ANSWERING REVIEWERS

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Dear Editor,

Please find enclosed our revised manuscript in Word format (file name: 4382-revised.doc).

Title: Variation in EAE scores in a mouse model of multiple sclerosis

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This manuscript has been revised according to the suggestions of the reviewers.

1. Revisions have been made according to the suggestions of the reviewers.

> *Reviewer 1: This is a nice mini-review pointing out a weakness in the EAE scoring across different labs. However, no suggestion for a homogeneous scoring approach is given. There should be at least a short discussion on how a more homogeneous scoring can be achieved.*

As suggested by the reviewer, we added the following sentences: To achieve the standard scoring system in EAE, it is necessary to define the terms for signs and to clarify the criteria for the signs in the EAE score. Furthermore, ambiguous representation, e.g., weak or strong weakness of the hind limb, must be eliminated *(page 11, line 19-page 12, line 1)*.

Reviewer 2:

Major concerns

1. Use (misuse) of correct medical terminology contributes to variation of EAE score. In this manuscript, the authors misused some terms, for example, "signs" versus "symptom." "Symptom" can be used only for humans, while "signs" can be used for both animals and humans. The authors need to discuss the following key words that are often misused by investigators: sign, symptom, paresis, paralysis, (para)plegia, coordination, flaccid (versus spastic), and ataxia, and should make a table or box including their definitions.

As suggested by the reviewer, we omitted the term "symptom" and replaced this term with "sign" (*page 3, line 9, page 8, line 1*).

In addition, regarding the misuse of terms related to EAE, the sentences "Many researchers misuse terms for evaluating EAE signs; for example, "EAE symptom" or "clinical assessment of EAE." The term "symptom" and "clinical" should only be used in the context of humans; and thus, the term "signs" must be used instead of "symptom." In addition, "clinical" must

not be used in EAE studies" have been added (*page 8, lines 4-9*).

We also mentioned the terms "paresis, paralysis, and paraplegia. We added the following sentences: The term "paralysis" indicates a complete or partial loss of voluntary movement. The prefix "para" means "both" and "plegia" means severe weakness. The term "paraplegia" is defined as a severe symmetrical muscle weakness of both lower limbs. "Paraparesis" commonly means slight or partial paralysis of both lower limbs; however, the definition of these terms is subjective and indistinct (*page 9, lines 3-8*).

Furthermore, terms such as "coordination, flaccid, spastic, and ataxia" have been initially used to explain neurological deficits observed in humans. Systematic neurological examination with the patient's cooperation must be obtained to make a neurological diagnosis. Thus, it is difficult to judge the diagnosis of EAE mice using coordination or muscle tonus due to the limited information provided by visual observation. Then, we did not add the explanation about the terms such as coordination, flaccid, spastic, and ataxia observed in humans in this mini review.

2. Page 4, second paragraph. The authors described EAE, as if there were differences between mouse EAE versus rat EAE. As far as this reviewer knows, there are no reports showing the difference between the two species. These sentences should be rewritten to avoid misleading the readers.

As suggested by the reviewer, we revised these sentences to the following: Commonly used murine EAE models manifest motor

dysfunction as ascending flaccid paralysis, beginning with a flaccid or limp tail[7, 10, 11]. The paralysis progresses from the hind limbs to the fore limbs and is occasionally followed by urinary incontinence and fecal impaction (classical EAE models)[7, 10]. Lesions are predominantly localized to the spinal cord in classical EAE (*page 6, lines 16-20*).

3. *Page 4, last paragraph (Page 5, first paragraph). The authors need to define the different disease courses of EAE depending of the enecephalitogenic antigens and animal species and strains. MBP-induced EAE in PL/J mice do not develop relapsing-remitting EAE (see Liblau et al 1997, for example), and MOG33-55 EAE in B6 mice is not "chronic-progressive". Although many EAE researchers have described MOG-EAE in B6 mice as "chronic progressive," this is incorrect. The EAE is monophasic with incomplete recovery (usually no mice show real disease progression during the chronic stage). In contrast, there are several reports showing real primary progressive or secondary progressive EAE, where mice die with disease progression (for example, Tsunoda et al, 2000).*

As suggested by the reviewer, we revised this sentence to the following: The standard EAE mouse model is induced using myelin proteolipid protein (PLP) peptide (amino acids 139-151), which causes relapse-remitting EAE in SJL mice. In addition, the myelin oligodendrocyte glycoprotein (MOG) peptide (amino acids 35-55) causes monophasic EAE with an incomplete recovery in C57BL/6 mice[8]. Several reports have

demonstrated real primary progressive or secondary progressive EAE, in which mice die as a result of disease progression[14] (*page 6, line 20-page 7, line 6*).

4. Page 6. "righting reflex" needs a more detailed discussion. Impairment of righting reflex and its scoring system has been used in Theiler's murine encephalomyelitis virus infection, a viral model for multiple sclerosis (by groups of Drs. Moses Rodriguez and Robert S. Fujinami, for example). This scoring system is useful when animals do not show classical EAE signs. This scoring system should be introduced as a table.

In this review, we provide a discussion focused on the EAE score in classical EAE caused by active immunization. Thus, we do not mention Theiler's murine encephalomyelitis virus infection EAE model and atypical EAE. Impairment of the righting reflex is also seen in classical EAE models; thus, it has been evaluated using the EAE scoring system in many previous studies.

5. "Weight change" has been used to evaluate EAE in many EAE manuscripts. It has been shown that weight changes correlated with clinical signs during the acute stage of EAE. The authors need to discuss the weight changes and EAE scores in detail.

As suggested by the reviewer, we added the following: After

sensitization, in general, the mice are weighed regularly and scored for EAE signs. Body weight loss is a common feature of EAE that usually precedes paralysis, and low body weight remains during the recovery phase[30-32]. Body weight begins to increase during the chronic phase of the disease; thus, weight loss is an important sign during the acute stage of EAE (*page 10, lines 10-15*).

6. "Cumulative" clinical EAE scores have also been used in many EAE manuscripts. The authors should add a paragraph explaining the cumulative EAE score and its usefulness with references.

We do not have experience with the cumulative score or sufficient knowledge regarding this method. Thus, we could not provide additional details, so we added the following short sentence: In addition, the cumulative score is obtained by the sum of the daily EAE score using previously described methods (*page 10, lines 6-8*).

7. The authors discuss only the classical EAE signs and its scoring system. There is another type of EAE, "ataxic type" (Brown and McFarlin, Endoh et al, Greer et al, for example). The ataxic type of EAE should be explained with its scoring system.

In this mini review, we mainly discuss EAE scores in classical EAE. Thus, we would not discuss the ataxic form of EAE and the scoring system.

We added the following sentences: However, variations from the classical EAE phenotype, such as ataxia or the head rolling phenomenon rather than limb paralysis, have been described and are referred to as atypical EAE[15-17]. The clinical signs observed in atypical EAE models reflect an increase in inflammation in the brain compared to classical EAE models. The characteristics of spontaneous EAE mice are also defined as atypical EAE (*page 7, lines 6-11*).

Minor concerns

1. Page 3, the second sentence requires references.

As suggested, we added one reference, which is reference 1 in the current reference list (*page 5, line 4*).

2. Page 3, lines 6 and 7. What is the difference between "prevalence" and "incidence"?

The term "prevalence" means the proportion of a population that is affected by a particular disease, whereas the term "incidence" means the rate of occurrence of new cases of a disease in a population within a specified time period.

3. Page 3, line 13. "in time and space" instead of " in time and place"

As indicated, we have corrected this phrase as "in time and space."(*page 5, lines 13-14*)

4. Page 3, line 14. "three major type" is inaccurate. There are four subtypes in MS (see reference #6 by Lublin et al 1996).

As suggested, we have rewritten this sentence as the following: The clinical course of the disease is characterized by four major subtypes: relapse-remitting, secondary progressive, primary progressive, and progressive-relapsing MS[1, 4, 6] (*page 5, line 14-16*).

We have also added the following sentence: There is no consensus definition in progressive-relapsing MS[6] (*page 6, line 1-2*).

5. Page 3, line 20. "65% of patients." This phrase needs references.

As suggested, we added one reference, which is reference 4 in the current reference list (*page 5, line 21*).

6. Page 4, line 9. "myelin antigen" instead of "myelin component protein"

We replaced "myelin component protein" with "myelin antigen."(*page6, line 11*)

7. Page 4, line 10-11. "transgenic animals" needs more explanation.

We have rewritten the sentences as follows: In addition, T cell receptor transgenic mice have been generated as a spontaneous EAE model. The characteristics of these mice are variable, and thus, most of the spontaneous EAE mice are also defined as atypical EAE[8, 12, 13] (*page 6, lines 12-15*).

8. Page 5, line 4. EAE can be induced by not only "myelin component peptide" but also some other CNS antigens, for example, CNS homogenate, and whole myelin protein.

We used the term "myelin component peptide" because it has been commonly used in many laboratories in recent years. We replaced "myelin component protein" with "myelin antigen." (*page7, line 13*)

9. Page 5, second paragraph. The authors described that the disease onset of active and passive EAE is "9 to 14 days" and "10 and 15 days," respectively. This is incorrect. The disease onset of passive EAE is earlier than active EAE in general.

We added one reference, which is reference 11 in the current reference list (*page 8, line 2*).

10. Page 5, lines 9 and 17. "sensitization" instead of 'immunization"

We replaced "immunization" with "sensitization."(*page 7, line 18*)

11. Page 7, lines 4-5. "Non-continuity…" This sentence needs more explanation with references.

Because this sentence includes an expression that is confusing, we have omitted this sentence.

12. Page 7, "BBB score" needs more explanation.

We added the following sentences: The BBB sub-components include limb movement, trunk position, paw placement, walking, predominant paw position, trunk instability and tail position. BBB is rated on a scale from 0 (no observable hind limb movements) to 21 (consistent coordinated gait, consistent toe clearance, predominant paw position is parallel at initial contact and lift-off, tail consistently up, and consistent trunk stability), which represents the sequential recovery of spinal cord injury[34] (*page 11, lines 3-9*).

13. *Page 8, second paragraph. This is based on the authors' unpublished reference #29. The whole paragraph should be omitted until the authors publish the manuscript.*

As suggested, we omitted this paragraph (*page 11, second paragraph*) and added the following sentence: Our recent study suggests that the traveled distance is a sensitive and accurate marker of motor dysfunction in a MS mouse model (unpublished) (*page 11, lines 14-16*).

2. Our reference citation style has been corrected. We placed reference numbers in square brackets in superscript at the end each citation in the text as well as added a DOI citation to the reference list.

Thank you for your consideration of our manuscript for World Journal of Neurology.

Yours sincerely,

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