Supplemental information

**Methods**

**Near-infrared time-resolved spectroscopy and analysis**

We used a portable three-wavelength near-infrared TRS system (TRS-21; Hamamatsu Photonics K.K., Hamamatsu, Japan) and attached a probe to the head of each piglet. The light emitter and detector optodes were positioned in the parietal region at an interoptode distance of 30 mm. The TRS system at our institution uses a time-correlated single-photon-counting technique for detection and has been described in detail elsewhere [[19](#_ENREF_19)]. Oxyhaemoglobin and deoxyhaemoglobin concentrations were calculated from their absorption coefficients using equations that assume background absorption is due to only 85% (by volume) water. Total cerebral haemoglobin concentration, ScO2 and CBV were calculated as described previously [[20](#_ENREF_20)].

**Amplitude-integrated electroencephalography monitoring**

For amplitude-integrated electroencephalography (aEEG), we used the Nicolet One (Cardinal Health, Inc., Dublin, OH, USA). With this device, the signal is displayed on a semi-logarithmic scale at low speed (6 cm/h). In this study, measurements were recorded at 1-s intervals. The gold-plated electrode discs were placed at the P3 and P4 positions (corresponding to the left and right parietal areas on the head). LAEEG was defined as the maximum amplitude < 5 μV; the aEEG pattern was evaluated using the aEEG scoring system developed by Peeters-Scholte et al. [[21](#_ENREF_21)], which ranges from 4 (normal) to 0 (worst) and integrates the aEEG background pattern with seizure activity. Five distinct patterns can be discriminated in the aEEG backgrounds of human term neonates: flat trace, burst suppression, continuous low voltage, discontinuous normal voltage and continuous normal voltage. Seizure activity was categorised as follows: no seizures present; an irregular, spiky aEEG (confirmed as multifocal epilepsy on a standard EEG recording); single seizures (less than 3 seizures/h, with a maximal duration of 10 min each); repetitive seizures (more than 3 seizures/h); and status epilepticus (saw-tooth pattern).

**Hypoxic insult**

Hypoxia was induced by reducing the inspired oxygen concentration of the ventilator to 4% after at least 120 min of stabilisation from the initial anaesthetic induction. If required to obtain a low-amplitude aEEG pattern (LAEEG; maximum amplitude < 5 µV) using a Nicolet One (Cardinal Health, Inc.), the inspired oxygen concentration was further reduced to 2%. From the beginning of the LAEEG, the insult was continued for 30 min. For the final 10 min of the 30-min insult, if mean arterial blood pressure (MABP) exceeded 70% of baseline, hypotension was induced by decreasing the FiO2 until MABP declined to below 70% of baseline. During the HI insult, CBV was continuously monitored using TRS; we calculated the change in CBV as described previously [[17](#_ENREF_17)]. When CBV fell to 30% of the height between the peak and baseline during the final 10 min of the insult, resuscitation was started. Otherwise, if the change in CBV remained above it, the HI lasted for 10 min.

**Histology**

Coronal blocks of cortical grey matter (GM), subcortical white matter (WM), the CA-1 area of the hippocampus (HIPP) and the cerebellum (CERE) were embedded in paraffin and cut with a microtome at 4 µm. The sections were stained with haematoxylin and eosin (H&E) and three areas of the cortical GM and subcortical WM were studied: the dorsal cortex, sensorimotor cortex and mid-temporal cortex [[24](#_ENREF_24), [25](#_ENREF_25)]. In each area, three regions of interest (ROIs) were randomly sampled. In the HIPP, three adjacent ROIs were selected from the CA-1 area. In the CERE, three adjacent ROIs were selected from the Purkinje layers. Samples were examined by investigators (M.U., S.N., S.Y. and Y.H.) who were blinded to all clinical information.

The extent of damage in each of the five regions was graded in 0.5-unit intervals on a nine-step scale that ranged from 0.0 to 4.0. Grade 0 indicated no damage; grade 1 indicated that ≤ 10% of the area was affected with morphological changes that included individual necrotic neurons and small patchy, complete or incomplete infarcts; grade 2 indicated that 20–30% of the area was affected with partly confluent incomplete or complete infarcts; grade 3 indicated that 40–60% of the area was affected with large confluent and complete infarcts; and grade 4 indicated that > 75% of the area was affected with neuronal necrosis in the hippocampus and total disintegration of the cortex.

**Results**

After resuscitation, arterial blood pH (pHa), partial pressure of arterial blood O2 (PaO2) and partial pressure of arterial blood CO2 (PaCO2) recovered to baseline values within 1 h in each group (Table 1). Some significant differences in base excess were detected among the groups at 6 and 12 h after resuscitation, but these differences were not considered physiologically significant. Hypothermia produced a transient but highly variable hyperglycaemic response at 6, 12 and 24 h after the insult. In addition, the TH-EV group showed more hyperglycaemia than the TH group.

We confirmed the protocol using TRS and monitored and recorded CBV and ScO2 during and after the HI insult. However, one animal from each of the NT and TH+EV groups had missing data after the HI insult. As in our previous study [[22](#_ENREF_22)], we calculated the change in CBV and ScO2 from the end of the insult to evaluate how much the parameters changed after the insult. The TH and TH+EV groups showed more of a decrease in CBV than the NT group after the insult (Supplementary Fig. 3). In ScO2, the change in ScO2 from the end of insult was significantly smaller in the TH+EV group (p < 0.05, at 6 h) and TH group (*p* < 0.05 at 12 h, *p* < 0.01 at 24 h) than in the NT group after the insult (Supplementary Fig. 4). Hence, there was no significant difference in the change in CBV and ScO2 after the insult between the TH and TH+EV groups. For the aEEG score, we did not find significant differences among the three groups (Supplementary Fig. 5)**.**

**Histopathological score [mean (SD), NT, TH, TH+EV group (Fig. 2)]**

GM: 2.7 (1.4), 2.0 (1.2), 1.9 (1.7) (Fig 2a); WM: 3.1 (1.2), 2.4 (1.8),2.2 (1.7) (Fig 2b); HIPP: 2.4 (1.8), 2.2 (1.6),1.1 (1.7) (Fig 2c); CERE: 1.1 (1.7) 1.5 (1.7), 1.4 (1.8) (Fig 2d)

**Supplementary Fig 1. Changes over time in the mean arterial blood pressure (MABP) in the three groups (NT, n = 8; TH, n = 7; TH+EV, n = 6) at baseline, end of insult (0 h) and 3, 6, 12 and 24 h after the insult. **MABP was higher within 3 h after the insult in the TH (1 h, *p* < 0.05 vs NT) and TH+EV groups compared with the NT group. Values are shown as the mean ± SEM. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001 vs. NT.

**Supplementary Fig 2. Changes over time in heart rate (HR) in the three groups (NT, n = 8; TH, n = 7; TH+EV, n = 6) at baseline, end of insult (0 h) and 3, 6, 12 and 24 h after the insult.**

****HR was significantly lower in the TH (1 h) and TH+EV (1, 3, 6 and 12 h) groups than in the NT group after the insult. Values are shown as the mean ± SEM. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001 vs. NT.

**Supplementary Fig 3. Changes in cerebral blood volume (CBV) (ml/100g brain) in the three groups (NT, n = 7; TH, n = 7; TH+EV, n = 5) at the end of insult (0 h) and 3, 6, 12 and 24 h after the insult.**

****Values are shown as the mean ± SEM. One piglet in the NT group and one in the TH+EV group were missing data.

**Supplementary Fig. 4. Changes in cerebral Hb oxygen saturation (ScO2) (%) in the three groups (NT, n = 7; TH, n = 7; TH+EV, n = 5) at the end of insult (0 h) and 3, 6, 12 and 24 h after the insult. **

Values are shown as the mean ± SEM.\* *p* < 0.05, \*\* *p* < 0.01 vs the NT group. One piglet in the NT group and one in the TH+EV group had missing data.

**Supplementary Fig. 5. Time course of the aEEG score in the three groups (NT, n = 8; TH, n = 6; TH+EV, n = 6) at the end of insult (0 h) and 3, 6, 12 and 24 h after the insult.**

Values are shown as the mean ± SEM. One piglet in the TH group was missing data.