Augmented neuronal death in CA3 hippocampus following hyperventilation early after controlled cortical impact

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Minimizing secondary injury after severe traumatic brain injury (TBI) is the primary goal of cerebral resuscitation. For more than two decades, hyperventilation has been one of the most often used strategies in the management of TBI. Laboratory and clinical studies, however, have verified a post-TBI state of reduced cerebral perfusion that may increase the brain’s vulnerability to secondary injury. In addition, it has been suggested in a clinical study that hyperventilation may worsen outcome after TBI.

Object. Using the controlled cortical impact model in rats, the authors tested the hypothesis that aggressive hyperventilation applied immediately after TBI would worsen functional outcome, expand the contusion, and promote neuronal death in selectively vulnerable hippocampal neurons.

Methods. Twenty-six intubated, mechanically ventilated, isoflurane-anesthetized male Sprague–Dawley rats were subjected to controlled cortical impact (4 m/second, 2.5-mm depth of deformation) and randomized after 10 minutes to either hyperventilation (PaCO₂ = 20.3 ± 0.7 mm Hg) or normal ventilation groups (PaCO₂ = 34.9 ± 0.3 mm Hg) containing 13 rats apiece and were treated for 5 hours. Beam balance and Morris water maze (MWM) performance latencies were measured in eight rats from each group on Days 1 to 5 and 7 to 11, respectively, after controlled cortical impact. The rats were killed at 14 days postinjury, and serial coronal sections of their brains were studied for contusion volume and hippocampal neuron counting (CA1, CA3) by an observer who was blinded to their treatment group.

Mortality rates were similar in both groups (two of 13 in the normal ventilation compared with three of 13 in the hyperventilation group, not significant [NS]). There were no differences between the groups in mean arterial blood pressure, brain temperature, and serum glucose concentration. There were no differences between groups in performance latencies for both beam balance and MWM or contusion volume (27.8 ± 5.1 mm₃ compared with 27.8 ± 3.3 mm₃, NS) in the normal ventilation compared with the hyperventilation groups, respectively. In brain sections cut from the center of the contusion, hippocampal neuronal survival in the CA1 region was similar in both groups; however, hyperventilation reduced the number of surviving hippocampal CA3 neurons (29.7 cells/hpf, range 24.2–31.7 in the normal ventilation group compared with 19.9 cells/hpf, range 17–23.7 in the hyperventilation group [25th–75th percentiles]; *p < 0.05, Mann–Whitney rank-sum test).

Conclusions. Aggressive hyperventilation early after TBI augments CA3 hippocampal neuronal death; however, it did not impair functional outcome or expand the contusion. These data indicate that CA3 hippocampal neurons are selectively vulnerable to the effects of hyperventilation after TBI. Further studies delineating the mechanisms underlying these effects are needed, because the injudicious application of hyperventilation early after TBI may contribute to secondary neuronal injury.

KEY WORDS • head injury • hyperventilation • alkalosis • hippocampus • rat
vulnerable to secondary injury during this period and that additional reduction of CBF by hyperventilation may attenuate the delivery of important energy substrates. Yoshida and Marmarou reported that hyperventilation produces relative ischemia in cat brain after fluid-perfusion injury and demonstrated an increase in brain lactate and inhibition of recovery of the ratio of phosphocreatine to inorganic phosphate. Muizelaar, et al., also demonstrated a loss of brain interstitial bicarbonate buffer after sustained prophyllactic hyperventilation in rabbits. It has been reported that hyperventilation after TBI in animals and humans can reduce CBF to what traditionally have been considered ischemic levels. However, defining the ischemic threshold in injured tissue is problematic. Muizelaar, et al., reported that prolonged hyperventilation after TBI in humans may worsen functional outcome, raising questions regarding the appropriate indications and timing for the optimum application of hyperventilation after TBI. Recently published guidelines for the management of severe head injury state that “in the absence of intracranial hypertension, hyperventilation (PaCO₂ ≤ 35 mm Hg) therapy should be avoided during the first 24 hours after severe TBI...” although “hyperventilation therapy may be necessary for brief periods where there is acute neurologic deterioration...” Consistent with these guidelines, in the setting of acute neurological deterioration, aggressive hyperventilation is used by both emergency and critical care personnel. In addition, in the initial stabilization of the brain-injured patient, aggressive hyperventilation (appropriate in the setting of impending herniation, or iatrogenic) occasionally occurs in both the prehospital and acute care settings. The specific impact of hyperventilation during this early low-flow period remains to be determined. Despite the availability of well-characterized rodent models of TBI, which reproduce the early posttraumatic reduction in CBF, the effect of aggressive hyperventilation on histopathological and functional outcome has not, to our knowledge, been investigated. Using a rat model of focal percussive contusion, we hypothesized that aggressive hyperventilation, beginning immediately after TBI and continuing for 5 hours, would worsen functional outcome, expand the contusion, and promote neuronal death in selectively vulnerable hippocampal neurons.

Materials and Methods
Animals and Study Groups
All experimental protocols used in this report were approved by the Animal Care and Use Committee of the University of Pittsburgh. Twenty-six virus-free Sprague-Dawley rats weighing 346 ± 5 g were studied. Food and water were continuously available in their home cages. After TBI the rats were randomly assigned to one of two groups of 13 animals, one receiving normal ventilation (PaCO₂ ≤ 30–40 mm Hg) and one receiving hyperventilation (PaCO₂ ≤ 15–25 mm Hg).

Surgery and Brain Trauma Model
Anesthesia was induced using 4% isoflurane in N₂O/O₂ (2:1). The rats were endotracheally intubated and mechanically ventilated. The isoflurane concentration was reduced to 2% followed by sterile surgical placement of a femoral arterial catheter for continuous mean arterial blood pressure (MABP) and arterial blood gas monitoring. Intramuscular injections of penicillin (100,000 U) and gentamicin (10 mg/kg) were given to minimize the risk of infection. Pancuronium bromide was administered at dosages of 0.1 mg/kg/hour via the arterial line to control ventilation. The rats’ core temperature was monitored using a rectal probe.

After stereotactically guided head positioning, an incision was made and the scalp was retracted, exposing the left parietal bone. A craniotomy was made using a high-speed dental drill aided by a binocular operating microscope. A burr hole was made 5 mm anterior and 2 mm lateral to the bregma in the left side of the skull and a temperature probe (0.009-in outer diameter) was inserted through the burr hole and 2 mm into the left parietal cortex. The bone flap was left in place and the isoflurane was reduced to 1% followed by 30-minute equilibration period. The brain temperature was maintained at 37 ± 0.5°C. Normal arterial blood gas levels were achieved in all rats and PaO₂ was maintained at greater than 70 mm Hg.

The TBIs were produced using a controlled cortical impact device as recently described with minor modifications. Fifteen minutes before controlled cortical impact, an arterial blood sample was obtained for measurement of arterial blood gas levels, glucose concentration, and hematocrit. The bone flap was then removed and a vertical controlled cortical impact (4 m/second impactor velocity, 2.5-mm deformation depth) was delivered onto the exposed dura overlying the left parietal cortex. The bone flap was replaced and sealed with dental cement and the scalp was sutured.

Study Design
The study protocol was designed to mimic the aggressive use of hyperventilation (as evidenced in the early posttrauma period in the prehospital as well as early hospital setting. Ten minutes after controlled cortical impact, rats were randomized to either the normal ventilation group (13 animals, PaCO₂ range 30–40 mm Hg) or the hyperventilation group (13 animals, PaCO₂ range 15–25 mm Hg). The ventilator was adjusted to maintain normocarbia or hypocarbia for 5 hours after controlled cortical impact. Arterial blood gas readings were obtained at 30 minutes post–controlled cortical impact, then hourly. The MABP was recorded every 30 minutes after controlled cortical impact. Brain and rectal temperatures were recorded every 15 minutes.

At 5 hours after controlled cortical impact, anesthesia was discontinued. Temperature probes and the femoral artery catheter were removed and the rat was weaned from mechanical ventilation in the course of 1 hour and underwent extubation. The time to extubation was recorded. After extubation, supplemental O₂ was administered for 30 minutes. When it had fully recovered, the rat was returned to its cage with full access to food and water.

Functional Outcome and Behavior Assessment
Beam Balance. Vestibulomotor function was tested using the beam balance test in eight rats from each group. One hour before surgery, the rat was placed lengthwise on a 1.5-cm-wide beam suspended above the ground. The time the rat remained on the beam was recorded (up to 60 seconds). The rat was then removed from the beam and the procedure was repeated. Rats were considered trained when they remained on the beam for three consecutive periods of 60 seconds. Beam balance tests were also performed daily on Days 1 to 5 postinjury. Three trials were recorded and averaged each day for each rat.

Morris Water Maze. Cognitive function was tested in the same eight rats from each group using a standard variation of the Morris water maze (MWM) paradigm. A pool 180 cm in diameter and 60 cm deep was painted black and filled with water to a depth of 28 cm. A clear Plexiglas platform 10 cm in diameter and 26 cm high (2 cm below the water surface) was used as the hidden goal platform. The pool was located in a 2.5 × 2.5-m room with numerous extra-maze cues (for example, posters, pipes, bookcase) that remained constant throughout the experiment. Testing started 7 days after controlled cortical impact to avoid confounding effects of motor deficits. The rats underwent four trials per day for 5 consecutive days to assess spatial memory performance. The rats started each trial once from each of the four possible start locations.

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TABLE 1
Physiological values in two groups of rats treated with hyperventilation or normal ventilation after TBI*

<table>
<thead>
<tr>
<th>Value</th>
<th>Baseline</th>
<th>Postrandomization</th>
<th>Baseline</th>
<th>Postrandomization</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.39 ± 0.01</td>
<td>7.37 ± 0.01</td>
<td>7.38 ± 0.01</td>
<td>7.53 ± 0.01†</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>36.7 ± 1.1</td>
<td>34.9 ± 0.3</td>
<td>37.2 ± 0.9</td>
<td>20.3 ± 0.7†</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>165 ± 6</td>
<td>167 ± 4</td>
<td>168 ± 4</td>
<td>180 ± 3†</td>
</tr>
<tr>
<td>base deficit (mmol/L)</td>
<td>2.7 ± 3.4</td>
<td>4.2 ± 0.7</td>
<td>-0.6 ± 0.9</td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td>serum glucose (mg%)</td>
<td>189 ± 9</td>
<td>174 ± 6</td>
<td>158 ± 10</td>
<td>152 ± 9</td>
</tr>
<tr>
<td>hct (%)</td>
<td>36 ± 2.3</td>
<td>35 ± 0.6</td>
<td>32.3 ± 1.5</td>
<td>35 ± 0.6</td>
</tr>
<tr>
<td>time to extubate (min)</td>
<td>NA</td>
<td>28 ± 0</td>
<td>NA</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>brain temperature (°C)</td>
<td>36.7 ± 0.1</td>
<td>37 ± 0</td>
<td>36.6 ± 0.1</td>
<td>37 ± 0</td>
</tr>
<tr>
<td>rectal temperature (°C)</td>
<td>36.5 ± 0.6</td>
<td>37 ± 0</td>
<td>37.1 ± 0.1</td>
<td>37.1 ± 0.1</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>129 ± 4</td>
<td>123 ± 4</td>
<td>129 ± 8</td>
<td>128 ± 3</td>
</tr>
</tbody>
</table>

* All values are expressed as mean ± SEM. Abbreviations: hct = hematocrit; NA = not applicable.
† p < 0.05 at 30 minutes postrandomization compared with baseline.

Histopathological Studies
At 14 days after controlled cortical impact (after completion of all of the functional outcome testing), the rats were anesthetized with 5% isoflurane and killed by perfusion fixation using 10% buffered formalin. Their brains were removed and postfixed at 4°C for a minimum of 1 week, and then cryoprotected in sucrose and cut with a cryotome into 10-µm coronal sections at 1-mm increments from the occipital to the frontal lobe and stained with Cresyl violet.

Contusion Volume. We used a computerized image analysis system to outline the margin of the contusion and the sectional area of the contusion at each 1-mm increment was calculated by an observer (M.L.F.) who was blinded to the treatment group. Contusion volume in each rat was calculated as the sum of these sections.

Hippocampal Cell Counting. Neuronal loss in hippocampal regions CA1 and CA3 pyramidal layers was quantified. A coronal section cut from the dorsal hippocampus underlying the area of contusion, approximately 2.6 mm posterior to the bregma, was used for analysis in each rat. The regions were visualized at ×100 magnification, then localized and counted at ×400 by an observer (R.S.B.C.) blinded to treatment group. Only complete cells with a clearly defined body and nucleus were counted. Surviving pyramidal CA1 and CA3 hippocampal neurons were counted in six separate ×400 fields for each region in both hemispheres. Sections were excluded if the boundary of the contusion extended into the pyramidal layers of the hippocampus or if fixation artifacts precluded accurate counting. Data are reported as the average number of surviving neurons per high-power field for the CA1 and CA3 hippocampal regions in both the ipsilateral and contralateral hemispheres.

Statistical Analysis
Survival was compared between groups using Fisher’s exact test. Between group comparisons of physiological parameters, beam balance, and MWM latencies were made using one- or two-way analysis of variance (ANOVA) for repeated measures where appropriate and post-hoc tests with appropriate correction for multiple comparisons. Contusion volume was normally distributed and was compared between groups using Student’s t-test. Hippocampal neuronal survival in CA1 and CA3 was not normally distributed and was compared between groups using the Mann–Whitney rank-sum test. Significance was defined at a probability level of less than 0.05.

Sources of Supplies and Equipment
Pancuronium bromide and gentamicin were purchased from Elkins-Sinn, Cherry Hill, NJ, and penicillin was acquired from Upjohn, Kalamazoo, MI. The stereotactic head positioning system was obtained from David Kopf, Tujunga, CA. The temperature probe was purchased from Physitemp Corp., Clifton, NJ. The video tracking system (Poly-Trak) was acquired from San Diego Instrument, Inc., San Diego, CA, and the image analysis system (MCID) was from Imaging Research, St. Catherines, Ontario, Canada.

Results
Physiological Parameters
Baseline and 30-minute postrandomization physiological data are presented for all measured parameters in Table 1. After randomization, there was a marked increase in pH and decrease in PaCO₂ in the hyperventilation group (compared with baseline, p < 0.05). Hyperventilation was also associated with a small increase (12 mm Hg) in PaO₂ compared with baseline (p < 0.05). This difference was attributable to the increased minute ventilation and mean airway pressure in the hyperventilation group. At no time were any of the rats hypoxemic (PaO₂ < 100 mm Hg). The entire time course of PaCO₂, arterial pH, MABP, and brain temperature after TBI is given for both groups in Fig. 1. The PaCO₂ and pH levels differed between groups at all time points after randomization (p < 0.05). The MABP and brain temperature were similar in both groups.

Five of 26 rats died during the 14-day study, with all deaths occurring on the day of injury. Two rats remained unresponsive postinjury and were unable to demonstrate any spontaneous respiratory effort for 1 hour after discontinuation of anesthesia and were therefore killed. Three rats developed pulmonary edema and/or respiratory distress and died soon after extubation. There were no differences in mortality between groups (two of 13 in the normal ventilation group compared with three of 13 in the hyperventilation group). There were no differences between groups in time to extubation (Table 1).
Functional Outcome Assessment

**Beam Balance.** There was no difference between groups in motor performance latencies over time ($F_{1,15} = 0.17, p < 0.69, \text{Fig. 2}$). Maximum impairment of performance occurred on Days 1 or 2 in both groups, and eventually returned to baseline. Beam balance performance did not differ significantly between normal ventilation and hyperventilation groups.

**Morris Water Maze.** There was no difference between normal ventilation and hyperventilation groups in the time needed to find the hidden platform in the MWM test ($F_{1,15} = 0.50, p < 0.50, \text{Fig. 3}$). In addition, there was a statistically nonsignificant tendency ($t_{13} = 1.77, p < 0.065$) for the rats in the hyperventilation group to swim slower than the rats in the normal ventilation group (30.8 ± 1.0 compared with 35.4 ± 2.1 cm/second).

Histopathological Studies

**Contusion Volume.** At the injury level selected for this study, the contusion was generally restricted to the parietal cortex beneath the impact site. Contusion volume in both groups is shown in $\text{Fig. 4}$. There was no difference between groups ($27.8 ± 3.3 \text{ mm}^3$ in the normal ventilation group compared with $27.8 ± 5.1 \text{ mm}^3$ in the hyperventilation group) in this outcome parameter.

**Hippocampal Cell Counting.** Figure 5 shows the number of surviving neurons/hpf in the CA1 and CA3 regions of the dorsal hippocampus ipsilateral to the contusion. There were no differences in the number of surviving CA1 hippocampal neurons between groups after controlled cortical impact. There was, however, a further reduction in the number of surviving CA3 neurons in the hyperventilation group after controlled cortical impact compared with the normal ventilation group (normal ventilation 29.7, range 24.2–31.7 neurons/hpf, compared with hyperventilation 19.9, range 17–23.7 neurons/hpf; median [25th–75th percentiles], $p < 0.05$). Neuronal cell counts in the CA1 and CA3 regions of the hemisphere contralateral to the contusion did not differ in either the normal ventilation or hyperventilation groups (CA1 counts = 55.3, range 52.1–59...
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Discussion

In a model of controlled cortical impact–induced focal contusion in rats, aggressive hyperventilation for 5 hours after TBI augments neuronal death in the CA3 region of the hippocampus ipsilateral to the contusion. However, hyperventilation did not worsen motor function or cognitive outcome, as assessed using standard beam balance and MWM paradigms, respectively, and did not increase contusion volume.

Hippocampal CA3 neurons are selectively vulnerable to delayed neuronal death after TBI. Theories about the mechanisms underlying this process remain speculative. Potential mechanisms include ischemia, TBI-induced excitotoxicity, apoptosis, and inflammation.

Yamakami and McIntosh reported reduced CBF as early as 15 and 30 minutes after TBI. Using a piglet model of TBI, Pfenninger, et al., reported CBF reduction as early as 5 minutes post-TBI. Some flow levels were in the range consistent with ischemia. We have previously demonstrated that the hippocampus and cortex ipsilateral to the impact show marked flow reduction (at least 60%) at 2 hours after TBI in the controlled cortical impact model. Cerebral blood flow approaches ischemic levels in the core of the contusion at 2 hours postinjury. Although we have not evaluated the reactivity status of the cerebral circulation to changes in PaCO₂ at 2 hours after TBI in this model, we have reported that CO₂ reactivity is impaired, although still present (62–71% of baseline) in and around the contusion at 24 hours after controlled cortical impact in rats.

Hyperventilation rapidly reduces cerebral blood volume and intracranial pressure (ICP). In some studies, this intervention has been associated with CBF values consistent with ischemia or brain tissue hypoxia. After global cerebral ischemia in dogs, hyperventilation did not increase neuronal death; however, the brains were assessed at 8 hours after reperfusion, and neuronal death may be delayed. Although ischemia may be considered a contributing mechanism in the observed augmented neuronal death, ischemia alone is an inadequate explanation for our findings in light of the preservation of CA1 neurons. Although CA1 neurons are known to be selectively vulnerable to ischemic injury, they were not affected by hyperventilation in this paradigm. Furthermore, in our model, CA1 neurons are more proximal to the point of impact in the cortex compared with CA3 neurons. The lack of CA1 neuronal death in light of ischemic and (presumed) anatomical vulnerability weighs against ischemia and primary injury as putative mechanisms of neuronal death in the hippocampus in this model. One limitation in this study is that neuronal counting using traditional histological methods may underestimate cell loss because of a loss of hippocampal volume. We did not use stereological methods in this study. However, CA1 neuronal counts did not differ between groups and were equivalent to those observed in sham-injured animals studied in our laboratory in prior published and unpublished work. In addition, comparisons were only made between injured groups within this study.

Hyperventilation produces cerebral vasoconstriction
Alkalosis exacerbates N-methyl-d-aspartate receptor–mediated neurotoxicity.\textsuperscript{17,18,21,43} As a result of aggressive hyperventilation, the rats in our study were quite alkalotic as indicated by arterial pH measurements. Although we did not measure brain pH, a decrease in PaCO\textsubscript{2} immediately reduces brain interstitial pH.\textsuperscript{40} Although alkalosis appears to have deleterious effects on neurons, acidosis has been shown to have both beneficial and detrimental effects. Giffard, et al.,\textsuperscript{17} and Takadera, et al.,\textsuperscript{54} reported a neuroprotective effect of acidosis via an attenuation of the N-methyl-d-aspartate receptor activation in vitro. Rosner and Becker\textsuperscript{53} reported a deleterious effect of tissue acidosis after experimental TBI in cats. The spatial distribution of brain pH around the contusion and in the hippocampus has not been determined for either normal ventilation or hyperventilation conditions in our model.

Finally, the potential effects of hyperventilation on other mechanisms such as posttraumatic seizures or axonal injury may contribute to the enhanced vulnerability of CA3 neurons. The lateralization of the deleterious effects also raises the possibility that spreading wave depression may be a component of the neurotoxic milieu after TBI in this model of focal contusion.\textsuperscript{20} It could also be the case that the combined effect of alkalosis and further flow reduction by hyperventilation is deleterious in regions vulnerable to excitotoxicity such as CA3. Early, aggressive, or prophylactic hyperventilation, therefore, in the context of reduced CBF, may potentiate excitotoxic mechanisms and augment neuronal death.

Aggressive hyperventilation in the early low-flow period did not worsen functional outcome or expand the contusion, failing to support a significant portion of our initial hypothesis. Ultimate contusion size, in controlled cortical impact or other models of focal contusion, is relatively refractory to manipulation by a variety of interventions.\textsuperscript{44} however, application of hypothermia, particularly prior to injury, reduces contusion volume resulting from controlled cortical impact and lateral fluid-percussion injury.\textsuperscript{13,44} Although we chose rather aggressive hyperventilation in an attempt to produce a maximum effect, we did not test the effect of hyperventilation on a milder contusion, which may be more manageable to secondary insults. The contusion penumbra has not been clearly defined in either of the standard rodent TBI models (controlled cortical impact or fluid–percussion) for any level of injury. It is possible that selectively vulnerable CA3 hippocampal neurons are the only potential target for a deleterious effect of hyperventilation in our model. However, the effect of hyperventilation on the survival of neurons in the dentate gyrus or hilus (all vulnerable to TBI)\textsuperscript{9,29} was not assessed.

Hippocampal damage and memory deficits are common after TBI in humans.\textsuperscript{26,28} This study did not reveal any added effect of hyperventilation on functional outcome deficits as measured by beam balance and MWM latencies. A number of factors may have contributed to this. Our sample size may have limited statistical power; however, this sample size was adequate to detect the exacerbation of functional deficits by the addition of 30 minutes of moderate hypoxemia (PaO\textsubscript{2}, 40 mm Hg) in our model.\textsuperscript{5} Second, the cognitive deficits in this model are modest compared with those detailed in previous reports.\textsuperscript{15} Bilateral hippocampal damage may be necessary to create more marked functional deficits.\textsuperscript{36,37} In addition, CA3 damage may not mediate post-TBI memory deficits, as manifested in MWM test results. Finally, the specific functional outcome paradigm may not have the necessary sensitivity to detect subtle functional deficits. For example, more demanding MWM paradigms have been used by other investigators.\textsuperscript{27,53} However, in support of the testing strategy used, our hypothesis was that hyperventilation would worsen functional deficits.

This study does not completely address the uncommon situation in which, soon after severe head injury, marked intracranial hypertension is observed. Hyperventilation may in fact be life saving in its ability to impede herniation. Similarly, we did not measure ICP or titrate ventilation to control cerebral perfusion pressure, and we evaluated only one level of hyperventilation and injury severity. We did not attempt to model the clinical scenario of optimum titration of ventilation when ICP is increased. In the clinical setting, some investigators have demonstrated a wide variety of beneficial effects of hyperventilation under those conditions, such as homogenization of CBF, normalization of cerebral glucose uptake, and improvement in autoregulation.\textsuperscript{12,41,42} Rather, we chose the worst-case scenario, aggressive hyperventilation during the early posttrauma period when flow is already low and excitotoxicity is peaking.\textsuperscript{45} However, our study does show that hyperventilation is associated with a tangible risk to vulnerable neurons in the controlled cortical impact model. To our knowledge, this is the first in vivo study demonstrating that hyperventilation can augment neuronal injury after TBI, suggesting that there is indeed a tradeoff associated with this intervention.

Conclusions

We have demonstrated that aggressive, early hyperven-
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tilation after TBI augments neuronal death in CA3 hippocampus. The further reduction of CBF with hyperventilation during the low CBF state immediately after severe TBI, coupled with alkalinosis, may increase the vulnerability of selected neurons to traumatic injury. Further studies are needed to delineate the relative contributions of these mechanisms to the observed effects. The results of this study reinforce that meticulous attention is necessary to prevent secondary injury after TBI, and a risk in the use of hyperventilation is demonstrated.

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