We have added them to the text in Conclusion: "The axial growth, emmetropization rate and key aspects of retinal development in guinea pigs are similar to humans, and there is a significant binocular interaction in guinea pig brain(48-50). Guinea pig has potential to become an important mammalian model for studies of amblyopia" (see Page 17-18, Line 369-373).

[541], Fig. 3: Why don't you use this figure to illustrate the one parameter that you measured by A-scan: (total) axial length. (That should be very simple: add a double-headed arrow.) J ... Also, produce a higher-resolution trace for publication, and add labels on both axes.

Reply: We thank the reviewer for the helpful comments. We have produced a higher-resolution trace and added a double-headed arrow and labels on both axes in this figure (see Figure 4), and illustrate axial length in Figure Legends: "The double-headed arrow between C and R represents axial length". (see Page 27, Line 572-573).

[547], 'variation': Should this be 'deviation'?

Reply: We thank the reviewer for the suggestion. We have revised it as "standard deviation" (see Page 27, Line 580).

[Table 1]: (a) There is no such thing as 'p=0'. (b) 'and time corresponding normal control eyes' à '... and contralateral [or normal control] eyes after the same duration of FD': Is this what you mean?

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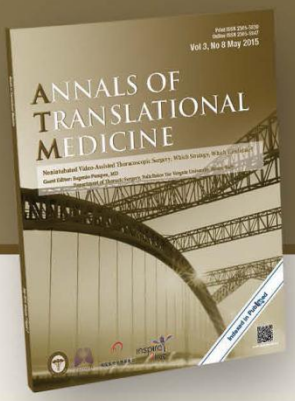

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Reply: We thank the reviewer raises an important consideration. We have revised it as “0.000” and “A indicates significant difference between form deprived eyes and contralateral eyes after the same duration of form deprivation; B indicates significant difference between form deprived eyes and normal control eyes after the same duration of form deprivation”. (see Table 2).

Reviewer B

Comment 1:

This manuscript presents data comparing refractive error, axial length and visual acuity in guinea pigs undergoing form deprivation. The authors show that the guinea pigs develop decreased visual acuity in the form-deprived eye at 1 weeks, but have normal refractive error and axial length. However, by 4 weeks, myopia has developed and visual acuity remains decreased. The authors conclude that amblyopia occurs prior to myopia and that the neuroplasticity of visual cortex may play an important role in FDM. However, the authors have no data on visual cortex plasticity or neuroplasticity, in general, and thus, their results do not support their conclusions. The authors need to carefully consider what their results mean and how to make conclusions that are fully supported by their current data.

In addition, the authors have used 3 timepoints to investigate changes with FD, 1, 4 and 8 weeks post-FD. There is a considerable gap between 1 and 4 weeks which makes it difficult to know if the myopia development was happening at 1.5 or 2 weeks post-FD which might suggest a closer connection between the changes in sweep VEP results and refractions and axial length.

Finally, the authors need to consult with a statistician about the proper reporting of the two-way ANOVA analyses. While it is helpful to see the F value and degrees of freedom reported, the interaction effect should be reported first and if significant, then the post-hoc comparisons used to compare groups. If the interaction effect is not significant, then the main effects of time and treatment can be reported.

Reply 1: We thank the reviewer for the helpful comments. We had deleted the speculation about “the role of neuroplasticity” and added additional references to support the results. These

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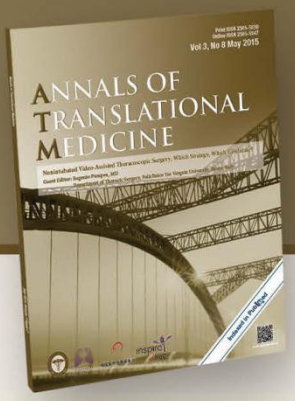

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changes will not influence the content and framework of the paper. Manuscript had been touched up and each change was highlighted in yellow color in the revised manuscript.

We thank the reviewer raises an important consideration on "a considerable gap between 1 and 4 weeks". There are some limitations to our study, this has been added to the text in Discussion: "Fourth, we only used 3 timepoints to investigate changes with form deprivation. It is unclear whether the sVEP changes of the deprived eyes occurred in a shorter deprivation time than 1 week, and it is also difficult to know if the myopia development was happening at 1.5 or 2 weeks after form deprivation, a further study is needed to determine the measurement changes at shorter intervals" (see Page17, Line 357-362).

We thank the reviewer raises an important consideration on "the proper reporting of the two-way ANOVA analyses". We have revised it as your suggestion. (see Table 2).

Comment 2:

Specific comments are below:

1) The title needs to be revised to remove "neuroplasticity" since this was not investigated in this study.

Reply: We thank the reviewer for the helpful comments. We have deleted it and revised the Title as: "Co-existence of myopia and amblyopia in a guinea pig model with monocular form deprivation". (see Page1, Line2-3)

2) The authors have used primary sources in most places, which is appropriate, but often only one reference is provided when many studies have contributed to the point being made. The addition of more citations would strengthen the manuscript.

Reply: We thank the reviewer for the helpful comments. We have made this paragraph brief and revised it as: "Animal experimental studies have established the importance of visual feedback in eye and refractive state development, and have demonstrated that form deprivation myopia is a graded phenomenon; increases in the degree of image degradation have been positively correlated with the severity of the induced axial myopia, the uncoordinated ocular growth has been found to be due to reduced retinal image contrast, and the absence of visual feedback has

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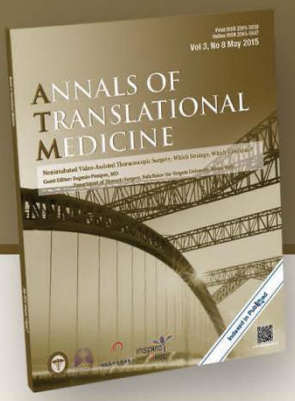

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been reported to be related to the effective refractive state of the eye (6-8). Many experiments regarding normal visual development in these animals have shown that the age of onset of form deprivation myopia in these animals coincided with the period of visual development and that larger refractive errors were present in younger animals and after a longer duration of form deprivation (9-13). For example, monocular form deprivation was performed in monkeys at the age of 2 weeks and led to the development of myopia of -13.5 diopters (D) after 18 months; performing monocular form deprivation at the age of one year led to the development of only -4.5 D myopia after 26 months. Eye development in the guinea pigs was basically completed by 3 weeks, and considerable myopia of approximately -3.4, -5.8, and -5.7 D was present after 6, 11, 16 days, respectively, of form deprivation performed at the age of 5 days. Similarly, any abnormal visual experience (e.g., monocular form deprivation, strabismus, or anisometropia) imposed during the period of visual development could result in abnormalities of the visual system (e.g., amblyopia); however, as this was initiated at progressively older ages, the resulting abnormality was smaller or even without visual impairment at all (14). For example, in rhesus monkeys, monocular form deprivation initiated during early life produced a severe degree of amblyopia, and the effect on spatial vision decreased systematically as the age of onset was delayed (15,16)**. Table 1** summarizes the onset time, duration, final induced diopter, and course of time from birth for emmetropization in each animal model of form deprivation myopia, as well as the relationship between the deprivation onset time and the amblyopia severity in rhesus monkeys"(see Page5-6, Line 93-120) .
We have added additional references to our manuscript in References:
"6. Troilo D, Smith EL, Nickla DL, et al. IMI – Report on Experimental Models of Emmetropization and Myopia. Investigative Opthalmology & Visual Science 2019;60:M31.
7. Schaeffel F, Feldkaemper M. Animal models in myopia research. Clin Exp Optom 2015;98:507-17.
8. Chakraborty R, Read SA, Vincent SJ. Understanding Myopia: Pathogenesis and Mechanisms. In: Ang M, Wong TY, editors. Updates on Myopia: A Clinical Perspective. Singapore: Springer Singapore; 2020. p. 65-94". (see Page , Line).

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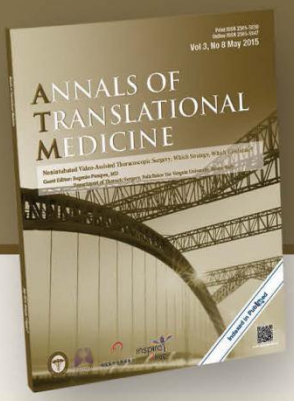

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3) The introduction is confusing. The authors appear to be making comparisons between species of when FD was induced at different ages. However, most of the species listed don't have more than one age of induction for FD, so it is difficult to understand what the overall conclusion. If the authors are trying to argue that these are critical periods of development, the studies cited do not support that as most haven't investigated the beginning and ending of the critical period. Additionally, a table or graph to better show these comparisons would help the reader.

Reply: We thank the reviewer for the helpful comments. We have made the Introduction brief and added a Table to show the onset time, duration, final induced diopter and time-course from birth for emmetropization in each animal model of form deprivation myopia, and the relationship between deprivation onset time and amblyopia severity in rhesus monkeys. (see Table1).

4) In lines 100-101, the authors state that "critical period of vision development" in mice. What aspect of "vision" was studied?

Reply: We thank the reviewer for the helpful comments. The "critical period of vision development" studied the developmental plasticity in the mouse primary visual cortex. We have deleted this paragraph.

5) Line 102-103. The authors have not provided evidence to support this conclusion.

Reply: We thank the reviewer for the helpful comments. We have deleted the word "critical period" and added Table1 and Reference "6. Troilo D, Smith EL, Nickla DL, et al. IMI – Report on Experimental Models of Emmetropization and Myopia. Investigative Opthalmology & Visual Science 2019;60:M31"to support this conclusion.

6) Line 105-177, the references show that visual development was affected but not that the critical periods of myopia and amblyopia overlap.

Reply: We thank the reviewer for the helpful comments. We have added **Table1** and Reference "14. Kiorpes L. Understanding the development of amblyopia using macaque monkey models. Proceedings of the National Academy of Sciences 2019;116:26217-23."to support this conclusion.

We have revised it as: "Similarly, any abnormal visual experience (e.g., monocular form

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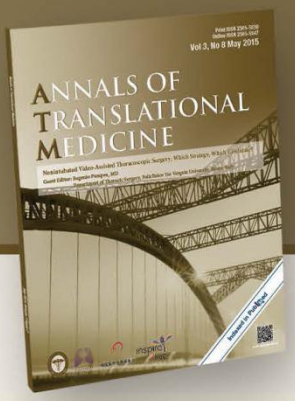

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deprivation, strabismus, or anisometropia) imposed during the period of visual development could result in abnormalities of the visual system (e.g., amblyopia); however, as this was initiated at progressively older ages, the resulting abnormality was smaller or even without visual impairment at all (14). For example, in rhesus monkeys, monocular form deprivation initiated during early life produced a severe degree of amblyopia, and the effect on spatial vision decreased systematically as the age of onset was delayed (15,16)" (see Page 5-6, Line 109-116).

7) Line 144. The authors should state the timepoints tested at first mention here.

Reply: We thank the reviewer raises an important consideration. We have revised it as "All animals underwent sweep VEPs and biometric measurement (refraction and axial length) prior to the experiment and at 1, 4, and 8 weeks after form deprivation induction". (see Page 7, Line 148-151).

8) Line 152. Provide the anesthesia used.

Reply: We thank the reviewer for the helpful comments. We have added and revised it as: "Guinea pigs were anesthetized by inhaling 2% isoflurane" (see Page 8, Line159).

9) Line 155. Change to "the active needle electrode"0

Reply: We thank the reviewer for the helpful comments. We have revised it as your suggestion (see Page 8, Line 165).

10) Acuity extrapolation. The authors approach to find the visual acuity threshold is not acceptable. The authors should examine methods used in other sweep VEP programs or only use the max amplitude obtained. The current method appears to artificially inflate the cpd values with the linear fit.

Reply: We thank the reviewer raises a thoughtful consideration. We have added reference "20. Ridder WH, 3rd. Methods of visual acuity determination with the spatial frequency sweep visual evoked potential. Doc Ophthalmol 2004;109:239-47"and detailed explanation for the method of acuity extrapolation: "The data were determined to be noise if the signal to noise ratio (SNR, open/filled circles) was less than 2. As seen in Figure 3a, the SNR did not exceed 2 for the 0.39, 0.50, 0.65, or 0.80 cpd data. Acuity was determined by fitting a line between the high spatial frequency data that were above noise (0.3 cpd) and the first spatial frequency that entered the

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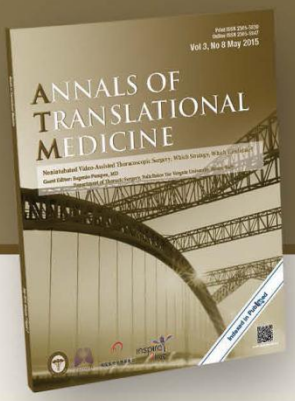

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noise (0.39 cpd) (solid arrows). The linear fit was extrapolated to the X-axis (zero amplitude) for visual acuity (dashed arrows).” (see Page9, Line181-187).
We have also revised the Figure (see Figure3).

11) Line 288-9. Since myopia and amblyopia are tightly linked, how would the authors prove that they aren't?

Reply: We thank the reviewer raises an important consideration. We have revised it as:
“ Although there are reports of the development of significant myopia following monocular form deprivation in many animal models and the development of form deprivation amblyopia in monkey and mouse models, there has not yet been any experiment to study the fact that form deprivation influences the acuity of form-deprived eyes in guinea pigs and that guinea pigs develop form deprivation amblyopia as a result of monocular form deprivation. The possible co-existence of amblyopia and myopia in form-deprived guinea pigs also remains unexplored. The present study was the first to use sweep VEPs to test the visual acuity of guinea pigs and to verify the co-existence of myopia and amblyopia after monocular form deprivation in guinea pigs” (see Page 13, Line 275-284)

12) Line 334-6. The authors have provided no evidence for this speculation of ocular dominance imbalance. More explanation is needed for the reader.

Reply: We thank the reviewer for the helpful comments. We have added a reference “14. Kiorpes L. Understanding the development of amblyopia using macaque monkey models. Proceedings of the National Academy of Sciences 2019;116:26217-23” and revised it as: “Based on the results and discussion above, we speculate that early brief monocular form deprivation, setting up competition between the eyes’ inputs to the cortex, leads to a dramatic shift in the ocular dominance distribution of primary visual cortex units in favor of the nondeprived eye and residual suppressive binocular interactions, resulting in amblyopia of the deprived eye(14), while after the retina receives the blurred visual stimulation input of long-term duration of form deprivation, the morphological structure of the retina and the content of a variety of bioactive

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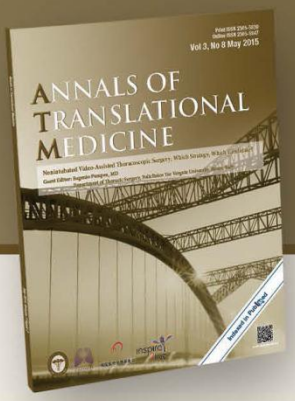

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substances are changed, leading to sclera remodeling, ocular elongation, and eventually the development of myopia" (see Page 15-16, Line 327-335).

13) Line 353. What is meant by "large crystal size"?

Reply: We thank the reviewer for the helpful comments. We have revised it as "relatively thick crystalline lens". (see Page 16, Line 344)

14) Line 354-356. The speed of stimulation is dependent on the retinal and visual cortex processing and not on the size of the eye.

Reply: We thank the reviewer for the helpful comments. We have revised it as "rodent eyes have characteristics of short axial length, relatively thick crystalline lens, lack of fovea, but high cell density region in retina, so the imaging of external objects in the retina is very small and they are unable to well fixation, therefore, the moving speed of stimulation must be relatively high to cause the change of retinal image and fast moving graphic stimulation can stimulate the maximum amplitude and the shortest latency of VEPs"(see Page 16, Line 343-348)

15) Line 373-375. The authors have not provided evidence to support this statement.

Reply: We thank the reviewer for the helpful comments. We have deleted the sentence.

16) In Figure 2, the authors should provide an example of both the FD and ungoggled eyes. Is the amplitude values shown from a FD eye?

Reply: We thank the reviewer raises an important consideration. We have added the example of both the FD and ungoggled eyes (see Figure 3a, b).

17) Figure 3 needs to include x- and y-axis labels

Reply: We thank the reviewer raises an important consideration. We have added labels on both axes in this figure (see Figure 4)

18) Table 1. This table is difficult to follow for the reader. The spacing is off for refraction, week 4

FD eyes. It is unclear what the F values in the timepoints versus under week 0 represent. This should be clarified. Also note that the comment above about interaction effects being reported.

Reply: We thank the reviewer for the helpful comments. We have revised it as your suggestion

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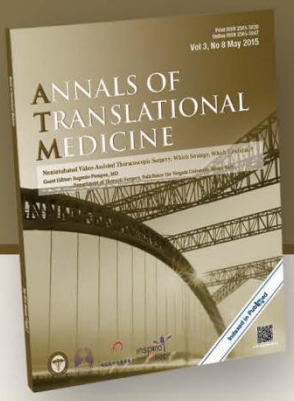

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(see Table 2).

Reviewer C

Comments to authors:

In the manuscript entitled “Co-existence of myopia and amblyopia in a guinea pig model with monocular form deprivation: the importance of neuroplasticity during critical period of visual development”, the authors explored the visual function during the monocular form deprivation in a strain of pigmented guinea pig. They found that the animals developed amblyopia when the animals were developing form-deprived myopia. This result should be expected because myopia researchers routinely induce myopia to accelerate eye growth during animals’ critical vision developing period. It would be easier to induce more myopia in younger animals.

Comment 1:

Introduction:

Instead of explaining what amblyopia is, the authors went straight into talking about what happened in several myopia animal models. More than half of the introduction was on listing the starting ages for deprivation treatment and the lasting time of deprivation on different animal models, and how their emmetropization happened in the critical visual development. It is the classic way to use vision deprivation to induce myopia; refractive error can cause amblyopia if not corrected during critical visual development, needless to say, vision deprivation can cause even worse amblyopia. Vision and refraction are two functional aspects of the eye; the point is you need to focus on the specific topic of what you are studying.

Then authors introduced that they established monocular form deprivation myopia model in guinea pigs (this was already documented by the Wenzhou myopia research group in 2006) and did VEP recording and found amblyopia occurred when inducing myopia in guinea pigs. In the current study, authors wanted to know whether myopia and amblyopia occurred simultaneously or successively, but they did not focus on detailed time-point design, they only turned to introduce how sweep VEPs worked on guinea pigs.

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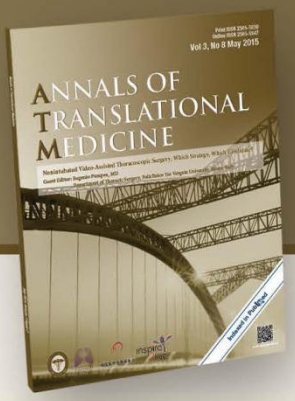

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The whole writing logic is not clear and there is no hypothesis, causing confusion to readers why authors are going to do what other researchers had already done.

Reply1: We thank the reviewer for the helpful comments. We have started the Introduction with a brief statement about FDM: “Form deprivation myopia is one of the ametropia in humans with identifiable causes such as congenital cataract, ptosis, and corneal opacity. In order to investigate the mechanisms of myopia in humans, form deprivation myopia has been induced in many animals(1-5). Animal experimental studies have established the importance of visual feedback in eye and refractive state development, and have demonstrated that form deprivation myopia is a graded phenomenon; increases in the degree of image degradation have been positively correlated with the severity of the induced axial myopia, the uncoordinated ocular growth has been found to be due to reduced retinal image contrast, and the absence of visual feedback has been reported to be related to the effective refractive state of the eye (6-8)” (see Page5, Line 90-99).

We have made Introduction brief and revised it as: “Many experiments regarding normal visual development in these animals have shown that the age of onset of form deprivation myopia in these animals coincided with the period of visual development and that larger refractive errors were present in younger animals and after a longer duration of form deprivation (9-13). For example, monocular form deprivation was performed in monkeys at the age of 2 weeks and led to the development of myopia of -13.5 diopters (D) after 18 months; performing monocular form deprivation at the age of one year led to the development of only -4.5 D myopia after 26 months. Eye development in the guinea pigs was basically completed by 3 weeks, and considerable myopia of approximately -3.4, -5.8, and -5.7 D was present after 6, 11, 16 days, respectively, of form deprivation performed at the age of 5 days. Similarly, any abnormal visual experience (e.g., monocular form deprivation, strabismus, or anisometropia) imposed during the period of visual development could result in abnormalities of the visual system (e.g., amblyopia); however, as this was initiated at progressively older ages, the resulting abnormality was smaller or even without visual impairment at all (14). For example, in rhesus monkeys, monocular form deprivation initiated during early life produced a severe degree of amblyopia, and the effect on

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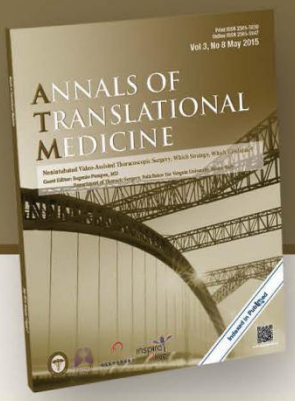

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spatial vision decreased systematically as the age of onset was delayed (15,16)" (see Page 5-6, Line 99-116) .
We have sdded a Table to summarize the onset time, duration, final induced diopter and time-course from birth for emmetropization in each animal model of form deprivation myopia, and the relationship between deprivation onset time and amblyopia severity in rhesus monkeys.(see Table1).
We thank the reviewer raises an important consideration on "detailed time-point design". There are some limitations to our study, this has been added to the text in Discussion: "Fourth, we only used 3 timepoints to investigate changes with form deprivation. It is unclear whether the sVEP changes of the deprived eyes occurred in a shorter deprivation time than 1 week, and it is also difficult to know if the myopia development was happening at 1.5 or 2 weeks after form deprivation, a further study is needed to determine the measurement changes at shorter intervals" (see Page 17, Line 357-362).
We have added "However, there have not been any studies on the effects of brief, moderate, and long-term form deprivation periods on the visual acuity of guinea pigs, and little attention has been paid to whether myopia and amblyopia occurred simultaneously or successively. Therefore, in this study, we established a monocular form deprivation model in guinea pigs with our own procedures, and then recorded refraction, axial length, and the amplitude of sweep VEPs at different intervals in the deprived eyes, contralateral eyes, and normal control eyes" to explain why we did this study. (see Page 6, Line 122-129).
We have also added additional references to our manuscript in References:
"6. Troilo D, Smith EL, Nickla DL, et al. IMI – Report on Experimental Models of Emmetropization and Myopia. Investigative Opthalmology & Visual Science 2019;60:M31.
7. Schaeffel F, Feldkaemper M. Animal models in myopia research. Clin Exp Optom 2015;98:507-17.
8. Chakraborty R, Read SA, Vincent SJ. Understanding Myopia: Pathogenesis and Mechanisms. In: Ang M, Wong TY, editors. Updates on Myopia: A Clinical Perspective. Singapore: Springer Singapore; 2020. p. 65-94". (see Page , Line).

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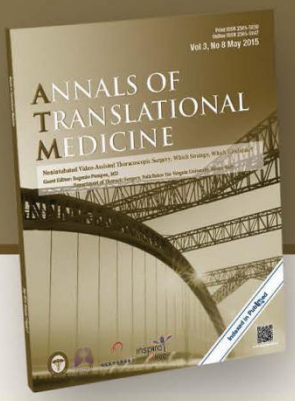

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14. Kiorpes L. Understanding the development of amblyopia using macaque monkey models. Proceedings of the National Academy of Sciences 2019;116:26217-23."(see Reference)

Comment 2:

Main concerns about Methods and Results:

From line 167 to line 174, authors need to explain more on the whole procedure of VEP operation since guinea pig is different from human, rats and mice.

The unit of stimulus as spatial frequency was expressed in cycle per degree (cpd), where the smaller the number, the bigger the stimulus, and the larger the amplitude of recorded neuronal responses, such as, showing larger amplitude with bigger stimulus. But in figure 2, it showed the opposite between 0 and 0.4, and suddenly all visual responses went down to the noise. It seems that the authors misunderstood the literature they cited to explain the extrapolation of the VEP recording data to the visual acuity. Authors should be very careful to check this description and give reasonable explanation.

Reply 2: We thank the reviewer raises a thoughtful consideration. This has been added to the text in Methods: "The stimuli were presented using a 21-inch CRT monitor. The stimulus contrast was 80% and the temporal reversal rate (grating wave) was 3 Hz (about 6 reversals/s). The mean luminance of screen was 100 cd/m^2. The stimulus screen was viewed monocularly at 20cm (100°wide ×82°high). Each spatial frequency was presented for 11 s and there was 1 s of adaptation before data collection. The entire sweep took 132 s (12 s ×11 spatial frequencies)" (see Page 8-9, Line171-177).

We have added a sample stimulus trace (see Figure2) and explained it in Figure Legends: "Figure 2: A diagram regarding the course of time and the eleven stimuli trace. Each spatial frequency was presented for 11 s and there was 1 s of adaptation before data collection. The stimulus contrast was 80% and the temporal reversal rate (grating wave) was 3 Hz (about 6 reversals/s)" (see Page 26, Line 552-555).

We have revised the figure of acuity extrapolation (see **Figure3**).

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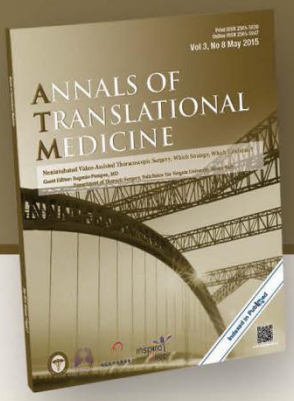

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We have added reference "20. Ridder WH, 3rd. Methods of visual acuity determination with the spatial frequency sweep visual evoked potential. Doc Ophthalmol 2004;109:239-47".
We have added detailed explanation for the method of acuity extrapolation: "The data were determined to be noise if the signal to noise ratio (SNR, open/filled circles) was less than 2. As seen in Figure 3a, the SNR did not exceed 2 for the 0.39, 0.50, 0.65, or 0.80 cpd data. Acuity was determined by fitting a line between the high spatial frequency data that were above noise (0.3 cpd) and the first spatial frequency that entered the noise (0.39 cpd) (solid arrows). The linear fit was extrapolated to the X-axis (zero amplitude) for visual acuity (dashed arrows)" (see Page 9, Line 181-187).

Comment 3:

Figure 4, there is a problem with the scales on X-axis, where there is one week from scale 0 to 1, three weeks from scale 1 to 4, and four weeks from scale 4 to 8. This will mislead readers to think that the changes of eye parameters induced by form deprivation for 3 or 4 weeks happened within one week.

Reply 3: We thank the reviewer raises a thoughtful consideration. We have changed it to different scales (time interval) on the X-axis. (see Figure 5).

Comment 4:

Discussion:

From line 333 to line 344, it is generally accepted that myopia development is a local eye growth event, it can happen without the involvement of central nervous system. Authors should discuss about the results obtained from the current study rather than speculating too much.

Reply 4: We thank the reviewer for the helpful comments. We have revise it as: "Based on the results and discussion above, we speculate that early brief monocular form deprivation, setting up competition between the eyes' inputs to the cortex, leads to a dramatic shift in the ocular dominance distribution of primary visual cortex units in favor of the nondeprived eye and residual suppressive binocular interactions, resulting in amblyopia of the deprived eye(14), while after the retina receives the blurred visual stimulation input of long-term duration of form deprivation, the morphological structure of the retina and the content of a variety of bioactive

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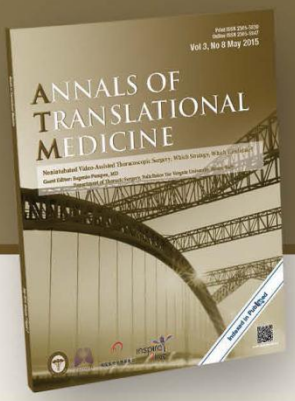

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substances are changed, leading to sclera remodeling, ocular elongation, and eventually the development of myopia" (see Page 15-16, Line 327-335)