A Novel Method for the Assessment of Facial Palsy in a Rat Model: the Black Light-Assisted Scoring System

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Abstract

Background: Facial palsy in humans is assessed using facial nerve grading systems. However, there is no standardized tool for the objective assessment of facial palsy in rat models. Here, we propose a novel black light-assisted scoring system (BASS) to assess facial palsy in rats.

Methods: BASS has three key components: blacklight, high frame rate videography (iPhone), and motion analysis software (Kinovea). The system can detect movement of a specific whisker and whisker base and automatically assess their angular velocity. Our study included 12 male Wistar rats: 6 with normal facial nerve function and 6 with a unilateral facial nerve crush injury. Four angular velocities were investigated using BASS to confirm the accuracy of the system and determine the best parameter to be used for BASS.

Results: The total velocity value (sum of maximum protraction velocity and maximum retraction velocity) had the least variation and highest reliability of all tested velocities.

Conclusions: BASS allows for repeated quantification of the whisker movements and weekly measurements of the whisker base. Our findings suggest that BASS is reliable, reproducible, and useful for the assessment of facial palsy.

Introduction

Several previous studies have used facial palsy scoring systems based on precise videographic recordings followed by subjective evaluation to assess the recovery of the facial nerve in rats.[1, 2] Shichinohe et al.[3] used a facial palsy scoring system to evaluate the orbicularis oculi muscle and vibrissal pad function in rats as a measure of facial nerve recovery. We have previously used this scoring system, but it depends to a certain degree of subjectivity.[4, 5] Recently, several studies have investigated the use of hybrid artificial nerve grafts, which combine artificial nerves and stem cells or growth factors, to promote nerve regeneration after injury to the buccal and marginal mandibular branches of the facial nerve in rats.[6–9] Hence, assessment of nerve recovery following these injuries requires a novel facial palsy scoring system designed specifically for evaluation of the midface function. Sasaki et al.[10] modified the Shichinohe score to assess the midface function, including the nose and whiskers. This scoring system is easy to use; however, because the videographic analysis involves a subjective assessment of behavioral parameters, it may lack objectivity and reproducibility. Previous psychophysiological studies have used high-speed videography to assess whisker movement in rats.[11–13] Although this method is objective, the system is complicated and difficult to use. Furthermore, since the video analysis technique was not developed to assess facial palsy, it is not suitable for the analysis of whisker and whisker base movements.

In humans, functional recovery of the facial nerve is assessed using facial nerve grading systems, such as the House-Brackmann[14]. As abovementioned, several previous studies in rats have used facial palsy scoring systems based on subjective interpretations of videographic recordings to assess facial nerve

recovery,[1–5, 8–10] but no standardized tool has been established. To be considered as a gold standard, a method must be economical, objective, reproducible, accurate, and convenient. We propose a novel facial palsy scoring system: the black light-assisted scoring system (BASS), which meets the criteria to be considered as a gold standard assessment tool. The goal of the present study was to provide evidence supporting the use of BASS as a gold standard scoring system for the assessment of facial palsy in rats.

Results

BASS scores in normal rats

The BASS scores for the normal group are shown in Fig. 5. In normal rats, BASS scores were predicted to be 100% because nerve function was normal on both the left and right sides. As expected, the scores were close to 100. The mean scores ($\pm$ SEs) for the angular velocities were $V_{\max}$, $115.0 \pm 6.0\%$; V_{pro} , $97.2 \pm 9.5\%$; V_{re} , $106.1 \pm 10.4\%$; and V_{total} , $101.6 \pm 6.6\%$.

BASS scores in the rat facial palsy model

We evaluated the crush injury group at 2 weeks after surgery. The BASS scores for the rats with facial palsy are shown in Fig. 6. The mean scores ($\pm$ SEs) for the angular velocities were $V_{\max}$, $65.5 \pm 4.2\%$; V_{pro} , $72.5 \pm 5.3\%$; V_{re} , $65.5 \pm 4.2\%$; and V_{total} , $68.0 \pm 3.6\%$ (Fig. 6). For both groups, the angular velocities were not significantly different within mice of the same group; however, some trends were observed: In the control group, the V_{total} score was the closest to 100%, whereas the $V_{\max}$ score had a maximum variance of 100%. The same result was obtained in the crush group. In summary, the variation was greater in V_{pro} and V_{re} scores than in $V_{\max}$ and V_{total} scores for both groups. V_{total} was the least variable and most reliable angular velocity value.

Discussion

Although many authors have used behavioral parameters to assess facial nerve regeneration in a rat model[1–3, 10], no universally agreed or standardized method for the assessment of recovery from facial paralysis has been previously proposed. Our novel method for the assessment of facial palsy in a rat model with crush injury reflects true facial movement by measuring the angular velocity of a specific whisker. Further, it allows for the quantification of the whisker movement and repeated measurement of the same whisker base.

BASS consists of three components that make it economical, accurate, objective, reproducible, and convenient. The first is the iPhone X. Normal videography is captured at 30 fps; however, 30 fps is not sufficient to record whisker movements, and the maximum movement of the whisker might be missed. Thus, a higher frame rate is necessary to accurately measure the whisker movement. Professional cameras that record at HFR for long periods of time are prohibitively expensive. With advances in smartphone technology, high-speed videos can be recorded for a short duration. However, despite being

less expensive, most smartphones can only record at HFR for a few seconds. In contrast, iPhone 8 and later models can record video at 240 fps for more than 10 min (Fig. 2). The second component is the Kinovea software. Kinovea can measure the whisker angle, although we could not choose the specific whisker in every case due to a lack of contrast. Therefore, black light (UV light), the third component of BASS, was used to help visualize the whisker to be assessed. UV paint is readily available and inexpensive. We used the paint to mark the points on a specific whisker and whisker base. This procedure made it possible to illuminate a specific whisker, create high contrast with surrounding whiskers, and detect a specific whisker and whisker base automatically using Kinovea (Fig. 4). When the whiskers move, the whisker base also moves. BASS can assess the movement in a specific whisker and its base; thus, the scoring system reflects true facial movement by tracking and measuring the whisker angle velocity. No previous studies have measured true facial movement. BASS allows the repeated quantification of whisker movement and measurement of the whisker base every week. Thus, by combining these tools, BASS achieves accuracy, objectivity, and reproducibility.

The second whisker base from the top was selected because its horizontal position allows both the right and left bases to be captured by a camera mounted above the head. The third whisker base from the top is in a blind spot that cannot be captured. Caudal whiskers move more than the rostral whiskers; thus, caudal whiskers are more suitable for analysis of the movement. However, most caudal whiskers contact the body and are therefore not suitable for the assessment of movements. The best choice for assessing the midface movement is the second whisker from the top. Whisker movements were assessed during the protraction (forward movement) and retraction (backward movement) phases. We compared four angular velocities in normal rats and rats with a crush injury. Here, V_{total} appeared to be the most appropriate indicator for BASS. A drawback of our method is that the behavior of the rat cannot be fully controlled. Nevertheless, we believe this method is a reliable means for determining the functional recovery after manipulation of buccal and marginal mandibular branches of the facial nerve in rats.

In conclusion, we propose a new evaluation method using three key components, namely, a black light, an iPhone, and Kinovea motion analysis software, in a rat model with facial palsy. The system can detect the movement of a specific whisker and whisker base and automatically assess the angular velocity. Our novel system is economical, objective, reproducible, accurate, and convenient. Therefore, this technique can be used as a gold standard for the assessment of facial nerve recovery after a crush injury in rats.

Methods

Animals

Twelve adult male Wistar rats (230–260 g) were included in this study. All animals were housed in a room with an artificial 12-h on/off light cycle and were allowed free access to standard laboratory food and water. This study was conducted in compliance with ARRIVE guidelines. All experiments were performed under anesthesia in accordance with the Guidelines for the Care and Use of Laboratory

Animals of Hokkaido University School of Medicine and were approved by the Animal Care and Use Committee of Hokkaido University School of Medicine.

Key components of BASS

Black light: We used UV paint to mark the specific whisker and whisker base to be assessed, allowing it to be easily visualized against the surrounding whiskers under black light (Fig. 1).

High frame rate (HFR) videography: The iPhone 8 and later models can shoot 240 fps/1080p slow-motion videos for longer than 10 min (Fig. 2). The iPhone X, which was released in 2017, was used for high frame rate (HFR) videography in this study. The HFR videography capability of the iPhone X (released in 2017) was used for accurately capturing whisker movements. We recorded at HFR for more than a few minutes because we were not able to completely control the rats' behavior.

2D motion analysis software: Kinovea is a free 2D motion analysis software distributed under the GPLv2 license. It was created in 2009 through a nonprofit collaboration of several researchers, athletes, coaches, and programmers from around the world. The software enables frame-by-frame analysis of distances, angles, coordinates, and spatial-temporal parameters from a video recording; it can also measure the whisker angle (Fig. 3). The illumination created by the black light and UV paint allowed the Kinovea software to automatically detect the movement of the whisker and whisker base (Video S1).

BASS evaluation and analysis

After the rats were immobilized with a restraint jacket (RTS 01–03, Lomir Biomedical, Inc., Quebec, Canada), they were marked with ultraviolet (UV) paint under light anesthesia. The whisker located second from the top and fourth from the nose was marked at the base (point B), at a point < 1 cm from the base (point A) on both sides of the face, and at the midpoint of the bilateral medial canthus (point O). The front of the face was then illuminated with black light (UV light).

After the rats recovered from anesthesia, the whisker movement was recorded using an iPhone X (Apple, Inc., Cupertino, CA, USA) mounted 30 cm above the rat's head. The video was recorded in slow motion at 240 frames per second (fps) for 3 to 4 min. During 1 second when minimal neck movement was registered, points A and B were recorded simultaneously and the whisker that moved was selected and trimmed. Subsequently, the 1-second, 240-fps movie was converted into an 8-second, 30-fps movie using iMovie (Apple, Inc.).

The 8-second video files were processed using Kinovea motion analysis software. After analysis with Kinovea, angular velocities were measured, and the data were exported to a Microsoft Excel spreadsheet. The facial palsy score was calculated as the percentage of movement on the side which had palsy compared to the movement on the normal side ($V_{\text{palsy}}/V_{\text{normal}} \times 100$) (Fig. 4).

The BASS score was defined as the ratio of the percentage of movement on the left side to the percentage movement on the right side (control). We classified the whisker movement into the protraction

(forward movement) and retraction phases (backward movement). The maximum protraction velocity (V_{pro}) and maximum retraction velocity (V_{re}) were calculated and added to obtain the total velocity ($V_{total} = V_{pro} + V_{re}$). The larger V_{pro} and V_{re} scores were defined as V_{max} . To determine which angular velocity measurement was the most appropriate for BASS analysis, we compared the V_{pro} , V_{re} , V_{total} , and V_{max} values in each group. Furthermore, the difference between each velocity was compared between the control and crush groups.

Determining BASS in rats

To determine the feasibility, applicability, and validity of the method, we first assessed the whisker movements of normal rats. We postulated that, in normal rats, the movement of the right and left whiskers movement should be identical; therefore, we hypothesized that the BASS score in these mice would be approximately 100%.

The remaining rats were subjected to unilateral facial nerve crush injury (left side). Briefly, adult Wister rats (10 weeks old) were anesthetized with 3% isoflurane using a nasal mask connected to an inhalation anesthesia unit, and a preauricular incision with a marginal mandibular extension was made on the left side of the face to expose the buccal and marginal mandibular branches of the facial nerve. Both nerve branches were crushed 7 mm distal to the anterior border of the parotid gland. The crush injury was achieved by applying consistent pressure for 30 s with a watchmaker's forceps. The rats were assessed 2 weeks after surgery using the BASS score. To reduce the variability in the BASS scores, all animals were tested within the same timeframe after surgery.

Statistical analyses

BASS scores are expressed as mean $\pm$ standard error (SE). A one-factor analysis of variance (ANOVA) was used to analyze the differences in parameters (V_{pro} , V_{re} , V_{total} , V_{max}) between each group, using the Tukey-Kramer multiple comparison test. A P-value < 0.05 was considered significant.

Declarations

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Author contributions:

Takahiro Miura and Emi Funayama wrote the main manuscript text and Taku Maeda and prepared figures 1–3 and Kosuke Ishikawa and Masayuki Osawa prepared figures 4–6. Yuhei Yamamoto is a supervisor of this study. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

Whisker motion was assessed using the Kinovea motion analysis software. Point A indicates the whisker, point B the whisker base, and point O the mid-point of the bilateral medial canthus.

Figure 2

Comparison of video image capture devices. Fast whisker movements cannot be captured at 30 frames per second (FPS) due to blurring; however, 240 fps can adequately capture fast movement.

Figure 3

Kinovea softwareenabled automatic frame-by-frame analysis of whisker angles. A: A captured image from high-speed videography. The marked whisker and whisker base are visualized by fluorescence under the UV light. B:Kinovea softwareenabled automatic frame-by-frame analysis of whisker angles.

$$\text{BASS Scores(\%)} = \frac{\text{V(Palsy side)}}{\text{V(Normal side)}} \times 100$$

Figure 4

BASS scores were defined as the ratio of the percent of movement on the palsy side to movement on the normal side.

Rat	Vmax	Vpro	Vre	Vtotal
A	122.8	121.6	122.8	122.4
B	90.0	83.3	90.0	88.0
C	134.3	70.6	134.3	107.3
D	116.1	115.1	116.1	115.7
E	109.7	75.1	109.7	94.1
F	117.2	117.2	63.5	81.8
Ave.	115.0	97.2	106.1	101.6
SE	6.0	9.5	10.4	6.6

Figure 5

BASS scores in normal rats according to angular velocity direction. The BASS score was calculated as the ratio of the percentage of movement on the left side to movement on the right side.

Rat	Vmax	Vpro	Vre	Vtotal
A	68.7	76.7	68.7	71.7
B	78.1	66.4	78.1	72.9
C	74.5	72.4	74.5	73.6
D	56.6	74.5	56.6	63.4
E	51.3	52.7	51.3	51.8
F	63.7	92.4	63.7	74.6
Ave.	65.5	72.5	65.5	68.0
SE	4.2	5.3	4.2	3.6

Figure 6

BASS scores in rats with a crush injury according to angular velocity direction measured 2 weeks after surgery.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [VideoS1.mp4](#)