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# Cerebral vasospasm and corticospinal tract injury induced by a modified rat model of subarachnoid hemorrhage

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ABSTRACT

*Objective:* Double-hemorrhage rat models of subarachnoid hemorrhages (SAH) are most effective at simulating delayed cerebral vasospasms (CVS). The present study modified the models to minimize additional trauma and investigated injury of the corticospinal tract (CST) using diffusion tensor imaging (DTI).
*Methods:* On the first day, 0.3 ml of autologous arterial blood was collected by puncturing the caudal artery and injected into the cisterna magna via percutaneous puncture; and the operation was repeated on the third day. The diameters of the basilar artery (BA), middle cerebral artery (MCA), and anterior cerebral artery (ACA) were measured by magnetic resonance angiography on days 3, 5, 7, 9, and 11post-SAH. Meanwhile, on days 3, 7, 11, 15 and 19, DTI was performed to evaluate the injury of the CST at cerebral peduncle (CP) and pyramidal tract (Py) by measuring fractional anisotropy (FA) value.
*Results:* Blood was deposited mainly in the basal cistern. Diameters of BA, MCA, and ACA were significantly reduced. FA value of the CP was lower in the SAH group than in the control group; but FA value of Py wasn't different between the two groups.
*Conclusion:* This is a minimally-invasive and high performance rat model of SAH. Additionally, the occurrence of CVS is firm and the axons in CP are injured.



## 1. Introduction

A subarachnoid hemorrhage (SAH) is a high-mortality form of stroke. Its mortality rate is about 15% at the first sign of bleeding and approximately 50% at the second time [1–4]. In living patients, cerebral vasospasm (CVS), which reduces the related regional blood supply or causes ischemic stroke after SAH, is an important complication that affects prognosis [5,6]. Additionally, high intracranial pressure, high-oxygen arterial blood, erythrocyte catabolism and many more are still important factors following brain injury [7–10]. Animal models are important tools for studying the pathophysiology and treatment methods of human diseases like SAH and CVS. Of the animals used for models, rats are relatively ideal subjects in SAH models for their similar cerebral vascular anatomical structure to humans [11]; and they are one of the most commonly used and economic experimental animals.

Presently, the commonly used rat SAH models include “cerebral arterial puncture model” and “double-hemorrhage model” [12]. The former is closer to the rupturing process of an aneurysmal subarachnoid hemorrhage, but its mortality rate is high, the amount of bleeding is not easy to control, and CVS is not constant after SAH [13]. Conversely, though the latter cannot simulate the main pathophysiological such as vessel rupture, sharp increase of intracranial pressure, and cerebral hernia in the acute phase, its advantages of lower mortality rate, controllable bleeding volume, and consistent appearance of CVS make it the ideal choice for researching CVS after SAH.

Although SAH models based on “double-hemorrhage” have many varieties according to the specific operation process, some short comings still need to be addressed. For example, the methods of autologous blood collection, mainly including femoral artery intubation, caudal artery incision, and tail vein intubation, vary in these different models [14–20]. Drawbacks of these methods include relatively serious trauma or the inability to get high-oxygen arterial blood. Injection protocol almost always includes dissecting the skin and muscle of the neck and then puncturing through the atlantooccipital fascia. To improve the survival rate, a certain amount of cerebrospinal fluid (CSF) is withdrawn

*Abbreviations:* SAH, subarachnoid hemorrhages; CVS, cerebral vasospasms; CST, corticospinal tract; DTI, diffusion tensor imaging; BA, basilar artery; MCA, middle cerebral artery; ACA, anterior cerebral artery; MRA, magnetic resonance angiography; CP, cerebral peduncle; Py, pyramidal tract; FA, fractional anisotropy; MD, mean diffusivity; λ//, axial diffusivity; λ⊥, radial diffusivity; CSF, cerebrospinal fluid; BBT, beam balance task; NSS, neurological severity scores.

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before injection [15–18]. Blood can be injected into the subarachnoid area with precision using this method, but surgery is traumatic for rats and may disrupt the pathophysiological changes of SAH and increase the risk of infection [21,22]. Removing CSF can reduce mortality rates by avoiding intracranial hypertension, which may trigger cerebral hernia, but intracranial hypertension itself is an important pathophysiological change after SAH [23]. Thus, the purpose of this study was to improve the "double-hemorrhage" rat model with less unnecessary trauma and higher efficiency.

Additionally, magnetic resonance (MR) diffusion tensor imaging (DTI) is regarded as the only technology that can provide microstructural information of axon in vivo [24]. Fractional anisotropy (FA) is the main indices of DTI and others include mean (MD), axial ($\lambda_{//}$) and radial ($\lambda_{\perp}$) diffusivities. These indices have been demonstrated successfully in assessing the white matter integrity [25]. However, few studies investigate white matter injury by this technology in patients or experiment animals with SAH [26,27]. In the current study, to our knowledge, it is the first time that DTI was performed on a rat model of SAH in vivo to dynamic observe the changes of corticospinal tract (CST).

## 2. Materials and methods

### 2.1. Practice before study

With the expectation of accelerating the learning curve, we performed an animal operation enhancement training within 6 weeks before the formal experiment. Two participants, a man and a women did the practice in accordance with the following steps in the same time. Twenty rats were operated every 2 weeks at the first 4 weeks, and 15 rats at the last 2 weeks. Each rat was planned for a two operation unless death was performed at the first operation. The primary characteristics of the learning curve were achievement ratio of blood sampling and paracentesis, mortality and operation time.

### 2.2. Animal preparation

All animals were supplied by the Dashuo Experimental Animal Center (Chengdu, Sichuan, China). A total of 20 adult male Sprague–Dawley rats (14 SAH, 6 controls, 300–400 g) were used in the experiments. All rats were acclimated in a standard 12:12-h light–dark condition room and allowed free access to food and water. The temperature in both the feeding room and the operation room was maintained at approximately 25 °C. On the first day, rats were anesthetized with chloral hydrate (300 mg/kg, i.p.) and allowed to breathe spontaneously. Adequate anesthesia made the animals lose consciousness within 3 to 5 min with no avoidance responses to acupuncture. Then, the fur around the occipital carina was removed, and the puncture point was marked on the dorsal midline about 6 mm from the occipital protuberance (Fig. 1A).

### 2.3. Blood collection

The caudal artery is on the midline of the ventral aspect of the tail, and collecting blood from it is a difficult task requiring much practice. The following steps were used to improve the success rate of blood sampling: A, the tail was washed and soaked with 40 °C warm water for 1 min; B, the rat was placed supine on the operation table with a 15-mm-high cushion under the back; C, the fur and stratum near the mid-low 1/3 position of the tail were scraped to clearly observe the arterial (suggested observation in relative subdued light) (Fig. 1B); D, the blood sampling site was wiped gently with 75% alcohol (the arterial might be hard to observe after scraping the cuticle of the tail surface because of capillary hyperemia, and alcohol caused capillary contraction and relieved congestion); and E, to see the caudal artery clearly, a 26G needle was slowly inserted into the tail about 2 mm toward the head at gentle angle. Blood flowed out once the puncture was successful (Fig. 1C). After being drawn gently, 0.3 ml of autologous arterial blood was gathered. The second operation after 48 h used only the D and E processes, and the blood sampling site was above the first.

### 2.4. Induction of SAH

The rat was flipped prone with cushion under the chest and the head flexed to 45°–60°naturally. A limiting sleeve was used on the needle to ensure that only the 4 mm tip of the needle could penetrate the skin. After fixing the head and neck skin, the 26G needle tip was aligned horizontally with the external auditory canals and the puncture point, and advanced until it punctured the atlantooccipital membrane (Fig. 1D). Withdrawing gently, when CSF could flow into the needle, the arterial blood was injected into the subarachnoid space of the cisterna magna for 3 min. The needle was left in for 1 min before being removed after injection, and then the puncture position was pressed with cotton for 1 min. Maintaining ahead-down position for 10 min, the rat was separately placed into a cage with soft wooden shavings pad in a quiet subdued light room to recover within 5 days. The injection was repeated after 48 h. The rats in the control group were injected with 0.3 ml of saline solution after blood was drawn from the caudal artery in the same manner. A subcutaneous injection of carprofen (4.0 mg/kg) was recommended to alleviate the suffering of animals at the end of the operation and on day 1.

### 2.5. Physical and neurological inspection

The rats were observed the state of consciousness, appearance, movement, and eating condition two times a day. Animals which cannot feed themselves were injected normal saline (4% body weight per day) through the tail vein or subcutaneous to try to save the life.

Beam balance task (BBT) [29] and neurological severity scores (NSS) [30] were used to characterize the behavioral deficits after SAH on days 1, 3, 5, 7, and 9 (D1, D3, D5, D7 and D9; n = 12) or sham on days 1(n = 6).

BBT: A task that assesses both motor and vestibular functioning by quantifying the animal's ability to balance on a narrow wooden beam (1.0 cm wide) for up to 60 s. The animals were placed thrice on the beam, and the mean score was calculated. Animals received 0 point for balances with steady posture, 1 point for grasping side of the beam, 2 points for hugging the beam with one limb falling down from the beam, 3 points for hugging the beam with two limbs falling down from the beam, or spinning on the beam (> 60 s), 4 points for attempting to balance on the beam but falling down (>40 s), 5 points for attempting to balance on the beam but falling down (>20 s) and 6 points for falling down with no attempt to balance or hang on the beam (<20 s).

NSS: 0 points: normal nerve function; 1 point: mild neurologic impairment (flexion of left anterior limb when raising the rat by the tail); 2 points: moderate neurological deficit (inability to walk straight); 3 points: moderate neurologic impairment (falling down to the one side); 4 points: No walking and consciousness deterioration; 5 points: the death associated with SAH.

### 2.6. Euthanizing rats

Two rats were euthanized 2 h after the second operation by cardiac perfusion, as previously described [20]. These animals were perfused with 250 ml of 0.1 mol/l phosphate-buffered solution (pH 7.4 at 37 8 C). After ensuring adequate perfusion, the cranium was removed to expose the brain. Then, the brain was carefully extracted from the skull base and placed on a tray for observation.

### 2.7. MRI protocol

Magnetic resonance imaging (MRI) experiments were performed on a 7.0 T animal scanner (Bruker Biospin, Ettlingen, Germany) with a