Potential long-term benefits of acute hypothermia after spinal cord injury: Assessments with somatosensory-evoked potentials*

Anil Maybhate, PhD; Charles Hu, BS; Faith A. Bazley, BS; Qilu Yu, PhD; Nitish V. Thakor, PhD; Candace L. Kerr, PhD; Angelo H. All, MD, MBA

Objective: **Neuroprotection by hypothermia has been an important research topic over last two decades. In animal models of spinal cord injury, the primary focus has been assessing the effects of hypothermia on behavioral and histologic outcomes. Although a few studies have investigated electrophysiological changes in descending motor pathways with motor-evoked potentials recorded during cooling, we report here hypothermia induced increased electrical conduction in the ascending spinal cord pathways with somatosensory-evoked potentials in injured rats. In our experiments, these effects lasted long after the acute hypothermia and were accompanied by potential long-term improvements in motor movement.**

Design: **Laboratory investigation.**

Setting: **University medical school.**

Subjects: **Twenty-one female Lewis rats.**

Interventions: **Hypothermia.**

Measurements and Main Results: **All animals underwent spinal cord contusion with the NYU-Impactor by a 12.5-mm weight drop at thoracic vertebra T8. A group (n = 10) was randomly assigned for a systemic 2-hr hypothermia episode (32 ± 0.5°C) initiated approximately 2.0 hrs postinjury. Eleven rats were controls with postinjury temperature maintained at 37 ± 0.5°C for 2 hrs. The two groups underwent preinjury, weekly postinjury (up to 4 wks) somatosensory-evoked potential recordings and standard motor behavioral tests (BBB). Three randomly selected rats from each group were euthanized for histologic analysis at postinjury day 3 and day 28. Compared with controls, the hypothermia group showed significantly higher postinjury somatosensory-evoked potential amplitudes with longer latencies. The BBB scores were also higher immediately after injury and 4 wks later in the hypothermia group. Importantly, specific changes in the Basso, Beattie, Bresnahan scores in the hypothermia group (not seen in controls) indicated regained functions critical for motor control. Histologic evaluations showed more tissue preservation in the hypothermia group.**

Conclusions: **After spinal cord injury, early systemic hypothermia provided significant neuroprotection weeks after injury through improved sensory electrophysiological signals in rats. This was accompanied by higher motor behavioral scores and more spared tissue in acute and postacute periods after injury. (Crit Care Med 2012; 40: 573–579)**

KEY WORDS: BBB score; hypothermia; neuroprotection; rat model; somatosensory-evoked potentials (SSEP); spinal cord injury (SCI)

Approximately 12,000 Americans sustain and survive spinal cord injury (SCI) each year with approximately 259,000 currently living with SCI (1). SCI is immediately followed by axonal disruption and vascular and metabolic changes (2). After the initial trauma, secondary injury cascades into extensive damage resulting from inflammation, adverse immune reactions, apoptosis-induced cell death, necrosis, and further nerve and axonal damage, which are also the result of demyelination (2–5). Among other effects, these responses lead to increased production of free radicals and endogenous opioids and excessive release of excitatory neurotransmitters. Cell death around the injury epicenter further promotes Wallerian degeneration and demyelination in somatosensory as well as motor pathways. The central nervous system is known to have a limited regenerative capacity and as a result, the secondary damages resulting from SCI significantly reduce the likelihood of long-term recovery. Therefore, a critical aspect of post-SCI therapeutic intervention is to either prevent or reduce the secondary injury, and recent evidence suggests that this is critical during the first few hours after injury (2, 4–7). For example, a recent retrospective clinical study by Levi et al (7) compared small groups of patients with and without post-SCI intravascular modest hypothermia (48 hrs, 33°C). The study suggested that early systemic cooling may have long-term rehabilitation benefits measured by the Abbreviated Injury Scale.

There have been numerous reports on the potential beneficial effects of hypothermia after injury to the central nervous system (4, 6–11). Whereas most recent clinical reports have focused on the use of cooling for head injury, stroke, cardiac arrest, cardiac surgery, and cerebral or aortic aneurysm repair (12–18),

***See also p. 691.**

From the Departments of Biomedical Engineering (AM, CH, FAB, NVT, AHA), Geriatric Medicine and Gerontology (QY), and Obstetrics and Gynecology (CLK), the Institute for Cell Engineering (CLK), and the Department of Neurology (AHA), Johns Hopkins University School of Medicine, Baltimore, MD.

The financial support for this work came from the Maryland Stem Cell Research Fund (MSCRF Grant #2007-MSCRFII-0159-00 and Grant #2009-MSCRFII-0091-00 awarded to Dr. All) and National Institutes of Health (NIH Grant #R21HD057487-01A1 awarded to Dr. Kerr).

This study was performed at The Johns Hopkins University School of Medicine, Biomedical Engineering Department.

The authors have not disclosed any potential conflicts of interest.

For information regarding this article, E-mail: hmn@jhu.edu

DOI: 10.1097/CCM.0b013e318232d97e

increasing interest has developed for its potential use after SCI in animal models as well as clinical investigations (4, 6, 19–25). Many of these studies reported beneficial effects of hypothermia with gait improvement and reduction in histopathologic damage to gray and white matter structures. Although a few studies have reported on the descending pathways with monitoring the motor-evoked potentials, the ramifications of acute cooling on the somatosensory function have not been reported in detail. Importantly, the afferent somatosensory pathways are an integral part of the complete sensorimotor network and their integrity is essential for successful locomotion. Thus, the electrophysiological consequences of injury to these pathways are critical to determining the neuroprotective role of hypothermia in the spinal cord. We describe the effects of acute, systemic hypothermia on contused spinal cord using a rat model. We present evidence that a single, acute administration of hypothermia can potentially provide long-term functional benefit as measured by somatosensory electrophysiological measurements and motor behavior scores (26, 27). Our results indicate that the neuroprotection provided by 2 hrs of early moderate hypothermia initiated within 2 hrs after injury leads to sustained improvements in somatosensory conduction indicated by somatosensory-evoked potential (SSEP) waveforms, higher BBB scores, and reduced tissue damage up to 4 wks after injury. Preservation of sensory pathways early after injury suggests that acute hypothermia may be potentially beneficial for long-term recovery.

MATERIALS AND METHODS

Animals

A total of 21 adult female Lewis rats (200–220 g; Charles River Laboratories, Germantown, MD) were used for the contusion injury model. The animals were housed individually in cages and had free access to food and water throughout the experimental period. All the procedures were approved by the Institutional Animal Care and Use Committee at the Johns Hopkins University.

Anesthesia

The anesthesia for all surgical procedures, except for the SSEP recordings, was a mixture of 30.4 mg/kg ketamine, 4.3 mg/kg xylazine, and 0.9 mg/kg acepromazine maleate administered through intraperitoneal injection (0.12 mL).

To induce general anesthesia for SSEP monitoring and recording, the rat was held in a transparent chamber with a mixture of 3% isoflurane gas and room air until the onset of drowsiness. The rat's mouth and nose were then placed within an anesthesia mask with a well-fitting rodent-sized diaphragm. A mixed flow influx of 1.5% isoflurane, 80% oxygen, and room air was delivered to the mask at a rate of 2 L/min. This mask was connected to a C-Pram circuit designed to deliver and evacuate the gas through one tube. An adequate level of anesthesia was determined by monitoring hindlimb withdrawal to painful stimuli and the corneal reflex. Rats continued spontaneous breathing; anesthesia depth was maintained throughout the recording. The rats were also placed on a homeothermic blanket (Harvard Apparatus Ltd., Kent, UK) to maintain body temperature at 37 ± 0.5°C as measured by a rectal probe throughout the experiment. Lacrilube ophthalmic ointment (Allergan Pharmaceuticals, Irvine, CA) was applied to the rats' eyes to prevent drying.

Contusion SCI

After the intraperitoneal injection to induce general anesthesia, the back region of the rat was shaved and aseptically prepared with chlorhexidine (Phoenix Pharmaceuticals, Inc., St. Joseph, MO). A midline incision was made along the thoracic vertebrae and the skin was opened. The paravertebral muscles at the region of interest (T6–T12) were retracted. A laminectomy was performed at thoracic vertebra T8 to expose the dorsal surface of the spinal cord without opening the dura mater. The spinous processes of the vertebrae at T6 and T12 were secured in stabilization clamps to reduce the motion of the spinal column during impact. The exposed dorsal surface of the spinal cord at the T8 level was then contused with the NYU weight-drop device by dropping a 10-g rod with a flat circular impact surface from precalibrated height of 12.5 mm. Biomechanical parameters such as the impact velocity of the rod, the distance of cord compression, the cord compression rate, and the dynamic force applied to the cord were precisely monitored using the computer to assure consistency among all rats. There was $<0.05\%$ variation among rats.

Acute Hypothermia

A group of rats (n = 10) was randomly assigned after the injury for hypothermia treatment. After contusion SCI, anesthesia was maintained and rectal temperature was monitored with an anal probe digital thermometer (Physitemp Instruments, Clifton, NJ) and maintained at 37 ± 0.5°C with the help of an electric heating pad for 2 hrs after the time of contusion (Fig. 1). Two hrs after contusion, general hypothermia was induced by spraying the rat with alcohol mist (70% ethanol) and using an electric fan until the rectal temperature dropped to 32 ± 0.5°C. The typical cooling duration was 20 ± 5 mins. The temperature was then maintained at 32 ± 0.5°C for the next 2 hrs. After hypothermic exposure, rats were gradually rewarmed to 37 ± 0.5°C using the heating pad. This rewarming period typically lasted 28 ± 5 mins and was followed by a 90-min observation period without a heating pad to ensure that the animals regained the capability to regulate body temperature on their own. The injured rat was then returned to its cage and was given easy access to food and water.

Postinjury Care

After injury, the muscles were sutured in layers using absorbable 2-0 suture, and the skin was closed with 4-0 suture. After the hypothermia induction in rats under that group, all rats were allowed to recover in a warmed cage and food and water was kept easily accessible. The antibiotic gentamicin (5 mg/kg, intramuscular; Abbott Laboratories, Abbott Park, IL) was administered immediately after surgery and then daily for 7 days. The analgesic, Buprenex (0.01 mg/kg of 0.3 mg/mL, intramuscular; Reckitt Benckiser Pharmaceuticals, Inc., Richmond, VA) was delivered after surgery and daily for 4 days. After surgery, the rats' bladders were expressed two times a day for the first 4 days or until they regained control of the urination. There were no complications or other infections to report. No signs of autotomy or autophagy were observed. The rats were housed for 4 wks after injury and thereafter anesthetized and euthanized by transcardial perfusion with 4% formaldehyde.

SSEP Recording

Electrode Implantation. One wk before injury, the rats were anesthetized and their head region was shaved and aseptically prepared with chlorhexidine (Phoenix Pharmaceuticals, Inc., St. Joseph, MO). A local anesthetic of 2% lidocaine HCl (Abbott Laboratories, North Chicago, IL) was injected under the skin and 1 min later an incision was made along the midline. The cranium bone was cleaned by removing the tissue under the skin. A standard dental drill (Fine Science Tools, North Vancouver, British Columbia, Canada) was used to drill five burr holes into the exposed part of the cranium. Four holes were located on the somatosensory cortex corresponding to the hind- and forelimbs in each hemisphere. On each hemisphere, the forelimb recording sites were located 0.2 mm posterior to bregma

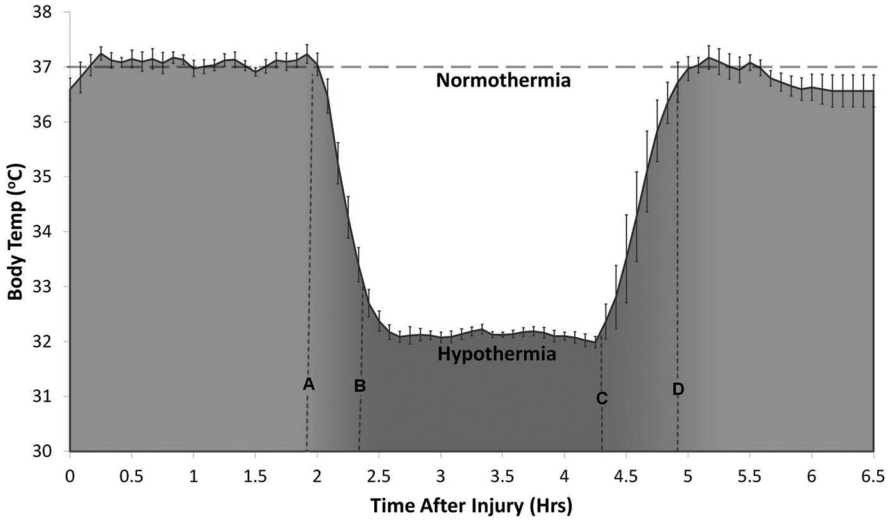

Figure 1. Recorded rectal temperature (mean ± SEM) in a group of ten rats after induction of spinal cord contusion. The temperature control was initiated (t = 0 in the plot) within 30 mins of a contusive spinal cord injury (T8) with 12.5-mm weight drop using the NYU impactor. For the first approximate 2 hrs, the temperature was held constant (37 ± 0.5°C). Systemic hypothermia was induced with cooling down to 32 ± 0.5°C (*A–B*) after which the temperature was again held constant at 32 ± 0.5°C for approximately 2 hrs (*B–C*). This was followed by gradual warming (*C–D*) back to 37 ± 0.5°C and then removal of the heating pad beyond line *D*. A normothermia group (n = 11) with constant temperature 37 ± 0.5°C was used as controls.

and 3.8 mm laterally from the bregma, and the hindlimb recording sites were located 2.5 mm posterior to bregma and 2.8 mm laterally from the bregma. A fifth hole drilled on the right frontal bone, situated 2 mm from both the sagittal and coronal sutures, served as the intracranial reference. Transcranial screw electrodes (E363/20; Plastics One, Inc., Roanoke, VA) were then screwed into the holes such that they made very light contact with the dura mater without causing compression of the brain tissue. The distal end of each electrode was inserted into one of the slots of an electrode pedestal (MS363; Plastics One Inc., Roanoke, VA). To secure the electrodes for long-term recording of cortical SSEPs, carboxylate dental cement (Durelon Carboxylate Cement; 3M ESPE, St. Paul, MN) was used to hold the screw electrodes and the electrode pedestal in place. After hardening of the cement, the skin incision was closed with a 4-0 suture.

SSEP Acquisition. Subdermal needle electrode pairs (Safelead F-E3-48; Grass Technologies, West Warwick, RI) were used to electrically stimulate the tibial nerves of both left and right hindlimbs without direct contact with the nerve bundle. An isolated constant current stimulator (DS3; Digitimer Ltd., Hertfordshire, U.K.) was used for the electrical stimulation of the limbs. A personal computer was interfaced with the stimulator. A neurologic monitoring system (Tucker-Davis Technologies, Alachua, FL) was used to set the stimulation parameters and trigger the stimulator. Positive current pulses of 3.5-mA magnitude and 200-μsec duration at a frequency of 1 Hz were used for limb stimulation, which sequentially stimulated each of the four limbs at a frequency of 0.25 Hz using a custom demultiplexer. Cortical SSEPs from the transcranial electrodes were amplified by an optically isolated biopotential amplifier (Tucker-Davis Technologies). The analog signal from each hemisphere was transferred to a personal computer through an optical data acquisition system with four input channels at a sampling rate of 5 kHz. The electroencephalogram of each hemisphere, containing the SSEP for the respective hemisphere, the stimulation pulse signal, and the stimulated limb number, was recorded on separate channels for postoperative data analysis. Contralateral SSEP recordings were used for analysis. All signal processing was performed using custom algorithms developed with MATLAB7.4 (MathWorks Inc., Natick, MA). The signal-to-noise ratio was improved by ensemble averaging of 100 stimulus-locked sweeps. The first 5 msec in the SSEP signals were generally corrupted by the stimulation artifact and were discarded from the analysis. Furthermore, only the SSEP signal between 5 msec and 30 msec was considered for analysis because a response beyond 30 msec has negligible amplitude and is corrupted with noise.

SSEP Analysis. In the postexperimental SSEP analysis, the signal-to-noise ratio was improved by detrending, subtracting the waveform mean, and applying a 60-Hz notch filter for all waveforms for stimulation of each limb. A custom peak detection algorithm was developed for computing the peak-to-peak mean amplitude and the latency within a window between 5 and 30 msec after the stimulation artifact, a window that corresponds to the nervous systems response to the stimulus. The mean amplitudes and latencies for both the experimental groups were computed for each day of the recording and were subject to statistical comparisons.

Behavioral Tests

Locomotor function was assessed using the BBB locomotor rating scale before injury and every 3 days postinjury. This scoring scale is sensitive to rat lower limb joint movements, hindlimb motion, trunk position and stability, coordination, stepping, paw placement, and tail position; and the details of the scoring scheme can be found in Basso et al (26, 27) and All et al (28). The increasing BBB scores correspond to an increasing level of locomotor recovery and can be categorized into three phases: early, intermediate, and late. Rats were placed individually in a 90-cm plastic open field. Two examiners observed and scored each rat for exactly 4 mins, giving a separate score for each hindlimb. The final given score for each limb was the minimum between the two observers. The rats were scored in a range 0–21, 0 signifying no hindlimb movement and 21 signifying perfect hindlimb movement with extensive joint movements, including ankle, knee, and hip of hindlimbs, consistent plantar stepping, consistent toe clearance, no rotation in stepping, and tail consistently up (26–28).

Histology

On postinjury day 3 and day 28, three rats from each group (normothermia and hypothermia) were randomly chosen for histologic analysis. These animals were euthanized through transcardial perfusion with DPBS (14,190; GIBCO, Grand Island, NY) and with paraformaldehyde solution (4%; 15,713-S; Electron Microscopy Sciences, Hatfield, PA). The spinal cord was then carefully extracted from the vertebrae column and postfixed in 4% paraformaldehyde followed by 30% sucrose solution for 24 hrs and embedded in paraffin for sectioning. Tissue sections were stained with hematoxylin and eosin to assess the morphology of the site of injury.

Statistical Analysis

All data reported are presented as mean ± SEM. Statistical analysis was implemented using commercial software (Stata; StataCord LP, College Station, TX). For each rat, BBB scores from each hindlimb were averaged to yield one score per test session. BBB scores and mean amplitudes and latencies for SSEPs in the hyporthermia and control groups were compared at each time point using the Student's *t* test (two-tailed; unpaired). Differences were considered statistically significant at $p < .01$ to adjust for multiple comparisons.

RESULTS

As described, we used SSEP monitoring, motor behavioral testing, and histologic examination to study the effects of acute hypothermic treatment on the progress of contusive SCI in rats. Rats with normothermia were used as controls.

Figure 2 shows example SSEP sweeps from two rats illustrating the effects of spinal cord contusion on controls and rats subjected to acute hypothermia immediately after injury. Each cluster of waveforms is composed of five SSEP sweeps overlaid, in which each is a moving average of individual time-locked 20 epochs. The baseline SSEP is characterized by latency after stimulus and a peak-to-peak amplitude in both experimental groups. After injury, SSEPs were measured on days 4, 7, 14, 21, and 28. The left panel shows the effects of spinal cord injury on the SSEPs from an untreated rat (normothermia). The SSEP is virtually absent on day 7 and shows little to no recovery thereafter up to 28 days after injury. The right panel shows an example rat exposed to hypothermia immediately after SCI. After exposure to hypothermia, this rat showed a partial retention of somatosensory conduction 1 wk after injury, as illustrated by the greater amplitude of the SSEP compared with the normothermic rat; this difference continued over the 4-wk observation period.

Figure 3 shows the group results for weekly variation in SSEP amplitudes and latencies (mean ± SEM) for a total of 15 rats. The two panels correspond to the SSEP amplitudes recorded on stimulation of the two hindlimbs. The colors correspond to the two experimental groups (dark gray: normothermia, n = 8; light gray: hypothermia, n = 7). All rats underwent baseline recordings before injury. After the baseline recording, the rats were randomly divided into two experimental groups for hypothermia treatment (n = 10) and normothermia control (n = 11). Three randomly chosen rats from both groups were euthanized for acute histologic assessments on day 3. Thus, the weekly SSEPs were monitored for 15 rats (n = 7 in the hypothermia group and n = 8 in the normothermia group).

Four days after injury, the mean SSEP amplitudes for normothermia group decreased from 166.38 ± 13.85 μV to 31.05 ± 3.73 μV and 143.13 ± 18.02 μV to 26.63 ± 7.25 μV for the left and right hindlimbs, respectively. The hypothermia group showed a comparatively smaller decrease in amplitude from baseline to day 4 after injury to 46.54 ± 9.13 μV and 46.49 ± 6.90 μV for the left and right limbs. The difference between the two groups was statistically significant ($p < .01$). In the follow-up period, the mean SSEP amplitudes from the hypothermia group were consistently higher than the normothermia group. The difference between the two groups was found

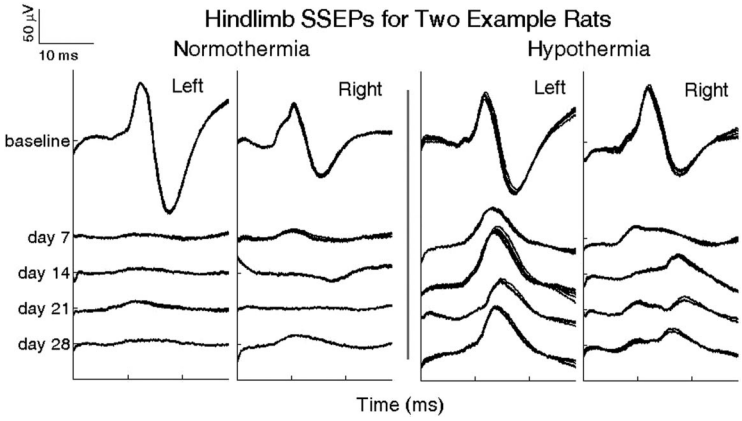

Figure 2. Panels show changes over 4 wks in somatosensory-evoked potentials (*SSEPs*) on hindlimb stimulation in two representative rats after a thoracic spinal cord contusion (T8) with a NYU impactor (12.5 mm impact height). *Left*, A rat with normothermia; *Right*, A rat subjected to an acute 2 hr systemic hypothermia (32 ± 0.5°C) initiated 2 hrs after the contusion.

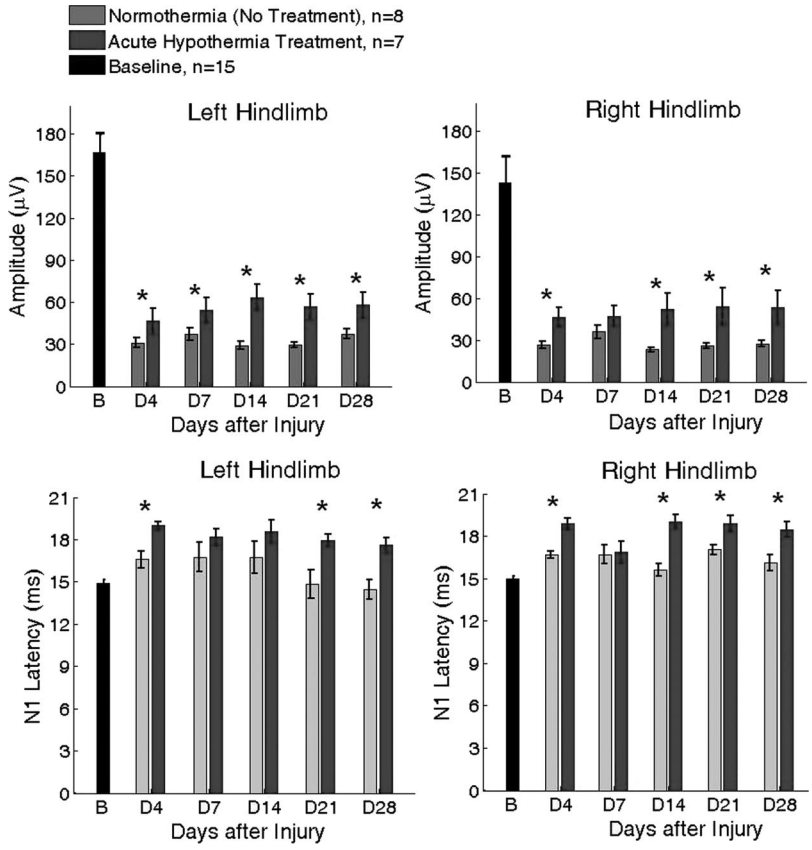

Figure 3. Somatosensory-evoked potential amplitudes (*top panels*) and N1 peak latencies (bottom panels) on stimulation of the two hindlimbs for two groups of rats after spinal cord injury at T8 with impact height 12.5 mm. *Dark gray*: acute, 2-hr hypothermia (n = 7; 32 ± 0.5°C); *light gray*: normothermia (n = 8; 37 ± 0.5°C). Baseline, *B*; days 4, 7, 14, 21, 28 after injury, *D4–D28*. It is important to note that no deleterious effects from hypothermia were detected in any rat as would have been indicated by decreases in somatosensory-evoked potential amplitude (*$p < .01$ between treatment groups; mean ± SEM).

statistically significant for most weekly recordings ($p < .01$). As the bottom panels in Figure 3 show, the SSEP latencies were longer in hypothermia group compared with controls and this difference was found statistically significant on most days between both groups ($p < .05$). These results may be indicative of potentially long-term changes in conduction speed along neural fibers resulting from acute hypothermia. However, a detailed neurobiologic understanding of lasting increases in SSEP latencies months after acute hypothermia needs further investigation.

In addition to SSEP recording, standard BBB motor scoring was performed every 3 days after injury. Figure 4 shows the mean BBB scores for the two experimental groups over the time course of the experiment. All rats were healthy before surgery and exhibited BBB scores of 21 (data not shown). Both groups showed motor function improvement 1 day after injury, yet the hypothermia treated group exhibited better total recovery by day 28, scoring an average of 15.2 ± 2.1 points, than did the control group, scoring only 11.1 ± 1.9 points ($p < .0001$). It is important to note that significant improvements in locomotor activity and balance occur from 11 to 14 points on the BBB score scale (26, 27). A score of 10 indicates only occasional weight-supported plantar steps and no front–hindlimb coordination, whereas a score of 14 means consistent weight-supported plantar steps and consistent front–hindlimb coordination (27). Thus, it is especially interesting to note that the hypothermia group and not the control group exceeded this threshold.

In addition, the two groups showed a significant difference in motor activity 1–4 days after injury with higher SSEP amplitudes in the hypothermia group ($p < .002$). This is consistent with the SSEP data and suggests that hypothermia promotes recovery from the initial or acute impact of the injury itself.

On the postinjury day 3 and day 28, three rats from each group (normothermia and hypothermia) were randomly chosen for histologic analysis as described in the methods. Figure 5 shows representative examples of the acute and postacute effects of hypothermia on the rat spinal cord. The results showed that rats with early postinjury hypothermia showed significantly lower damage on day 3 compared with controls. The dorsal and ventral horns of the gray matter in these rats was preserved to a large extent. In contrast, the normothermia group showed near-complete destruction of the dorsal and ventral horns of the spinal cord. These were clear indications of lower secondary damage in hypothermic rats. The benefits of hypothermia were less discernible on day 28, but the extent of preserved gray matter was still greater in the hypothermia group than in the normothermia group, as shown in the two bottom panels of Figure 5. This is consistent with the normal progression of SCI in which the greatest extent of secondary damage occurs within the first week after injury. Thus, the initial benefit of hypothermia on gray matter preservation does not completely alleviate all of the effects of secondary damage arising in the weeks after injury.

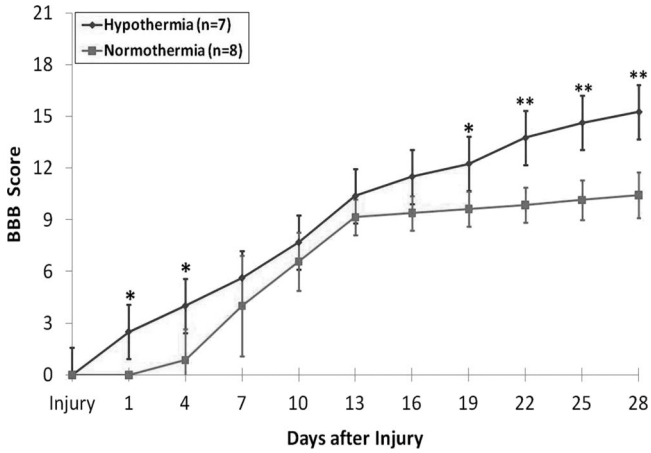

Figure 4. Variation in BBB scores (mean ± SEM) over a period of 28 days in the two groups, normothermia (n = 8) and hypothermia (n = 7). It should be noted that a change in the BBB score from 11 to 14 is a critical milestone in recovery. It is the difference between an animal having almost no front–hindlimb coordination with only occasional weight-supported plantar steps and a consistent coordinated front–hindlimb motion with consistent plantar steps. The hypothermia group exceeded this benchmark in recovery, whereas the normothermia group did not. (*$p < .002$; **$p < .00004$).

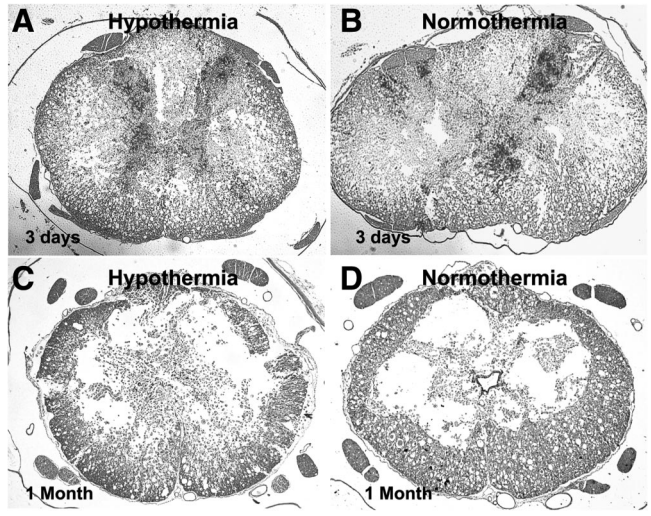

Figure 5. Four representative spinal cord histologic sections at the site of injury of each treatment group at 3 days (*A–B*) and at 1 month (*C–D*) stained with hematoxylin and eosin. At 3 days, better preservation of morphology was observed in the hypothermia-treated rat (*A*) than in the untreated rat (*B*). Tissue analysis showed that preservation of gray matter at 3 days postinjury was significantly better in the hypothermia-treated rat than in the untreated rat. At 1 month, there were no significant difference in observable morphology between the hypothermia-treated rat (*C*) and the untreated rat (*D*), which is reflected in the analysis of gray and white matter tissue-sparing.

DISCUSSION AND CONCLUSIONS

Hypothermic treatment after central nervous system injury has been used successfully as a neuroprotective intervention for both spinal cord and brain trauma in rodents (3, 21, 29–32) and in humans (4, 7, 9, 23). However, the mech-

anisms underlying the neuroprotection have eluded researchers. Most of the animal model research in this context has focused on the two components of motor function, usually assessed by behavioral testing and histologically identified tissue preservation. Few studies have reported the descending tract electrophysiology after hypothermia in rats after SCI with motor-evoked potentials as the outcome measure (3, 23, 31), which focus on the integrity of the descending pathways from the brain to the periphery. In contrast, we have reported here the electrophysiological effects of acute hypothermia after SCI by SSEPs that assess the functional integrity of ascending sensory pathways from the peripheral nerves to the somatosensory cortex.

We presented the potential long-term benefits of acute hypothermia after contusion SCI and its effect on the preservation of ascending somatosensory pathways using SSEP monitoring. Our results showed improvements lasting for several weeks in SSEP amplitudes. This was also accompanied with increased motor behavioral scores and histologic preservation, indicative of neuroprotection. Importantly, early enhancements of SSEP amplitudes in hypothermia-treated rats indicated that the benefits lie in the preservation of somatosensory conductivity after injury and reducing the secondary damages.

Interestingly, hypothermia-treated rats also demonstrated longer latencies. It is possible that longer latency is a direct result of slower somatosensory conduction post-SCI. Another possibility is change in the neural connectivity and compensation indicative of different plastic responses posthypothermia. In a diversity of contexts, few previous studies have explored the effects of moderate or severe cooling on the somatosensory-evoked potentials, for example, in healthy rats (33, 34), in rats immediately after compressive SCI (35); in patients undergoing open heart surgery (36); and in rats after cardiac arrest (16). All of these studies have reported acute elongation of SSEP latencies resulting from hypothermia. Given that the SSEP stimulus travels through the neuronal axons and synapses in a serial manner and the effects of cooling on these are additive (35), the effects of cooling on SSEP may be the result of changes in conductivity as well as connectivity.

These results necessitate future studies to determine the mechanisms of hypothermia after SCI in the SSEP pathways. Indeed, studies in other systems have shown that hypothermia tends to reduce the overall inflammatory response in tissues (37, 38). This is consistent with other studies of hyperthermia in which body temperatures are elevated after injury. In these cases, posttraumatic hyperthermia was evaluated after lower thoracic SCI (39), which demonstrated worsened behavioral and histopathologic outcomes compared with normothermia suggesting a role of body temperature in SCI recovery.

Therefore, it seems reasonable to suggest that at the cellular level hypothermia may reduce redox and heat shock-induced proteolytic activity that causes cell-mediated apoptosis. However, it is not known at this time which cells or which tracts of the spinal cord are preserved or "targeted" by hypothermia. A study by Morino et al (37) suggested that hypothermic treatment is effective for the amelioration of delayed motor dysfunction through inhibition of microglial inflammatory responses. Our results demonstrate that like the motor tracts, the sensory tracts are affected by hypothermic treatment. This is important because sensory functionality is a vital part of the complete sensorimotor functional circuitry. Without recovery of the sensory functionality, motor commands through the descending pathways alone will not be sufficient for successful locomotion.

Many experimental SCI studies have reported the benefits of both local and systemically administered hypothermia on SCI, specifically focusing on the behavioral tests and demonstrations of tissue preservation by histopathology (3, 5). For example, a study by Yu and colleagues (21) showed benefits of early, systemic hypothermia (33°C) in improving motor function assessed by the open-field locomotor test (26, 27). Also, compared with normothermic SCI rats (37°C), the degree of gray and white matter damage was significantly reduced in cooled rats. These initial results emphasized the beneficial effects of mild cooling on white matter integrity and long tract function. Most hypothermia studies have however relied on the open-field behavioral test, which may be subjective and highly variable in many types of SCI (40). Furthermore, BBB testing while assessing locomotor activity does not necessarily correlate with the functional integrity and neurologic conduction of the spinal cord. Unlike in rodents, these are the processes that define recovery in clinical settings with patients (23). Therefore, the importance of our findings demonstrates how short-term hypothermic treatment after SCI can help preserve the sensorimotor tract immediately after injury and that this benefit persists 1 month afterward in terms of stabilized SSEP signals and significant improvements in BBB scores. Preservation of SSEP pathways early after injury also suggests that acute hypothermia may be beneficial for long-term recovery and may provide a unique window of opportunity for other interventions such as stem cell therapies for remyelination of neuronal axons or regeneration of neurons.

ACKNOWLEDGMENTS

The authors thank Neil O'Donnell and Nikta Pashai of JHU for their help with the behavioral scoring of animals and animal care, Amir Pourmorteza of JHU for insightful discussions, and Liye Zhou of JHU for histologic preparations.

REFERENCES

1. National Spinal Cord Injury Statistical Center: National Spinal Cord Injury Statistical Center Annual Statistical Report. Birmingham, AL, University of Alabama at Birmingham, 2009
2. Gupta R, Bathen ME, Smith JS, et al: Advances in the management of spinal cord injury. *J Am Acad Orthop Surg* 2010; 18: 210–222
3. Dietrich WD, Atkins CM, Bramlett HM: Protection in animal models of brain and spinal cord injury with mild to moderate hypothermia. *J Neurotrauma* 2009; 26:301–312
4. Dietrich W, Cappuccino A, Cappuccino H: Systemic hypothermia for the treatment of acute cervical spinal cord injury in sports. *Curr Sports Med Rep* 2011; 10:50
5. Kwon BK, Sekhon LH, Fehlings MG: Emerging repair, regeneration, and translational research advances for spinal cord injury. *Spine* 2010; 35:S263–S270
6. Marion D, Bullock M: Current and future role of therapeutic hypothermia. *J Neurotrauma* 2009; 26:455–467
7. Levi AD, Casella G, Green BA, et al: Clinical outcomes using modest intravascular hypothermia after acute cervical spinal cord injury. *Neurosurgery* 2010; 66:670–677
8. Dietrich WD: Therapeutic hypothermia for spinal cord injury. *Crit Care Med* 2009; 37: S238–S242
9. Cappuccino A, Bisson LJ, Carpenter B, et al: The use of systemic hypothermia for the treatment of an acute cervical spinal cord injury in a professional football player. *Spine* 2010; 35:E57–E62

10. Kao C-H, Chio C-C, Lin M-T, et al: Body cooling ameliorating spinal cord injury may be neurogenesis-, anti-inflammation- and angiogenesis-associated in rats. *J Trauma* 2010; XX:1–9
11. Batchelor PE, Kerr NF, Gatt AM, et al: Hypothermia prior to decompression: Buying time for treatment of acute spinal cord injury. *J Neurotrauma* 2010; 27:1357–1368
12. Krieger DW, De Georgia MA, Abou-Chebl A, et al: Cooling for acute ischemic brain damage (cool aid): An open pilot study of induced hypothermia in acute ischemic stroke. *Stroke* 2001; 32:1847
13. Bernard SA, Gray TW, Buist MD, et al: Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med* 2002; 346:557–563
14. Fehrenbacher J, Siderys H, Shahriari A: Preservation of renal function utilizing hypothermic circulatory arrest in the treatment of distal thoracoabdominal aneurysms (types III and IV). *Ann Vasc Surg* 2007; 21:204–207
15. Madhok J, Iyer S, Thakor N, et al: Characterization of neurologic injury using novel morphological analysis of somatosensory evoked potentials. *In:* Engineering in Medicine and Biology Society (EMBC), 2010 Annual International Conference of the IEEE. Buenos Aires, Argentina, IEEE, 2010
16. Madhok J, Maybhate A, Xiong W, et al: Quantitative assessment of somatosensory-evoked potentials after cardiac arrest in rats: Prognostication of functional outcomes. *Crit Care Med* 2010; 38:1709
17. Walters JH, Morley PT, Nolan JP: The role of hypothermia in post-cardiac arrest patients with return of spontaneous circulation: A systematic review. *Resuscitation* 2011; 82: 508–516
18. Callaway CW: Refining the use of therapeutic hypothermia after cardiac arrest. *Crit Care Med* 2011; 39:201
19. Martinez-Arizala A, Green B: Hypothermia in spinal cord injury. *J Neurotrauma* 1992; 9:S497
20. Westergren H, Yu W, Farooque M, et al: Systemic hypothermia following spinal cord compression injury in the rat: Axonal changes studied by -APP, ubiquitin, and PGP 9.5 immunohistochemistry. *Spinal Cord* 1999; 37:696–704
21. Yu WR, Westergren H, Farooque M, et al: Systemic hypothermia following spinal cord compression injury in the rat: An immunohistochemical study on MAP 2 with special reference to dendrite changes. *Acta Neuropathol* 2000; 100:546–552
22. Inamasu J, Nakamura Y, Ichikizaki K: Induced hypothermia in experimental traumatic spinal cord injury: An update. *J Neurol Sci* 2003; 209:55–60
23. Kwon BK, Mann C, Sohn HM, et al: Hypothermia for spinal cord injury. *Spine* 2008; 8:859–874
24. Levi AD, Green BA, Wang MY, et al: Clinical application of modest hypothermia after spinal cord injury. *J Neurotrauma* 2009; 26: 407–415
25. Lo TP, Cho K-S, Garg MS, et al: Systemic hypothermia improves histological and functional outcome after cervical spinal cord contusion in rats. *J Comp Neurol* 2009; 514: 433–448
26. Basso DM, Beattie MS, Bresnahan JC: A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma* 1995; 12:1–21
27. Basso DM, Beattie MS, Bresnahan JC: Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. *Exp Neurol* 1996; 139:244–256
28. All AH, Agrawal G, Walczak P, et al: Evoked potential and behavioral outcomes for experimental autoimmune encephalomyelitis in Lewis rats. *Neurol Sci* 2010; 31:595–601
29. Marsala M, Galik J, Ishikawa T, et al: Technique of selective spinal cord cooling in rat: Methodology and application. *J Neurosci Methods* 1997; 74:97–106
30. Chatzipanteli K, Yanagawa Y, Marcillo A, et al: Posttraumatic hypothermia reduces polymorphonuclear leukocyte accumulation following spinal cord injury in rats. *J Neurotrauma* 2000; 17:321–332
31. Dimar JR, Shields CB, Zhang YP, et al: The role of directly applied hypothermia in spinal cord injury. *Spine* 2000; 25:2294–2302
32. Truettner JS, Suzuki T, Dietrich WD: The effect of therapeutic hypothermia on the expression of inflammatory response genes following moderate traumatic brain injury in the rat. *Mol Brain Res* 2005; 138:124–134
33. Budnick B, McKeown K, Wiederholt W: Hypothermia-induced changes in rat short latency somatosensory evoked potentials. *Electroencephalogr Clin Neurophysiol* 1981; 51: 19–31
34. Oro J, Haghighi SS: Effects of altering core body temperature on somatosensory and motor evoked potentials in rats. *Spine* 1992; 17:498
35. Jou I: Effects of core body temperature on changes in spinal somatosensory-evoked potential in acute spinal cord compression injury: An experimental study in the rat. *Spine* 2000; 25:1878
36. Markand ON, Warren C, Mallik GS, et al: Effects of hypothermia on short latency somatosensory evoked potentials in humans. *Electroencephalogr Clin Neurophysiol* 1990; 77:416–424
37. Morino T, Ogata T, Takeba J, et al: Microglia inhibition is a target of mild hypothermic treatment after the spinal cord injury. *Spinal Cord* 2008; 46:425–431
38. Kao C-H, Chio C-C, Lin M-T, et al: Body cooling ameliorating spinal cord injury may be neurogenesis-, anti-inflammation- and angiogenesis-associated in rats. *J Trauma* 2011; 70:885–893
39. Yu CG, Jagid J, Ruenes G, et al: Detrimental effects of systemic hyperthermia on locomotor function and histopathological outcome after traumatic spinal cord injury in the rat. *Neurosurgery* 2001; 49:152
40. Barros Filho TEP, Molina AEIS: Analysis of the sensitivity and reproducibility of the Basso, Beattie, Bresnahan (BBB) scale in Wistar rats. *Clinics* 2008; 63:103–108