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Master's Thesis  
석사 학위논문

# Quantitative Analyses on Gait-training Effect of Stroke Rats

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**2017**

# Quantitative Analyses on Gait-training Effect of Stroke Rats

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A thesis submitted to the faculty of DGIST in partial fulfillment of the requirements for the degree of Master of Science in the Department of Robotics Engineering. The study was conducted in accordance with Code of Research Ethics<sup>1</sup>

01. 06. 2017

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# Quantitative Analyses on Gait-training Effect of Stroke Rats

Joong-Kwang Ko

Accepted in partial fulfillment of the requirements  
for the degree of Master of Science

11. 30. 2016

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## ABSTRACT

Brain stroke is serious disease in modern society which has been not overcome yet. Thus, various researches and therapies about the stroke have been undergone to solve this problem. Recent stroke researches with rats have been represented the researches with human because of better controllability and homogeneous population. Many challenging researches have been applied to stroke rats, especially monitoring methods. Laser monitoring might be the most remarkable methods, since these monitoring systems obtain various information such as blood flow, blood oxygenation with *in-vivo* state. However, previous laser monitoring systems were inappropriate to be practical uses because of their penetration depth. Diffuse speckle contrast analysis (DSCA) is a monitoring system which measures blood flow index that is calculated by image contrast due to the speckle effect. DSCA could measure the signal under 5mm of tissue. In this study, possibility of recovery monitoring through gait-training of stroke rats was estimated by using DSCA. For statistically validated biological experiment, 30 rats are divided into rehabilitation group (n=10), stroke group(n=10), and normal group(n=10). All experimental groups had blood flow measurement and other quantitative analyses during 14 days and rehabilitation group had treadmill training 8m/min with 30 minutes daily. Results show the cerebral blood flow in infarction of rehabilitation group increases more than that of stroke group. Blood oxygenation monitoring was also applied, however, there are no significant difference between experimental group with 635 nm wavelength. Not only blood flow but also sensorimotor function of rehabilitation group is recovered more during recovery period. Histological analysis shows less infarction volume in rehabilitation group compared to stroke group. There are proportional correlation between blood flow and sensorimotor function recovery. We expect that development of DSCA could offer self diagnosis for stroke patients in near future.

**Keywords:** Brain stroke, Gait-training, DSCA, Blood flow, Rat

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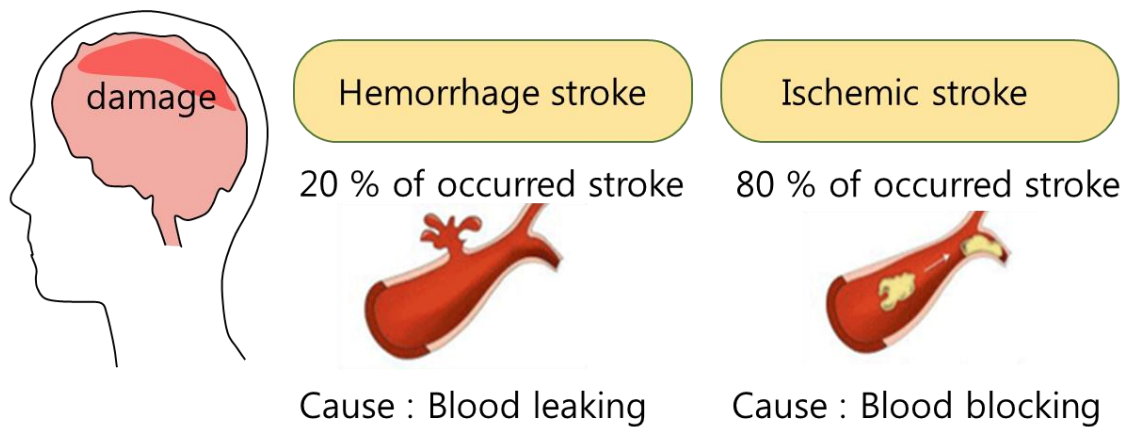
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# 1. Introduction

## 1.1 Study Background

Brain stroke is a serious disease that causes malfunction of body by brain damage. Not only high mortality which is referenced from 3 principal causes of death (heart disease, cancer, brains stroke), but also long lasting impairment of sensorimotor function is considered to serious problem [1]. Usual stroke is induced from ischemia by blood vessel blocking and some of other scale, stroke can be also induced from hemorrhage by blood vessel leaking as shown in Fig. 1. In modern society, various factors such as dietary habits and lack of exercise results to increase of stroke patients as shown in Fig. 1. Principal symptoms for stroke patients are hemiplegia, aphasia and apraxia [2]. These impairments influenced to social activity negatively, moreover, stroke patients became big burden for their family. As well as other pathological research, brain stroke also has been studied and therapies about this disease also have been developed to overcome the problem. Physical therapy and pharmacotherapy are widely used for stroke recovery until now. In case of physical therapy, treadmill gait-training and upper limb exercise are utilized for recovery of sensorimotor function [3, 4]. In case of pharmacotherapy, thrombolytic agent or coagulant are used depend on stroke type whether ischemic stroke or hemorrhage stroke to improve blood circulation [5, 6]. However, brain has numerous mechanism of recovery from the damage and still continuous researches have been required. Despite of this requirement, research with human had several limitations such as moral problem, strict IRB test, impossibility about control the variables. To compensate these issues, many experiments which is represented by animal especially rodent have been suggested due to the economic advantage, high controllability of variables, populations (Fig. 2). This study would like to know recovery effect of gait-training from stroke by using rat.



- Symptoms : impairment of sensorimotor function  
( ex) Aphasia, hemiplegia, apraxia)
- Around 15 % of the principal causes of death

**Figure 1.** Characteristics of brain stroke

#### Human stroke research



##### Limitations

- Moral problem
- Heterogeneous
- Strict IRB

#### Rodent stroke research



##### Advantages

- Controllability
- Population
- Low cost

**Figure 2.** Stroke research with rodents

## 1.2 Previous Studies

There are various studies such as stroke inducing technology, rehabilitation method, and histological analyses and monitoring in previous researches related with stroke rat. Stroke model preparation is the first step to proceed animal experiment about stroke.

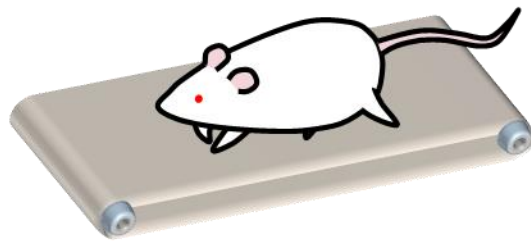
To prepare stroke rat model, various methods have been developed such as middle cerebral artery occlusion surgery, endothelin-1, and photo thrombosis [7-9]. Most of all middle cerebral artery occlusion (MCAO) is widely used for experiments.

In research area, treadmill gait-training is universal rehabilitating methods so that applied to not only rodents but also human [10-12]. Several studies show that sensorimotor function of stroke rat which is measured by behavior test was improved through the treadmill training. Infarct volume measurement also proved effect of rehabilitation. Other studies also show gait-training effect by using spontaneous wheel running, bicycle [13, 14]. In case of rat, gait-training follows protocol of that speed is usually 5~15 meter/minutes and training time is 10~30 minutes. Challenging studies with development of robot is also applied to rat rehabilitation such as rat exoskeleton [15]. For instance, Bogdan et al. presented a robotic system for complex motor training in the stroke rat [16].

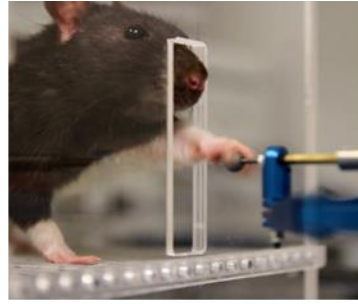
About monitoring methods, there are behavior tests, histological analyses, laser hemodynamics etc. Since some reports on treadmill training for the stroke rats said that high intensity training could aggravate an infarcted lesion, monitoring methods are required to modulate the training intensity and give feedback [17]. Actually, effects of physical therapy for the stroke patient has been evaluated with behavior test by empirical inspection of therapist [18]. There are various types of behavior test such as Garcia test, escaping maze, swimming and keeping position at a rotary treadmill [19, 20]. However, most behavior test requires empirical and subjective decision for evaluator. Histological analyses are widely used in

biological area since they can offer definite image to prove the research result. Not only infarction volume, even neuron activity or synthesis activity for angiogenesis were different between stroke group and rehabilitation group in previous studies [21-23]. However, those results could be acquired after autopsy which means *in-vivo* imaging is not available. To be possible to monitor *in-vivo* state, laser monitoring systems have been developed such as diffuse optical tomography, laser speckle contrast imaging, laser Doppler flowmetry and optical coherence tomography etc. Laser speckle contrast imaging (LSCI) showed angiogenesis effect by treadmill training of stroke rat which means recovery of stroke [24]. Diffuse optical tomography acquired various hemodynamic parameters such as blood oxygenation, blood flow, and metabolic rate of oxygen consumption showed the hemodynamic differences among brain tissues during the MCAO surgery, for example, measured blood flow near to occluded middle cerebral artery was lower than contralateral site [26]. Laser Doppler flowmetry measures blood flow and it is widely used so that many stroke rat researches have been already reported several results by using laser Doppler flowmetry [27]. However, those inspection methods had critical problem to be practical uses since they require invasive approach such as craniotomy, incision of scalp due to the short penetration depth. Non-invasive inspections such as magnetic resonance imaging (MRI) and computed tomography (CT) are commonly used for diagnosis of the stroke, however, these modalities have limitations on real time monitoring of the neurophysiological change during rehabilitation and expensive [28].

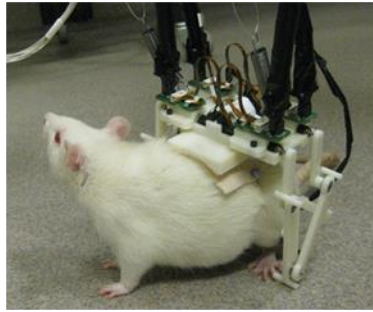
Non-invasive and quantitative monitoring system would be valuable for continuous monitoring during the gait-training. Furthermore, hemodynamic information can provide significant index of brain state. That is the why non-invasive hemodynamic monitoring systems is required to monitor the stroke rat brain.



(a) Treadmill training



(b) Rat manipulandum



(c) Rat Backpack

**Figure 3.** Previous methods for rehabilitation of rat (a) treadmill training method, (b) 3 DOF parallel manipulator for training of forelimb [16], (c) rat backpack type exoskeleton [15]

### 1.3 Study Purpose

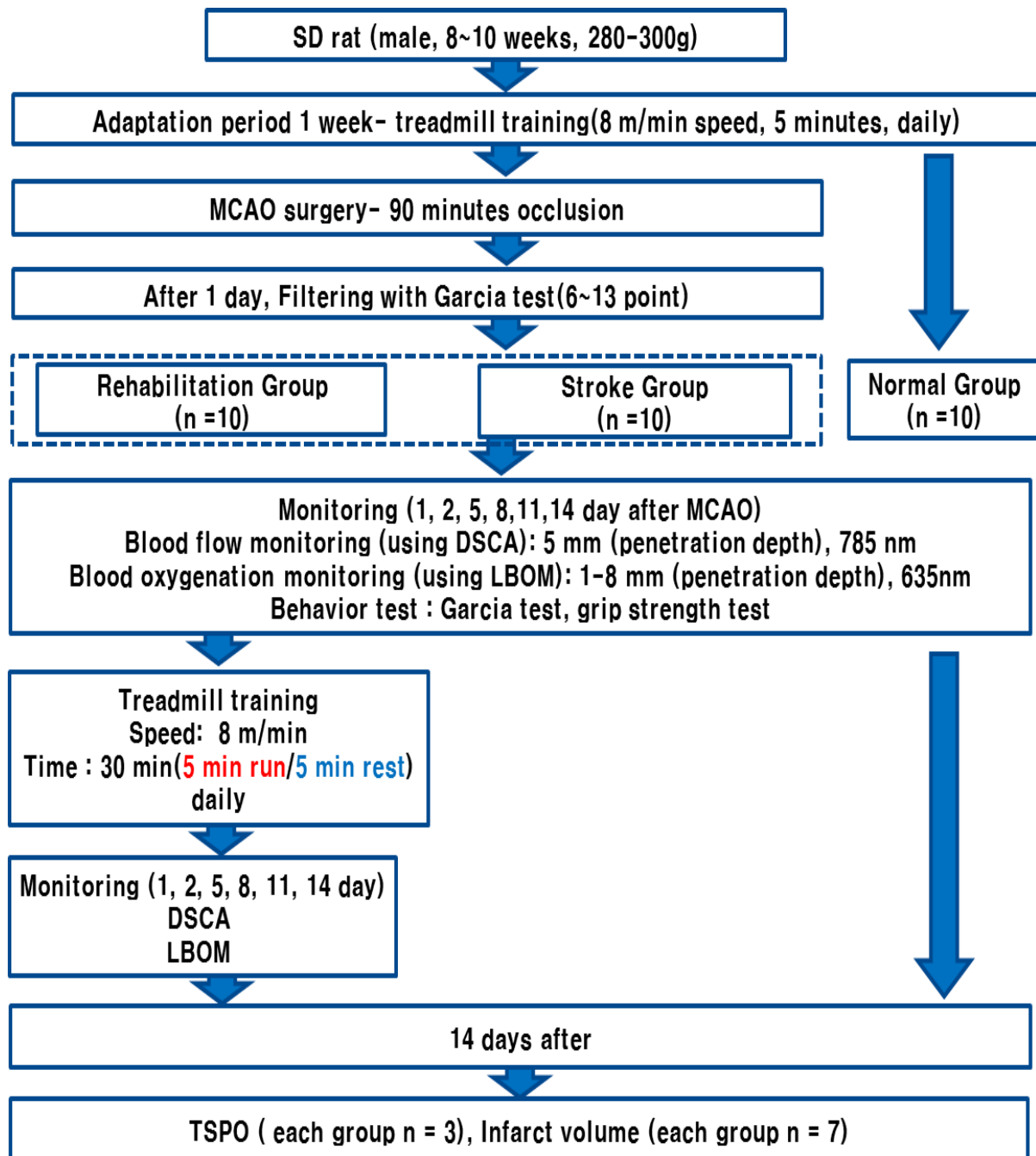
Probably everyone might know that early exercise after stroke is helpful for recovery of stroke through the previous studies. However, most of previous studies are not appropriate to be practical monitoring modality since they are invasive, or require human resource for evaluation, or are not real time monitoring. In this study, Diffuse speckle contrast analysis (DSCA) system is introduced which is novel deep tissue flowmetry system with the advantages of a low cost, easy analysis, fast image acquisition time, and a simple experimental setup. DSCA system proved validation for the blood flow monitoring through chick embryo vascularization experiments, cuff-occlusion experiments [29, 30]. Now, we would like to apply this system to cerebral blood flow monitoring. Here, we analyzed the gait-training effect by comparing stroke rats between rehabilitation group and stroke group. As previous studies show different blood flow in infarction between rehabilitation group and stroke group, if DSCA system show similar tendency, it could prove the validation for cerebral blood flow monitoring and could be practical monitoring system even for human. In this experiments, the quantitative measurements such as blood oxygenation, blood flow index (BFI), and sensorimotor function, grip strength, infarction volume are achieved by using laser blood oxygenation monitoring (LBOM), diffuse speckle contrast analysis (DSCA), Garcia test, grip strength test and histological analyses, respectively. Through this experiments, we would like to show correlation of blood flow compared to other recovery level evaluating factors. In the future, we expect DSCA system became the useful self-diagnostic device for stroke patients which provide cerebral blood flow information.



## **2. Method and Materials**

### **2.1 Experimental protocol**

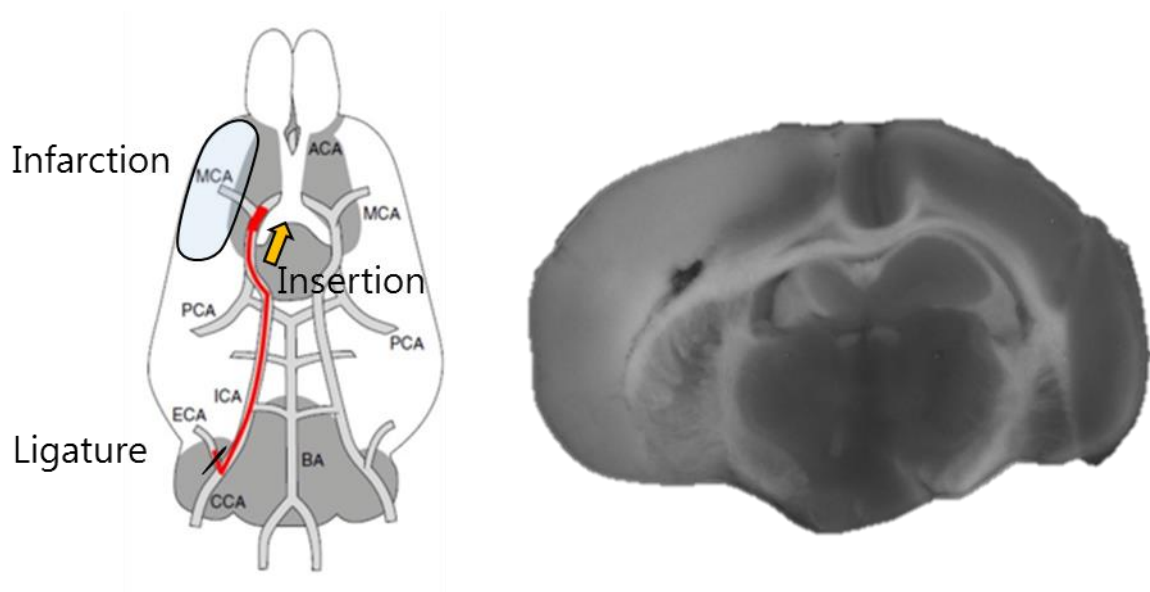
To know effect of gait-training for stroke recovery, Sprague-Dawley rats were subjected to this experiment due to high survival rate compared to mice. 30 rats are subjected to experimental groups for statistical convincing experimental data. All rats are divided into three groups, rehabilitation group (n=10), stroke group (n=10), and normal group (n=10), respectively. Rats had adaptation period during 1 week. After the adaptation period, 20 rats were subjected to middle cerebral artery occlusion (MCAO) surgery. After 24 hours, operated rats were classified to rehabilitation group and stroke group randomly which were filtered through the Garcia behavior test. Laser monitoring including DSCA and LBOM were applied to all groups. Behavior tests including Garcia test and grip strength were applied to all groups too. Since frequent monitoring could be stressful for rats, monitoring was done on 1, 2, 5, 8, 11, 14 day post MCAO. Rehabilitation group had laser monitoring at pre-training and post-training. Experimental period is 14 days to compare rehabilitation effect of previous studies. After 14 days, all rats were sacrificed and under the histological analyses.



**Figure 4.** Experimental protocol

### 2.1.1 Animal model

To make ischemic stroke rat model, Sprague-Dawley rats (male, 8-10 weeks, 270~300g) were subjected to transient middle cerebral artery occlusion (MCAO) surgery with nylon suture (4-0 fine MCAO suture, DOCCOL). The rats were anesthetized with respiration by using isoflurane during 30 minutes. The body temperature of rat was maintained at 37°C by heating pad during the surgery. After 90 minutes of occlusion time, reperfusion was applied to the rats. Thus, focal ischemia is occurred to the rat brain. After 24 hours of the surgery, the ischemic stroke rats were filtered by the Garcia behavior test for homogeneous sample. As Fig.5 shows, brain tissue would be infarcted after the blocking middle cerebral artery so that penumbra will be appeared after TTC staining of the brain slice. The animal experiment was approved from experimental animal center of DGIST, Korea.



**Figure 5.** MCAO surgery

### **2.1.2 Treadmill training**

All rats are subjected to daily treadmill training during adaptation period (1 week) with 8 m/minute speed for 5 minutes. After the MCAO surgery, rats in the rehabilitation group are forced to run on the treadmill (800 series, IITC) with a speed of 8 m/minute for 30 minutes daily. Electric shocker behind the treadmill motivated to run for rats. During 30 minutes, the rats are allowed to 5 minutes break after each 5 minutes of running. Rehabilitation group rats start the gait-training after 24 hours post-MCAO. The gait-training period is 14 days to compare the result with other conventional experimental result about stroke rat rehabilitation.

### **2.1.3 Laser monitoring**

Laser monitoring methods measured blood flow and blood oxygenation by using diffuse speckle contrast analysis and laser blood oxygenation monitoring, respectively. In the blood flow measurement, DSCA system with a laser wavelength of 785 nm is used for detecting blood flow index. Since the laser diode output power is 65 mW, optical 1x4 coupler divided laser power so that let incident laser power be around 15 mW which follows laser safety for biological sample. Distance between an emitter and a detector is 10 mm for 5 mm penetration depth which is enough to monitor from striatum to cortex of rat. The BFI is fluctuating even though a rat doesn't move during monitoring periods because of low frequency oscillation in normal blood flow regulation. Thus, 100 seconds of measurement time is applied to obtain average BFI. In addition, more than 100 second for measurement time was not available with consideration about stress and endurance of rat. In the blood oxygenation measurement, LBOM (BOM-L1TRSF, OMEGA-WAVE) with an incident laser wavelength of 635 nm, 655nm, and 690nm is used for detecting blood oxygenation. Distances between an emitter and two detectors in the optical probe of the LBOM are 5mm and 8mm, respectively so that penetration depth is 1~8 mm. Since the obtained signal is almost step response, data acquisition time could be under the 10 second, also, to minimize stress of rat during the measurement. Laser monitoring is achieved before and after gait-training, respectively. Because of biological differences between experimental animals, hemodynamic indices should be normalized to be compared easily. As equation (1), (2) show, normalized hemodynamic parameters are achieved under the assumption that both ipsilateral blood flow and blood oxygenation of stroke rat would be lower than that of normal rat, since normal rat doesn't have circular impairments so that both lateral hemodynamic parameters are expected to be similar which means average data is equal to both data from lateral brains. Thus, normalized blood flow and blood oxygenation is achieved from

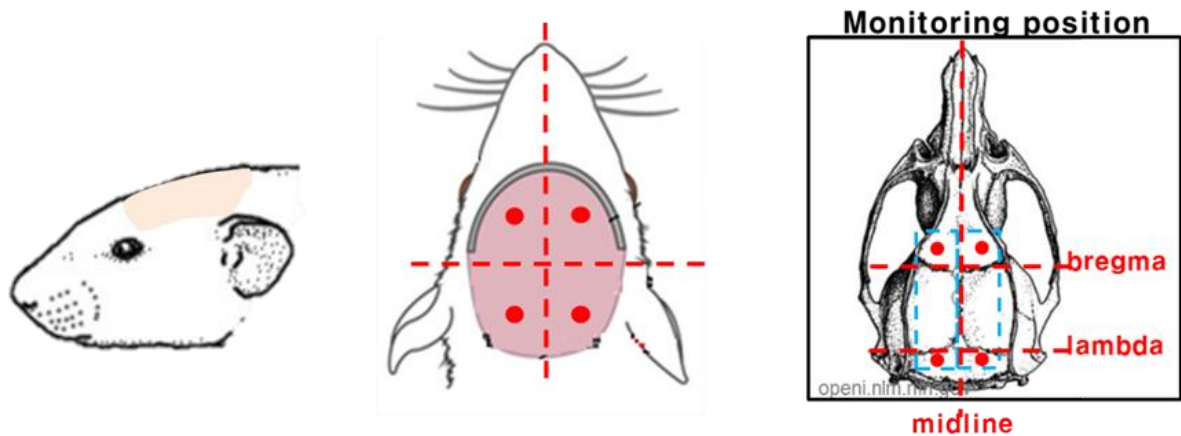
lateral blood flow of measured group per average BFI of normal group on each measurement day.

$$\text{Normalized blood flow (\%)} = \frac{\text{Lateral BFI of measured group}}{\text{Average BFI of normal group}} \quad (1)$$

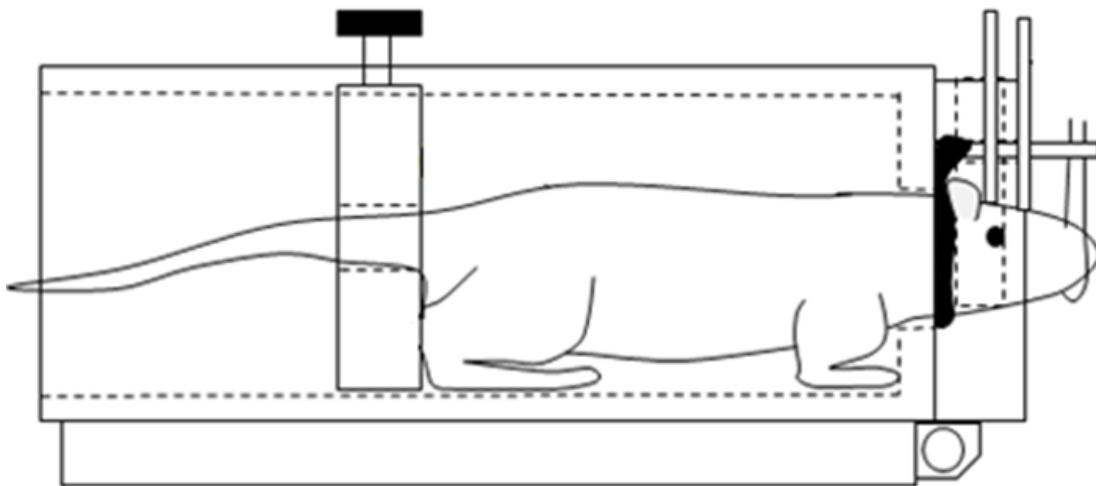
$$\text{Normalized blood oxygenation (\%)} = \frac{\text{Lateral StO2 of measured group}}{\text{Average StO2 of normal group}} \quad (2)$$

As Fig. 6 shows, the monitored positions are on the left and right head. Irradiation point is located on the 5mm lateral to midline and 1mm posterior to lambda. Detection point is located on the 5 mm lateral to midline and 1 mm frontal to bregma. Middle cerebral artery might be located near to the bregma. To minimize attenuation of the laser, hair on the head are shaved with remaining scalp only and fiber with ferrule is directly contacted to the scalp on the point. During the monitoring, the rats should be awake state since significant different neural activities of anesthetized rat and awake rat were reported. Also anesthesia can affect training performance and neural activity so that the monitoring was proceeded under the awake state during this experiment [21]. Although non-invasive laser monitoring requires static state of the rat since the laser monitoring modalities are sensitive to its motion artifacts, rat usually couldn't endure static state during monitoring so that restrainer was necessary for the effective monitoring without motion artifact. As Fig. 7 shows, restrainer is designed to prevent rat motion during blood flow monitoring without anesthesia. To increase proximity of the measurement probe, this restrainer is assembly type. While rat is forced to insert inside of the cylinder, U-shape neck holder and tail side disk compress whole body for immobility. Head restrainer is applied after that. To sustain contact state of measurement probe on the scalp, two side rubber band strapped neck and maxilla (Fig. 8). During the monitoring, only few rats resisted about this restrained state. As Fig. 9 shows, this implementation can help a reduction of rat's motion artifact during blood flow monitoring without the anesthesia. By strapping neck

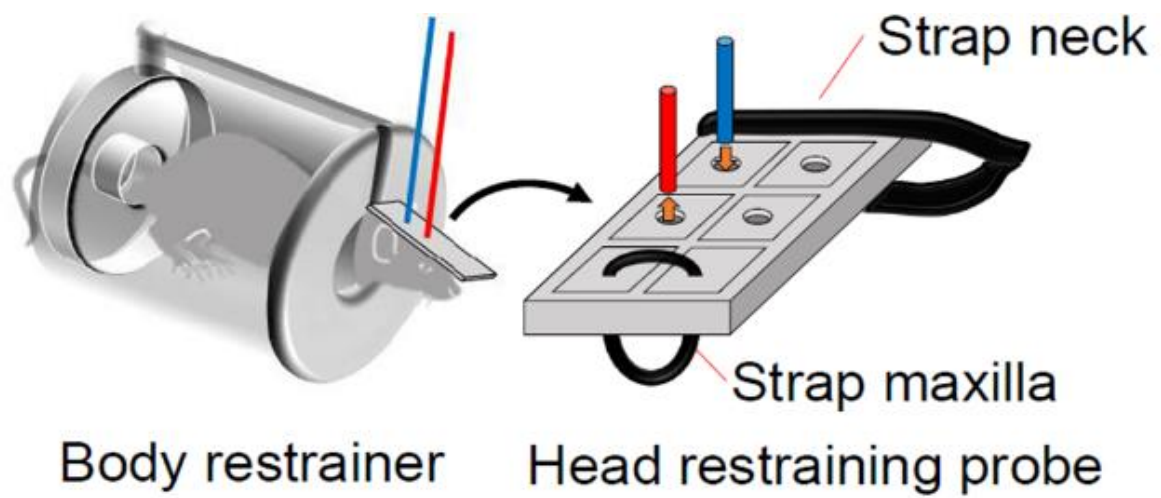
and maxilla, probe positioning is stable with 10 mm distance between laser source and detector. According to midline on the rat head, the position of midline of head restraining probe also could be set. An optical fiber probe is fixed because of the restrainer structure. In this structure, sponge acts as a damper that preventing vibration of the probe, as described in Fig. 9.



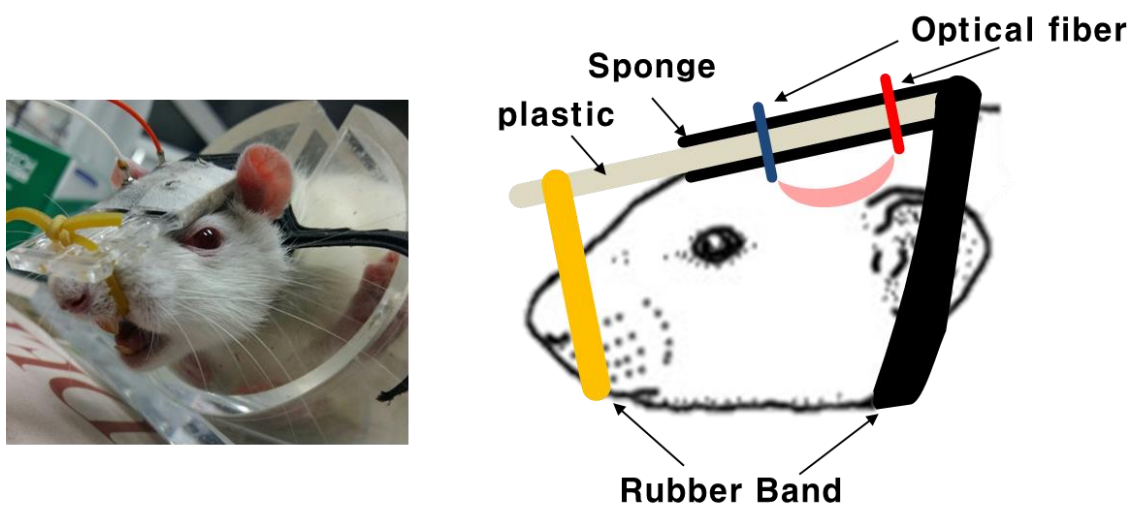
**Figure 6.** Measurement point



**Figure 7.** Rat restrainer



**Figure 8.** Component of rat restrainer



**Figure 9.** Head restrainer



## 2.2 Diffuse speckle contrast analysis

Diffuse speckle contrast analysis (DSCA) system is novel flowmetry that allows deep tissue monitoring. When a coherent light is illuminated to a tissue through the fiber, movement of scatterer within a tissue reduce diffused speckle contrast during exposure time of CCD camera, as shown in Fig. 10. Slow blood flow inside the tissue would offer little scattering effect so that high contrast on the region of interest (ROI) would be obtained from CCD camera, while fast flow would be appeared as a low contrast image in specific ROI. In this experiment, DSCA system components are CCD camera, 1X4 coupler, laser and probes as Fig. 11 shown. Speckle contrast ( $K$ ) indicates distribution of speckle intensity during given exposure time. Laser speckle imaging system also computed speckle contrast value as a ratio of standard deviation per average of speckle intensity at spatial domain as described in Eq. (3) [31]

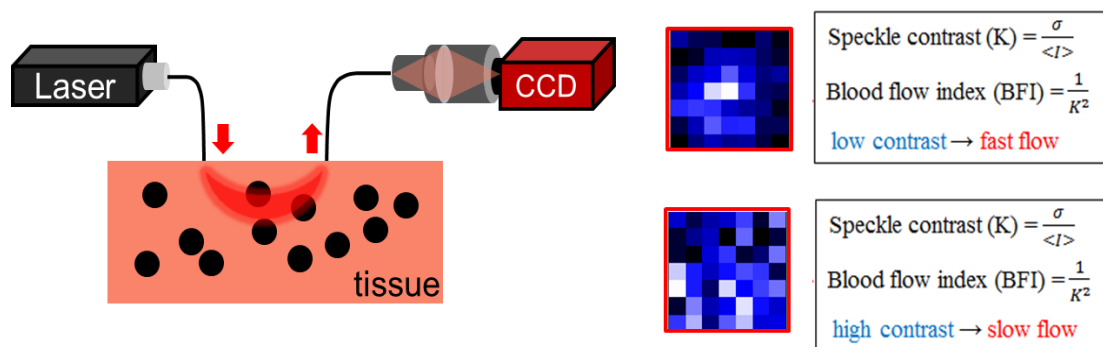
$$K = \frac{\sigma}{\langle I \rangle} \quad (3)$$

The speckle contrast value at the given exposure time is derived from below equation [32].

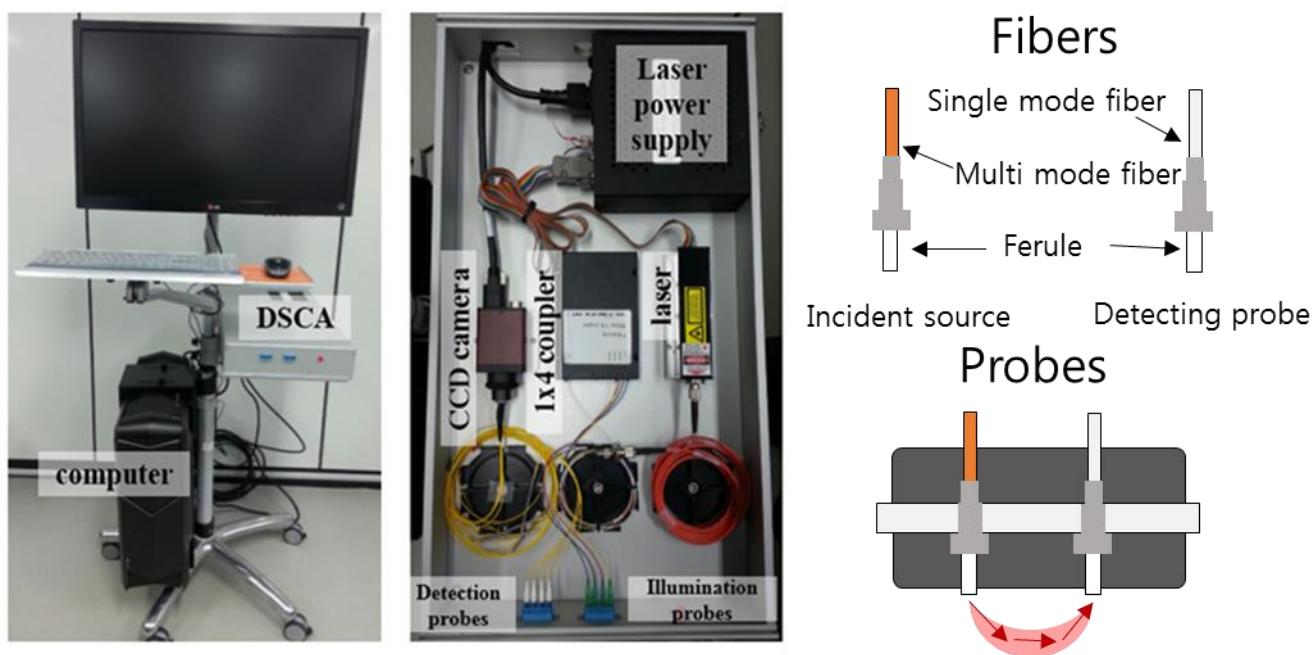
$$K^2(T) = V_N(T) = \frac{2p}{T} \int_0^T \left(1 - \frac{\tau}{T}\right) [g_1(\tau)]^2 d\tau \quad (4)$$

where  $V_N$  is the normalized variance,  $\tau$  is delay time,  $T$  is exposure time,  $p$  is constant coefficient related with ratio between size of CCD chip and size of speckle, and  $g_1(\tau)$  is autocorrelation function. Blood flow index (BFI) could be derived from the speckle contrast  $K$  and  $\alpha D_b$  which is following Brownian motion model. The linear relationship between  $1/K^2$  and  $\alpha D_b$  is already demonstrated [33].

$$BFI = \frac{1}{K^2} \quad (5)$$



**Figure 10.** Diffuse speckle contrast analysis principle



**Figure 11.** DSCA system for rat brain monitoring [34]

## 2.3 Laser blood oxygenation monitoring

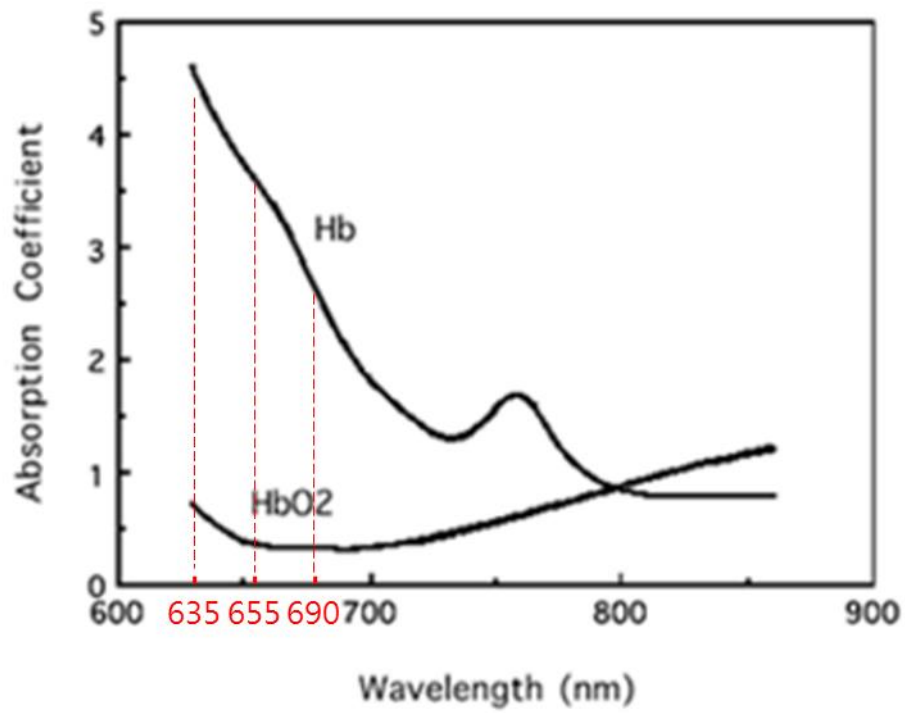
From the modified Beer-Lambert law, blood oxygenation in the cerebral vessels is calculated by a light attenuation ratio due to the inherent light absorption of the each tissue such as a hemoglobin [35]. According to the absorption spectrum of oxygenated hemoglobin (HbO<sub>2</sub>) and deoxygenated hemoglobin (HbR) as shown in Fig. 12, they have different absorption coefficients at the specific wavelengths. The volume of hemoglobin is calculated from the light attenuation ratio with given boundary condition.

$$I = \eta I_0 e^{(-\alpha V_0 - \beta V_d - \mu)L} \quad (6)$$

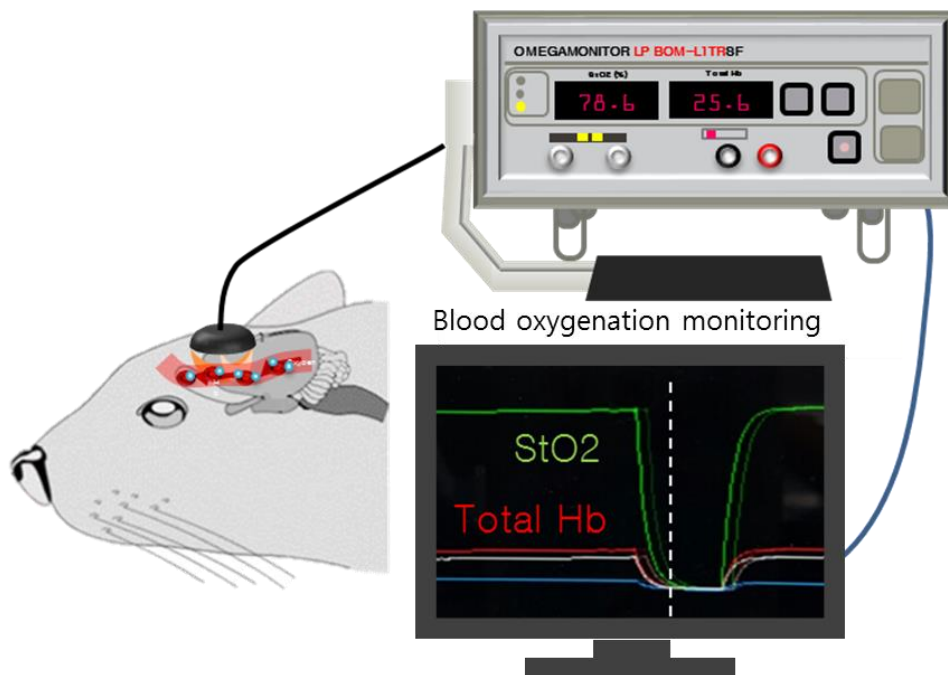
where the parameters  $\alpha$ ,  $\beta$ ,  $\mu$ ,  $\eta$ ,  $I$ ,  $I_0$ , and  $L$  are the absorption coefficient of HbO<sub>2</sub>, the absorption coefficient of HbR, the attenuation coefficient of tissue, the coefficient of measurement system, the detected light intensity, the irradiated light intensity, and the distance between the incident point and the receiving point, respectively [36]. When the laser illuminates the rat brain, the volume of HbO<sub>2</sub> ( $V_0$ ) and the volume of HbR ( $V_d$ ) are calculated from the equation (6). Saturated oxygenation ( $StO_2$ ) is calculated by the blood volume ratio following equation (7).

$$StO_2 = \frac{V_0}{V_0 + V_d} \quad (7)$$

Fig. 13 describes the monitoring of blood oxygenation in the rat brain. A distance between an emitter and a detector can determine a penetration depth of light during blood oxygenation monitoring. Three wavelengths are required to detect the volume of hemoglobin since each similar wavelength (635 nm , 655nm, 690nm) from lasers had scattering and absorption by tissue, therefore, attenuation effect can be considered as the absorption by oxy-Hemoglobin and deoxy-Hemoglobin.



**Figure 12.** Absorption ratio of hemoglobin on wavelength domain



**Figure 13.** Blood oxygenation monitoring

## 2.4 Behavior test

### 2.4.1 Garcia behavior test

The Garcia behavior test is proceeded by three evaluators to identify sensorimotor function of the rats. Evaluation is usually achieved at night because the rats are nocturnal animals which show actual activities at the night. Table 1. shows the Garcia score chart to evaluate the neurological score of the stroke rat. Following this score, if rat obtains high score, it means the rat is close to normal. Oppositely, if rat obtains very low score, it means the rat has severe stroke disease. For the objectivity, blind test for each evaluator was required and three times of repetitive evaluation was done.

**Table 1.** Garcia score chart [19]

Test	Score			
	0	1	2	3
1. Spontaneous activity (in cage for 5 min)	No movement	Barely moves	Moves but does not approach at least three sides of cage	Moves and approaches at least three sides of cage
2. Symmetry of movements (four limbs)	Left side: no movement	Left side: slight movement	Left side: moves slowly	Both sides: move symmetrically
3. Symmetry of forelimbs (outstretching while held by tail)	Left side: no movement, no outreaching	Left side: slight movement to outreach	Left side: moves and outreaches less than right side	Symmetrical outreach
4. Climbing wall of wire cage		Fails to climb	Left side is weak	Normal climbing
5. Reaction to touch on either side of trunk		No response on left side	Weak response on left side	Symmetrical Response
6. Response to vibrissae touch		No response on left side	Weak response on left side	Symmetrical response

### 2.4.2 Grip strength test

Grip strength is measured by force meter which is contained with grid. During the force measurement, evaluator hang the rat tail to make the rat grab the grid. In case of stroke rat grip strength experiment, there is an assumption that one side forelimb might become weak since the stroke brings hemiplegia. To figure out this assumption, other side forelimb was forced to be taped so that rat couldn't grip grid with whole forelimb. Fig. 14 shows grip strength measurement experiment with one side forelimb only. Each forelimb grip strength measured 3 times repetitively during 14 days. Measuring day was at day 1, 5, 8, 11, 14.

$$\text{Relative force} = \frac{\text{Left forelimb grip strength (N)}}{\text{Right forelimb grip strength (N)}} [\%] \quad (8)$$

To know force imbalance of stroke rat, the equation (8) is required to figure out the balance. Under the assumption of hemiplegia effect in stroke rat, normal rat is expected to have similar grip strength in both left forelimb and right forelimb. Thus, following the assumption, normal rat might have 100 % relative force and stroke rat might have relative force quite less than 100%. The reason of why left forelimb grip strength is on numerator is because of hemiplegia side. Through the MCAO surgery, left body become hemiplegia so that left side forelimb activity is reduced dramatically. This symptom can be seen even in Garcia test. While tail is hung on the hand, rat usually tries to move forward, however, due to the impairment of left forelimb, rat turned left side as a result of right forelimb stretching only. Finally, grip strength would be appeared with ration between left forelimb strength and right forelimb strength.

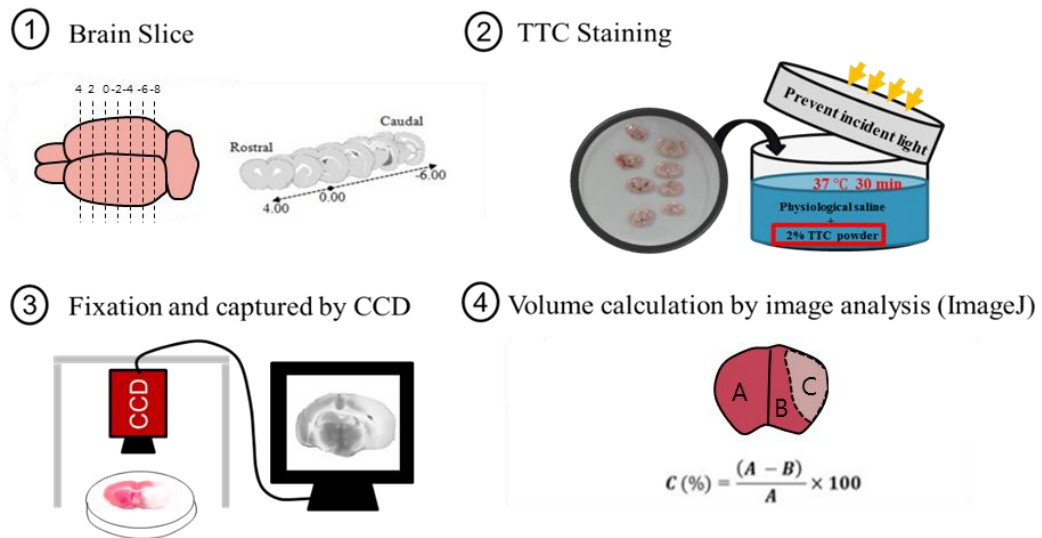


**Figure 14.** Vertical posture to measure one side limb

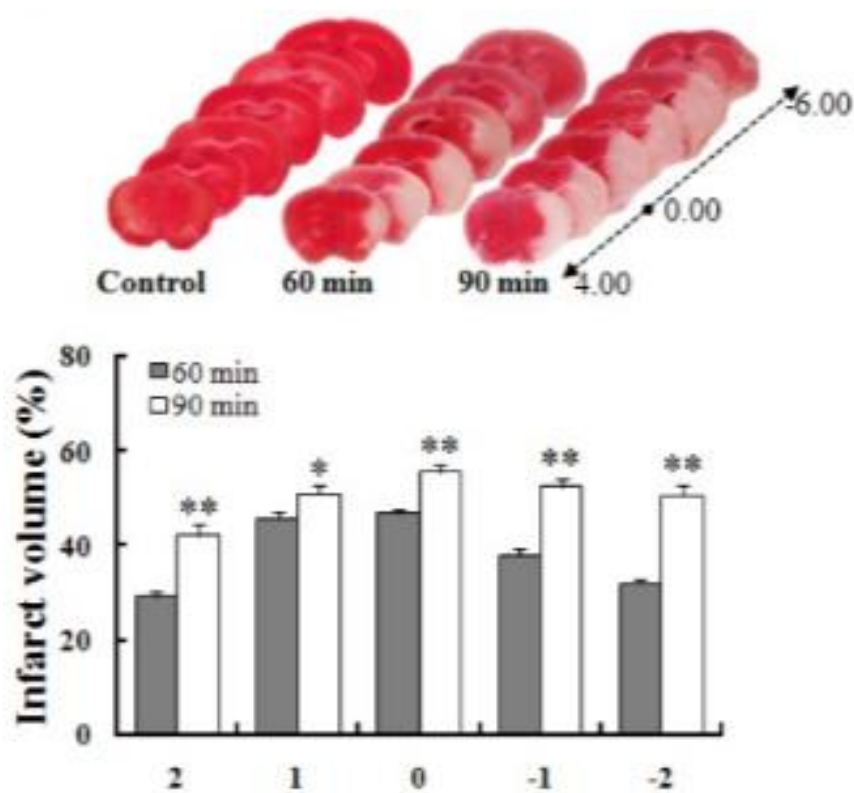
## 2.5 Histological analysis

Infarct volume is measured by several process as shown in Fig. 15 [23]. First, brain tissue should be sliced with 2mm thickness. For precise slice, brain should be directly freeze right after autopsy for few hours. After the freezing, brain is sliced with razor with fixed state in frame. Second, each brain slice should be embedded in TTC solution. TTC solution is made of 2 % w/v TTC powder. The solution temperature is maintained at 37 °C. During the 30 minutes of staining time, light should be prevented by covering the solution vessel. After the staining, tissues are submerged by 10% phosphate buffer saline (PBS) solution at 4°C. Any light penetration is not allowed in this process also. After the tissue cleaning by PBS solution about 1 hours, paraformaldehyde (PFA) fixation should be applied to the brain slice. The brain slices are under the 4% PFA solution at 4°C with 1 hours. After this procedure, all brain slice become fixed so that handling become much easier than previous stage. Third, after the fixation, brain slices are positioned on the slide glass and scanned by CCD camera (DISSEM co.). From the taken picture, image analysis is applied by image analysis program (Image J). From the scanned image, infarction area could be distinguished as a white color area. To calculate the infarct volume, we counted the pixel number of image. As Fig. 15 shows, by counting pixel number of A, B and C, infarct volume could be calculated through equation  $(A-B)/A * 100$ . Previous studies about infarct volume measurement shows different infarct volume size could be modulated by occlusion time as shown in Fig. 16 [25]. In this experiment, 90 minutes occlusion is chosen to prove definite effect of rehabilitation compared to stroke group.





**Figure 15.** Process of the infarction volume measurement



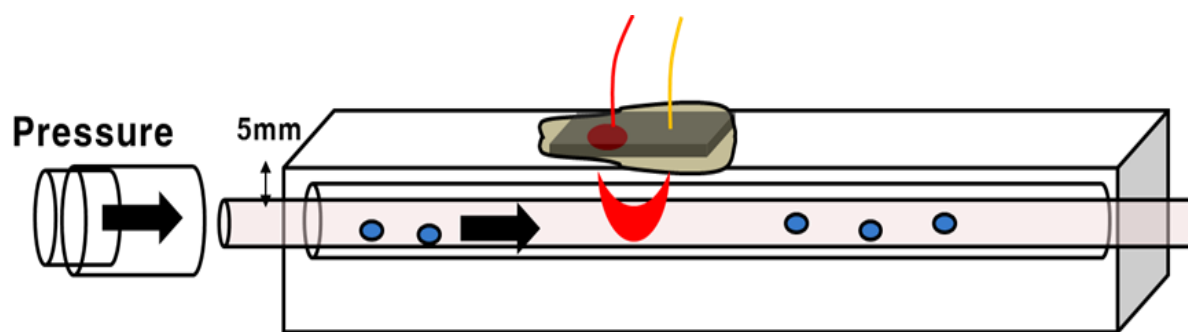
**Figure 16.** Example of infarct volume by occlusion time [25]

## **3. Experimental Results**

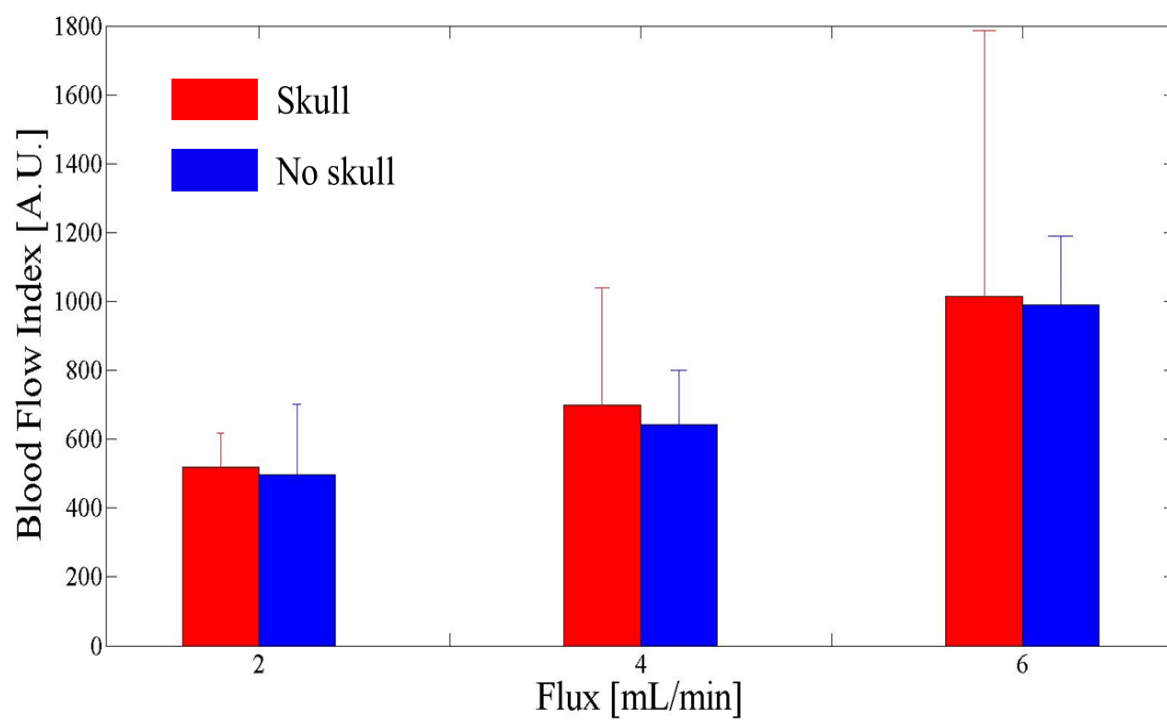
### **3.1 Blood flow**

#### **3.1.1 Validity experiment of DSCA**

Fig. 17 shows the phantom experiment for the validation of skull-covered blood flow monitoring. Since modeled phantom mimics human tissue, PDMS is a brain tissue, intra lipid is a blood, and micro size bead is hemoglobin. In this experiment, this phantom model could be considered as a brain tissue such as gray matter and white matter, while plastic pipe could be considered as a cerebral blood vessel which is located under the 5 mm as same as target point of real rat brain. Fluidic pressure is occurred by micro pump so that the intra lipid flows like real blood flow. To be measured, skull is flattened and two optical fibers were contacted on the skull. This experiment is undergone 3 times repeatedly. Comparison experiment between non-covered state and skull covered state would show the validation of the laser monitoring even in the skull covered state. Three different flow rates are given in this validation evaluation experiment which each flow rate is 2 ml/min, 4 ml/min and 6 ml/min, respectively. Fig. 18 shows the result of the validation experiment. As flow rate increases, both non-covered state and skull covered state show increase of blood flow index. Even though the experimental result doesn't show linearity, increasing tendency could be observed from DSCA modality. As a result, laser monitoring on the skull is observable at 785 nm wavelength with 15 mW.



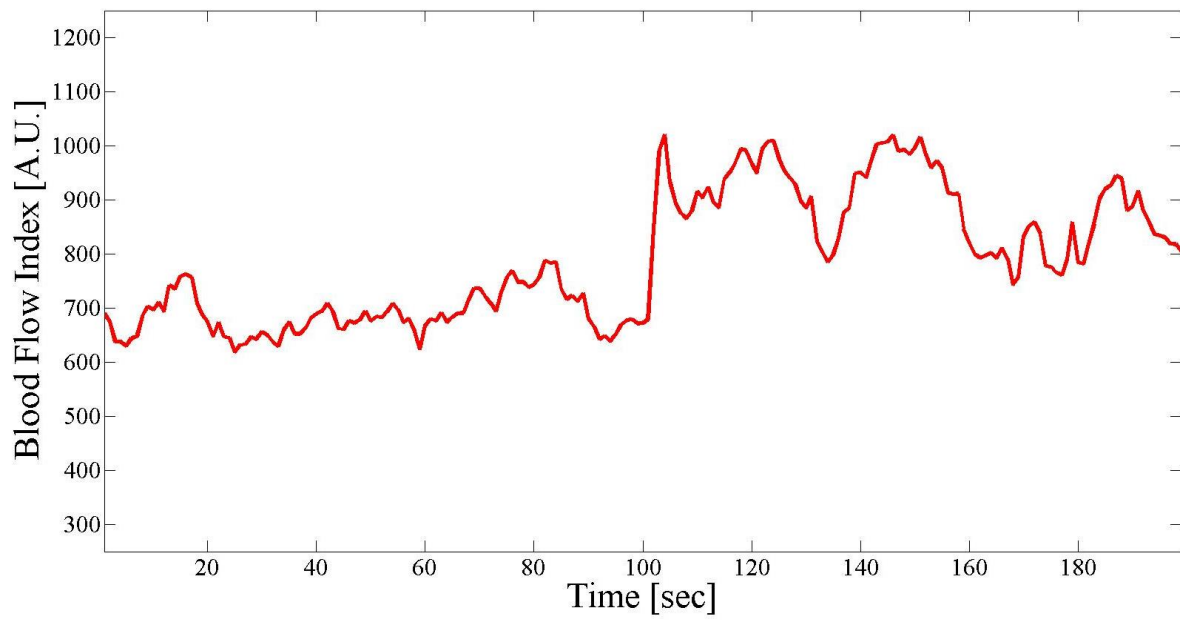
**Figure 17.** Phantom with rat skull



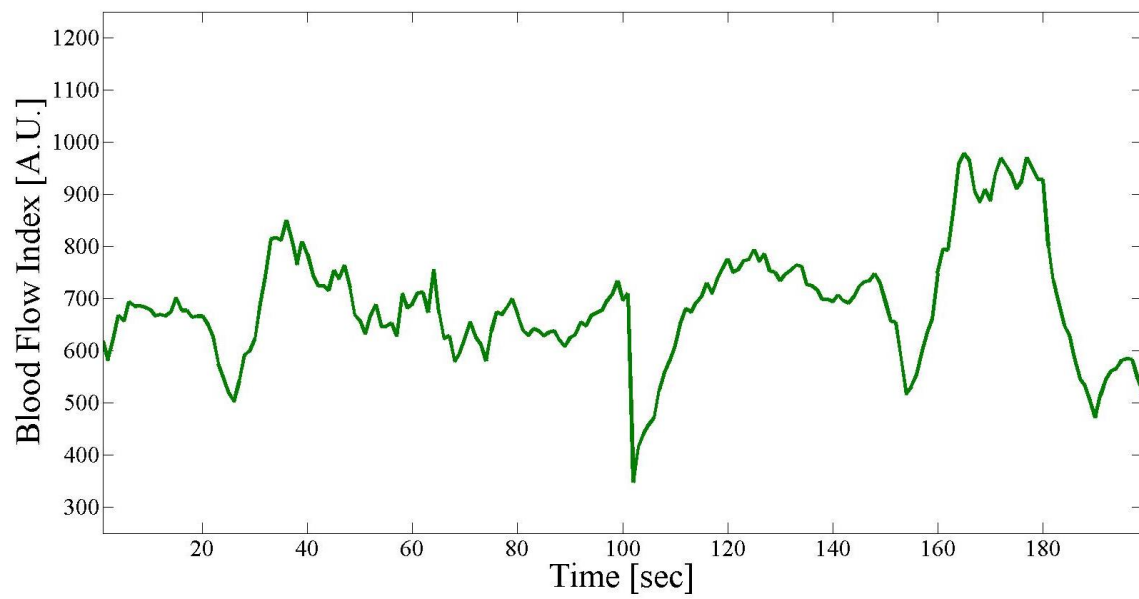
**Figure 18.** Validity of DSCA with skull

### **3.1.2 Real time blood flow monitoring of stroke rat**

Fig. 19 and Fig. 20 are representative measured row data that showing different blood flow between left hemisphere and right hemisphere. Left hemisphere for stroke rat is infarcted by MCAO surgery. During the measurement, significant difference between ipsilateral and contralateral blood flow could be monitored. Both figures show 200 seconds monitoring which is divided into 100 seconds each for each measured point. In this experiments, during 0 to 100 seconds, ipsilateral blood flow was measured while contralateral blood flow was measured during 100 to 200 seconds. During the 100 second data acquisition time, blood flow in left hemisphere is lower than blood flow in right hemisphere. This indicates that left hemisphere is infarcted lesion as shown Fig. 19. On the other hand, average value difference between left hemisphere and right hemisphere was not significantly big in normal group rat as shown in Fig. 20. This indicates that left hemisphere has similar blood flow circulation with right hemisphere which means left hemisphere is not infarcted.



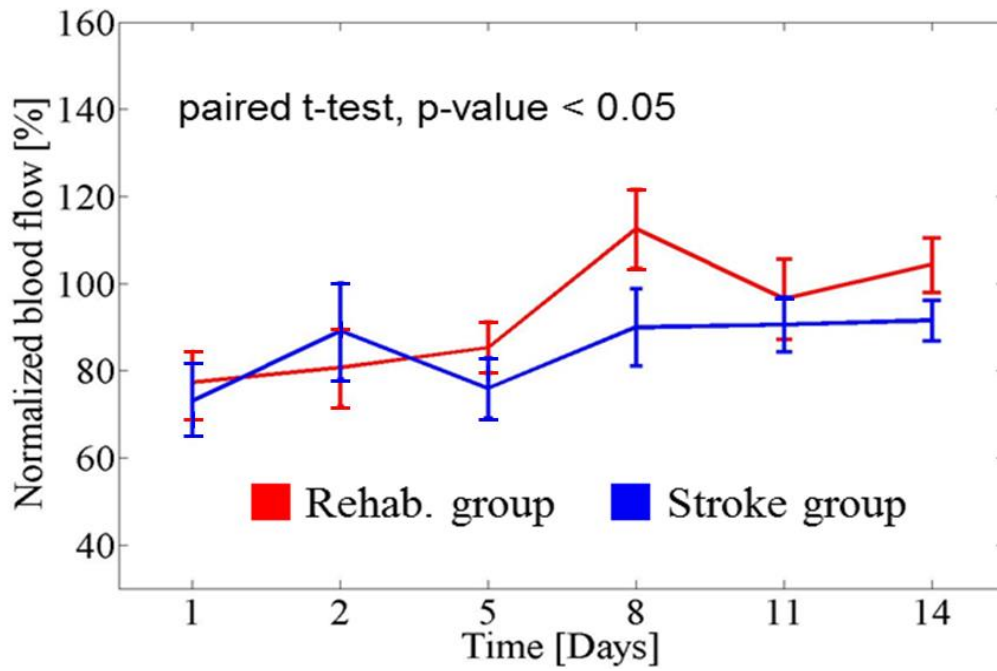
**Figure 19.** Blood flow of left hemisphere (infarction) and right hemisphere in stroke rat brain



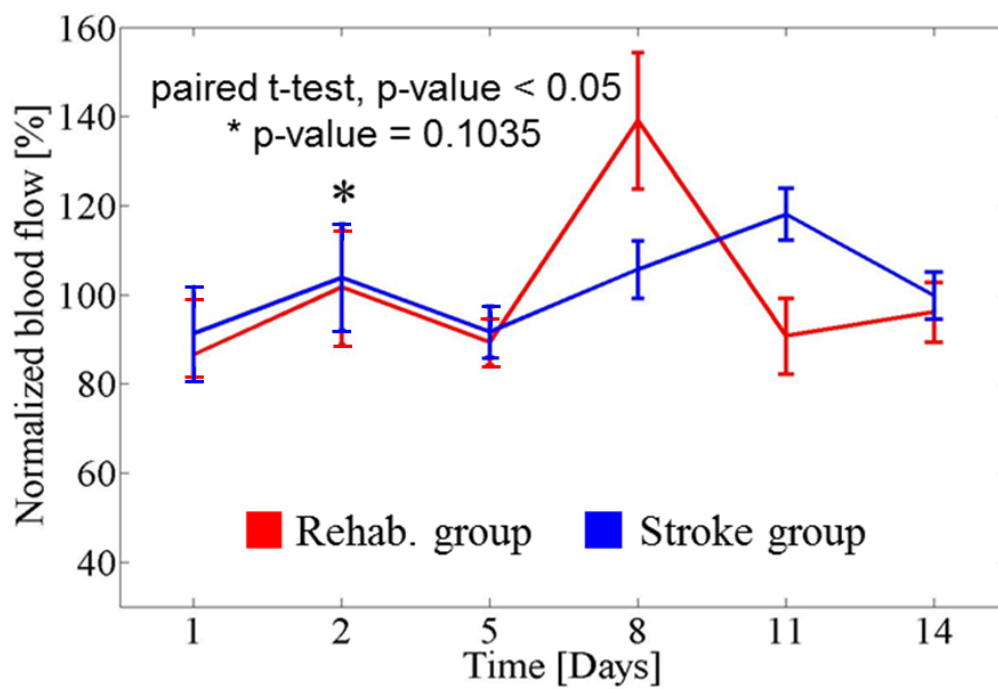
**Figure 20.** Blood flow of left hemisphere and right hemisphere in normal rat brain

### **3.1.3 Cerebral blood flow change during recovery period**

Fig. 21 depict blood flow change in ipsilateral brain during 14days. In Fig. 21, two groups show different increase tendency in left hemisphere which is infarcted side. Normalized blood flow index 100% means blood flow of normal group. Thus, blood flow that relatively lower than 100% means there are some infarction still remains so that disturb the flow. While rehabilitation group shows gradual increase, stroke group shows less increase during 14 days. Fig. 22 shows blood flow change in contralateral brain during 14 days. In Fig. 22, two groups show little difference at the day 14 and their blood flow is close to 100 % which means they are not different with normal group. However at day 8, rehabilitation group shows much higher blood flow index that is almost 140 %. It would be discussed in discussion chapter of this study. In brief, contralateral blood flow is usually higher than ipsilateral blood flow and the blood flow index is close to 100 % except day 8 value of rehabilitation group. However, since overall normalized blood flow is almost 100 %, it could be considered as a normal tissue which is not damaged. Most of calculated p-value from measured blood flow indices between two groups on each day were under the 0.05 except day 2 in contralateral blood flow. This means blood flow differences between both groups is significant. Although even contralateral blood flow differences between both groups is significant, however, gap between measured value of day 1, 2, 5, and 14 is not quite big compared to measured ipsilateral blood flow of same day.



**Figure 21.** Blood flow change in infarction

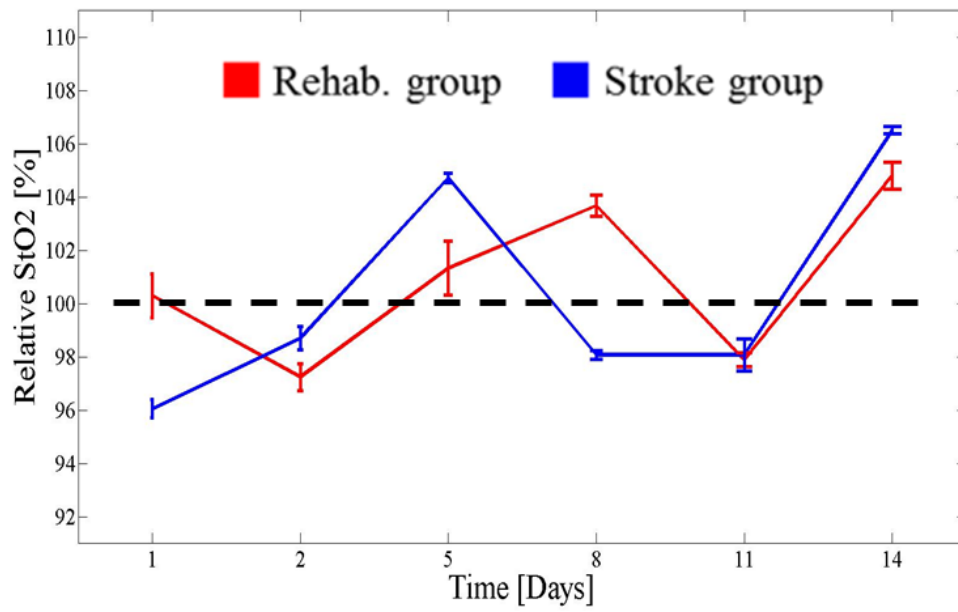


**Figure 22.** Blood flow change in contralateral

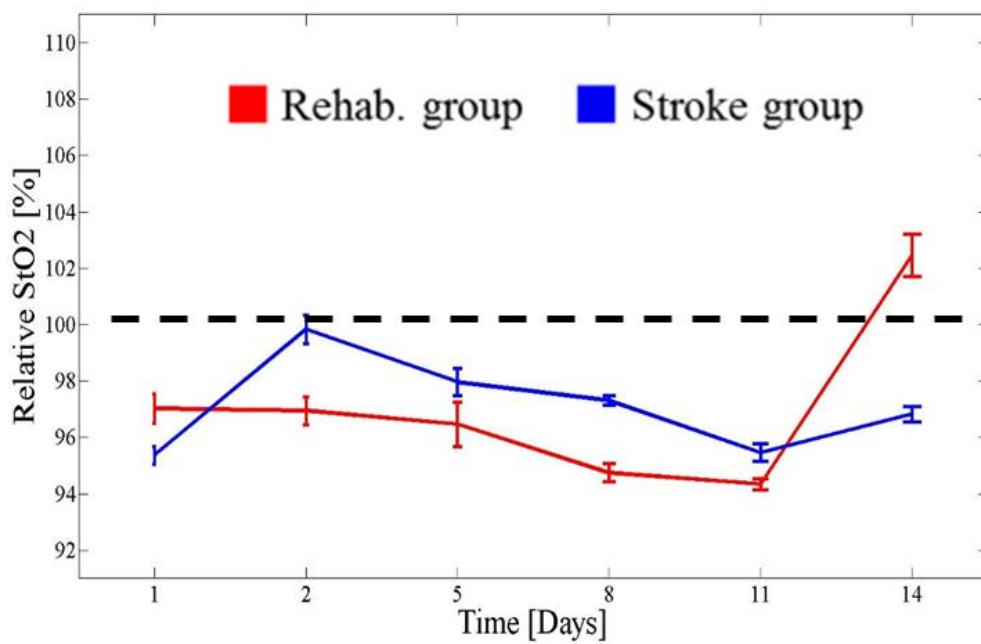
### **3.2 Blood oxygenation**

As Fig. 23 shows, ipsilateral StO<sub>2</sub> of stroke group and rehabilitation group finally increased during the recovery period more than normal group. Although hypoxia was expected to MCAO surgery rat at the first time, rehabilitation group marked similar StO<sub>2</sub> with normal rat on day 1. Both group showed volatile change of StO<sub>2</sub> in ipsilateral brain during recovery period even higher StO<sub>2</sub> than normal group. On the contrary, contralateral StO<sub>2</sub> of both stroke and rehabilitation group were decreased during recovery period even though contralateral brain was not infarcted as Fig. 24 shown. Furthermore, there were no significant differences between normal rats and stroke rats about measured StO<sub>2</sub>. While Normal rats usually marked around 70% of StO<sub>2</sub>, stroke and rehabilitation rats usually marked close to 66% of StO<sub>2</sub>. Thus, normalized StO<sub>2</sub> difference between normal stroke group and rehabilitation group is under the 5 % which may not significant value. We will discuss about the reason of the result later.





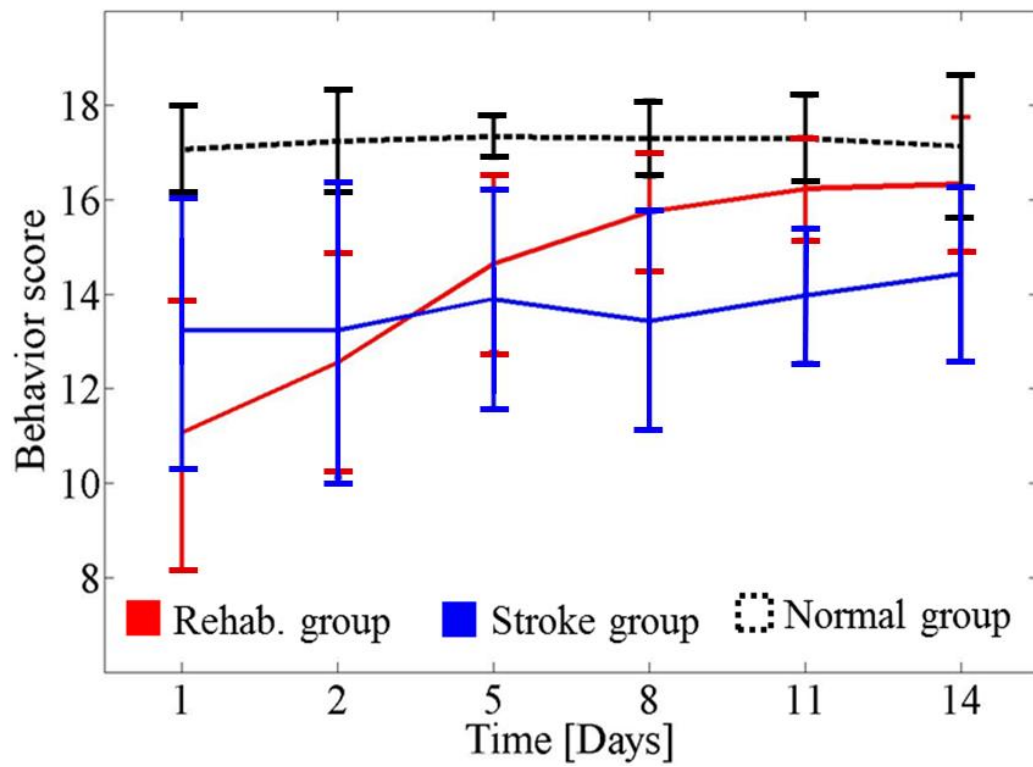
**Figure 23.** Blood oxygenation change in ipsilateral



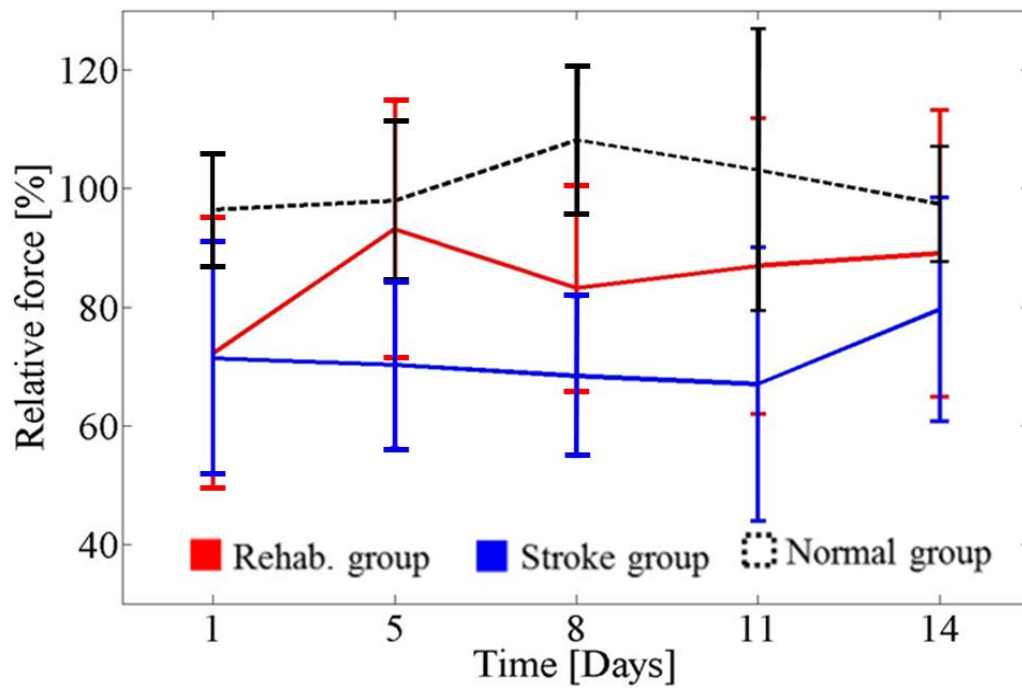
**Figure 24.** Blood oxygenation change in contralateral

### 3.3 Behavior test

Fig. 25 shows the Garcia behavior test result. While normal group marked almost 18 score, rehabilitation group and stroke group marked almost 13 score. 7~13 score indicates stroke states. Thus, we could judge that rehabilitation group and stroke group get the stroke following the behavior test. During the recovery period, rehabilitation group score is increase and finally marked 16 score which means almost recovered as much as normal group. Otherwise, stroke group marked 14 score which indicates there is almost no increase of the behavior score. Compared to two groups, rehabilitation group increased 5 point, and stroke group increased less than 1 point in Garcia behavior test. Fig. 26 shows grip strength test result. Follow the equation (8), relative force of normal group was almost 100% initially. During the measurement day, normal group grip strength was always close to 100 %. In case of some change was not considerable compared to other stroke group. In case of stroke rats, both rehabilitation group and stroke group showed quite decrease of grip strength in left forelimb at day 1. However, as time goes by, rehabilitation group grip strength increases while stroke group doesn't show specific increase during 14 days. At day 14, the grip strength of the rehabilitation group marked around 90 % which means 15 % increase of grip strength in left forelimb. It means that rehabilitation group rats have more balanced forelimb motor function than stroke group rats.



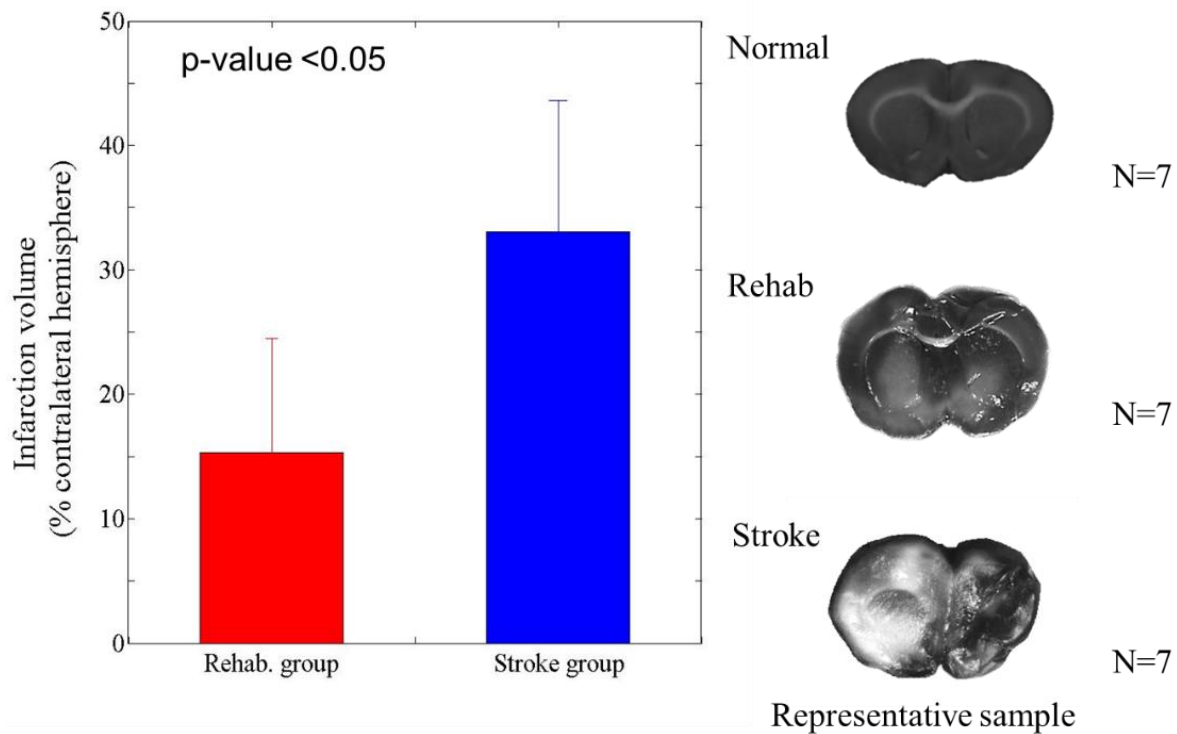
**Figure 25.** Result of Garcia behavior test



**Figure 26.** Result of Grip strength test

### 3.4 Histological analysis

Brain infarction is distinguishable among those three brain slice. Larger infarction volume could be seen in stroke group. However, rehabilitation group showed less infarction volume than that of stroke group as Fig. 27 shows. Thus, Fig. 27 shows obvious difference between infarction size of stroke group and that of rehabilitation group. Infarction size of rehabilitation group is around 15 % while stroke group marked more than 30 % of infarction size. Significant difference is proved by t-test ( $p < 0.05$ ) so that rehabilitation group could be classified to more recovered group.



**Figure 27.** Comparison of infarction size

## 4. Conclusion

### 4.1 Conclusion

The purpose of this study is to suggest probability of DSCA modality for stroke recovery monitoring. Several complementary experiments are applied to prove the performance of DSCA system for stroke recovery monitoring such as behavior test, blood oxygenation monitoring, and infarction volume measurement. Animal model, especially SD rat is chosen for the stroke recovery monitoring and those 30 rats are under the experiment due to the population and controllability. Three groups which are divided into rehabilitation group (n=10), stroke group (n=10), normal group (n=10) had blood flow monitoring, blood oxygenation monitoring, Garcia behavior test, grip strength test, and infarction volume measurement following the protocol during 14 days.

In blood flow monitoring, validity evaluation experiment by using skull covered phantom show the effectiveness of deep tissue monitoring of DSCA even considering absorption effect by skull. Thus, blood flow monitoring on rat scalp could be available for non-invasive *in-vivo* monitoring. In real time blood flow monitoring of stroke rat (Fig. 19) show different blood flow index between left hemisphere and right hemisphere while similar blood flow index between both hemispheres was found in normal rats (Fig. 20). Therefore, statistical result with 30 rats showed in Fig. 21 and Fig. 22. About infarction lesion which is located in left hemisphere, significant difference of blood flow index between rehabilitation group and stroke group became bigger as time goes on during the recovery period. On the contrary, contralateral blood flow index difference was not too much between two groups that can inform non-infarction tissue. As other previous studies shows, DSCA system also showed that overall cerebral blood flow of rehabilitation group has increased more than stroke group.

Blood oxygenation is measured by using LBOM (635 nm laser wavelength) to monitoring hypoxia of stroke rats. However, significant difference could not be found from experimental rats. This problem is expected to be solved with longer wavelength (785 nm) which could penetrate skull depth. Further study will be undergone with different wavelength.

Behavior tests in this study are two, Garcia behavior test and grip strength test. As already referred from above, Garcia behavior test evaluates sensorimotor function by evaluators. As a result, Garcia behavior test showed significant increase of sensorimotor function of rehabilitation group rather than stroke group while sensorimotor function of normal group was maintained similar score during recovery period. However, deviation value was quite big which means some stroke group rat could be better than rehabilitation group rat about sensorimotor function. Since Garcia behavior test is proceeded by subjective decision of evaluator, other complementary methods is also required. In this experiment, grip strength test is the complementary method of Garcia test since power meter records grip strength of rat. However, individual rat grip strength is different each other so that deviation is also quite big. Grip strength test also showed significant difference between stroke group and rehabilitation group during the recovery period. Infarction volume measurement also showed significant difference between stroke group and rehabilitation group which are indicated as a lower infarction volume rate compared to stroke group.

From the result, we could watch that blood flow increase from the low blood flow level state to normal rat blood flow level state in rehabilitation group. Furthermore, while blood flow is increased remarkably, also other parameters such as Garcia behavior score, grip strength, and infarction volume show correspondent result. This study proves that gait-training is effective to recover the stroke disease with in terms of blood flow, behavior test, and infarction volume. Also, this study proved that effectiveness of the DSCA system for brain stroke monitoring with blood flow measurement. While conventional behavior test method showed huge deviation that

can induce wrong evaluation of recovery level, blood flow measurement showed less deviation among measured groups and this could be standard monitoring method for correct evaluation. DSCA monitoring could distinguish infarction area and non-infarcted area with high resolution. Even the penetration depth performance is superior to laser Doppler flowmetry so that invasive craniotomy is not required anymore. We expect that DSCA system can represent the LDF and even represent behavior test for stroke monitoring in near future. As a result, we suggest that blood flow can be key parameter to know recovery level of stroke and DSCA system is promising implement for blood flow monitoring.

## 4.2 Discussion

Results show blood flow increase during recovery period. However, relative cerebral blood flow of rehabilitation group at day 8 recorded the highest value among recovery day, while linear increase of blood flow was expected. The reason of high value of blood flow is speculated about angiogenesis or monitoring system problem. Pengyue Zhang et al. reported that cerebral blood flow of rehabilitation group increased more than normal group through Laser Speckle Contrast Imaging modality [37]. Since Matthias Endres et al. have reported that physical activity increases activity of endothelial nitric oxide synthase which is known as essential matter for a healthy cardiovascular system, even cerebral blood flow increase might be related with that [38]. Thus, blood flow increase of rehabilitation group which is more than that of normal group can be explained with angiogenesis since DSCA system could monitor not only flow velocity but also vascularization. In contralateral hemisphere, higher blood flow index compared to blood flow in ipsilateral hemisphere was observed so that contralateral brain could be estimated as a non-infarction tissue or less infarction. Contrary to expectation that blood flow during recovery period in contralateral brain would be similar so that graph would be flat, the value was not as Fig. 23 shows. We could speculate that even though contralateral brain didn't have direct damage by occlusion, it could be influenced by occlusion of ipsilateral side also or because of the DSCA system instability. To investigate about this problem, repetitive experiment and stability test must be required.

Fig. 24 and Fig. 25 show unpredictable tendency of blood oxygenation change. As culver et al. mentioned, light whose wavelength is less than 670 nm can't penetrate the skull so that thinned or removed skull must be prepared. Moreover, center wavelength of the laser blood oxygenation monitoring system in this study is 635 nm. From the reference, this modality is decided as an inappropriate for non-invasive blood oxygenation monitoring. Jobsis reported



that light in the 690 nm to 850 nm range permits non-invasive hemoglobin oxygen saturation monitoring which is able to penetrate skull. Thus, different wavelength especially higher than 690 nm should be applied for non-invasive blood oxygenation monitoring in later.

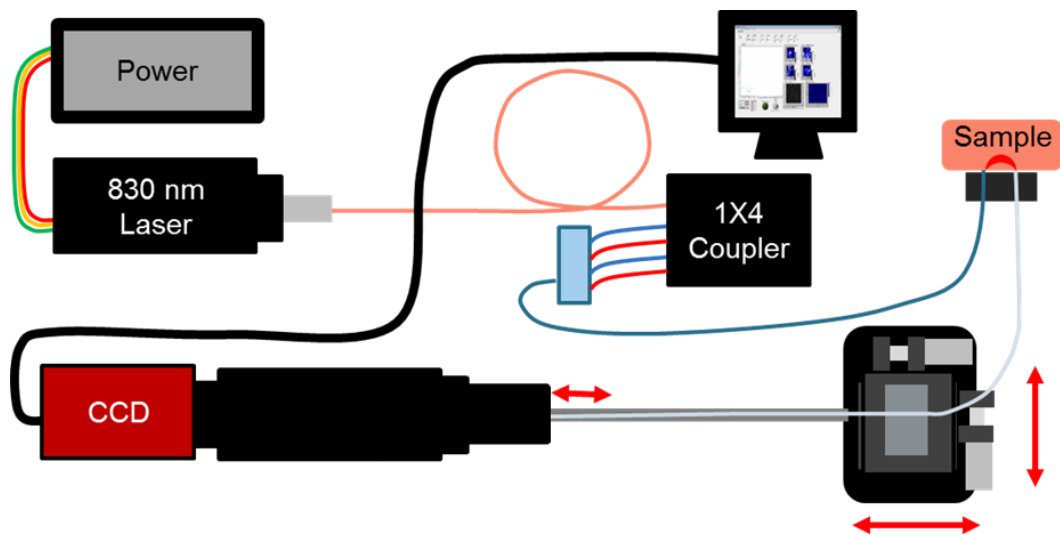
Fig. 26 and Fig. 27 show each behavior test result of Garcia test and grip strength test. Although average values were distinguishable, large deviation value range means that some sample had no difference between stroke rat and rehabilitated rat. For example, in the Garcia test result, stroke group marked the score 12 point to 16 point and rehabilitation group marked the score 14 point to 17.5, while normal group marked the score 16 point to 18 on day 14. It means some rats who scored 16 point can be affiliated to stroke group, rehabilitation group and normal group. This might be caused of biological sample inherent characteristics and limitation of behavior test itself by subjective inspection. Various behavior test such as rotarod experiment can be applied to compensate above problem. Finally behavior test results prove the necessity of quantitative analyzing method such as laser monitoring due to the deviation problem of the behavior test. Anyway behavior test also could prove the effectiveness of gait-training for recovery of stroke.

Infarction volume showed significant difference between stroke group and rehabilitation group. However, irregular infarction was observed in this experiment, while general infarction is expected to be generated from cortex area in ipsilateral side only with clear border. For example, we could observed infarction which is appeared even on contralateral side in brain section or located on striatum only not on a cortex. This may be due to uncertain middle cerebral artery occlusion or cortical recovery.

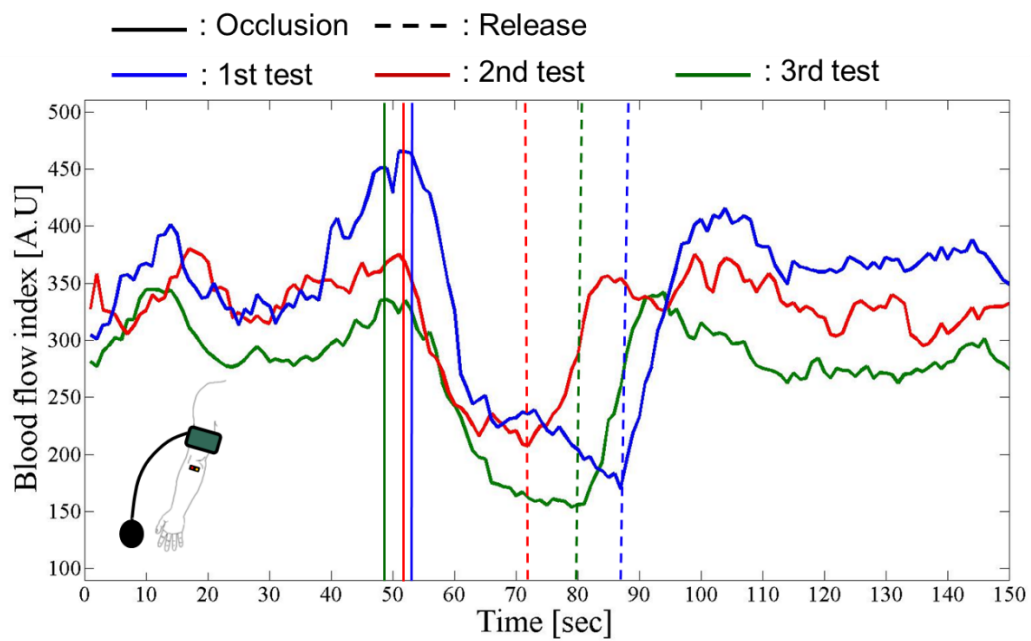
Above results show correlation among them as a recovery indicating factors. We expect DSCA become practical use for stroke recovery monitoring.

### **4.3 Future study**

DSCA system should be evaluated from another wavelength laser for various applications. In the future work, developed DSCA system would be required with various wavelength range. In this study, 830 nm laser was applied to confirm that effectiveness of DSCA monitoring. As Fig. 29 shows, micro stage manipulate optical focal length between CCD chip and fiber. From the focused image which would be appear as small dot, speckle contrast image in specific ROI is available to obtain by the computer. Fig. 30 shows the validation of 830 nm wavelength DSCA system in Fig. 29. Preliminary cuff-occlusion experiment was used to prove effectiveness of the DSCA system. 3 times repeated experiment shows when occlusion is applied to arm, blood flow index dropped radically and after the release, blood flow index increased dramatically and recovered previous blood flow index level. Since present DSCA system didn't show linearity for flow rate measurement, further study should contain purpose for the increasing linearity of the measurement system.



**Figure 28.** 830 nm DSCA set up



**Figure 29.** Preliminary result of cuff-occlusion experiments

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## 요 약 문

### 뇌졸중 쥐의 보행 훈련 효과에 대한 정량적 분석

뇌졸중은 현대사회에서 아직까지 인간이 극복하지 못한 질병이다. 이에 따른 다양한 치료법 개발과 뇌졸중에 대한 연구가 계속되고 있지만 인간을 대상으로 한 연구에는 한계가 있어 설치류를 이용한 동물 실험이 많이 이용되고 있다. 설치류를 이용한 뇌졸중 연구는 그 자율성 덕에 도전적인 검사 방법들이 적용되고 있다. 그 중에서도 가장 주목 받는 검사방법은 레이저를 이용한 방법으로, 이를 이용해 혈류를 비롯한 다양한 정보를 생체 내 측정으로 얻을 수 있다는 장점이 있다. 하지만 기존 레이저 측정장치는 투과 깊이의 문제로 인해 침습적 방법을 동원하여 실용적인 모니터링으로서는 가치가 떨어졌었다. 확산 스펙클 대비 분석 시스템(이하 DSCA)은 레이저의 스펙클 현상을 이용하여 이미지의 명도 대비를 통해 역으로 동적인 움직임을 알아내는 시스템으로 5mm 정도의 깊은 조직 내 에서도 측정이 가능하다. 본 연구는 이러한 DSCA 장점을 살려 뇌졸중 쥐의 보행 훈련을 통한 회복 모니터링이 가능한지 보고자 하였다. 레이저를 장비로 이용하여 혈류 및 혈중 산소포화도를 측정하였다. 통계적 유의성을 가진 생체 실험을 위해 30 마리의 SD 랫을 이용하였고, 실험 그룹은 재활그룹(n=10), 비 재활그룹(n=10), 정상그룹(n=10)으로 나뉘어져 혈류 측정 및 기타 정량적 분석이 진행되었다. 실험 결과 회복기간인 14 일 동안 경색부위에서 뇌졸중에 걸린 재활 그룹 쥐의 혈류증가가 비재활 그룹보다 더 증가하는걸 볼 수 있었고, 혈류가 증가하는 동안 감각운동기능도 개선된 것을 볼 수 있었다. 다만 혈중 산소포화도는 그룹간의 큰 차이를 볼 수 없었으며, 이는 산소포화도 측정장치의 파장대가 낮아서 발생한 원인으로 판단된다. 그 밖에도 경색부위 크기 비교를 통해 재활 그룹의 경색부위 크기가 비재활 그룹의 경색부위보다 작다는 것을 통해 통상적인 실험과의 연관성도 보여주었다. 본 실험을 통해 회복에 영향을 미치는 다양한 인자와 회복 정도를 나타내는 지표들을 알 수 있었다. 예를 들어 혈류의 증가와 회복 정도는 행동평가 실험 및 경색부위 측정을 통해 상관관계가 있음을 보여주었다. DSCA 의 발전을 통해 바야흐로 뇌졸중 환자가 자신의 상태를 자가 진단할 수 있도록 하는 첨단 진단장비로 발전할 것으로 기대해본다.

핵심어: 뇌졸중, 보행훈련, 확산광 스펙클 대비 분석, 혈류, 설치류