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# Objective assessment of CNS function within 6 hours of spinal cord ischemia in rabbits

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**Abstract**
*Purpose.* To develop a neurologic scoring (NS) system to objectively assess CNS function shortly after spinal cord ischemia.
*Methods.* Spinal cord ischemia was induced by temporarily clamping the infrarenal aorta in 27 rabbits anesthetized with isoflurane/$N_2O/O_2$ without muscle relaxants. Animals were divided into group I, normothermic ischemia [I-a, 11 min ($n$ = 8); I-b, 12 min ($n$ = 8)], and group II, 60 min hypothermic ischemia targeted to II-a, 29.5°C ($n$ = 5), and II-i, 30.0°C ($n$ = 6). Postischemic neurologic function was scored from 0 to 6.
*Results.* Seventy-five percent of each group I subgroup ended with paraplegia. Function in the I-b group tended to be worse than in I-a (NS = 1.7 *vs* 1.9; $P > 0.05$). Hypothermia of 29.9 ± 0.1°C protected partially (NS = 2.8), whereas 29.4 ± 0.1°C resulted in significantly higher NS, starting at 150 min ($P < 0.05$ *vs* IIi) with total recovery 5.5 hours ($P < 0.0001$) post re-perfusion.
*Conclusions.* Protection of the spinal cord from ischemia can be objectively quantitated by our system. Protection strategies can be compared within 6 h of the ischemia-insult.

**Key words:** Functional scoring, Spinal cord ischemia

## Introduction

Spinal cord function in humans is usually assessed either by electrophysiologic measurements of varying complexity (sensory evoked or motor evoked potentials) or simply by clinical observation of the integrated sensory-motor function. Electrophysiologic studies in experimental animals are cumbersome, and function after spinal cord ischemia has usually been assessed 2 or 3 days after the insult, following the Tarlov score [1]. The waiting period has been necessary mostly because intravenous anesthetics and/or muscle relaxants, which preclude immediate evaluation, have been used. Because the Tarlov scoring method has an inherent observer's subjective variability, to avoid potential bias in the assessment, a consensus must be reached by more than one trained observer blinded to the experiment [1–3]. The anatomical characteristics of the spinal cord in the rabbit have provided a simple and economical model of neural injury, highly reproducible and relatively easy to produce by temporary infrarenal abdominal aortic clamping; it is regarded as a very practical model of CNS injury [1–4]. However, if the rabbit model is to be used for testing protective strategies of the CNS for eventual human use, the functional assessment method needs to be reevaluated. An objective method, devoid of subjective bias, quantitative, and easily reproducible is highly desirable.

A novel neurologic scoring (NS) system, based on the ability of the animal to clear progressively more difficult specific tasks, was developed by us for evaluation of CNS function (see Appendix). In this research we tested the feasibility of using this NS to discern the degrees of injury caused by two different normothermic ischemic periods and compared the protection afforded by two different hypothermic temperatures.

## Materials and Methods

Experiments were performed on 27 male Japanese white rabbits (KBT-JW, 2.7 to 3.0 kg). The rabbits were initially anesthetized with 5% isoflurane in 65%–70% $N_2O$ and 25%–35% $O_2$ and placed supine on a specially designed table equipped with a heat-exchanging system to allow temperature control. Anesthesia was maintained via tracheotomy tube with 1.5%–3.0% isoflurane without the administration of any muscle relaxant. Ventilation was controlled with a mechanical ventilator

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(SN-490-5-T2, Shinano, Japan) to maintain end-expiratory $CO_2$ concentrations of 4.5%–5.0% (Respina, IH26, San-ei, Japan) during normothermia or closer to 5.5%–6.0% during hypothermia, especially below 32°C, to facilitate control of the hypotension caused by hypothermia [5].

The spinal cord temperature measured with a thermocouple probe in the epidural space of the ischemic cord region in preliminary experiments was found to be stable during the entire ischemic period and practically equal to the esophageal temperature (measured 2 cm above the diaphragm) at the time of aortic clamping. Therefore only esophageal temperatures (Mon-a-therm/Model 6500, Mallinckrodt Medical, St. Louis, MO, USA) were used in this study.

The proximal aortic pressure was recorded using a Polygraph system (Nihon Kohden, Tokyo, Japan) from an 18 Fr polyethylene catheter introduced into the left carotid artery towards the aortic arch, which also served as the access for obtaining samples for arterial blood gas measurements. A 16 Fr polyethylene catheter inserted into the right jugular vein was advanced into the right atrium for administration of maintenance fluids ($5\,ml \cdot kg^{-1} \cdot h^{-1}$ of Hartman's solution). The acid-base status was determined periodically before clamping the aorta to adjust the ventilatory rate, but was corrected with infusion of 1 M $NaHCO_3$ solution [following the formula: 0.3 body weight (kg) × base deficit = $NaHCO_3$ (ml)] only after declamping the aorta, and maintained by additional amounts as required according to hourly arterial blood gas measurements during the entire 6 h of observation. Chlorpromazine (0.1 to $0.2\,mg \cdot kg^{-1}$) was given intravenously, if required, 2 to 2.5 h after the inhalational anesthesia was terminated. Additional sedation (chlorpromazine 0.3–$0.5\,mg \cdot kg^{-1}$ or pentobarbital $15\,mg \cdot kg^{-1}$) was given at the end of 6 h of observation, when the animals were allowed to survive for 24 h. The neck was freed from restraint whenever an NS of 6 was reached or after the 6-h observation period if the animal had not recovered but the animals were allowed to survive for 24 h.

Normothermic ischemia (group I) was used to evaluate the capability of the scoring system to discern degrees of injury, and hypothermic ischemia (group II) was used to compare protective strategies.

In group I animals ($n = 16$), the esophageal temperature was controlled at the target temperature of 38.5°C by heating the table. The animals were subdivided into group I-a (8 rabbits undergoing ischemia for 11 min mean temperature 38.1 ± 0.58°C) and I-b (8 animals for 12 min, mean temperature 38.6 ± 0.21°C).

In group II animals ($n = 11$) undergoing spinal cord ischemia for 60 min, the esophageal temperature was targeted to 30.0°C (group II-i) or 29.5°C (group II-a). All animals were given a bolus of hydroxyethyl starch (isotonic sol) $10\,ml \cdot kg^{-1}$ as soon as the jugular venous access line was secured. Animals were ventilated to maintain at least the normothermic end-expiratory $CO_2$ concentration regardless of the temperature. This is equivalent to the pH-stat management of acid-base balance during cardiopulmonary bypass.

In group II animals, systemic surface cooling was induced with plastic bags containing iced water placed on the chest and the abdomen after completion of all neck surgical preparations. Allowing for the after-drop (usually of 1.5°–1.8°C), the bags were sequentially removed (abdominal bags first) at a temperature slightly above the target temperature so that the nadir of the after-drop would coincide with the targeted temperatures of 29.5°C or 30.0°C.

A midline laparotomy and transperitoneal approach provided access for clamping the aorta distal to the renal artery to induce spinal cord ischemia. In the normothermic groups the clamp was applied for 11 or 12 min, and in the hypothermic groups for 60 min. Laparotomy in the hypothermic groups was performed shortly after removing the chest bag, close to the nadir temperature, which was reached by the time of aortic clamping. The target temperature was maintained during the spinal cord ischemic period by controlling the ambient temperature and the specially designed table temperature. Rewarming by heating the table (excluding a 5.0-cm width of the paraspinal region) to 38°–43°C followed the release of the vascular clamp and abdominal closure in the hypothermic groups. Care was taken to maintain a temperature gradient between the water recirculated through the heat exchanger and the esophageal temperature below 8°C.

Ninety minutes after declamping, when the esophageal temperature had reached 36°–37°C in the hypothermic groups, or 30 min after declamping in the normothermic groups, the anesthetic mixture was terminated and was substituted with 25% to 30% $O_2$. Thirty minutes later, the rabbits were transferred to a temperature-controlled specially designed observation cage. To assess the hind limb movements properly, the rabbit was restrained with a stationary collar but with both hind limbs kept free. The cage was equipped with means to provide ventilatatory support (mechanical ventilation, positive end-expiratory pressure, or $O_2$-enriched air), as well as with interchangeable hurdles to be inserted under the hip area. A progressively higher hurdle (3.5 or 6.5 cm high) was introduced between the abdomen and the table under the hip area as the animal's ability to clear the imposed task increased (See Appendix).

Because recovery after 6 h could not be documented in the first four animals observed for 8 h post-declamping, in the remaining animals the NS was determined every 15 min for a total of 6 h after reperfusion.

Statistical analysis was performed using the commercially available Stat View Ver 4 software (Abacus Concepts, Berkeley, CA, USA). All values are expressed as mean ± SD. Factorial ANOVA followed by Fisher's protected least significant difference were used to determine significant differences in multiple comparisons. Statistical significance was defined as $P < 0.05$.

Experimental protocols were approved by the institutional animal care committee, and animals were cared for in compliance with the Guiding Principles in the Care and Use of Laboratory Animals approved by the Council of the Japanese Physiologic Society.

## Results

All animals tolerated the procedures to allow postoperative neurologic evaluation. The mean blood pressure during the early periods of reperfusion, coinciding with the rewarming period of the hypothermic animals, was significantly higher in the normothermic animals than in the hypothermic animals (Table 1), but there were no significant intragroup differences.

Though two animal in each of the group I subgroups had recovered function to NS 6 at 6 h after reperfusion, the NS of those not recovering (75% of the animals) was consistently lower throughout the entire 6 h of observation (12 min = 1.7 *vs* 11 min = 1.9) with longer ischemic periods, as would be intuitively anticipated, however, the differences were not statistically significant ($P > 0.05$).

None of the II-i rabbits (29.9 ± 0.13°C, range 29.7°–30.1°C) recovered neurologic function. In contrast, the II-a group (29.4 ± 0.15°C, range 29.1°–29.5°C) had reached significantly higher NS ($P < 0.05$ *vs* II-i) as early as 150 min, and all recovered spinal cord function with no neurologic impairment 330 min after reperfusion ($P < 0.0001$ *vs* II-i) (Fig. 1).

Two animals in each group were allowed to survive for 24 h. Those that had reached NS 6 at 6 h after reperfusion remained recovered 24 h later, and those with neurological impairment by 6 h (a score of <5) did not have further improvement 24 h later, if any had worsening of the score.

## Discussion

In order to allow assessment of functional recovery early after the ischemic insult, the use of volatile anesthetic agents without muscle relaxants is mandatory. Isoflurane and $N_2O$ were used at concentrations not significantly protective [6,7] and similar to those used for anesthetic procedures in humans. Intramuscular or intravenous agents often used for experimental work, such as ketamine [3,8] and barbiturates [3,9], are potentially protective by themselves.

The functional recovery progressed with reperfusion time after emergence from anesthesia, and at the latest, by the 6th hour of reperfusion, all animals that had not sustained irreversible changes or were adequately protected had completely recovered. No further recovery was observed beyond 6 h of reperfusion in either normothermic or hypothermic animals. Thus, the selected period of observation of 6 h seems to be adequate for early neurologic evaluation when volatile anesthetics and no muscle relaxants are used.

**Table 1.** Mean arterial blood pressure ± SD (mmHg)

| Period | Time (min) | Hypothermic | | Normothermic | |
|---|---|---|---|---|---|
| | | 29.5°C | 30°C | 11 min | 12 min |
| Laparotomy | | 69 ± 25 | 66 ± 13 | 66 ± 17 | 62 ± 6 |
| Reperfusion | 15 | 42 ± 5* | 42 ± 5* | 63 ± 12 | 64 ± 10 |
| | 30 | 44 ± 8** | 49 ± 7*** | 59 ± 10 | 62 ± 9 |
| | 45 | 44 ± 7 | 49 ± 7 | 60 ± 8 | 71 ± 14 |
| | 60 | 53 ± 11 | 54 ± 9 | 74 ± 12 | 74 ± 14 |
| | 75 | 54 ± 11 | 59 ± 9 | 83 ± 21 | 102 ± 14 |
| | 90 | 54 ± 6 | 60 ± 9 | 94 ± 10 | 108 ± 20 |
| | 105 | 108 ± 8 | 100 ± 12 | 100 ± 25 | 115 ± 23 |
| | 120 | 115 ± 6 | 108 ± 11 | 103 ± 19 | 111 ± 23 |
| | 135 | 119 ± 7 | 112 ± 17 | 105 ± 14 | 112 ± 23 |
| | 150 | 121 ± 9 | 112 ± 14 | 107 ± 17 | 112 ± 21 |
| | 165 | 121 ± 12 | 111 ± 12 | 104 ± 17 | 107 ± 24 |
| | 180 | 121 ± 12 | 114 ± 12 | 104 ± 20 | 104 ± 26 |

* $P < 0.0005$ *vs* 11 and 12 min; ** $P < 0.01$ *vs* 11 and 12 min; *** $P < 0.05$ *vs* 11 min. The mean arterial blood pressure during the first 30 min of reperfusion, before anesthesia washout, could be compared between hypothermic and normothermic groups, and found to be significantly lower, but once anesthetics are washed out, the pressures are not different.

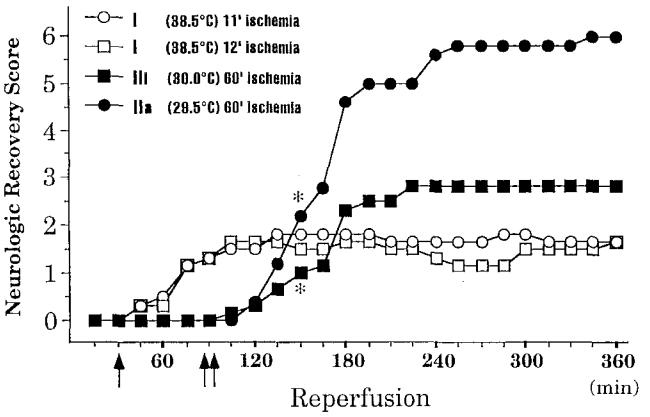


**Fig. 1.** Time course of neurologic recovery as expressed in score from 0 to 6. See Appendix for neurologic score (NS) definitions. The normothermic animals apparently start to recover earlier than the hypothermic animals, but when corrected for their respective anesthesia termination time (*one arrow* = normothermic animals; *two arrows* = hypothermic animals), that difference is abolished. *Earliest time when neurologic scores became significantly different ($P < 0.05$) between the two hypothermic groups. For purposes of clarity, the subsequent increasingly significant $p$ values of the hypothermic animals and SD bars of all groups have been omitted. No efforts were made to compare normothermic and hypothermic animals, since the ischemic periods were so different

Lower blood pressure following aortic declamping in the hypothermic animals theoretically might place them at a disadvantage, and yet group II-a animals had no neurologic deficit.

This newly developed NS seems to reflect the extent of injury. Although the differences were not statistically significant, the longer ischemic period of 12 min indeed resulted in worse NS than 11 min of ischemia, as would be anticipated a priori. Given the fact that two rabbits in each normothermic ischemic group recovered to an NS of 6, and because the differences of those not recovering were not statistically significant, additional studies with different ischemic periods or a larger number of experiments might be needed to illustrate better the ability of this scoring method to discern the extent of injury. What can be said with the available data is that the extent of injury caused by 11 or 12 min of normothermic ischemia is such that functional differences were so subtle as to be beyond the sensitivity of this method.

Whether the use of alpha-stat strategies during hypothermic perfusion and circulatory arrest with nonpulsatile systems would duplicate the present results obtained in animals with pulsatile perfusion and with acid-base management close to pH-stat strategies is not addressed by this study. Acid-base management following pH-stat strategies was recently reappraised in humans and found to be, not surprisingly, more protective than the commonly used alpha-stat management [10]. Nevertheless, hypothermia has been regarded for many years as the gold standard of tissue protection in general [7,11]. Because hypothermia alone reduces energy consumption of any tissue in direct proportion to the degree of cooling [12], circulatory arrest at profound levels of hypothermia, near 20°C or lower, has been used as a very effective protective means and has proven to adequately protect the spinal cord from ischemic time periods close to one hour [4].

In our study, a temperature of 29.4° ± 0.15°C (range, 29.1°–29.5°C) was found to totally preserve (NS = 6) the function of spinal cord subjected to 60 min of ischemia induced by aortic clamping, and a temperature only 0.5°C higher (29.9° ± 0.13°C; range, 29.7°–30.1°C) resulted in partial protection (NS = 3) with delayed recovery or no protection at all (NS = 1). The sharp inflection imposed by only 0.5°C difference suggests that the temperature level of 29.5°C is the critical temperature when protection from 1 h of ischemic period is being sought.

The ability of this scoring method to discern differences in protective strategies has been well demonstrated. The difference in the extent of protection expressed in terms of recovery of the NS afforded by only 0.5°C lower hypothermia is significantly different even as early as 2.5 h after reperfusion. Evaluation of the function in the early phases of reperfusion to assess the rate of recovery might be the important discriminating parameter when comparing protection strategies.

In the past, evaluation using the Tarlov scoring method 2 or 3 days after the insult missed the most informative time frame. Observer's bias with the Tarlov scoring method could only be avoided in animals with total recovery, which is an easy objective end point to identify. Recognizing the limitations of not having sham animal groups without ischemia to evaluate the effects of anesthesia per se, anesthesia length, or hypothermia on the recovery of this new objective NS, the rate of recovery assessable objectively by this new method, nevertheless, made it possible to discern quantitatively the quality of protection afforded by two hypothermic temperatures differing by only 0.5°C, even before full recovery was attained, without being influenced by the subjective bias of the observer. The score is determined by the animal's ability to clear specific tasks, in turn dependent on the degree of injury or recovery from the insult.

This newly described objective scoring makes possible to compare the protection afforded by more than two dissimilar strategies. It is quite obvious that 29.9°C resulted in better, albeit not perfect, neurologic function (NS = 2.8) even after 60 min of ischemia than 11 min (NS = 1.9) or 12 min (NS = 1.7) at normothermia, and hypothermia of only 0.5°C lower (29.5°C)

could totally preserve spinal cord function after 60 min of ischemia.

Although no biochemical markers of neurologic injury were determined and no histological studies were performed to objectively substantiate it otherwise, the rate of recovery was clearly dependent on the quality of protection. Neurologic recovery could only be assessed properly if the animal was totally awake, free from the effects of anesthetics and muscle relaxants, and observed in a properly designed temperature-controlled cage that allowed objective assessment of hind limb movements over progressively higher hurdles, independently of the trunk and front limbs.

Complex electrophysiologic monitoring devices that are cumbersome to fit in experimental animals and immunohistochemical staining methods that are expensive, tedious, time-consuming, and not necessarily more accurate or free from the observer's bias are not absolutely essential to compare different CNS protective agents or strategies if function can be properly assessed, such as with the present objective functional scoring method. This NS system is most appropriate for early quantitative evaluation of spinal cord function, but it may be equally applicable to brain (the other arm of the CNS) function as well.

Our results confirmed that: (a) profound levels of hypothermia are not essential to protect the spinal cord against 60 min of ischemia; a hypothermia level of 29.5°C protected adequately using pH-stat acid-base management; (b) the recovery process following spinal cord ischemia in rabbits can be followed quantitatively by this newly developed simple objective functional scoring method, and be adequately evaluated within 6 h of the ischemia-reperfusion injury without requiring complex electrophysiologic monitoring devices; and (c) this scoring system is particularly suited for comparison of different protective strategies.

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## Appendix: Objective neurologic scoring (NS) system

Rabbits are observed restrained by a stationary collar in a special temperature-controlled cage, equipped with means to provide respiratory care (end-expiratory $O_2$ and $CO_2$ monitoring, mechanical ventilation, positive end-expiratory pressure or $O_2$-enriched air as required, usually the first 60–90 min following anesthesia washout) as well as graded interchangeable hurdles (3.5 cm and 6.5 cm high) to be placed under the abdomen (hip portion). Each recovery stage is defined by the objectively measurable ability of the rabbit to clear the specific height hurdle and not by the observer's subjective evaluation, as follows:

0. Unresponsive. Still under the effects of anesthesia.

1. Assessed in (a) supine position: responsive to stimuli; moves ears and/or head; front limb movements present, but unable to move hind limbs; might be able to flex pelvis and abdomen over the chest spontaneously or in response to any stimuli, by contraction of abdominal muscles, but not the hind limbs proper; or (b) prone position: able to hold the head up, but hind limbs are either flaccid or spastic in extension and unable to arch the trunk, tail movements may be present.

2. Vigorous extension of hind limbs (to the point of being able to arch the trunk) might be present but unable to retract (flex) them under the abdomen when placed prone with the hind limbs extended on flat surface in the cage.

3. Capable of retracting (flexing) both hind limbs under the abdomen if hind limbs are extended on a flat, even surface, either spontaneously or in response to

painful tail pinching. One-half-point credit for each hind limb.

4. Capable of retracting (flexing) both hind limbs extended over a hurdle 3.5 cm high, either spontaneously or in response to painful tail pinching. The back is still straightened and unable to take the normal (rounded back) posture. Falls to one side unless supported.

5. Vigorous retraction (flexion) of both hind limbs in response to painful tail pinching over a 6.5-cm-high hurdle. The back is still straightened and unable to keep the normal (rounded back) posture, although it may be capable of repositioning the body to walk. Lateral support no longer needed.

6. Vigorous retraction (flexion) of both hind limbs over a 6.5-cm-high hurdle spontaneously. Able to keep the normal (rounded back) posture, able to reposition the body to walk, able to kick, able to hop when neck restraint is removed.