Author’s response to reviews

Title: Therapeutic effect of vasoactive intestinal peptide on form-deprived amblyopic kittens

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Version: 1 Date: 20 Jul 2019

Author's response to reviews:

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers’ comments of our manuscript entitled “Therapeutic effect of vasoactive intestinal peptide on form-deprived amblyopic kittens” (ID : BOPH-D-19-00316). Those comments are all valuable and very helpful in revising and improving our paper, the comments are of huge implication for guiding our studies. We have studied comments carefully and have made revisions which we hope to meet with approval. The revised portions are marked in red in the paper. The revisions in the paper and the responses to the reviewer’s comments are as follows:
Responses to the reviewer’s comments:

Reviewer#1:

1. Response to comment: (Page numbers are lacking.)

   Response: Thank you for your comment. We have already marked the page number and the line number of the manuscript.

2. Response to comment: (Page 4, lines 1-24: These information should be in the methods (first paragraph) and in the results (second paragraph) sections.)

   Response: We have deleted partial lengthy text in introduction (line 1-13 in page 4), to make it more consistent with the journal's requirements.

3. Response to comment: (Page 5, line 1: "...keep enough toys, cat scratching boards and cat litter in.." Please revise the sentence.)

   Response: We have revised “keep enough toys, cat scratching boards and cat litter in the room (provided by the Experimental Animal Centre of North Sichuan Medical College)” as “provided with enough toys, cat scratching boards and cat litter in the room (provided by the Experimental Animal Centre of North Sichuan Medical College)”. line 6-10 in page 5.

4. Response to comment: (Please give the exact p values rather than giving p<0.05.)

   Response: We have substituted the specific number for all P-values in the result of manuscript, we have substituted specific number for all P-values, as demonstrated in page 9-12.

5. Response to comment: (Discussion, second paragraph, line 37: "....., thereby inhibiting the physiological effect of vascular endothelial growth factor on visual development." Are vascular endothelial growth factor and VIP related?)

   Response: Regretfully, we have misspelled the words in this sentence, which caused your misunderstanding. We have revised this sentence to: “Down-regulated expression of VIP in the LGBd will lead to a reduction in the binding of VIP to its associated receptors, thereby probably inhibiting the expression of the corresponding function”. Line 16-18 in page 13.

Special thanks to you for good comments.
Reviewer#2:

1. Response to comment: (In the introduction, I would like to see more details describing if the neurotransmitters involved in visual development are decreased or increased and what the normal pattern of expression should be during development (i.e. do they increase and peak during normal development or are they at a constant level of expression). This is particularly important for VIP.)

   Response: We are grateful for your comments. We have cited a document (twelfth document, line 18-20 in page 3) in the background to elucidate VIP expression in the cerebral cortex. Since we have not yet found relatively complete studies on the expression variations of kitten’s cerebral cortex VIP in recent years, we have cited the progressive research of VIP expression in rat cerebral cortex given that both cats and rats fall under the class of mammals, hoping that it may help readers to understand our manuscript. (The cerebral cortex of rats began to express VIP at birth, and VIP expression in the cortex was significantly up-regulated 42 days ago, and the expression was significantly down-regulated from 42 days to adulthood [12]).

2. Response to comment: (It would be good to illustrate the deprivation procedure using a schematic diagram)

   Response: We have added Figure 1 to illustrate the covering method of deprivation group (A) and presented the grouping process of the kitten in the whole experiment (B) to make readers understand the experimental process more easily.

3. Response to comment: (I was unclear what Sefsol was until much later in the manuscript. It would be advisable to explain what this is much earlier in the manuscript otherwise, a reader could think that this is an alternative treatment as opposed to being the solvent being used to deliver the VIP solution.)

   Response: We have marked Sefsol as a type of VIP solvent when it was first mentioned in the abstract. (Line 24, page 1).
4. Response to comment: (It would be good to illustrate the VEP results by showing the mean +/- SEM VEP waveforms for each of these groups side by side on the same scale, in order to facilitate a visual comparison of the results.)

Response: We have revised Table 1 to make it easier to compare the result of PVEP intuitively.

5. Response to comment: (Were there any side effects or evidence of toxicity in the treated kittens? This would be important to include in the results.)

Response: Thank you very much for your comments. We have added a description of the results: “no obvious changes in the habits of kittens were observed, and the feeding process was normal.” (Line 21-22 in page 9). At the end of the study, we have not found the obvious abnormal behavior in kittens, whereas the side effect of VIP requires further studies.

6. Response to comment: (Would recommend labelling each panel with the group subtype so that it is easier for the reader to interpret.)

Response: We have added a grouping diagram (Fig. 1 B) to help the reader understand the paper.

7. Response to comment: (Some minor language correction is needed. I have attached an annotated version of the manuscript with the errors that I have spotted highlighted. Please check for any others.)

Response: Thank you very much for pointing out the language expression errors in our manuscript, and we have revised them all. We have made the following revisions in the resubmitted manuscript: 1. Line 3 in page 3, "the" has been deleted; Line 6-8 in page 3, the expression variations of these neurological factors in amblyopia have been described. 2. Line 6 in page 5, we have revised "keep enough" as "provided with enough"; Line 22 in page 5, we have revised "pay" as "paying". 3. Line 1 in page 6, we have revised "use" as "using"; Line 2 in page 6, we have revised “After the” as “After confirmation that the”, and “has” as “had”; Line 7 in page 6, we have increased the cover model and grouping diagram of kittens in the study; Line 8 in page 6, we have revised “wrap the kittens” as “the kittens were wrapped”; Line 9 in page 6, we have revised “wait for them to wake up” as “pay attention to their status”; Line 12 in page 6, we have added “and”. 4. Line 7 in page 8, we have revised “A gene pen was then used to mark the tissue, which was then” as “After natural cooling, ”. 5. Line 5 in page 9, we have revised
“does” as “did”. 6. Line 18-22 in page 13, we have split “At the same time, the decrease in VIP reduces the diurnal discharge frequency of neurons, affecting the long-term electrical activity in the suprachiasmatic nucleus of the central system [20,21] and inhibiting electrical transmission between neurons through the VPAC2-mediated cAMP pathway [22], thereby blocking the transmission of information in the visual nervous system.” as “At the same time, the decrease in VIP reduces the diurnal discharge frequency of neurons, thereby affecting the long-term electrical activity in the suprachiasmatic nucleus of the central system [20,21]. The down-regulation in VIP also inhibits electrical transmission between neurons via the VPAC2-mediated cAMP pathway [22], thereby hindering the information transmission in the visual nervous system.”. 7. Line 5 in page 14, we have revised “metastasis” as “translocation”.

Special thanks to you for good comments.

Reviewer#3:

1. Response to comment: (1. Please add the access number of animal (cats) approval.)

Response: According to your requirement, we have attached the relevant approval number for the kitten. Production approval number: SCXK (Liao) 2018-0003. Application approval number: SYXK (Chuan) 2019-215 (Line 22 in page 4 and line 1 in page 5).

2. Response to comment: (Did pento. interfere with "PVEP" functional studies? Please also show the typical curves of PVEP.)

Response: The action mechanism of pentobarbital sodium is primarily associated with the blocking brain stem reticular ascending activating system, which acts on the postsynaptic membrane of the central nervous system. It has the effect of quasi-γ-aminobutyric acid, which can reduce the excitability of the post-synaptic neuron, thus resulting in sedation, hypnosis, analgesia and anesthesia in the experimental animals. PVEP reflects the electric activity changes of the visual signal from the retina to the visual center. Given the effect of sodium pentobarbital on the central system, it is bound to affect the PVEP waveform acquisition. Besides, since kittens are not well matched, we must give them a certain level of anesthesia to complete the test. Hence, we have used an anesthetic with a ratio of 35mg/Kg for each kitten, hoping to minimize the effect of the anesthetic. We have added Figure 2 to show the typical curves of PVEP in each group of kittens.
3. Response to comment: (What is the real scale of Fig. 1 and 2? Please also show the arrow marker for positive cells (IHC))

   Response: We have indicated the dyeing method and magnification next to the picture title, both DAB×200 (line 17 in page 10, and line 16 in page 11). Also, we have marked the typical positive cells in the two images (Fig3 and Fig4).

4. Response to comment: (The legends of Figures and Tables need to be re-written. Also, it should be more descriptions of Results section)

   Response: We have revised the legends of the figures to give a more detailed description of the results section (pages 25-27 of the manuscript and the table file).

5. Response to comment: (Please use WB to elucidate VIP signalings on LGBd.)

   Response: Thank you so much for your comments. The WB experiment can more accurately reflect the differential expression of VIP, and it is also one of the commonly used methods to study protein differential expression in the world. The reason why we applied the method of Immunohistochemistry is that we hope to conduct location and semi-quantitative research preliminarily. In the meantime, due to the limited amount of specimens, WB was not applied to accurately quantify. In the next phase, we are ready to study the long-term intervention of VIP, and then we can apply WB to accurately quantify VIP.

6. Response to comment: (Please try IHCs on VIP-R to evaluate the crucial role of VIP on amblyopia.)

   Response: Thank you very much for your guidance on the study. The VIP-R study can more fully and accurately reflect the difference in VIP expression in LGBd and the therapeutic effect of VIP on amblyopia. Since the use of VIP-R was not considered in the initial experimental design, we will add VIP-R to the study in the subsequent VIP long-term intervention experiment.
7. Response to comment: (The early mechanism (PKA activation) need to be studied for VIP between pathological process of amblyopia.)

Response: According to the 17th document, in the central nervous system, VIP can facilitate the synthesis of cAMP by activating adenylate cyclase, and cAMP can further react with PKA, thereby phosphorylating the substrate, which is defined as neuronal metabolism. This document may elucidate the partial relation between the VIP in visual development and PKA activation, whereas the detailed relation and other interactions require further studies.

8. Response to comment: (Please evaluate visual acuity on amblyopia by treatment of VIP inhibitor (L-8-K).)

Response: Thank you for your valuable comments on the study. Since our experiment was originally designed, L-8-K was not involved. Thus, the present study is a long-term intervention study of VIP. We will add L-8-K treatment to this project, to make more convincing results.

9. Response to comment: (Please discuss the interactions between inflammation and VIP.)

Response: The 25th document and “Delgado M, Leceta J, Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit the production of inflammatory mediators by the activated microglia. J Leukoc Biol. 2003;73:155-164.” pointed out that activation of microglia is a histopathological hallmark of several neurodegenerative diseases. Pathological microglial activation is believed to contribute to progressive damage in neurodegenerative diseases through the release of proinflammatory and/or cytotoxic factors, including tumor necrosis factor α (TNF-α), interleukin (IL)-1β, IL-6, IL-12, and nitric oxide (NO). VIP inhibit TNF-, IL-1, IL-6, and NO production by lipopolysaccharide (LPS)-activated microglia. The specific type 1 VIP receptor mediates the inhibitory effect of VIP, and cyclic adenosine monophosphate is the major, second messenger involved. VIP regulate the production of these proinflammatory factors at a transcriptional level by inhibiting p65 nuclear translocation and nuclear factor-kB-DNA binding.

Special thanks to you for good comments.