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Original Article

소동물 전정안구반사 측정용 마커 어레이의 최적화

[1]가톨릭관동대학교 국제성모병원 이비인후-두경부교실, 가천대학교 [2]이비인후과학교실, [3]뇌과학연구원

김미주[1], 이지연[1], 홍의재[2], 이은지[2], 민유진[1], 이동주[2], 김남범[3], 한규철[2]

Vestibulo-Ocular Reflex Recordings of Small Rodents using a Novel Marker Array

Mi Joo Kim[1], Jiyeon Lee[1], Eui Jae Hong[2], Eun Ji Lee[2], Yu Jin Min[1], Dong Ju Lee[2], Nam Beom Kim[3], Gyu Cheol Han[2]

[1]Department of Otorhinolaryngology, Catholic Kwandong University International ST. Mary's Hospital, Incheon; [2]Department of Otolaryngology-Head and Neck Surgery, Gachon University of Medicine and Science, Graduate School of Medicine; [3]Neuroscience Research Institute, Gachon University, Graduate School of Medicine, Incheon, Korea

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· Corresponding Author:
Gyu Cheol Han
Department of Otolaryngology-Head and Neck Surgery, Gachon University of Medicine and Science, Graduate School of Medicine, Simgok-ro 100gil 25, Seo-gu, Incheon 22711, Korea
Tel: +82-32-460-3324
Fax: +82-32-467-9044
E-mail: hangckr@gmail.com

Objective: Recording the nystagmus of small experimental rodents is an integral technique in vestibular research. Theoretically, the size and the shape of markers strongly affect the analysis of 3 dimensional nystagmus.
Methods: The nystagmus of 6 healthy ICR mice were recorded and their gain values were compared using 200 μm, 300 μm, 400 μm, and 600 μm isosceles triangle markers at the peak velocity of 60°/sec and 100°/sec with the rotational stimulations of 0.1 Hz, 0.2 Hz, and 0.5 Hz.
Results: The gain values of 3 different sizes of the markers showed no significant differences in horizontal- vertical-torsional component. However, it was unable to record the nystagmus with 200 μm markers since the markers were too small to be placed and stayed on the center of the pupils.
Conclusion: Technicians can decide the size of the markers from 200 to 600 μm to record the nystagmus of mice, depending on the technicians' skills.
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Keywords: Vestibulo-ocular reflex; Marker; Video-nytagmography; Mice

INTRODUCTION

Non-primate vertebrates are widely used to study the vestibular system because their vestibular reflexes are very similar to those of humans.[1] However, recording the vestibulo-ocular reflex (VOR) in laboratory animals, such as mice, requires sophisticated technical skills.[2,3]

Small rodents have small eye balls and the weak contrasts of iris and sclera.[4] Sclera-search-coil technique[5,6] and video-nystagmography[2,3] are commonly used to analyze the values of VOR accurately. Even though sclera-search-coil-technique has advantages with detecting micro-saccades, the usage of the technique is invaluable and limited due to its major side effects.[6] It is an invasive method, requiring the glue to be

placed on the pupil which can cause infections and leave scars, and furthermore, it can limit the eye movement; therefore, retaining the precise values might fail. Using the video-nystagmography (VNG) works with the light reflexes also has side effects and fastidious conditions because the eyeballs should always be moistened to detect and record nystagmus accurately.[2] Its disadvantage is also that it barely measures the circumnutating of the eyeballs. Complementing these disadvantages, the markers were used to detect the variations in the vector values of axis rotation and to record the eye movements. To record the nystagmus of small rodents, the markers are supposed to be placed on the pupil.[3]

Until now, the variable sizes of rectangular markers have been used for rabbits, guinea pigs, or chinchillas. However, the rectangular marker has the limit when it comes to reducing its size and this limit causes the lower precision since the rectangular markers are too large for small rodents such as mice.[5] Diminishing the marker size can bring out some restricted values, and it will not be accurate enough to evaluate the effects. There are pros and cons of using the various sizes of the markers. If the marker is too small, it will be a demanding job to place the marker on the pupil, and if the marker is bigger than the rodent's eye, the eye movements can be limited. Another disadvantage with the small rectangular markers is that a high-definition video equipment can be required to detect the marker, and it would increase the budget.

In order to analyze the 3D-eye-movement in a video-based technique, it required only two detection points. However, a single attachable marker with the multiple detection points at three vertices of a triangle can be used with a high yield and a minimization of measurement error using a high performance computing system (Figures 1, 2).[7] Since 2007, we have been using markers for VOR recordings (Figure 3).[7] Throughout the trials and the studies, we have concluded that the different sizes of the markers can cause some changes in results when analyzing VOR recordings. Consequently, we manufactured and substituted 600 μmisosceles triangles with 200 μm, 300 μm, and 400 μmisosceles triangles to evaluate its efficiency by 3-dimensional analysis (Figure 1).

MATERIALS AND METHODS

1. Subject

Six healthy, adult imprinting control region mice (ICR, Narabiotec, Seoul, Korea) with the weight of 22–25 g, and the age of 12–16 weeks were used without distinction of their genders. The surgical procedures and the experimental protocols were in accordance with the recommendation of Institutional Animal Care and Use Committees of Gacheon University of

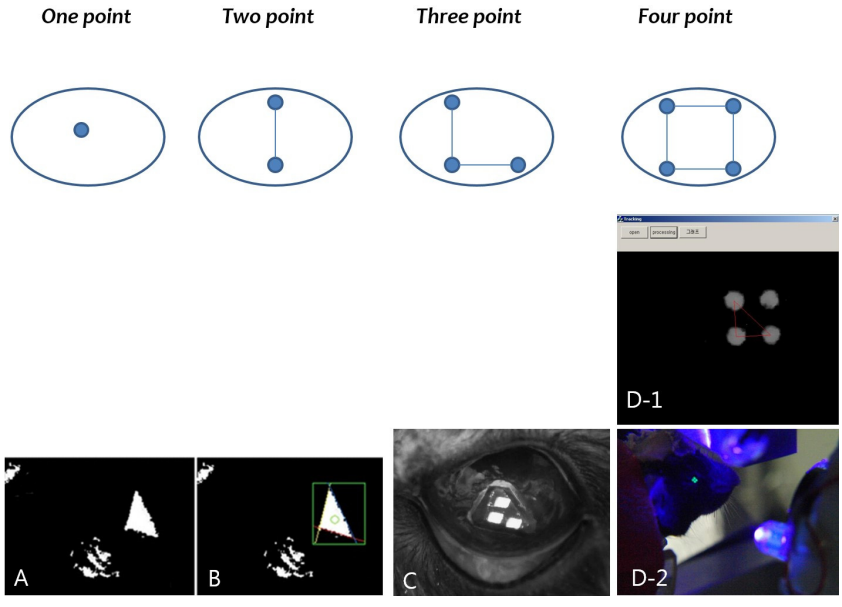

Figure 1. Based on the theory, comparing 1–4 tracking points. Single tracking points are only able to track 2-dimensionally. For two or more tracking points, tracing in 3-dimensions is available. If the multiple tracking points are assigned, it would cause calculation errors since the ranges of the calculations increase as the marker size gets bigger. In pictures (A), (B) and (C), each has the same triangle markers but each calculates different mechanisms. With picture (B), it calculates the lengths of sides; in picture (C), it calculates a little square inside of the triangle. The picture (D-1) and (D-2) represent an example of 4 points marker.

• ***Rotation angle:*** *the angle of long side in isosceles triangle*

• ***Translation position:*** *the crossing point from half of each side*

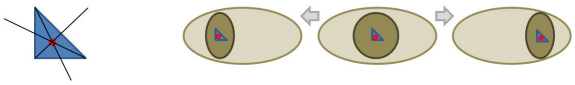

Figure 2. A theory of measuring the rotational movements when using an isosceles triangle marker. Since an isosceles triangle has two equal sides, the other side can be used in distinguishing the degrees of rotational movements, and also two equal sides can be used in measuring the linear motion.

Medicine and Science (GIACUCR-003). All laboratory animals were housed under 100 lux lights with the day-to-night ratio of 12:12 hours in a day. The temperature, humidity, and noise level of the laboratory have been maintained at 23°C, 40%, and 50 dB, respectively.

2. VOR recording procedures

With VOR recording,[7] we used the same methods by recording nystagmus irrigated by rotational stimulation.[7] Mice were physiologically awake, and the stimulation was under the peak velocity fixed at 0.1 Hz, 0.2 Hz, and 0.5 Hz, each in 60°/sec and 100°/sec.

3. Marker

The marker has been designed with Adobe Illustrator CS4, ver. 14.00 (Adobe Systems Inc., Mountain View, California, USA) as a white isosceles triangle with the equilateral length of 200 μm, 300 μm, 400 μm, and 600 μm. The markers were printed out on premium glossy photo papers (S041285, Seiko Epson Co. Suwa, Nagano, Japan), using Canon Pixma pro-1 (Canon Inc., Tokyo, Japan) with the resolution of 9,600 dpi.

Figure 3. The basic appearance of the markers. The markers, used in vestibulo-ocular reflex recordings, have the two same sides of 500 μm, and they all are white isosceles triangles.

4. Analysis of Nystagmus

The triangular marker was recognized so that the center of three vertexes was found which can be considered as the center of the pupil. The coordinate of the pupil was finally plotted in graphs which were separated into horizontal, vertical, and torsional movements.

Minimum three of each cycle was summated on a one-cycle-scaled chart to calculate the average of the three cycles. The average of slow-phase sine curves was, then, compared to that of the head velocity sine curve to calculate the gain values.

5. Statistical analysis

We used the average of slow-phase sine curves for the statistical analysis. We checked the normality of the data with a Kolmogorov-Smirnova test before performing a parametric test. For the repeated measurements of gain and phase, one-way repeated measurement analysis of varianc was applied to test the within-subjects main effect. The Greenhouse-Geisser method was also applied to correct the violation of sphericity of the covariance. In Post-hoc analysis, the polynomial contrast and the pair-wise comparisons were applied in within-subjects factor with the multiple comparison adjustments using Bonferroni correction to identify the order of the trend of the repeated measurements and the mean difference between the initial and the following measurements. For the repeated measurements of symmetry, Friedman test was applied to test the within-subjects main effect. In Post-hoc analysis, pair-wise Wilcoxon signed-rank test was applied with the multiple

comparison adjustments using Bonferroni correction to compare central tendency between initial and following measurements. Corrected *p*-value (Greenhouse-Geisser or Bonferroni correction) ≤ 0.05 was considered statistically significant. All statistical analyses were performed with SPSS ver. 21.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

In horizontal-vertical-torsional component, the different sizes of the markers were tried under 60°/sec and 100°/sec, with the peak velocity fixed at 0.1 Hz, 0.2 Hz, and 0.5 Hz, but for each nystagmus, its gain value showed no significant differences. However, with the 200 μm marker, recording the nystagmus was impracticable due to its diminutive size. It was strenuous to attach the 200 μm marker on the exact middle of the pupil, since the marker was significantly small, and the marker would easily detach itself. With the 300 μm marker, the vertical component was omitted because it was barely detectable on the recordings (Figure 4).

DISCUSSION

Tracking the eyeball movement of mice is an essential

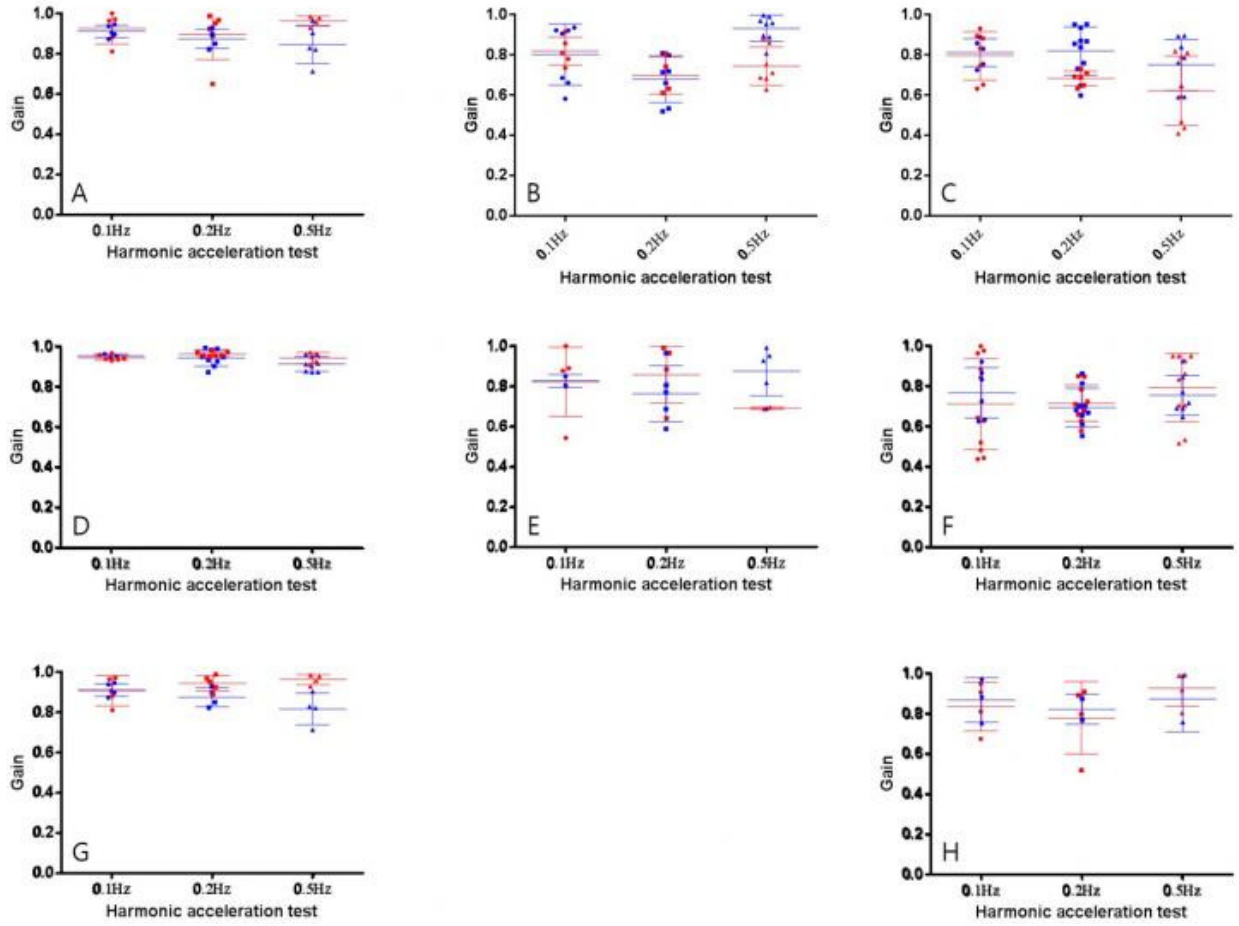

Figure 4. Two different gain values were compared. The nystagmus shown in rotational stimulation were measured in 60°/sec (blue) and 100°/sec (red) at the peak velocity of 0.1 Hz, 0.2 Hz, and 0.5 Hz. Graphs (A–C) show the comparison of the gain values of horizontal-vertical-torsional component in use of the 600 μm markers. Graphs (D–F), with the 400 μm markers, and graphs (G–H), used the 300 μm markers, represent each measurement of those of the sizes. With the 200 μm markers, measuring nystagmus was impracticable since it was demanding to attach the marker on the exact middle of the pupil when the marker was significantly small, and would easily detach itself.

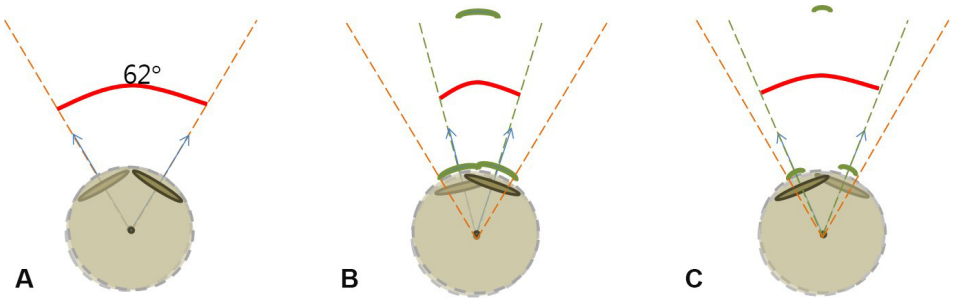

Figure 5. According to the various sizes of the markers, the measurement of an eye movement shows in different angles. Picture (A) shows normal eye movements. Picture (B) shows the limitations in the eye movements when bigger size of marker is used. Meanwhile, picture (C) shows a normal eye movement without any limitations when the smaller markers are used.

method in measuring physiologic characteristics and the response of the vestibular organ.[1] However, it has been difficult to apply the 3D VNG system to mice.[5] The reasons are: The small size of the mouse eyeball.[4] A periodic serial measurement in a mouse is needed to trace the changes of the gain values after an intervention. In laterally-eyed animals, objects are perceived through the individual tecto-fugal visual system by a selective internal attention mechanism.[8] To address these issues, the attempts to improve the conventional VNG system have been made. In VNG, using the marker array technique, the design and the size of the markers are very important in achieving a high resolution. The size of the eyeball of mouse is approximately 3.32 mm in a diameter. Even the range of its rotational angle of the eyeball in mouse was different according to the rotation axis: the horizontal rotation angle was 62^{o}, and the value was bigger than other axes. The circular arc of the 600 μm marker occupied 20.85^{o} [(0.6/2π×1.66)×360] in the mouse eyeball, but it is decreased to 17.37^{o} with a 500 μm marker, to 13.90^{o} with a 400 μm marker, and to 6.91^{o} with a 200 μm marker (Figure 5). This suggests that the marker can disrupt the eyeball's movement, especially vertically, even if the computer can calculate the eye position as the center of the marker. In other words, a smaller marker is necessary to accurately measure the eye movements in small rodents. In contrast, in rats, the 600 μm and 500 μm markers occupy 10.75^{o}, 8.95^{o} respectively [(0.6/2π×3.20)×360], and these angles appear to be enough to measure the eye movements.

As based on the fundamental theories, the gain values in six ICR mice showed no significant differences and also had no relations to the sizes of the markers. This result proves that any sizes of the markers can be applied to measure nystagmus in small rodents, and recording the eye movements of mouse will be still limited no matter what size of the marker an analyst uses. Having the limited ranges of the eye movements is one of the unique characteristics in lateral eyed animals.

CONCLUSION

Technicians can decide the size of the markers from 200 to 600 μm to record the nystagmus of mice, depending on the technicians' skills.

중심 단어: 전정안반사, 마커, 비디오 안진계, 마우스

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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