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PERSPECTIVE

Three-dimensional motion analysis for evaluating motor function in rodents with peripheral nerve injury

Peripheral nerves act like networks of "wires" that conduct signals from the brain and spinal cord to organs throughout the body. Damage to these nerves leads to numbness, pain, and weakness that eventually reduces the patient's quality of life. Fortunately, peripheral nerves have a high regenerative capacity and, in most cases, recover from mild disorders and injuries. However, there have been continuous challenges facing the development of treatments for peripheral nerve conditions such as disorders that occur through chronic mechanical stimulation like carpal tunnel syndrome, disorders due to side effects from drugs and intractable diseases like chronic inflammatory demyelinating polyneuropathy. Furthermore, self-regeneration becomes difficult when trauma causes a large gap in the axon, leading to the patient needing nerve transplantation. While autologous nerve transplantation is the current gold standard, only approximately half of the cases obtain satisfactory functional recovery (Yang et al., 2011). More endeavors are needed to achieve better therapeutic effect with this treatment method.

In order to develop a more effective treatment for peripheral nerve injury, an appropriate indicator for evaluating the treatment outcomes is essential. Patients with peripheral nerve damage generally desire functional improvements, such as the ability to move the body, improvement in sensation, and pain relief, rather than regenerating as many axons as possible. Thus far, pre-clinical research conducted using rodents has primarily evaluated the therapeutic effect of treatments through histology, electrophysiology, or molecular biology-based analysis of the regenerated nerves. Here, the problem is that the results of evaluation through histology and electrophysiology may not necessarily match the function desired by patients (Munro et al., 1998). However, whether this is because of discrepancies between these evaluations or flaws in motor function evaluation has not been clarified. Methodologies for evaluating the function of experimental animals, motor function in particular, have been inadequate thus far. Presently, human motor function evaluation is the primary focus of biomechanical research. We evaluated motor function in rodents and conducted a more detailed assessment of the functional recovery process through gait motion analysis (**Figure 1A** and **B**). The results reveal that in the rat sciatic nerve crush injury model, there is a high correlation between the toe angle at the time of toe-off during gait and the histological findings of the peripheral nerve (Wang et al., 2018) (**Figure 1C**). Furthermore, evaluating the angular velocity of the hind legs and tracking the movement of the virtual center of the pelvis allowed comprehensive evaluation of the disorder (Tajino et al., 2018).

The sciatic nerve injury model in rodents has often been used to develop treatments for peripheral nerve regeneration (Wood et al., 2011). Evaluation of motor function in animal models with sciatic nerve crush injury has relied mostly on the sciatic functional index (SFI) (Bain et al., 1989; Sarikcioglu et al., 2009). The advantage of the SFI is that it allows for the evaluation of motor functions in a facile and non-invasive manner over time. SFI has been extensively applied in experiments because it permits ease of comparison and reference to past data. However, the SFI also has many drawbacks (Monte-Raso et al., 2008; Sarikcioglu et al., 2009; Lee et al., 2013), including low reproducibility of the SFI during the early stages after injury, low reliability of the SFI between testers, the fact that autotomy (the animals biting off their own toes) makes evaluation impossible, and problems with reliability of the value from toe contracture. Footprints are significantly affected by the stride and gait speed of the animal, and these factors are difficult to unify because the experiments allow for free gait, therefore causing variation in data. Furthermore, although dependent on the extent of the injury, the SFI shows rapid recovery values at 2–3 weeks after injury in the sciatic nerve crush injury model, returning to the original state prior to injury at 4 weeks and reaching a plateau thereafter (Wang et al., 2018). However, at 4 weeks after injury, histologic recovery of the nerves is only approximately 60% in terms of the number and diameter of myelinated nerves and 45% in terms of the myelin thickness compared to the control, which is very different from the recovery values of the SFI (Wang et al., 2018). The SFI shows a ceiling effect in an even earlier stage. Lastly, the SFI value is calculated indirectly using a formula and the particular function that has recovered is unclear.

In order to resolve this issue with the SFI, evaluation using motion analysis has been considered (Lee et al., 2013; Rui et al., 2014). We applied three-dimensional (3D) motion analysis, as used in humans, to rodents. The advantage here is that detailed analysis methods used in humans are applicable in almost the same way in rodents. For example, it is possible to calculate not only the gait cycle and the stride, but also the joint angles during movement and the angular velocity of joints. Since it is possible to calculate the relationship and cooperativeness of the motion of each joint, it is possible to evaluate the motor function from the perspective of the smoothness and efficiency of movement. The advantage of using 3D motion analysis is that the values are more accurate than those from 2D analysis. Since the images are taken by multiple cameras, an accurate value can be calculated using relative spatial recognition. This makes it possible to measure parameters that would be difficult assess using 2D, such as rotational movement. Furthermore, a virtual center of gravity can now be calculated from each marker and be applied to the evaluation of pain and compensatory movement, *etc.* by examining which side the center of gravity inclines. As pain cannot be directly measured in animals, alternative methods are often used to assess behaviors that reflect pain such as reduced weight bearing. Additionally, 3D motion analysis enables to estimate a rodent's balance shifting from several parameters (*e.g.*, stance phase, pelvic tilt, and shift of the center of gravity). These parameters can be comprehensively measured at once and necessary data extracted later at any time.

We measured histologically and kinematically, the peripheral nerve regeneration process in a sciatic nerve crush injury rat model (Wang et al., 2018). When the relationship between histologic parameters, such as the number of regenerated axons and diameter of myelinated nerves, and angle of each joint and the SFI was examined, there was a very high correlation coefficient between the toe joint angle at the time of toe-off and the number of regenerated nerves and diameter of myelinated nerves (**Figure 1C**). As the toe joint angle at the time of toe-off reflects the kicking off of the hind legs from the ground, we hypothesize that the muscle action of the flexor digitorum and plantar flexion involving muscles of the ankle are primarily involved in the action and believe that there has been regeneration of the tibial nerve dominating these parts. Although ankle dorsiflexion and toe extension movement are required in the swing phase to ensure toe clearance, in the sciatic nerve injury model, 3D motion analysis shows that the movement is significantly restricted (Tajino et al., 2018). The SFI, which uses footprints, cannot analyze such motor function in the swing phase. Furthermore, owing to the highly positive correlation with the histologic regeneration of nerves, measurement of the toe angle alone would allow for the non-invasive estimation of the nerve regeneration process over time and contribute to a reduction in the number of animals used. Several problems associated with the SFI can also be solved. For example, while the SFI shows sudden recovery during specific periods, the toe angle tends to recover in a relatively linear manner with time. The loss of toes due to autotomy affects the fourth and fifth toe in most cases, and it is therefore not problematic to measure at the third toe. As we observe the gait motion of animals at a constant speed on the treadmill, there is little error associated with data in the gait speed. Furthermore, since the animals had not recovered to their original state prior to injury even 4 weeks after the sciatic nerve crush injury, and the ceiling effect was slower than that in the SFI, it is possible to analyze the motor function effect for an even longer period of time (**Figure 1C**). In fact, we showed that compared to the SFI, toe angle had a greater statistically significant correlation with the number and size of regenerated myelinated nerves (Wang et al., 2018). Additionally, 3D motion analysis allows for not only the direct motor function analysis of the nerve injury site but also motion analysis to compensate it. Such as, the movement of the pelvis to compensate for the toe clearance of the paralyzed hind leg in the sciatic nerve injury model could be evaluated by analyzing the trajectory of motion. By estimating the center of gravity from the pelvic marker, we could evaluate

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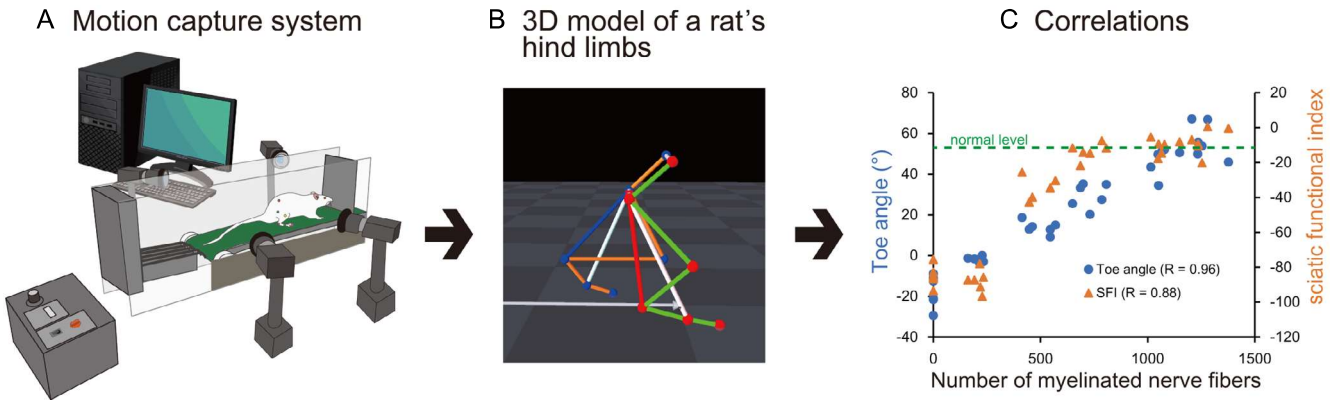

Figure 1 Three-dimensional (3D) motion capture system.
(A) Illustration of the 3D motion capture system. (B) A 3D model of a rat's hind limb built with photos taken by four cameras. Red circles connected by a green line and blue circles connected by an orange line are bone landmarks of the right and left sides, respectively. These landmarks represent the following anatomical sites: anterior superior iliac spines, greater trochanters, knee joints, lateral malleoli, fifth metatarsophalangeal joints, and the tips of the fourth toes. White lines are auxiliary lines that connect the greater trochanter and fifth metatarsophalangeal joint. The red and blue lines are also auxiliary lines that connect the greater trochanter and ipsilateral lateral malleolus, respectively. (C) Correlations between the sciatic functional index and toe angle with the number of myelinated nerve fibers (Spearman's rho correlation analysis, both $P < 0.01$).

the left-right deviation in the center of gravity throughout the gait cycle (Tajino et al., 2018).

Some problems with 3D motion analysis should be noted. Measurement is somewhat complicated because it requires a marker to be attached to each landmark. In order to obtain reproducible data, becoming familiar with marker attachment is necessary. The animals will also require training. In order to have the animals perform a reproducible gait on the treadmill, it is necessary to familiarize them with the treadmill even before the injury. Furthermore, pronounced shifting of the skin during the movement of parts of the body, particularly the knee joint of the hind legs of rodents, will result in deviation from the actual movement of the animal (Filipe et al., 2006). Besides, the cost of 3D motion analysis may limit its usage in experiments involving rodents. Until recently, 3D motion analysis devices for humans were very expensive and the analysis was complicated. However, with the development of technology and extensive use of such devices, the cost has reduced, and the devices have become relatively more affordable and accessible with simpler handling. Markerless devices have also been proposed. These benefits may be possible in the near future for small animal 3D motion analysis devices.

Hereafter, we require characteristic motions in each animal disease model to be clarified, as shown in the rat sciatic nerve crush injury model, how good an indicator the toe joint angle at the time of toe-off is. We believe this would provide useful motor function indicators to determine the effect of drugs, rehabilitation, and cell therapy. As motion capture can only analyze "movement", estimation of "force and torque" by combination with a floor-reaction force meter, as seen in human studies, is also starting to be applied for motor function evaluation in animal experiments. The results obtained by applying these methodologies from human research to animal experiments can then be reapplied to humans. Thus, 3D motion analysis of rodents represents an excellent motor function evaluation in a variety of diseases, particularly in nerve diseases.

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References

Bain JR, Mackinnon SE, Hunter DA (1989) Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. Plast Reconstr Surg 83:129-138.
Filipe VM, Pereira JE, Costa LM, Mauricio AC, Couto PA, Melo-Pinto P, Varejao AS (2006) Effect of skin movement on the analysis of hindlimb kinematics during treadmill locomotion in rats. J Neurosci Methods153:55-61.
Lee JY, Giusti G, Wang H, Friedrich PF, Bishop AT, Shin AY (2013) Functional evaluation in the rat sciatic nerve defect model: a comparison of the sciatic functional index, ankle angles, and isometric tetanic force. Plast Reconstr Surg 132:1173-1180.
Monte-Raso VV, Barbieri CH, Mazzer N, Yamasita AC, Barbieri G (2008) Is the Sciatic Functional Index always reliable and reproducible? J Neurosci Methods 170:255-261.
Munro CA, Szalai JP, Mackinnon SE, Midha R (1998) Lack of association between outcome measures of nerve regeneration. Muscle Nerve 21:1095-1097.
Rui J, Runge MB, Spinner RJ, Yaszemski MJ, Windebank AJ, Wang H (2014) Gait cycle analysis: parameters sensitive for functional evaluation of peripheral nerve recovery in rat hind limbs. Ann Plast Surg 73:405-411.
Sarikcioglu L, Demirel BM, Utuk A (2009) Walking track analysis: an assessment method for functional recovery after sciatic nerve injury in the rat. Folia Morphol 68:1-7.
Tajino J, Ito A, Tanima M, Yamaguchi S, Iijima H, Nakahata A, Kiyan W, Aoyama T, Kuroki H (2018) Three-dimensional motion analysis for comprehensive understanding of gait characteristics after sciatic nerve lesion in rodents. Sci Rep 8:13585.
Wang T, Ito A, Aoyama T, Nakahara R, Nakahata A, Ji X, Zhang J, Kawai H, Kuroki H (2018) Functional evaluation outcomes correlate with histomorphometric changes in the rat sciatic nerve crush injury model: A comparison between sciatic functional index and kinematic analysis. PLoS One 13:e0208985.
Wood MD, Kemp SW, Weber C, Borschel GH, Gordon T (2011) Outcome measures of peripheral nerve regeneration. Ann Anat 193:321-333.
Yang M, Rawson JL, Zhang EW, Arnold PB, Lineaweaver W, Zhang F (2011) Comparisons of outcomes from repair of median nerve and ulnar nerve defect with nerve graft and tubulization: a meta-analysis. J Reconstr Microsurg 27:451-460.

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