# A Novel Brainstem Hemorrhage Model by Autologous Blood Infusion in Rat: White Matter Injury, Magnetic Resonance Imaging, and Neurobehavioral Features

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*Background:* Primary brainstem hemorrhage (BSH) has the highest mortality and morbidity as a subtype of intracerebral hemorrhage. A major limitation of BSH research is the lack of a corresponding animal model. The purpose of this study was to establish a novel rat model of BSH and to characterize the resulting brain injury, especially focusing on white matter injury. *Methods:* BSH was produced by stereotactically injecting autologous whole blood into the pons. Time course of hematoma resolution was observed by 7-T magnetic resonance imaging. White matter injury was evaluated in detail by multiple parameters including diffuse tensor imaging (DTI), demyelination, axonal injury, oligodendrocyte degeneration, and oligodendrocyte precursor cell proliferation. Brain water content and neurobehavior were also evaluated. *Results:* Blood infusion (30 μL) led to a stable, reproducible hematoma in the right basotegmental pons. The hematoma absorption started, became obvious, and was nearly completed at 7, 14, and 30 days, respectively. Hematoma caused obvious brain edema at 3 days. White mater injury was observed pathologically, which was in line with decreased fractional anisotropy (FA) in DTI in the pons. FA reduction was also noticed in the cerebral peduncle and medulla. Behavioral abnormality persisted for at least 14 days and neurofunction was recovered within 1 month. *Conclusions:* This novel model can produce a stable hematoma resulting in brain edema, white matter injury, and neurofunctional deficits, which could be useful for future investigation of pathophysiological mechanisms and new treatment evaluation after BSH. **Key Words:** Brainstem hemorrhage—animal model—autologous blood injection—white matter injury.


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## Introduction

Intracerebral hemorrhage (ICH) can be divided into supratentorial and inferatentorial brain hemorrhage according to the anatomy. Supratentorial ICH primarily involves basal ganglion and inferatentorial ICH in the brain stem and cerebellum.[1] Brainstem hemorrhage (BSH) accounts for approximately 10% of all types of ICH,[1,2] which exclusively occurs in the pons, with the highest mortality and morbidity.[2,3] It has been reported that 61.8% of patients with BSH die in the first year and 73.7% in the following 3 years.[4] Most of those who survive are severely disabled. The optimal treatment for BSH is still unknown,[5] despite guidelines recommending supportive management based on the information from basal ganglia hemorrhage.[6] On the one hand, BSH research of large populations is difficult because of the rarity of the disease. Moreover, as the majority of BSH patients are in critical condition, it is impractical to perform randomized clinical trials to obtain high-level evidence regarding the management.[7] On the other hand, due to the totally distinct blood supply system and the possible different neuronal and endothelial cell reactions to stroke,[8,9] it is inappropriate to copy the findings from basal ganglia hemorrhage to BSH. Therefore, it is very urgent to establish a BSH animal model to understand the pathophysiological mechanisms and for better translational research.

There are mainly 2 animal models of ICH widely used. One method produces ICH by injecting bacterial collagenase and the other by infusing autologous blood into basal ganglia where ICH occurs most commonly in patients.[10] Each model has its own advantages and disadvantages, and only partly mimics the pathophysiological features in patients after ICH.[11,12] Therefore, it has been suggested that the efficacy of any therapy targeting ICH should be confirmed in both models in preclinical studies.[11,13] ICH models have been developed using several animals including cats, dogs, rabbits, primates, mice, and rats, among which rats are relatively the ideal subjects due to the similarity of their cerebral vascular anatomical structure to humans and their being one of the most commonly used and economic experimental animals.[12]

Collagenase-induced BSH in rat has been reported by Zhang et al in 2013.[14] This model is relatively easy to establish but had its innate limitations just like in basal ganglia ICH. One of the disadvantages is that the exogenous collagenase injected altered the composition of the extracellular matrix and the migration of inflammatory cells, so the use of this model is limited to fully explore the inflammation, which is an important pathological mechanism following BSH.[14] In addition, the importance of ICH-induced white matter injury has been recognized recently.[15-17] As the brain stem contains more white matter consisting of many vital conduction fibers such as pyramidal tract, spinothalamic tract, and ascending reticular projecting fibers,[18] it is equally important to investigate the white matter injury caused by BSH, which has been unmentioned previously.[14]

To our knowledge, no BSH rat model by autologous blood injection is reported. The purpose of the present study was to establish a novel BSH model by injecting autologous blood in rats and to characterize the resulting brain injury. We focused on the BSH-induced white matter injury by evaluating neurobehavioral, magnetic resonance imaging (MRI), and histopathological features.

## Materials and Methods

### *Animals*

The present study was approved by the Animal Care and Use Committee of West China Hospital, Sichuan University. Adult male Sprague–Dawley rats weighing 300-340 g were used (Dashuo Laboratory Animal Co., Ltd, Chengdu, China). The rats were housed in our center with free access to food and water and were kept at normal daily light/dark duration. The rats are randomly divided into BSH (n = 32) and sham groups (n = 15).

### *Model of BSH*

Under intraperitoneal pentobarbital sodium anesthesia (5 mL/kg, 1%), the rats were secured prone onto a stereotactic frame (RWD, Shanghai, China), and an incision was then made about 2 cm over the posterior scalp. The right pontine basotegmentum was localized by the following stereotactic coordinates: 1.3 mm lateral and 2 mm posterior to the posterior fontanelle, and 9.3 mm ventral to the dura. After a 1-mm-diameter burr hole was made in the skull, each rat received a 30 μL injection of autologous whole blood with a microinfusion pump at a constant rate of 5 μL/minute (Fig 1). Blood was taken and collected by puncturing the central tail artery. The needle was slowly withdrawn 30 minutes after infusion. The burr hole was then sealed by bone wax and the wound sutured. Rectal temperature was maintained at 37 + .5°C using a feedback-controlled heating blanket during the operational procedures and during MRI exams. Surgical rats were housed as routinely done after complete anesthesia recovery in an incubator. Sham rats had only needle insertion.

### *MR Imaging*

We performed all MRI experiments on a 7.0-T magnetic resonance scanner (Bruker Biospec, Ettlingen, Germany) to confirm the presence, extent, and location of BSH 2-4 hours after blood injection and to evaluate the dynamic resolution of hematoma at days 3, 7, 14, and 30, and white matter injury at day 3. All rats were anesthetized with 2% isoflurane throughout the whole examination. A T2 spin–echo sequence (repetition time/echo time = 4000/11 ms) and a 4-shot, spin–echo, echo-planar diffuse tensor imaging (DTI) sequence were used