Int. Adv. Otol. 2012; 8:(3) 453-462

ORIGINAL ARTICLE

"Tail-Hanging Test" Behavioral Parameter of Vestibular Deficit and Compensation in Labyrinthectomized Mouse Model

Mi Joo Kim, Nambeom Kim, Eun Ji Lee, Gyu Cheol Han

Departments of Otorhinolaryngology Head & Neck Surgery, Yonsei University, College of Medicine, Seoul, Korea (MJK)
Neuroscience Research institute, Gachon University, Graduate School of Medicine, Incheon, Korea (NK)
Department of Otolaryngology-HNS, Gachon University of Medicine & Science, Graduate School of Medicine, Incheon, Korea (EJL, GCH)

Purpose: We are introducing a method to quantify the balance function in mouse with unilaterally damaged vestibular organ. The findings of this method were previously described in 1987 and were used to confirm the acute deficient status of rodents with unilateral or bilateral vestibular dysfunction. The method named as Tail-Hanging Test (THT) could be the useful method to test mostly vestibular system by confusing the somatosensory system and obstructing the visual input as like as like as posturography in human study.

Materials and Methods: Fifteen mice underwent unilateral labyrinthectomy and five mice of control group underwent sham operation. After the surgery, the tail of mouse was fixed at the experiment stand and spinning motion was recorded at postoperative 24, 72, and 144 hours. In control group, recording was done at 24 hours. The recorded images were processed with the digital-video-based tracking system using image subtraction technique. Several numerical parameters were compared among different postoperative periods and control group.

Results: Minimum angle (MA), mean angle changing velocity (MACV) and mean spinning velocity (MSV) showed the equal variance ($p < 0.05$, Bartlett's test) and study group showed statistically different result from those of control group ($p < 0.05$, ANOVA), which means that these three parameters can be indicators to distinguish the normal mouse from the mouse with significant vestibular damage. The results about MA and maximum spinning width (MSW) among three different periods showed the statistically significant difference with equal variance ($p < 0.05$, ANOVA, Bartlett's test), and it suggests two parameters reflects vestibular compensation depending on the elapsed times after the unilateral damage to the vestibular system.

Conclusion: We successfully quantified the balance function in animal. It will be helpful & easy to estimate vestibular function and compensation status at laboratory. MA of THT could be used for evaluation of vestibular damage and compensation of it simultaneously.

Submitted : 04 September 2012 *Accepted : 19 October 2012*

Introduction

The vestibular system plays an important role in detecting the motion of head-in-space and in turn, produces reflexes crucial for the normal behaviors of vertebrates, such as stabilizing the visual axis and maintaining the head and body postures.[1, 2] In animal model for vestibular dysfunction, several well-known methods using vestibular reflex have been introduced as a useful marker to determine the severity of vestibular damage and the grade of central compensation. To estimate vestibular dysfunction on vestibule -ocular reflex (VOR), spontaneous nystagmus or VOR during sinusoidal rotation by search-coil method or videonystagmography have been used in animal model.[3-5] In term of vestibulo-spinal reflex (VSR) of animal research, there is measuring method like head-tilt degree at static status.[5] But there could be some timing difference

Corresponding address:
Gyu Cheol Han
Department of Otolaryngology-Head & Neck Surgery, Gachon University of Medicine & Science,
Graduate School of Medicine, 1198 Guwol-dong, Namdong-gu, Incheon, 405-760, Korea
Tel +82-32-460-3324
Fax +82-32-467-9044
E-mail hangckr@gmail.com

between VOR and VSR in a view point of compensation.[6-8] Observing the behaviors of animal, circling, rearing, and several body angles (cranio-cevical, cervico-thoracic and thoraco-lumbar angle) were utilized as behavioral parameters showing dynamic vestibular deficits.[2]

In 1987, Hunt et al. described that animal rotates along the longitudinal axis of the body when lifted by the tail in unilateral labyrinthectomy model. And then animal bends ventrally and crawls up toward its tail when lifted by tail in bilateral labyrinthectomy model.[9] We named these interest findings as "Tail-Hanging Test" (THT) and they have been informally used to only determine the acute status of animal model with unilateral and bilateral vestibular dysfunction in animal experiments. We tried to quantify the results of THT using numerical parameters and to find change and difference between the parameters according to the time elapsed after the unilateral labyrinthectomy in mouse model.

Materials and Methods

Animals

Twenty C57BL/6 mice (male, 15 mice in study group, male, 5 mice in control group, Narabiotec, Seoul, Korea) aged 10-12 weeks and weighing 18-25 g were used in this study and were treated in accordance with the animal use guidelines of Gachon University Institutional Animal Care and Use Committee. Animals were housed under 14:10 day and night cycle in 100 lux and dark room in which temperature was kept at 23°C, humidity at 40%, and noise level under 50dB.

Surgical labyrinthectomy and sham operation

In study group, mice were anesthetized by inhalation with isoflurane gas (Aerane, O2 5L/min, 2.0, Ilsung Pharm.co, Seoul, Korea). Labyrinthectomy was operated on: incision at the posterior portion of left auricle, about 0.5 cm behind the auricular sulcus, identification of the horizontal and posterior semicircular canal, drilling of the horizontal semicircular canal to make a hole, confirmation of perilymph leakage and suction out, opening the vesitibular lateral wall, and filling the hole with collagen (Helitene, Intergra Life Sciences Co., New Jersy, USA) to prevent the closer of opening and to close the incision wound. In control group, undergoing sham operation, procedure consisted of the same sized incision at the left retroauricular area and closure of the wound.

Tail-Hanging Test

Authors manufactured an experiment stand. Posterior wall, left lateral wall and superior wall were treated with white foam board (HF, 1T, 600×900 mm, Hyunjin art, Goyang, Korea). The bar for fixing tail was also finished as white with the white stickling plaster. From anterior and right lateral directions, two infrared video cameras (Pleomax PWC-2200 web cam ,Samsung, Seoul, Korea) were established about 30 cm away from mouse body to capture the image including the whole length from tail end to snout at full resting position. The experiment stand was kept on horizontal plane as much as possible (Figure 1). Captured images were stored for data analysis in laptop computer, which has IBM compatible mobile CPU 1.5 GHz, 1.00 GB RAM and Windows® XP (service pack 3). The capture setting of video camera was as follows; Black/White mode was used, the flicker frequency of frame was adjusted to 60 Hz, the value of gamma, contrast and brightness was set respectively at 0.07, 1.13 and 0, white balance and USB bandwidth were applied as automode, video capture resolution was adjusted to 800 x 600, frame rate was 30 frames/second, recording time was limited to 20 seconds, and video file was saved as *.avi file. Testing environment remained under the following constant conditions; room temperature was about 25°C,

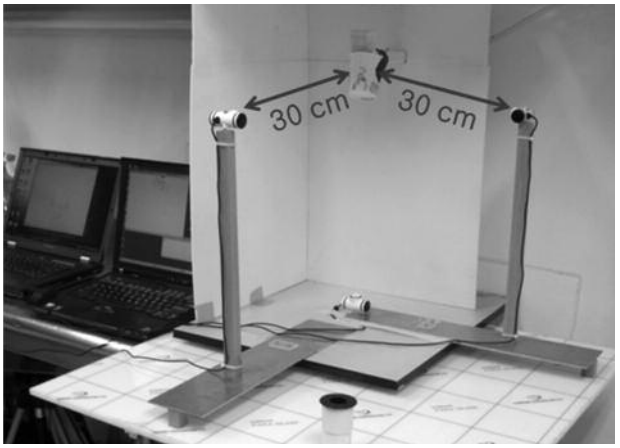


Figure 1. The experiment stand for the Tail-Hanging Test and recording the movement.

humidity was about 30% in room air, light provided an illumination of 90 lux and environmental noise was kept under 50dB. In study group, the movement of mice during THT was recorded at postoperative 24, 72 and 144 hours. About 1cm length of tail end was fixed at the experiment stand using the white stickling plaster. To prevent the visual input, eyeball was treated with terramycin ophthalmic ointment (Terramycin eye ointment®, Pfizer Pharm. Korea, Seoul, Korea) only at the testing periods. At each testing, body rotation was recorded three times repeatedly and each recording was carried out at interval of at least fifteen minutes to exclude the exhaustion. Based on the findings observed, mouse labyrinthectomized on left side always turned clockwise along the longitudinal axis of the body when lifted by tail. In control group, same testing protocol was applied at postoperative 24 hours.

Tracking of coordinates (x, y) and imaging processing

Visual Studio 6.0 and Open CV v1.0 (Microsoft®, Redmond, WA, USA) were used as programming tools. The basis of the tracking algorithm is the image subtraction of a reference video frame from subsequent video frames containing a moving object. Authors will consider tracking in a digital video recording with each pixel value representing a brightness level ranging from black (0) to white (255). We denote the frame being analyzed I and the background reference B, with I and B being matrices whose elements are pixel values. Then an image for analysis, A, can be computed by subtracting I and B:

$A = |I - B|$

With this new image, A, the threshold technique can be used (Figure. 2. A, B).

Tracking and imaging processing was followed as, (1) A captured RGB image (horizontal x vertical, 320 x 240 pixels) is transformed into gray image. (2) All pixels of gray image has its own value between 0 (black) and 255 (white) according to the value of brightness. (3) By the threshold technique using the threshold value of 210, the first binary image was taken to determine the coordinate of tail end after the pixels of value below 210 were processed as white (255) and the pixels of value above 210 as black (0) (Figure.2. A). (4) By threshold technique using the threshold value of 80, the second binary image was taken to determine the coordinates of snout and the contact portion between tail and body after the pixels of value below 80 were processed as white (255) and the pixels of value above 80 as black (0) (Figure 2. B). (5) In quadrant area including only the tail end (horizontal x vertical, 80~240 x 50 pixels), the selected area is scanned using the image of Figure 2. A and the pixel value of 255 is stored as the coordinate of tail end (TC) (Figure.2. C). (6) Using the image of Figure 2. B, image of mouse body is scanned from the top to the bottom of body contour. The first and the last pixel value of 255 are stored selectively as the coordinate of the contact portion between tail and body (CC), and the coordinate of snout (SC) (Figure 2. D). (7) The values of three coordinates (SC, CC, TC) of one frame (1/30 second @ 1 frame), are calculated and are stored. And the coordinates' values of the next frame are stored, and the several parameters like angle, moving distance, rotating velocity, length et al. were calculated using the two different values of three each coordinate from before and next frames.

Statistical analysis

ANOVA test was used to compare the achieved results of 24, 72 and 144 hours period within the study group. ANOVA test was also used to compare the achieved results of 24, 72, 144 hours period and control group. The results were expressed in graph using Prism® 4 for windows (Graphpad Software Inc., La Jolla, CA, USA). The Bartlett's test was used to determine the equal variance of achieved parameters. Statistical analysis was performed using a commercially available statistical software package (SPSS Version 12.0; SPSS Inc, Chicago, IL, USA). Statistical significance was accepted at a confidence level of 5%.

Results

Angle (Minimum angle, Mean angle changing velocity)

Angle is defined as the degree being formed between tail and body during the rotation of mouse. Two parameters about angle were analyzed. Minimum angle (degree, MA) is the lowest value of angle during total rotation of 20 seconds (Figure 3. A). The measured values of 24 hours, 72 hours and 144 hours period showed the equal variance ($p = 0.0461$) and there was statistically significant difference among the

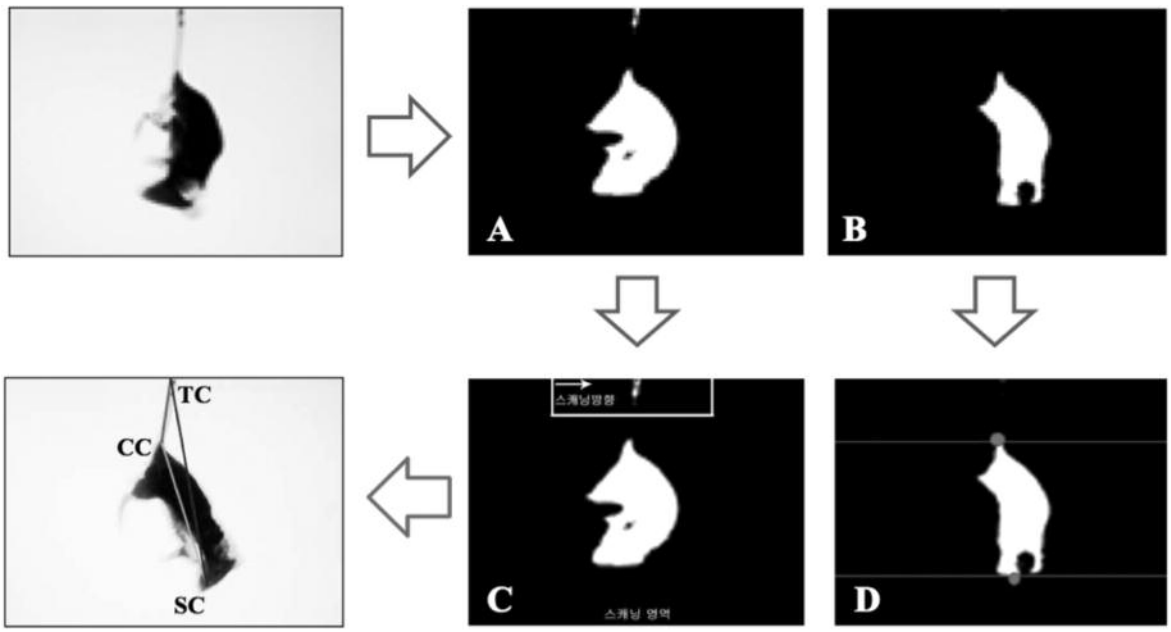


Figure. 2. Imaging processing and determining of three coordinates using two binary images. (**A**) First binary image by threshold value of 210. (**B**) Second binary image by threshold value of 80. (**C**) Within the quadrant area including only tail end, the coordinate of tail end (TC) is determined. (**D**) The coordinates of the contact portion between tail and body (CC), and the coordinate of snout (SC) are determined.

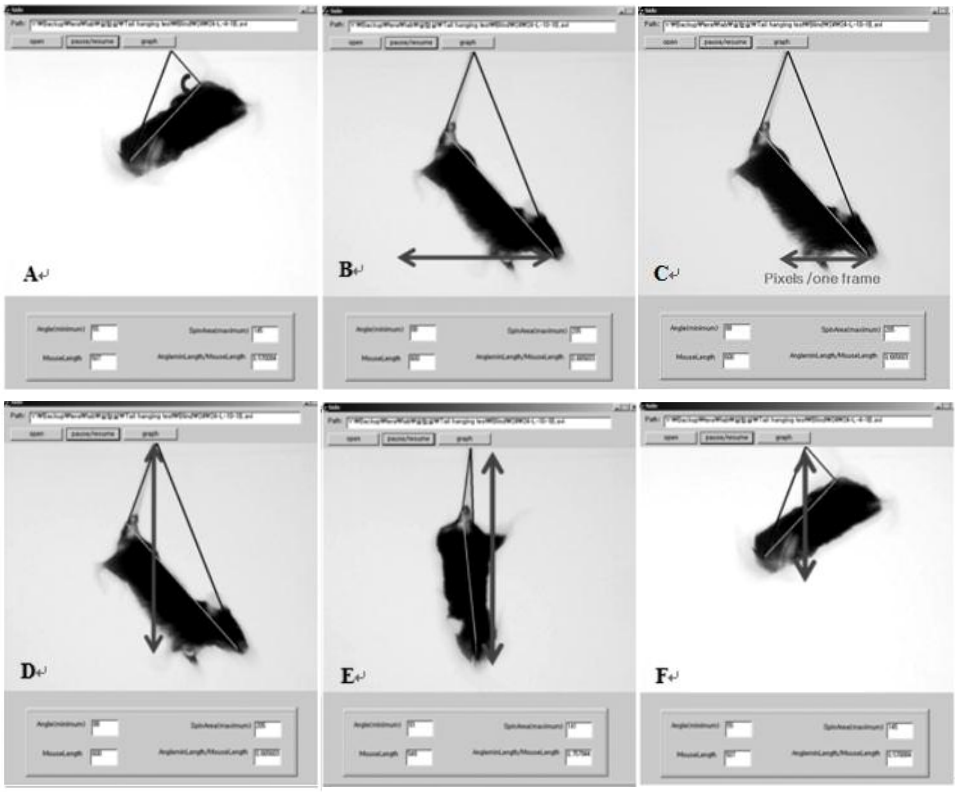
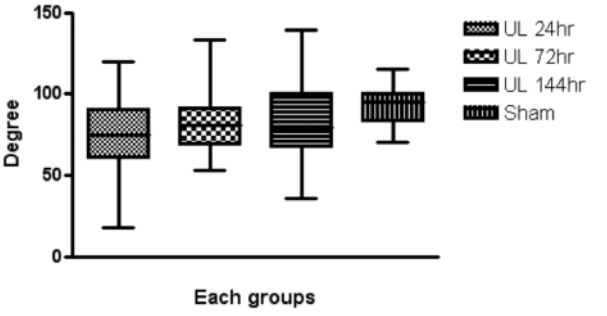


Figure 3. Photographs explaining the measured parameters during Tail-Hanging Test. (**A**) Angle formed between tail and body during rotation. (**B**) Spinning width (pixels, 1cm ≅ 30 pixels) between the snout's coordinates of the far right and left sides. (**C**) Spinning velocity is the number of pixels between the coordinates of moving snout from two serial frames (1 frame ≅ 1/30 second). (**D**) Mouse length (pixels, 1cm ≅ 30 pixels) between the end of tail and the snout. (**E**) Maximum mouse length at resting. (**F**) Mouse length at minimum angle.

results of three different periods (p = 0.0024) (Figure 4). The measured values of 24, 72, 144 hours period and sham operation showed the equal variance (p = 0.0113) and there was also statistically significant difference between the results of study and control group (p = 0.0113) (Figure 4). Mean angle changing velocity (degree/30s, MACV) is the change of angle between two serial frames (1/30 second). The mean value of these angle changing velocities during 20 seconds is defined as MACV. The measured values of 24 hours, 72 hours and 144 hours period showed the equal variance (p = 0.0009) but there was no statistically significant difference among the results of three different periods (p = 0.3457) (Figure 5). The measured values of 24 hours, 72 hours, 144 hours period and sham operation showed the equal variance (p = 0.0006) and there was statistically significant difference between the results of study and control group (p = 0.0002) (Figure 5).

Spinning width (Maximum spinning width, Total spinning width)

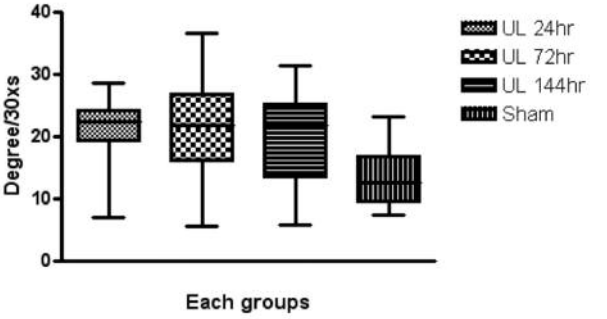
Spinning width is defined as the width (pixels, 1cm @ 30 pixels) between the snout's coordinates (SC) of the far right and left sides. Two parameters about width were analyzed (Figure 3. B). Maximum spinning width (pixel, MSW) is the largest value of spin width during rotation. The measured values of 24 hours, 72 hours and 144 hours period showed the equal variance (p = 0.0481) and there was statistically significant difference among the results of three different periods (p = 0.0055) (Figure 6). The measured values of 24 hours, 72 hours, 144 hours period and sham operation didn't show the equal variance (p = 0.0955) and there was statistically significant difference between the results of study and control group (p = 0.0069) (Figure 6). Total spinning width is the sum of spin width during 20 seconds. The measured values of 24 hours, 72 hours and 144 hours period didn't show the equal variance (p = 0.7563) and there was no statistically significant difference among the results of three different periods (p = 0.6894) (Figure 7). The measured values of 24 hours, 72 hours, 144 hours period and sham operation showed the equal variance (p = 0.0029) but there was no statistically significant difference between the results of study and control group (p = 0.2686) (Figure 7).



	ANOVA	Bartlett's test
Among 24hrs, 72 hrs and 144 hrs group	p = 0.0024*	p = 0.0461*
Among 24hrs, 72 hrs, 144 hrs and sham group	p = 0.0113*	p = 0.0113*

UL: Unilateral labyrinthectomy, Sham: Sham operation, significance was accepted for p values of <0.05

Figure 4. Minimum angle



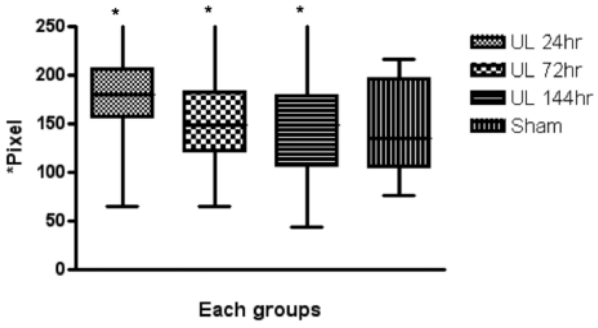
	ANOVA	Bartlett's test
Among 24hrs, 72 hrs and 144 hrs group	p = 0.3457	p = 0.0009*
Among 24hrs, 72 hrs, 144 hrs and sham group	p = 0.0002*	p = 0.0006*

UL: Unilateral labyrinthectomy, Sham : Sham operation, significance was accepted for p values of <0.05

Figure 5. Mean angle changing velocity

Spinning velocity (Mean spinning velocity)

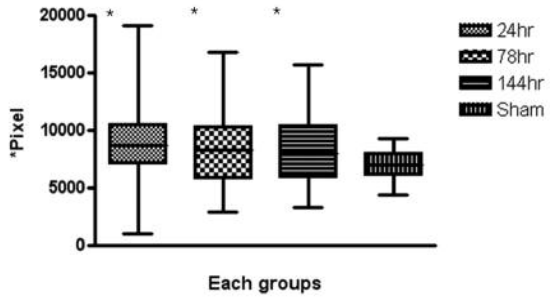
Spinning velocity is the number of pixels between the coordinates of moving snout from two serial frames. Mean spinning velocity (degree/30s, MSV) is the mean value of these values during total 20 seconds (Figure 3. C). The measured values of 24 hours, 72 hours and 144 hours period didn't show the equal



	ANOVA	Bartlett's test
Among 24hrs, 72 hrs and 144 hrs group	p = 0.0055*	p = 0.0481*
Among 24hrs, 72 hrs, 144 hrs and sham group	p = 0.0069*	p = 0.0955

UL: Unilateral labyrinthectomy, Sham: Sham operation, 1cm ≅ 30 pixels, significance was accepted for p values of <0.05

Figure 6. Maximum spinning width



	ANOVA	Bartlett's test
Among 24hrs, 72 hrs and 144 hrs group	p = 0.6894	p = 0.7563
Among 24hrs, 72 hrs, 144 hrs and sham group	p = 0.2686	p = 0.0029*

UL: Unilateral labyrinthectomy, Sham: Sham operation, 1cm ≅ 30 pixels, significance was accepted for p values of <0.05

Figure 7. Total spinning width

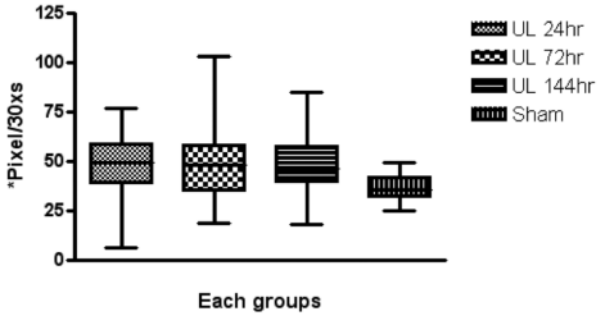
variance (p = 0.7188) and there was no statistically significant difference among the results of three different periods (p = 0.9533) (Figure 8). The measured values of 24 hours, 72 hours, 144 hours period and sham operation showed the equal variance (p = 0.0063) and there was statistically significant difference between the results of study and control group (p = 0.0370) (Figure 8).

Mouse length (Length ratio)

Mouse length is the length (pixels, 1cm @ 30 pixels) between end of tail and snout. Maximum mouse length is the longest value of mouse length at resting state (Figure 3. D). Length ratio is defined as mouse length at the minimum angle / maximum mouse length during rotation (Figure 3. E, F). The measured values of 24 hours, 72 hours and 144 hours period showed the equal variance (p = 0.0388) but there was no statistically significant difference among the results of three different periods (p = 0.4052) (Figure 9). The measured values of 24 hours, 72 hours, 144 hours period and sham operation showed the equal variance (p = 0.0252) but there was no statistically significant difference between the results of study and control group (p = 0.4534) (Figure 9).

Discussions

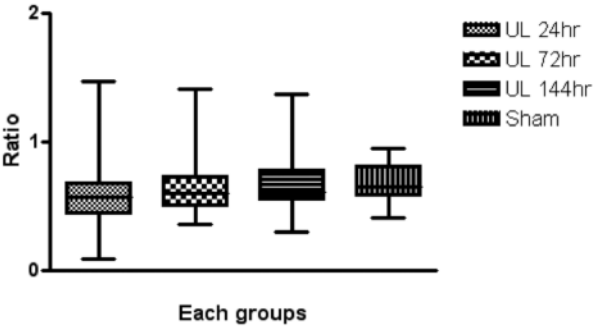
In animal and human, posture is controlled by visual, somatosensorial and vestibular inputs, which are integrated in the vestibular nucleus. In turn, locomotion & posture center selects and adjusts muscle and contractile patterns, and finally generates dynamic and static body movement.[10] From previous studies for animal model with vestibular dysfunction, nystagmus has been used to determine the vestibular damage of animal and to estimate the degree of central compensation. But, the method using horizontal nystagmus only reflects the function of lateral semicircular canal and has limitation in representing dynamic vestibular deficit.[11] Other tests in animal model, such as head-tilt degree, resting posture, circling, running were introduced.[2] Although these means are useful in animal study, all methods are applied at situation with four limbs on the ground,[2] so they have some limitation to estimate the vestibular system primarily because the effect of somatosensory input cannot be excluded. And as previously mentioned, the compensation of static vestibular deficits are not consistent with that of dynamic deficits, so the complete compensation of static deficits does not mean the compensation of dynamic



	ANOVA	Bartlett's test
Among 24hrs, 72 hrs and 144 hrs group	p = 0.9533	p = 0.7188
Among 24hrs, 72 hrs, 144 hrs and sham group	p = 0.0370*	p = 0.0063*

UL: Unilateral labyrinthectomy, Sham: Sham operation, significance was accepted for p values of <0.05

Figure. 8. Mean spinning velocity



	ANOVA	Bartlett's test
Among 24hrs, 72 hrs and 144 hrs group	p = 0.4052	p = 0.0388*
Among 24hrs, 72 hrs, 144 hrs and sham group	p = 0.4534	p = 0.0252*

UL: Unilateral labyrinthectomy, Sham: Sham operation, significance was accepted for p values of <0.05

Figure 9. Length ratio

vestibular deficits. We considered that vestibular system could be effectively evaluated by THT because somatosensation may fall into disorder due to the loss of ground, and furthermore, visual sensation can be in confusion by blocking visual information. Furthermore, THT can be applied to other kinds of rodent with the proper length of tail, including rat, if the experiment stand is somewhat modified to stand the weight of animal and image processing program is revised considering the color of animal and the contrast between animal and background. The second advantage is that brief time required for test. In our study, testing duration is only 20 seconds. It is much shorter than the recording times of other representative methods such as circling, which needs recording time of about one hour,[12] and so THT may be a timesaving experiment method. By allowing the animal to have interval time enough for resting, repeated test is possible. In our study, three serial tests were performed within 40~50 minutes although mouse was allowed to have two resting periods of 30 minutes. Third, THT has a cost-effective merit. Test equipment, programming and result interpretation cost is relatively low. Above all things, the change of vestibular function can be easily understood by identifying the numerical change of parameters.

Behavior is an important parameter in several neuroscience studies. It is central to studies of neurobehavioral genetics, and brain function, and the number of behavioral studies is vast. Assessment of locomotor behavior is especially crucial in neuro and psychopharmacology. Automated recording system is more reliable and accurate method in behavior study. Several automated systems for laboratory use have been developed to quantify rodent locomotor behavior, which was based on photo beams[13,14], infrared[15] or ultrasonic[16] motion sensors, sensors detecting of mechanical vibration[17] ,mechanical devices with joy stick connected to neck collar[18] and video-based tracking systems.[19-22] Automated methods for recording locomotor behavior in rodents based on photo beams likewise suffer from having poor resolution in time and space. Tracking an animal is more precise, and requires a system with high resolution. The principles of video-tracking analysis are often based on image-subtraction algorithms[23,24], grayscale threshold[20,24], or color.[24] The use of an image-subtraction algorithm is more advantageous in this study because the color of mouse is almost black, so if the background is white, certain degree of contrast between the moving object and the tracking background always exists. In the presented study, we

chose to use a tracking algorithm based on image-subtraction techniques. With high contrast between the mouse and the background, a simple threshold in brightness value can be estimated that separates the animal from its background. However, this is not possible if the contrast is low if the color of the animal is close to that of the testing environment. However, if a reference frame containing only the background, without the animal, is available, it is possible to use the threshold technique with a slight modification. We have developed inexpensive software for quantifying the parameter of mouse THT.

Authors tried to quantify the values of several numerical parameters and to select the parameters useful to confirm the vestibular damage or to predict the degree of vestibular compensation. Angle between body and tail was supposed to show the lowest value in 24 hours period than angles in other groups, and as more vestibular system is compensated, the value of angle is expected to increase. MA tended to increase as time elapses after unilateral labyrinthectomty, and the value of control group was higher than those of study group. The MA of three postoperative periods and control group ranged with the equal variance and the differences among these groups were statistically significant. It suggested that lower value of MA means the prominent vestibular damage of study group compared to the state of control and within study groups. We could estimate the extent of vestibular damage. So, MA could be not only the parameter to determine damaged or normal but also the indicator to reflect how much the vestibular system has been compensated.

MACV is the change of angle between two serial frames (1/30 second @ 1 frame). When mouse with unilateral vestibular damage was fixed by tail at the experiment stand, mouse started to rotate mostly with latency, which is within 1 second. Considering the spinning of mouse after latency, MACV could be regarded as acceleration. With hypothesis that angle changes faster in the mouse with more acute vestibular damage, we compare the results of MACV among four groups. All data were distributed equally and the results of study group differ from those of control group significantly. But, there was no statistically significant difference among three periods of study group. We thought that MACV may be the parameter to discriminate the damaged vestibular system from normal vestibular system.

MSW is defined as the width expressed as a unit of pixels (1cm @ 30 pixels) between the snout's coordinates (SC) of the far right and left sides and MSW means the maximum diameter of circle made by turning snout. The faster mouse rotates, the larger scale of the value of spin width is assumed to appear. The data of three periods within study group were distributed equally except the result of control group and the difference within study group was statistically significant. The results of control group didn't show normal distribution because unexpected movement of mouse such as lateral bending or twisting might result in extremely high value out of distribution. Our result implies that MSW can be used as parameter for vestibular compensation within study group. If the mentioned unexpected motion of normal mouse is excluded from the recording in future, MSW can be utilized as the parameter about damaged or normal system.

Total spinning width is defined as the sum of spin width during total 20 seconds. The difference of results was not significant between study and control group, and within study group. It is predicted results because mouse doesn't rotate during full recording time. Mouse usually showed a crescendo-decrescendo tendency during rotation and latent period before rotating; for example, mouse in 24 hours period rotates faster with making larger circle, but the exact rotation time of total recording period could be shorter than that of other groups. In such case, total spin width of acutely damaged state could be shorter than the results of compensated state and control group, less likely.

MSV is the value of pixels between the coordinates of moving snout from two serial frames. MSV is the mean value of these results during total 20 seconds. The results within study group didn't show the equal variance and there was no statistically significant difference among the results of three different periods. The data of control group showed the equal variance and there was statistically significant difference between the results of study and control group. It is suggested that MSV could be the parameter indicating the definite vestibular damage such as MACV.

Conclusions

We believe that THT will be utilized as valuable experimental method for the animal study about vestibular system. In THT, MA, MACV and MSV can be utilized as useful indicator to judge the significant dynamic vestibular deficiency in mouse model with unilateral vestibular dysfunction. Furthermore, MA and MSW can be important indicators to reflect the vestibular compensation depending on elapsed time after the damage to the vestibular system.

Acknowledgment

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0011858)

References

1. Cullen KE, Roy JE. Signal processing in the vestibular system during active versus passive head movements. J Neurophysiol. 2004; 91:1919-33.

2. Vidal PP, Degallaix L, Josset P, Gasc JP, Cullen KE. Postural and locomotor control in normal and vestibular deficient mice. J Physiol. 2004; 559:625-38.

3. Kitahara T, Nakagawa A, Fukushima M, Horii A, Takeda N, Kubo T. Changes in foes expression in the rat brainstem after bilateral labyrinthectomy. Acta Otolaryngol. 2002; 122:620-6.

4. Stahl JS, van Alphen AM, De Zeeuw CI. A comparison of video and magnetic search coil recordings of mouse eye movements. J Neurosci Methods. 2000; 99:101-10.

5. Kim MS, Kim JH, Jin YZ, Kry D, Park BR. Temporal changes of c Fos-like protein expression in medial vestibular nuclei following arsanilate-induced unilateral labyrinthectomy in rats. Neurosci Lett. 2002; 319:9-12.

6. Curthoys IS. Vestibular compensation and substitution. Curr Opin Neurol. 2000; 13:27-30.

7. Darlington CL, Dutia MB, Smith PF. The contribution of the intrinsic excitability of vestibular nucleus neurons to recovery from vestibular damage. Eur J Neurosci. 2002; 15:1719-27.

8. Black FO, Shupert CL, Peterka RJ, Nashner LM. Effects of unilateral loss of vestibular function on the vestibulo-ocular reflex and postural control. Ann Otol Rhinol Laryngol. 1989; 98:884-9.

9. Hunt MA, Miller SW, Nielson HC, Horn KM. Intratympanic injection of sodium arsanilate (atoxyl) solution results in postural changes consistent with changes described for labyrinthectomized rats. Behav Neurosci. 1987; 101:427-8.

10. Rama-Lopez J, Pérez N, Martinez Vila E. Dynamic posture assessment in patients with peripheral vestibulopathy. Acta Otolaryngol. 2004; 124:700-5.

11. Nashner LM, Peters JF. Dynamic posturography in the diagnosis and management of dizziness and balance disorders. Neurol Clin. 1990; 8:331-49.

12. Kaufman GD, Shinder ME, Perachio AA. Correlation of Fos expression and circling asymmetry during gerbil vestibular compensation. Brain Res. 1999; 817:246-55.

13. Gapenne O, Simon P, Lannou J. A simple method for recording of the path of a rat in an open field. Behav Res Methods Instrum Comput. 1990; 22:443–8.

14. Minematsu S, Hiruta M, Taki M, Fujii Y, Aburada M. Automatic monitoring system for the measurement of body weight, food and water consumption and spontaneous activity of a mouse. J Toxicol Sci. 1991; 16:61–73.

15. Kramer KM. Inexpensive motion detectors for quantification of animal activity. Biotechniques 1998; 25:385–8.

16. Vatine J-J, Ratner A, Dvorkin M, Seltzer Z. A novel computerized system for analyzing motor and social behavior in groups of animals. J Neurosci Methods 1998; 85:1–11.

17. Van de Weerd HA, Bulthuis RJA, Bergman AF, Schlingmann F, Tolboom J, Van Loo PLP, et al. Validation of a new system for the automatic registration of behaviour in mice and rats. Behav Process. 2001; 53:11–20.

18. Brodkin J, Nash JF. A novel apparatus for measuring rat locomotor behavior. J Neurosci Methods 1995; 57:171–6.

19. Torello MW, Czekajewski J, Potter EA, Kober KJ. An automated method for measurement of circling behavior in the mouse. Pharmacol Biochem Behav 1983; 19:13–7.

20. Hoy JB, Koehler PG, Patterson RS. A microcomputer-based system for real-time analysis of animal movement. J Neurosci Methods 1996; 64:157–61.

21. Spink AJ, Tegelenbosch RAJ, Buma MOS, Noldus LPJJ. The etho vision video tracking system—a tool for behavioral phenotyping of transgenic mice. Physiol Behav 2001; 73:731–44.

22. Marchand A, Luck D, DiScala G. Evaluation of an improved automated analysis of freezing behaviour in rats and its use in trace fear conditioning. J Neurosci Methods 2003; 126:145–53.

23. Wu BM, Chan FHY, Lam FK, Poon PWF, Poon AMS. A novel system for simultaneous monitoring of locomotor and sound activities in animals. J Neurosci Methods. 2000; 101:69–73.

24. Noldus LPJJ, Spink AJ, Tegelenbosch RAJ. Etho vision: a versatile video tracking system for automation of behavioral experiments. Behav Res Methods Instrum Comput. 2001; 33:398–414.