**Hemorrhagic Shock**

To induce severe HS in rats, we utilized the fixed-pressure model as previously described. Briefly, rats were anesthetized by isoflurane (5% isoflurane for induction; 2-2.5% during the surgery; 1-1.5% maintenance) and then allowed to stabilize with spontaneous respiration on a heating pad to maintain a body temperature at 37±0.5 °C. Right and left femoral arteries were cannulated with PE-50 catheters. They were then placed in a stereotaxic frame with a heating plate to maintain body temperature at 37±0.5 °C (Physitemp TCAC-2LV closed-loop controller) and breathed spontaneously (20/80 oxygen/nitrogen). HS was initiated by withdrawing blood from the right femoral artery to achieve a mean arterial blood pressure of 27±1 mmHg for the first 5 minutes and then maintained at 27±2 mmHg for 30 minutes (**Supplemental Digital Content 2**, http://links.lww.com/CCM/E430). In order to make sure that the rats experienced a similar shock condition in all the experimental groups, both blood glucose and lactate were measured at the HS onset (0 min). Only the animals with blood lactate levels between 9.5-12 mM and glucose levels higher than 450 mg/dL were included in the analysis. After the HS onset, isoflurane level was maintained at 1.0-1.25%. Time to death after the completion of the hemorrhage was recorded. Any rat that survived for 2 hours from the onset of HS was euthanized by decapitation.

**Experimental Design**

Animals were randomly assigned to the following four groups: Control group #1 with neurectomy of trigeminal nerve branches (infraorbital nerve and anterior ethmoidal nerve), Control group #2 with non-specific stimulation of facial nerves, HS control group (Vehicle) and with TNS-treatment group (TNS). Both vehicle and TNS groups were further randomly divided into four different experimental groups (survival test, brain monitoring with neural implants, and sample collection at 15 min and 30 min after HS) to investigate the effect of TNS on hemodynamics, ANS activity, brain perfusion, catecholamine release, metabolic acidosis and systemic inflammation in HS rats. All the groups underwent the same surgery procedures described above including placing electrodes, but stimulation was not applied to vehicle group. Blood pressure, body temperature, and electrocardiogram (ECG) were continuously monitored and recorded for all the rats. The catheter placed in the left femoral artery was connected to a blood pressure transducer (MLT0670, ADInstruments, USA) to monitor blood pressure. Body temperature was measured using an IT-18 thermocouple microprobe connected to the temperature pod (T-type pod, ADInstruments, USA). Needle-type electrodes (MLA1213, ADInstruments, USA) were inserted into the subcutaneous area in both the front and hind limbs to continuously record the ECG.

**Trigeminal Nerve Stimulation**

Immediately after HS had been induced, intermittent TNS (1 minute in every 4 minutes) was delivered for 60 minutes in the TNS treatment group. Two Teflon-coated bipolar wires (bipolar electrodes), with only the cut surface exposed (diameter: 0.3 mm, distance between wire: 1 mm), were inserted bilaterally at the inner edge of the eyebrows to a depth of approximately 6 mm under the skin. Rectangular biphasic pulses (25 Hz, 0.5 ms) with amplitude of 7 V were delivered by an electrical stimulator (Isolated Pulse Stimulator Model 2100, A-M Systems, Sequim, WA). To confirm the inserted position of the bipolar electrodes, we placed a microneedle in the same position as the electrodes and injected 4% Evans blue dye. As indicated by the blue area, the inserted wires can stimulate both the infraorbital nerve (ophthalmic branch of V1) and the anterior ethmoidal nerve (maxillary branch of V2) (**Supplemental Digital Content 3**, http://links.lww.com/CCM/E431). The vehicle group had the electrodes inserted, but didn’t receive any electrical charge.

**Neurectomy of Trigeminal Branches**

An anterior-posterior skin incision approximately 9 mm long was made 3 mm above the left eye, following the curve of the frontal bone. The fascia and muscle were then gently teased laterally from the bone and the eye was retracted to reach the infraorbital nerve (ION) and anterior ethmoidal nerve (AEN) within the orbit. Once visible, at least 7 mm of the ION was cut starting from AEN. After that, the AEN, which crosses perpendicularly above the ION, was cut. Bipolar electrodes were inserted to a depth of approximately 6 mm under the skin and the electrical stimulus was delivered for Control group #1.

**Non-specific Stimulation of Facial Nerves**

The bipolar electrodes were inserted under the skin approximately 3 mm below the left eye. This placement non-specifically targeted the facial nerves and did not disturb the trigeminal nerve. Electrical stimulus was delivered following the same conditions as Control group #1 and TNS treatment group.

**Brain Probe Implantation**

Animals underwent a 1-mm craniotomy (ML: +2 🡪 +3 mm; AP: -2 🡪 -3 mm) to implant a multimodal neural probe. A single neural probe, that we have developed and that can measure CBF, brain temperature and brain oxygen tension (PbrO2), was implanted to a depth of 5 mm and fixed with dental acrylic cement (16, 17). A waiting period of 60 min was used following implantation of the neural probe to record the baseline cerebral signals.

**Measurement of Blood Norepinephrine and Cytokines**

Arterial blood samples were collected from the femoral artery for analysis at baseline (-35 min), during HS (-15 min), at the onset of HS (0 min), at post-HS (15 min and 30 min). The plasma was separated by centrifugation (10,000 rpm, 10 min) and stored at -20 °C until subsequent analysis. Levels of norepinephrine in the plasma from different time points were extracted using a cis-diol-specific affinity gel, acylated, converted enzymatically and then detected and measured by the enzyme-linked immunosorbent assay according to the manufacturer’s instructions (Ref: KA1891, Abnova Corporation, Taiwan). IL-6 levels in the plasma samples were measured by using the specific rat Quantikine ELISA (enzyme-linked immunosorbent assay), cat: kits following the manufacturer’s manuals (R6000B, R&D Systems Inc., USA).

**Measurement of Blood Lactate and Glucose**

Arterial blood samples were collected from the femoral artery for analysis at the baseline and the HS onset (0 min). Lactate levels were measured using i-STAT test cartridges (i-STAT CG4+ Cartridge, Abbott Point of Care Inc., USA) and glucose levels were measured using Contour blood glucose meter.

**Heart Rate Variability Analysis**

The ECG analysis was performed off-line. For each time point, 1 sec, 10 sec or 1 min observation periods were analyzed and R-R intervals were generated. The overall variability of the R-R interval was assessed in the time and frequency domains by spectral estimation as described elsewhere (24). The frequency-domain metrics included very low frequency (VLF: 0.0-0.2 Hz), low frequency (LF: 0.2-0.75 Hz), and high frequency (0.75-2.5 Hz) band powers. The LF and HF components are first expressed in absolute values and then normalized to the total power, because of the large inter-animal variability in absolute values of the spectral data. Normalization was achieved by dividing the integrated LF and HF spectra by the total power and multiplying this value by 100. Here, LF/Total was taken as an index of SNS activity whereas HF/Total was taken as an index of PNS activity (18, 19).

**Data Collection and Processing**

All data were digitized at 1 KHz with PowerLab digitizer (Powerlab 16/SP analog/digital converter, ADInstruments, USA). Data were stored and analyzed with LabChart 7.0 software (ADInstruments, USA).

**Supplemental Digital Content Legends**

**Supplemental Digital Content 2.** One of the representative recordings of blood pressure from each sham, vehicle, and TNS groups.

**Supplemental Digital Content 3.** (**A**) AEN and ION are the targeted trigeminal nerve branches for resuscitation. (**B**) To confirm the inserted position of the bipolar electrodes, Evans blue dye was injected at the same position as bipolar wires. As indicated by the blue area, the inserted electrodes stimulate both ION and AEN. (**C**) Neurectomy of ION and AEN for Control #1 group. The component of the ION and AEN was marked with \*.

ION: Infraorbital nerve; AEN: Anterior ethmoidal nerve.

**Supplemental Digital Content 4.** (**A**) The representative recordings of electrical stimulation response on blood pressure for Control #1, Control #2, and TNS groups. There were no significant increases in MAP for both control groups. (**B**) Three examples of the blood pressure recordings from TNS treatment group for an hour. MAP responded to repeated TNS treatment for an hour (1-min TNS ON; 3-min TNS off).

Control #1 group: neurectomy of trigeminal nerve branches; Control #2 group: non-specific stimulation of facial nerves.

**Supplemental Digital Content 5.** Effects of TNS on low-frequency blood pressure oscillation. 1-min TNS after HS (0 🡪 1 min) significantly increased MAP compared with the baseline (-1 🡪 0 min) and initiated low frequency (0.2-0.75 Hz) oscillations of systemic blood pressure. TNS triggered a tightly coupled and dynamic relationship between MAP and LF/Total. Expanded view of dashed red box shows that 1-sec TNS initiates descending arterial pressure which results in increased sympathetic nerve activity (LF/Total). The compensatory elevation in MAP and feedback reduction in sympathetic outflow were produced during 1-sec TNS Off period. Gray box: during HS; Green box: during TNS.

**Supplemental Digital Content 6.** Effects of TNS on autonomic nervous system activities. (**A**) 1-min TNS after HS (0 🡪 60 sec) significantly increased MAP compared with the baseline (-60 🡪 0 sec). LF/Total increased significantly during TNS whereas HF/Total decreased. However, immediately after TNS (60 🡪 240 sec), LF/Total significantly decreased and HF/Total increased compared with the baseline. (**B**) There were no significant changes in MAP, LF/Total, and HF/Total without TNS treatment. Data were expressed as mean ± SD and analyzed using one-way repeated measures ANOVA. @p<0.05 vs TNS-off phase, &p<0.05 vs TNS-on phase. When p<0.05 means there are statistical significant changes between TNS-on and TNS-off phases.

Green box: during TNS-on phase.

TNS: trigeminal nerve stimulation; MAP: mean arterial pressure; SNS: sympathetic nervous system; PNS: parasympathetic nervous system.

**Supplemental Digital Content 7.** One of the representative recordings of TNS effects on cerebral perfusion. (**A**) CBF. (**B**) PbrO2.