Effects of a Small Acute Subdural Hematoma following Traumatic Brain Injury on Neuromonitoring, Brain Swelling and Histology in Pigs

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Abstract

An acute subdural hematoma (ASDH) induces pathomechanisms which worsen outcome after traumatic brain injury, even after a small hemorrhage. Synergistic effects of a small ASDH on brain damage are poorly understood, and were studied here using neuromonitoring for 10 h in an injury model of controlled cortical impact (CCI) and ASDH. Pigs (n = 32) were assigned to 4 groups: sham, CCI (2.5 m/s), ASDH (2 ml) and CCI + ASDH. Intracranial pressure was significantly increased above sham levels by all injuries with no difference between groups. CCI and ASDH reduced $ptiO_2$ by a maximum of 36 ± 9 and 26 ± 11%, respectively. The combination caused a 31 ± 11% drop. ASDH alone and in combination with CCI caused a significant elevation in extracellular glutamate, which remained increased longer for CCI + ASDH. The same two groups had significantly higher peak lactate levels compared to sham. Somatosensory evoked potential (SSEP) amplitude was persistently reduced by combined injury. These effects translated into significantly elevated brain water content and histological damage in all injury groups. Thus, combined injury had stronger effects on glutamate and SSEP when compared to CCI and ASDH, but no clear-cut synergistic effects of 2 ml ASDH on trauma were observed. We speculate that this was partially due to the CCI injury severity.

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Introduction

Traumatic brain injury (TBI) is the leading cause of death and severe disability among young people and affects up to 2% of the overall population [1, 2]. A prevalent problem of TBI is the development of subdural hematoma, which leads to complications in as many as 45% of mild-to-severe TBI cases [1]. The immediate primary injury due to deformation of the brain in the moment of mechanical impact is not amenable to therapy, but is followed by a multitude of delayed secondary events which continue to cause neuronal damage and ultimately lead to secondary expansion of the primary lesion [3]. Trau-

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matic ruptures of bridging veins or hemorrhage from pial arteries lead to acute subdural hematoma (ASDH) that affects expansion of tissue damage by increasing intracranial pressure (ICP) and decreasing cerebral perfusion pressure (CPP) and local cerebral blood flow (CBF). This worsens outcome despite early therapeutical interventions [1, 4–7].

These data indicate that the management of patients needs to be improved. This includes development of neuroprotective drugs, but also monitoring of neuropathological events by clinically applied multi-parametric neuromonitoring [1, 8–16]. Only a few attempts have been made to study effects of brain injury in clinically relevant settings, such as large animals [17–19]. We established a porcine model of controlled cortical impact (CCI) [17] as well as of ASDH by injecting three different volumes of autologous venous blood into the subdural space (2, 5 and 9 ml) [19] and applied extended multi-parametric monitoring in both models. Already the smallest volume of 2 ml which represented ~3% of intracranial volume produced short-term effects on the assessed monitoring parameters and caused significant brain swelling and tissue damage 12 h after the onset of injury. The influence of subdural blood volume on neuropathophysiology and outcome has been demonstrated in experimental studies of ASDH as well as in patients [1, 20–22]. In general, large subdural blood volumes are associated with higher mortality, whereas the effect of smaller volumes is less clearly understood. An important factor of large ASDH for lesion development after TBI is a further ICP elevation. However, the degree of damage also depends on blood itself [23, 24], which activates detrimental effects if ICP is allowed to build up after hemorrhage [25] or after a primary TBI [26]. This suggests that even small ASDH could potentiate pathophysiological parameters and outcome in some cases.

Therefore, the goal of the present study was to combine a CCI injury with a subdural hemorrhage to test the hypothesis that even a small subdural hematoma can aggravate brain damage and that this can be detected by extended neuromonitoring.

The present experiments and experimental design were part of a randomized study investigating blood-volume-dependent effects of ASDH [19] and effects of CCI [17] in combination with ASDH. For the combination of CCI with ASDH, a subdural volume of 2 ml has been chosen. This small subdural volume did affect neuromonitoring and histology, but had little effect on ICP. A potentiating effect of a small ASDH should be detected by extended neuromonitoring, brain swelling and histology.

Materials and Methods

Animals and Anesthesia

All experiments were carried out in accordance with the Animal Welfare Guidelines and were approved by the local ethics committee. Juvenile male pigs (n = 32, 4 months old; German breed), weighing on average 28.67 ± 0.37 kg were assigned to 4 different groups: a CCI group (n = 5); a group with subdural blood volume (ASDH 2 ml, n = 8); a combination group that received cortical impact and subdural blood injection (CCI + ASDH 2 ml, n = 8); a sham operated group (n = 11) that was compared to all other groups.

Anesthesia was performed as previously described [17, 19]. Briefly, initial sedation was achieved by intramuscular injection of ketamine (15 mg/kg), azaperone (3 mg/kg) and atropine (1 mg), then anesthesia and analgesia induced through peripheral vein injection of 15 mg piritramide (Dipidolor, Janssen-Cilag) and 40–50 ml α-chloralose [5 g (Physalis, France) + 4 g $Na_2B_4O_7$ (Merck) in 100 ml saline solution 0.9% (adjusted pH 7.4)] and maintained by a slow continuous infusion of piritramide (1 mg/ml): 2–5 ml/h via ear-vein and α-chloralose (2 g/40 ml) 10–40 ml/h through ear (initial dose) and femoral vein (after catheterization). All animals received a continuous infusion of saline, but no pharmacological treatment to stabilize blood pressure. Pigs received an oral endotracheal intubation and artificial ventilation adapted to maintain physiological arterial parameters (Servo 900B, Siemens-Elema, AB, Sweden). Rectal temperature was monitored and held constant at 38°C (Homeothermic Blanket, Harvard Apparatus, South Natick, Mass., USA).

Surgical Preparation

Animals were prepared for sham-operation and trauma as previously described in detail [19]. Briefly, the left femoral artery and vein were cannulated to monitor blood pressure, to withdraw venous blood for blood gas analysis, or for subdural infusion and to administer medication. After surgical suprapubic catheterization of the bladder, the animal was turned. The head was fixed in a stereotactic frame and the skull was exposed. Thereafter, a large craniotomy (diameter 30 mm) for application of TBI (ASDH, CCI) was drilled carefully over the left parietal cortex. At a distance of 5 mm posterior to the craniotomy ipsilaterally and at the same position on the contralateral hemisphere, small burr holes were prepared for neuromonitoring catheters. Brain tissue oxygen (ptiO₂, Licox), temperature, microdialysis (CMA/70) and ICP catheters were inserted as described in detail previously [17]. Monitoring probes were lowered into the parenchyma to a depth of at least 15 mm. After probe insertion, burr holes were closed using bone wax, whereas a cover made out of d^S-Alginat Quick, an alginate-based hydrocolloid formulation (Demedis Dental Depot GmbH, Düsseldorf, Germany) was used to close the craniotomy. This cover was kept in place by a metal cylinder fixed to the stereotactic frame in order to prevent the release of pressure during and after injury. For somatosensory evoked potential (SSEP) monitoring, two extradural electrodes were screwed into the scull with the recording electrode right above the ipsilateral prefrontal cortex, i.e. frontal to the craniotomy and the reference electrode about 2–3 cm further medio-frontal.

Fig. 1. Schematic drawing of position of neuromonitoring catheters, large craniotomy, area of CCI bolt impact and placement of tubing for subdural blood infusion. The monitoring setup included microdialysis, ptiO$_2$, brain temperature, ICP and medianus nerve SSEP. Note that positions could vary slightly due to the shape of each individual skull.
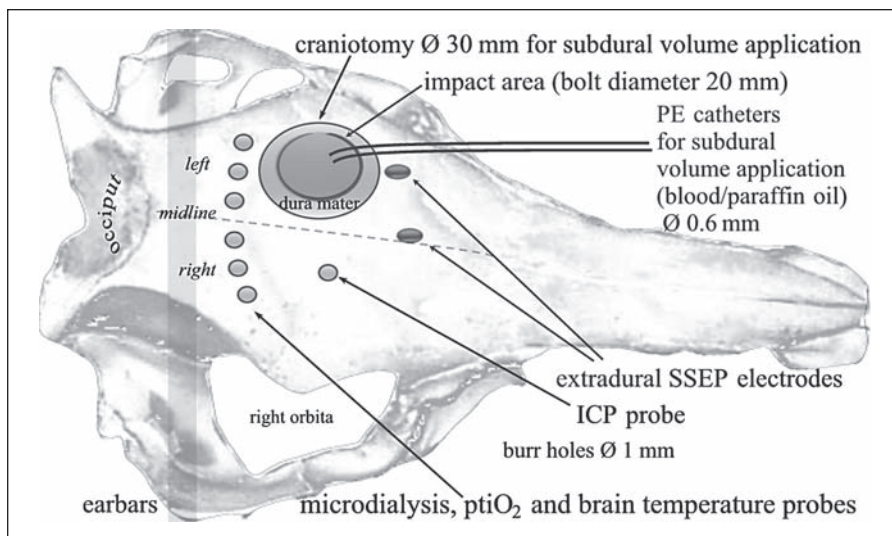
Multiparametric Monitoring
Physiological Parameters
Physiological monitoring included ventilation-parameters (CO$_2$, FIO$_2$), arterial blood gas analyses (paO$_2$, paCO$_2$, pH, lactate, glucose, electrolytes, hematocrit), rectal temperature, heart rate and mean arterial blood pressures (MABP).

Neuromonitoring
ICP was measured by intraventricular catheter (saline filled) in the right lateral ventricle. CPP was calculated by MABP-ICP. ptiO$_2$ pressure and brain temperature were measured with Licox® probes (Licox CC1 SB, Licox LT, Integra Neurosciences Limited Hampshire, UK). ptiO$_2$, ICP, CPP and intracerebral temperature were recorded every minute.

Samples for the assessment of biochemical markers such as glutamate and lactate were collected through microdialysis catheters (CMA/70, flow rate 1 µl/min), immediately frozen at –20°C and analyzed with an enzymatic analyzer (CMA 600, Axel Semrau GmbH). Dialysate samples were taken every 15 min during the baseline period and the first 3 h post-TBI induction (CCI, ASDH or both), and thereafter every hour until the end of the experiment.

For the SSEP of the left cortex, the right foreleg median nerve was stimulated with 1 Hz and 5 mA (each value was created by the average of 20 stimulations) every 15 min. We analyzed latency and amplitude of the first cortical response to stimulation (N20).

Histology and Water Content
At the end of each experiment, the craniotomy was opened and the location of the subdural blood clot was checked visually. As described in detail elsewhere [17, 19], animals were euthanized 12 h after TBI under deep α-chloralose anesthesia and brains were removed immediately. One brain slice was taken underneath the injury for determination of cortical water content using the wet-dry-weight method (drying at 110°C for 24 h). The rest of the brain was sliced into blocks (I–IV) and was immersed in paraformaldehyde for several days, then embedded in paraffin. Coronal sections (10 µm thick) were cut from blocks containing the anterior and

posterior brain coordinate of 12.5 and 4.5 mm, respectively [27]. From each coordinate, 2 sections were stained with HE and analyzed for brain damage using a lesion index (LI) [for detailed description, see 19]. The LI is derived by adding several injury parameters – such as intracerebral bleeding (ICB, values range: 0–3), subarachnoid hemorrhage (SAH, 0–2), intensity of edema (0–3) and surface contusion (0–1) – to the Contusion Index [28], which grades pyknotic and eosinophilic neurons (e.g. dead or dying cells) by depth and extent (range from 0–12: injury depth 1–4 and injury extent 1–3). Thus, LI is calculated as follows: (extent × depth) + ICB + SAH + edema + contusion. An LI was calculated for two anatomical brain locations (A12.5, P4.5) in order to standardize histological analysis. They were added for each hemisphere and pig. The final LI per hemisphere and animal ranges from 0 to 42. LI includes contusional and ischemic damage (fig. 1) [18, 28, 29].

Traumatic Brain Injury
Two types of TBI were performed, which have been described in detail previously [17, 19]. Sham pigs received all interventions, but no TBI.

Controlled Cortical Impact
Briefly, a pneumatically driven bolt (diameter 20 mm) was placed over the craniotomy. The injury was produced at velocity of 2.5 m/s, an injury depth of 9 mm and 400 ms dwell time. This moderate injury did not lacerate the dura in any animal of this group.

Acute Subdural Hematoma
The ASDH was applied by a perfusion syringe via polyethylene catheters. They were inserted horizontally into the subdural space only to 2 mm in order to prevent pushing catheters into the parenchyma. They were fixed and sealed with acrylic glue (Histoacryl®; B. Braun, Melsungen, Germany) followed by immediate closure of the craniotomy. The infusion of 2 ml non-heparinized autologous venous blood lasted 4 min. We checked for the location of the blood clot and possible leakage during infusion at the end of the experiment.
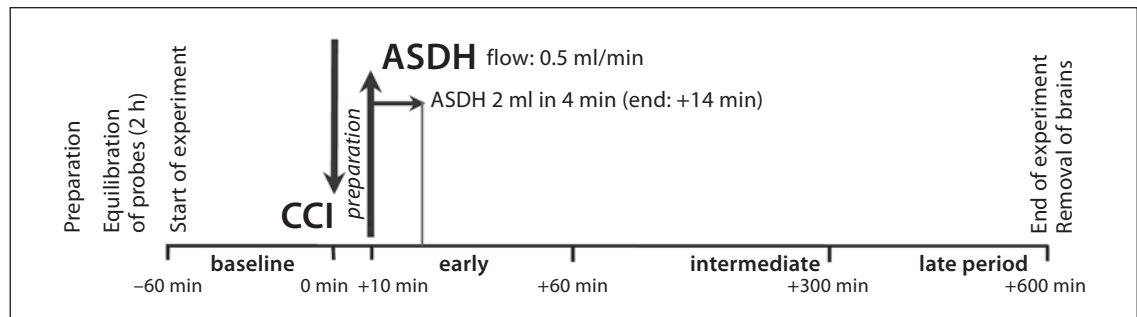
Fig. 2. Time course of the experiment: After a 1-hour baseline period the craniotomy was opened shortly before time point 0. Trauma was induced either by CCI (velocity 2.5 m/s, depth 9 mm, dwell time 400 ms) at time point 0, ASDH (2 ml venous blood) at time point +10 min, or a combination of both (CCI + ASDH 2 ml). In all groups, craniotomy was left open for 6–7 min in order to allow CCI and insertion of tubing for ASDH. After trauma, multi-parametric neuromonitoring continued for 10 h (600 min). After removal of all sensors, the brain was removed and processed for histological analysis and measurement of water content (+12 h). Statistical analysis was performed for 4 pre-defined time periods, namely a baseline (–60 to 0 min pre-trauma), an early (0 to +60 min post-trauma), an intermediate (+180 to +300 min post-trauma) and a late period (+480 to +600 min post-trauma).

Study and Monitoring Protocol

After stabilization of physiological parameters and ptiO$_2$ values, a 60-min baseline period followed. This was followed by the trauma (time point 0) and a 10-hour observation period (fig. 2). Pigs were randomly assigned to different injury groups to study ASDH as co-injury; two groups received a focal CCI. A trauma group with a focal cortical contusion (CCI, n = 5) was compared to a group with a combination of CCI and ASDH with 2 ml blood (CCI + ASDH 2 ml, n = 8) and the ASDH 2 ml group (n = 8), without contusion. CCI was performed at time-point 0, and at +10 min the subdural infusion was started. The experimental time-course for each group is depicted in figure 2. In all groups, after induction of TBI, the craniotomy was closed and all parameters were recorded for 10 h.

Data Analysis and Statistics

According to our previous studies, we selected 4 different time periods within the entire monitoring time frame of 11 h. We analyzed a 60-min baseline period (pre-injury time point –60 to 0 min), an early (post-injury time point +10 to 60 min), an intermediate (post-injury time point +180 to 300 min) and a late (post-injury time point +480 to +600 min) period [17]. In order to compare both ASDH groups, end of baseline was considered time point 0 in all groups, although ASDH infusion started 10 min after end of baseline or after CCI. Area under the curve (AUC) was calculated for each animal and time period separately. Comparisons between groups for all neuromonitoring parameters (i.e. calculated AUC), for water content and for histological damage were made using one-way ANOVA with Student-Newman-Keuls post-hoc tests. Peak values, i.e. the lowest or highest absolute value which was found within the first 2 h after injury, do not peak out at the same time point for different groups and parameters. Therefore, peak values of each injury group were compared to the appropriate sham value using a t test. A paired t test was used to compare data within study groups (e.g. hemispheric water content). Due to large baseline differences between animals in microdialysis, ptiO$_2$ and SSEP parameter values were normalized to baseline and expressed as relative or percentage change to baseline. The mean of the last three measurements before injury was used for calculations. We used Sigma Stat 3.0 for all statistics. Values are given as means ± SEM.

Results

Physiological Parameters

Physiological data are shown in table 1. All values were within physiological ranges. Despite saline solution 0.9% being administered to stabilize MAP, hematocrit values were not affected over time. Baseline and 10-hour post-injury values changed from 29.2 ± 0.7 to 27.9 ± 0.8% for sham, from 27.8 ± 0.6 to 26.9 ± 0.2% for CCI, from 29.5 ± 0.6 to 28.9 ± 0.4% for ASDH and from 31.5 ± 0.8 to 30.9 ± 0.8% for the CCI + ASDH group [for comparison, see 30].

ICP and CPP

Analysis of the AUC for ICP indicated that all injury types caused a significant increase in ICP during the early observation period (0–60 min post-trauma). At the intermediate time periods (180–300 min post-trauma), only the ASDH 2 ml group was still significantly elevated compared to sham-operated pigs (p < 0.05). AUC for ICP did not differ between groups in the late observation period (480–600 min post-trauma). Analysis of peak ICP values is depicted in figure 3. ICP rose to a maximum of 14.40 ± 6.2 mm Hg at 11 min post-CCI in the CCI group. Subdural hematoma alone elevated ICP to 22.25 ± 1.8 mm Hg at 15 min post-CCI (= post time
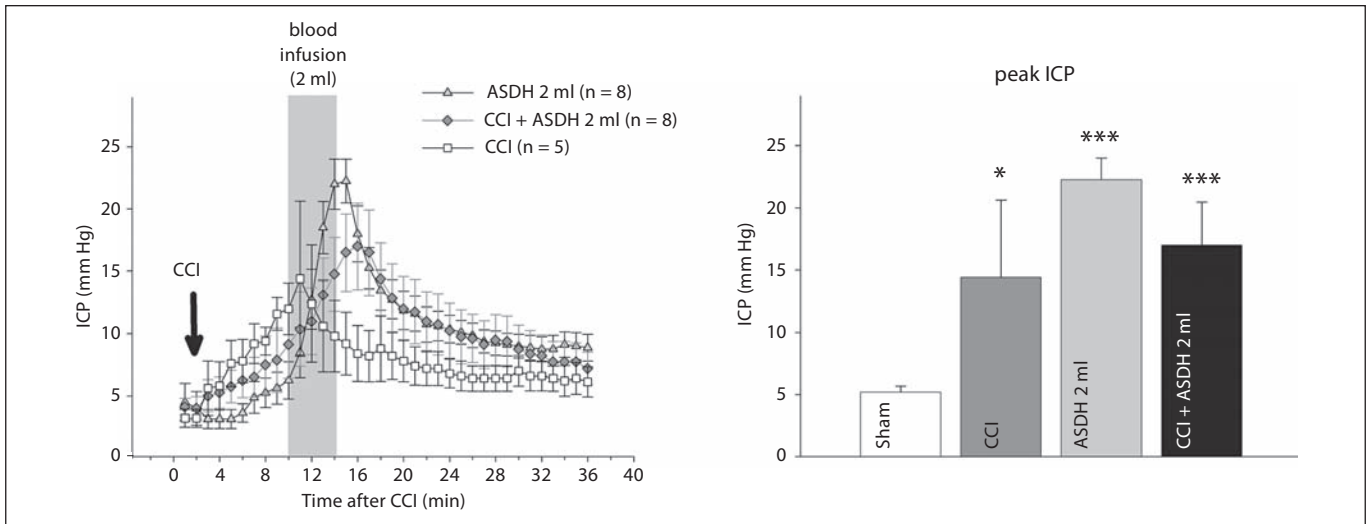
Fig. 3. Early monitoring period. Time-course and peak values of ICP changes following CCI (n = 5), ASDH 2 ml (n = 8) and CCI + ASDH 2 ml (n = 8). Peak values were reached at 11, 15 and 16 min post-trauma for the CCI, ASDH 2 ml and combined injury groups, respectively. Values are given as means ± SEM. * p < 0.05, *** p < 0.001 vs. sham (t test). Note that data analysis of AUC for the early monitoring period (0 to +60 min post-trauma) revealed significant differences of all trauma groups to sham for ICP, but not for CPP (see 'Results').

Table 1. Physiological parameters before (baseline) and after (+60, +600 min post-trauma) brain injury or sham operation

	MABP mm Hg	Rectal temp. °C	pH	paO_2 mm Hg	$paCO_2$ mm Hg	Glucose mg/dl	Lactate mM
Baseline							
Sham	91.4 ± 5.1	37.2 ± 0.3	7.46 ± 0.01	139.0 ± 6.0	46.0 ± 1.2	79.7 ± 9.9	0.8 ± 0.1
CCI	95.3 ± 4.0	37.3 ± 0.2	7.46 ± 0.02	127.4 ± 6.4	46.2 ± 2.3	82.2 ± 6.0	0.8 ± 0.2
ASDH 2 ml	97.1 ± 5.4	37.3 ± 0.2	7.46 ± 0.02	141.4 ± 4.0	47.2 ± 0.9	89.0 ± 7.3	0.8 ± 0.1
CCI + ASDH 2 ml	89.0 ± 5.4	37.4 ± 0.2	7.46 ± 0.01	137.0 ± 5.1	46.8 ± 0.6	90.4 ± 9.7	0.9 ± 0.1
+60 min post-trauma							
Sham	92.2 ± 7.4	37.7 ± 0.2	7.45 ± 0.01	142.2 ± 5.7	45.3 ± 1.8	73.3 ± 13.4	0.8 ± 0.2
CCI	91.5 ± 3.2	37.6 ± 0.3	7.45 ± 0.01	134.1 ± 6.1	46.2 ± 1.7	85.0 ± 5.8	0.6 ± 0.1
ASDH 2 ml	96.5 ± 6.3	37.7 ± 0.2	7.46 ± 0.02	145.8 ± 4.9	46.8 ± 0.7	92.3 ± 6.4	0.7 ± 0.1
CCI + ASDH 2 ml	87.7 ± 5.3	37.5 ± 0.3	7.46 ± 0.01	138.7 ± 5.2	46.1 ± 1.4	81.3 ± 7.4	0.9 ± 0.1
+600 min post-trauma							
Sham	73.3 ± 4.4	37.4 ± 0.2	7.43 ± 0.02	120.0 ± 16.1	41.1 ± 3.6	70.5 ± 6.8	0.6 ± 0.1
CCI	84.0 ± 7.0	37.4 ± 0.2	7.46 ± 0.01	117.4 ± 6.8	44.4 ± 1.2	80.0 ± 5.3	0.6 ± 0.1
ASDH 2 ml	80.1 ± 5.2	37.7 ± 0.2	7.45 ± 0.01	134.6 ± 12.4	45.7 ± 0.7	88.5 ± 10.1	0.7 ± 0.1
CCI + ASDH 2 ml	76.9 ± 6.4	37.6 ± 0.3	7.45 ± 0.004	118.31 ± 7.3	45.0 ± 1.6	78.6 ± 5.9	0.8 ± 0.1

All values are given as means ± SEM. MABP = Mean arterial blood pressure; paO_2/CO_2 = arterial blood O_2/CO_2.

point 0) and in combination with CCI to a maximum of 17.0 ± 3.5 mm Hg at 16 min post-CCI. ICP values of the sham-operated group varied between 4.0 ± 0.7 and 5.6 ± 0.5 mm Hg during the entire monitoring period (fig. 3).

CPP before injury were 88.8 ± 2.6 mm Hg (sham), 87.1 ± 1.6 mm Hg (CCI), 92.8 ± 1.6 mm Hg (ASDH 2 ml) and 86.0 ± 2.0 mm Hg (CCI + ASDH). After CCI, there is a peak around 110 mm Hg in both the CCI and the combination group that is caused by an acute rise in

Fig. 4. Early monitoring period: Relative $ptiO_2$ values (means ± SEM) in the ipsilateral hemisphere during the early monitoring period (0 to +60 min post-trauma) for the sham operation (n = 11), CCI (n = 5), ASDH (2 ml, n = 8) or the combination of both (CCI + ASDH 2 ml, n = 8) group. The arrow shows the time point of CCI and the gray bar indicates the time of blood infusion (4 min). Data analysis of the intermediate (180–300 min post-trauma) and late observation period (480–600 min post-trauma) revealed no significant difference between injury groups.
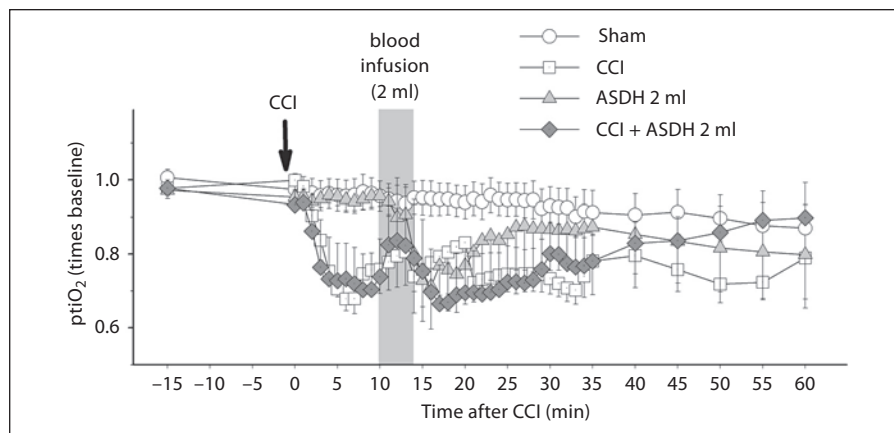
MABP (Cushing reaction). Due to differences in baseline CPP and moderate injury-induced ICP changes, CPP of injury groups were not significantly different from sham values at time-points of highest ICP (fig. 3). Similarly to the ICP values, the CPP of the sham-operated group remained constant throughout the experiments at 81.06 ± 0.53 mm Hg. Analysis of the AUC for the early, intermediate and late observation periods showed no long-lasting significant drop in CPP when compared to sham values.

Tissue Oxygen Tension

We analyzed the relative changes in oxygen levels within the tissue, due to large differences in baseline values between animals and in the ipsi- and contralateral hemispheres.

Relative values were calculated as a ratio between each absolute value and the mean of the last three baseline values of the appropriate parameter.

Relative values for baseline and early monitoring period (0 to +60 min post-injury) are plotted in figure 4. The absolute baseline values for $ptiO_2$ were as follows: ipsilateral: sham = 26.00 ± 1.06 mm Hg; ASDH 2 ml = 25.86 ± 1.68 mm Hg; CCI = 26.54 ± 1.55 mm Hg; CCI + ASDH 2 ml = 21.47 ± 1.19 mm Hg; contralateral: sham = 29.17 ± 1.67 mm Hg; ASDH 2 ml = 19.57 ± 0.99 mm Hg; CCI = 25.58 ± 1.53 mm Hg; CCI + ASDH 2 ml = 23.19 ± 1.23 mm Hg. Although the AUCs for the different statistically analyzed periods were not significant between groups, CCI injury alone or in combination with ASDH 2 ml reduced $ptiO_2$ directly after impact. A one-way ANOVA analysis of time-point +10 min (just before infusion of subdural blood) revealed significant differences between both CCI groups with all other groups ($p < 0.05$). After the end of the blood infusion (+15 min),

groups did not differ from each other anymore. With start of blood infusion, $ptiO_2$ dropped again. No significant difference between groups could be found at 17 min post-trauma, at which time-point the lowest value for CCI + ASDH 2 ml was reached ($p = 0.088$, one-way ANOVA). However, the effect of combined injury was more pronounced in the contralateral hemisphere and the CCI + ASDH 2 ml group differed significantly from the other groups at +15 min post-injury ($p < 0.05$, data not shown).

Glutamate and Lactate in Dialysate

Absolute ipsilateral baseline and post-traumatic peak glutamate and lactate levels as well as total amount of extracellular release (AUC) during an early (0–60 min post-trauma), an intermediate (180–300 min post-trauma) and a late observation period (480–600 min post-trauma) are shown in table 2. We analyzed dialysates as relative changes from baseline (= 1), since we detected in 6 of 32 pigs unusually high baseline glutamate levels of >50 μM. Although these high baseline glutamate levels did not correlate with outcome parameters (histology, water content), these experiments were excluded from calculation of absolute microdialysis values in table 2.

Ipsilateral glutamate release reached sham levels within the first 60 min after injury for the CCI and ASDH group, and was not elevated significantly at later time points and in the contralateral hemisphere. Injection of 2 ml subdural blood alone (ASDH 2 ml) or in combination with CCI (CCI + ASDH 2 ml group) induced a significant extracellular glutamate peak immediately after injury. This effect was stronger and prolonged (AUC for early period) by a combined injury (all $p < 0.05$; table 2).

None of the different injuries elevated the total amount of lactate release in the ipsi- and contralateral hemisphere

Table 2. Ipsilateral concentration of glutamate and lactate in dialysates and ipsi- and contralateral brain water content

	Baseline μM	Peak μM	Early μM/h	Intermediate μM/h	Late period μM/h	Water content (ipsilateral/ contralateral), %
Glutamate release						
Sham	9.0 ± 4.1	8.0 ± 3.4	35.4 ± 16.0	5.9 ± 1.8	8.0 ± 4.2	79.2 ± 0.2 (ipsi.)
CCI	8.4 ± 3.2	40.1 ± 28.0	87.5 ± 35.8	3.4 ± 0.6	6.0 ± 1.7	80.8 ± 0.6[a] (ipsi.)
ASDH 2 ml	15.4 ± 6.1	30.4 ± 7.7[a]	126.3 ± 35.2	25.1 ± 11.9	22.0 ± 10.6	80.6 ± 0.4[a] (ipsi.)
CCI + ASDH 2 ml	18.8 ± 6.3	86.9 ± 29[a, b]	267.6 ± 97.7[a]	17.1 ± 8.6	35.5 ± 17.7	80.9 ± 0.2[a] (ipsi.)
Lactate release						
Sham	1.6 ± 0.5	1.4 ± 0.3	6.3 ± 1.8	1.5 ± 0.3	1.8 ± 0.5	79.6 ± 0.3 (cont.)
CCI	1.8 ± 0 6	2.9 ± 1.2	9.6 ± 3.1	1.3 ± 0.2	1.3 ± 0.2	80.1 ± 0.5[c] (cont.)
ASDH 2 ml	2.0 ± 0.3	2.6 ± 0.4[a]	9.3 ± 1.1	2.8 ± 0.5	2.8 ± 0.4	80.1 ± 0.2 (cont.)
CCI + ASDH 2 ml	1.8 ± 0.2	3.1 ± 0.6[a]	9.6 ± 1.1	2.7 ± 0.6	2.5 ± 0.6	80.4 ± 0.2[c] (cont.)

All values are given as means ± SEM. A baseline and peak value (in μM) were selected and total extracellular release was calculated as AUC and therefore expressed μM/h for the early, intermediate and late post-traumatic observation periods (see 'Materials and Methods'). Glutamate: post-traumatic peak values were reached at +15 min in all groups. Lactate: post-traumatic peak values were reached at +15 min (CCI), at +45 min (CCI + ASDH 2 ml) and at +90 min (ASDH).

[a] p < 0.05 vs. sham.
[b] p < 0.05 ASDH 2 ml vs. CCI + ASDH 2 ml.
[c] p < 0.05 ipsi- vs. contralateral.

significantly above that seen in sham animals. ASDH 2 ml alone and CCI + ASDH 2 ml produced a significantly higher peak lactate level during the acute monitoring phase.

Somatosensory Evoked Potentials

The bar chart (fig. 5) shows changes of amplitude L1-L2 in % baseline values. Sham operated animals maintained baseline SSEP amplitudes for up to 10 h, but only values from 3 pigs could be collected. Therefore, comparisons were made against baseline values only. All injury groups reduced their SSEP amplitude during the first hour after trauma (early period (0 to +60 min post-trauma). At the end of the monitoring period SSEP amplitudes did not differ from baseline anymore except for the group with the combined injury, where amplitudes remained significantly reduced throughout the post-traumatic observation phase.

Brain Water Content

As depicted in table 2, contusion, subdural hematoma and the combination of both did increase ipsilateral brain water content significantly when compared to sham-operated animals (p < 0.03), whereas the contralateral water content was not affected. There was a statistically significant difference in water content between the hemispheres within each group in the CCI and in the combi-
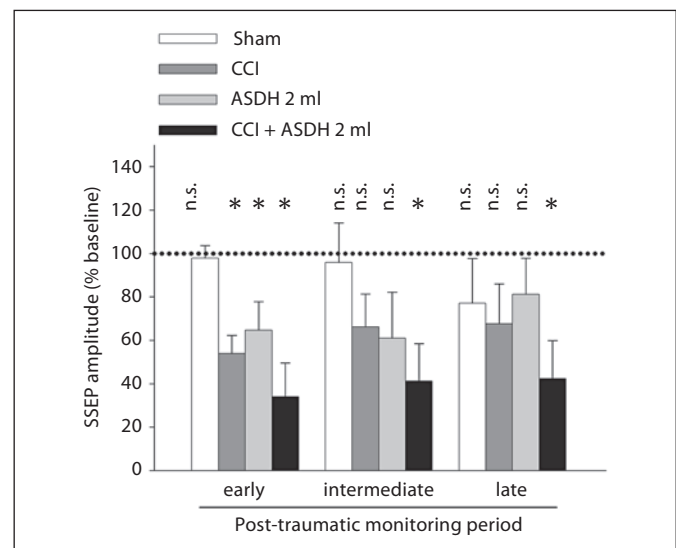

Fig. 5. Percent relative changes from baseline (100%, marked by dotted line) of SSEP amplitudes during the early (0–60 min post-trauma), intermediate (180–300 min post-trauma) and late observation periods (480–600 min post-trauma) after sham operation (n = 3), CCI (n = 4), ASDH (with 2 ml, n = 5), and a combination of injuries (CCI + ASDH 2 ml, n = 5). All values are given as means ± SEM. * p < 0.05 vs. appropriate baseline. n.s. = Not significant.
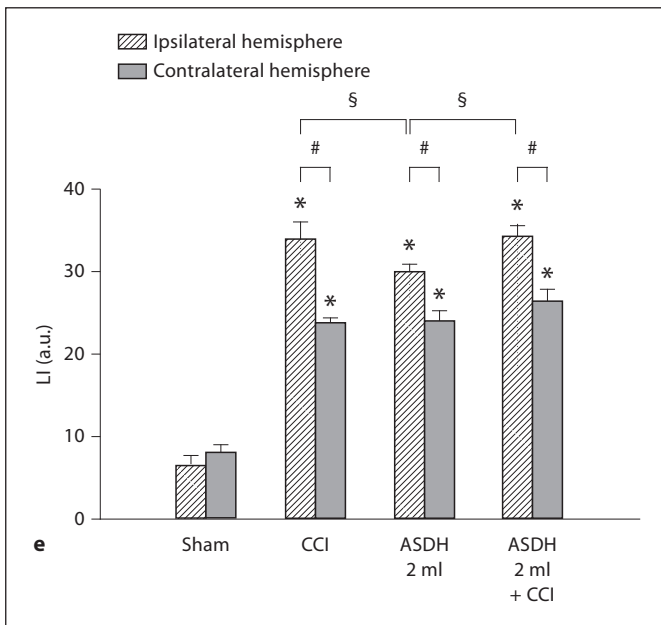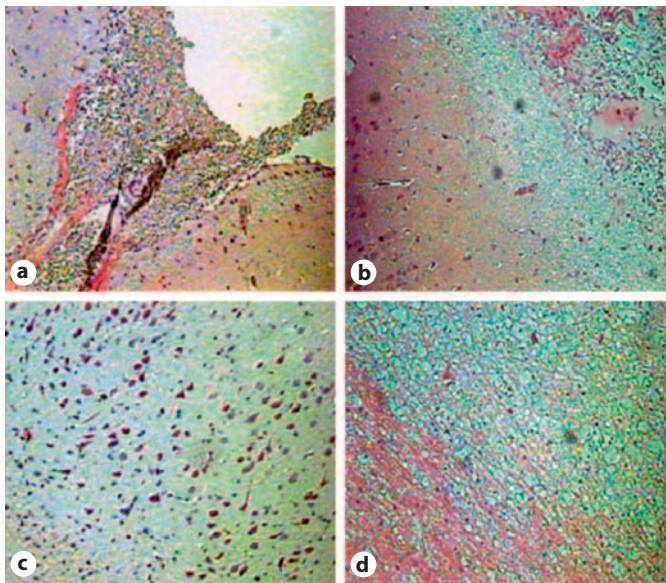
Fig. 6. Examples of HE-stained sections show SAH (**a**), contused tissue (**b**), pyknotic/eosinophilic cells (**c**) and edematous white matter (**d**). Contusion- and ischemia-induced histological changes were included in the LI (**e**; see 'Materials and Methods'). LI was calculated for sham operation, injury by CCI, ASDH (with 2 ml) or a combination of both injuries (CCI + ASDH 2 ml). All injury types produced a significantly increased LI in the ipsi- and contralateral hemispheres when compared to sham (* p < 0.001). # p < 0.05 ipsi- vs. contralateral LI; § p < 0.01 ASDH vs. CCI and CCI + ASDH groups. ASDH 2 ml produced almost no surface contusion and intracerebral hemorrhage which are prominent histopathological features in both CCI groups. All other indicators for tissue damage did not differ significantly between injured animals. Values are given as means ± SEM.

nation CCI + ASDH 2 ml groups (p < 0.05) indicating ipsilateral brain swelling after CCI, but not after ASDH 2 ml alone.

Lesion Index

As can be seen in figure 6, all injuries caused highly significant lesions in both hemispheres (p < 0.001 vs. sham; ipsilateral: sham 6.36 ± 1.41; ASDH 2 ml, 30.0 ± 0.95, CCI 34.6 ± 1.3; CCI + ASDH 2 ml 34.6 ± 1.2; contralateral: sham 8.18 ± 0.83; ASDH 2 ml 23.9 ± 1.4; CCI 23.6 ± 1.3; CCI + ASDH 2 ml 26.8 ± 1.1). All injury groups had a significantly bigger LI in the injured hemisphere when compared to the contralateral hemisphere. CCI and the subdural hematoma as secondary injury after CCI produced a significantly larger ipsilateral lesion than 2 ml blood alone. CCI and CCI + ASDH 2 ml did not differ from each other. Analysis of single parameters of the ipsilateral LI revealed that CCI produced more surface contusion, issue lacerations and intracerebral hemorrhages than ASDH 2 ml alone.

Markers for ischemic changes, pyknotic and eosinophilic cells, were situated percontusionally (in animals with CCI), but also disseminated in sulci and gyri all over the cortex in cases of subdural hematoma as a co-injury or as a single injury. Otherwise, distribution of damaged tissue and density of dead or dying cells were similar for all injury groups.

Discussion

A prevalent problem of TBI is the development of ASDH producing a secondary insult. This leads to complications in as many as 45% of mild-to-severe TBI cases [1, 5]. Although there is a relationship between subdural clot thickness and outcome [21, 31], van den Brink [22] could not confirm a close relationship between clot size and favorable versus unfavorable outcome. Since an acute subdural hemorrhage is normally secondary to TBI, positive correlations between clot volume or thickness and outcome may include a number of false-positive cases and even small subdural blood volumes are found to be detrimental. Thus, the goal of the present study was to determine whether a small subdural hemorrhage can aggravate the injury induced by CCI and whether this can be detected by multi-parametric neuromonitoring.

Traumatic ASDH

Most animal models of ASDH have investigated the effects of non-traumatic ASDH on brain damage. In the

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original model Miller et al. [32] used 400 μl subdural blood volume, i.e. around 20% of brain size, to induce a large ischemic lesion in rats. Using modified models even volumes of 100 μl (5% of brain volume) led to reproducible lesions [20]. Such a small subdural blood volume raises ICP only shortly to 15.2 mm Hg in rats, but causes significant brain damage. Comparably, infusion of 2 ml subdural blood volume in our porcine ASDH model did not lead to massive and long-lasting alterations in most neuromonitoring parameters. However, already this small ASDH with 2 ml which represents only ~3% of intracranial volume caused acute pathophysiological changes which translated into significant tissue damage and edema formation. Therefore, we decided on the basis of online parameters (ICP, ptiO$_2$) from our previous CCI study, in which a moderate contusion produced significant neuropathophysiological and histological changes [17], to combine these two injury strengths. We expected that the 2 ml blood volume is ideal to potentiate acute and outcome parameters since a 'ceiling effect' might be reached if both single injuries are too strong. It is well known that secondary insults such as hypoxia or hypotension exacerbate the initial traumatic brain damage [26, 33–37]. Sawauchi et al. [26] reported effects of trauma-induced ASDH in rats by combining diffuse injury with subdural hematoma. They manipulated the extent of injury by varying hematoma volume, severity of TBI, or by presence of hypoxemia. Their results revealed that either increasing TBI severity as well as subdural blood volume affected monitoring parameters dramatically. Unfortunately, a high mortality rate occurred and the extent of brain damage was not reported clearly at 5 h after injury. In addition, TBI was combined with relatively large subdural blood volumes and strong ischemia was induced underneath the blood clot. It is therefore still unknown how small and how increasing blood volumes influence monitoring parameters and histological outcome.

Effects of a Small ASDH on Cortical Contusion

A model using moderate cortical contusion and 2 ml subdural blood combines focal contusion with parenchymal damage and diffuse injury due to volume-induced local pathophysiological changes underneath the blood clot. Therefore, we hypothesized for the present study that a 2 ml-ASDH will potentiate CCI-induced brain damage and that some underlying mechanisms can be observed by means of multi-parametric neuromonitoring. Such pathomechanisms could be ischemic CBF, excitotoxicity, lactic acidosis or brain swelling. Other changes, which are not induced by the volume of the hemorrhage, are less eas-

ily accessible. Recently, we could demonstrate that blood constituents also define lesion growth [24]. This notion is in agreement with older studies [for example, 23]. When comparing effects induced by the same subdural volume of blood or an inert oil, different functional and histological outcome was found despite nearly identical patterns for ICP, CPP and CBF changes in rats. Thus, either blood volume or blood constituents can have an influence on lesion growth. In this way, even small ASDH volumes might exert an effect on brain damage. In our pig model, such an influence of blood itself on edema formation or histological outcome could not be demonstrated 12 h after injury. Subdural infusion of 5 ml blood or paraffin oil produced almost identical changes in neuromonitoring parameters and histological outcome [19].

These data suggest that other factors than blood constituents are responsible for the early histological damage due to 2 ml subdural blood infusion. These pathomechanisms could be the significant early release of excitotoxic glutamate and the drop of ptO$_2$ accompanied by significant early lactate elevation or increased early ICP in combination with brain swelling. Both subdural groups, ASDH 2 ml alone or in combination with CCI, showed similar pathophysiological changes, whereas the contusion alone produced only a tendency towards elevated glutamate and lactate. This suggests that the small subdural hemorrhage caused stronger ischemic changes than contusion alone. Although the early rise in glutamate was significantly higher in the CCI + ASDH 2 ml group when compared to contusion alone (table 2), analysis of most parameters revealed only a tendency towards stronger effects of combined injury compared to each single injury. ASDH as secondary insult had significant effects on ICP, glutamate, lactate, SSEP, brain edema and tissue damage when compared to control values, but failed to potentiate most parameters when compare to the effects of each single injury.

For instance, CCI + ASDH reached the lowest mean tissue oxygen level of all groups, which also remained decreased for the longest time. However, statistical analysis did not reach a significant level when compared to single injuries. A reason for this may be found in the strength of the contusion injury itself. For the present study, closure of the cranial window after CCI had to be delayed by 6 to 7 min when compared to our original porcine CCI study [17]. This delay was caused by the fact that we could place a subdural tubing in the CCI + ASDH 2 ml group only after contusion. In order not to produce flawed results, closure of the craniotomy had to be performed with a delay, even in the CCI group. This could

be considered as short-lasting decompression. Such a small change in our surgical protocol led to weaker effects on ICP, CPP, ptiO$_2$ and biochemical changes after CCI, and consequently on blood-brain barrier breakdown (water content) and tissue damage when compared to Alessandri et al. [17]. Since ICP was largest for the ASDH group, it seems that displacement of ventricular water after CCI influenced superimposed effects of ASDH. As a consequence ICP could not be increased by 2 ml anymore. Our originally moderate CCI might be considered only as mild injury. The lack of potentiating effects by ASDH would be in agreement with data of Sawauchi et al. [26]. They demonstrated that a mild diffuse injury only slightly alters the time-course of ICP, CPP or water content. Furthermore, mild fluid-percussion injury (FPI) in rats did not alter the decrease in hippocampal CBF or electrophysiology induced by transient forebrain ischemia [38]. In this study, mild TBI only eliminated the acute hyperemia after ischemia, but otherwise had no effects on measured parameters. Similarly, the combination of mild-to-moderate FPI with hemorrhagic shock delayed cognitive recovery, but did not influence the time-course of blood pressure, brain swelling, or functional and histological outcome when compared to TBI alone [39]. Thus, TBI causes tissue damage, but a superimposed secondary insult does not seem to worsen outcome if one of the injuries is too mild. On the other hand, Fritz et al. [40] demonstrated that blood withdrawal as secondary insult did not affect global CBF and CMRO$_2$ at 8 h after moderate FPI in pigs. At 24 h after injury, however, CBF was significantly increased. This suggests that time is an important factor for injury development. Therefore, we speculate that a longer survival period may have helped to detect potentiating effects of a small ASDH, at least on histological outcome, in the present study.

On the other hand, a further aspect for injury development and potentiation of effects of a primary insult concerns the type of secondary insult. For instance, hypoxia is a very potent secondary insult. A 10-min reduction in inspired O$_2$ (13%) already caused massive effects on ICP and CPP in a combined injury model [26]. Furthermore, large ASDH induces ischemic/hypoxic damage underneath the hemorrhage in rats [41, 42] or in a porcine model [43]. Measurements of tissue oxygen following 300 μl subdural blood infusion in rats showed a drop of ptiO$_2$ from 30 to <10 mm Hg that is paralleled by a local CBF decrease [Alessandri et al., unpubl. data]. Consequently, low ptiO$_2$ correlates with outcome in TBI patients [e.g. 44]. Furthermore, evolution of tissue degradation underneath an evacuated subdural blood clot is worsened the lower ptiO$_2$ and the higher extracellular lactate levels remaining [45]. Considering ptiO$_2$ and lactate as markers of hypoxia/ischemia, we did not reach a deep and long-lasting level of secondary insult. Tissue oxygen was decreased and extracellular lactate increased in all injury groups mainly during the first post-injury hour, with a slightly stronger effect for the combined injury. In terms of neuromonitoring, both ASDH groups seemed to produce more signs of ischemia when compared to CCI alone. In addition, glutamate that correlates with CBF [12] and outcome [46] in TBI patients was especially high in the combined CCI + ASDH 2 ml group. These effects translated at least partially into a different pattern of histological damage. Both single treatments led to significant brain damage, including pyknotic and eosinophilic cells, 12 h after injury. ASDH 2 ml did not cause tissue lacerations, which were found in both contusion groups. Contusion also induced more intracerebral hemorrhage than a subdural hematoma. Similar to our results with CCI, Manley et al. [18] reported little surface contusion but intracranial hemorrhages and some cytoskeletal damage with a contusion depth of 9 mm at 10 h post-CCI. The extent of damage (tissue lacerations, cell loss) increased with the depth of injury. We also expected such a relationship in our combined injury group when compared to single injuries. Comparing different variables of our LI only revealed that ASDH 2 ml did not produce surface contusion and intracerebral hemorrhage. No difference, however, was observed for all other parameters (fig. 6). However, despite an obvious difference in tissue damage and signs for ischemia between CCI and ASDH, injury extent and depth of regions with dead or dying cells were comparable in all groups. The expected more severe injury induced by CCI + ASDH 2 ml may only be detected with a more severe injury or longer survival. This would also allow a better distinction between ischemic and contusional damage in this model. Assessment of histology one or more days after FPI [47], cortical contusion [48] or ASDH [49] allows better distinction between healthy and injured tissue in piglets and pigs.

Whereas ptiO$_2$ and lactate showed a tendency towards a stronger effect of CCI + ASDH 2 ml, the effect of ASDH as second injury was more pronounced for glutamate and SSEP. Increased glutamate, but especially the longer lasting depression of SSEP in the combined injury group, suggest that the small ASDH influences the underlying contusion. SSEP was the only monitoring parameter that was affected throughout the entire monitoring period for the CCI + ASDH 2 ml, but not for the single-injury ani-

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mals. We analyzed N20 peak as first cortical response to peripheral motor nerve stimulation. The reduction in N20 amplitude may indicate that fiber tracks and neurons have been permanently damaged to a greater extent by the combined insults than by CCI or ASDH alone. Unfortunately, the design of our study (i.e. removal the brain 12 h after injury, and assessment of brain edema and histological damage in the same animal) is not suitable to detect differences in damaged fibers. Measuring sensory function after CCI in rats revealed a clear relationship between injury severity, brain damage and loss of sensory-motor function [50]. Reduction in SSEP amplitudes and time-course of changes were comparable for the CCI and ASDH 2 ml group, but longer lasting for the combined injury group, indicating a more severe injury in this group. However, the basis for a permanent reduction in SSEP amplitude after CCI + ASDH 2 ml is unknown, and may not be detected by measurements of neuromonitoring parameters in cortical regions alone. It could be that stronger ptiO$_2$ decreases, excitotoxicity by glutamate and energy crises, which are found in severe TBI patients [51–53], might not have been picked-up fully by our sensors due to their positions. This is a major problem when analyzing patient data. Mild or even moderate changes in neuromonitoring parameters are often difficult to interpret and to relate to outcome [see also, 54].

We combined a focal contusion injury with an ASDH as secondary insult into a new and clinically relevant porcine model. The goal was to find synergistic effects of a small hemorrhage as second injury and to detect these by multi-parametric neuromonitoring and histology. The secondary insult prolonged the significant acute glutamate elevation and reduced SSEP amplitude throughout the entire 10-hour monitoring phase when compared to the single injury. Only these two parameters suggest a synergistic effect of ASDH after TBI. All other neuromonitoring parameters seem to indicate that there is no difference between injury type on edema formation and histology. In line with other studies, the secondary insult failed to produce a clear-cut potentiation of brain swelling and tissue damage, which were our outcome parameters, 12 h after injury. We speculate that a threshold injury severity, reached either by the primary or the secondary insult, has to be passed in order to distinguish effects on neuromonitoring parameters due to the insult from each other and to relate changes to outcome. In the present study, 2 ml subdural blood seemed to be slightly below such a threshold volume which would potentiate neuromonitoring parameters and histological outcome. On the other hand, possible synergistic effects might have been confounded by the CCI strength which was reduced by delayed closure of the craniotomy when compared to our original CCI study [17]. Thus, further experiments varying CCI severity in combination with a small ASDH will be necessary to elucidate the risk of a small ASDH after TBI.

Disclosure Statement

No conflicting financial interests exist.

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