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Rectal temperature in the first five hours after hypoxia-ischaemia critically affects

neuropathological outcomes in neonatal rats

Running title: Early temperature and injury after HI

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ABSTRACT

Background: Hyperthermia after hypoxia-ischaemia (HI) in newborn infants is associated with worse neurological outcomes. Loss of thermoregulation may also be associated with greater injury.

Methods: In the postnatal-day 7 (P7) rat, the effect of 5h of graded hyperthermia (38°C or 39°C) immediately after unilateral HI was compared to normothermia (NT, 37°C), and therapeutic hypothermia (TH, 32°C). Early (negative geotaxis) and late (staircase test) behavioural testing was performed, as well as neuropathology scoring in adulthood. Separately, P7 rats were exposed to HI, and individual nesting temperatures monitored before analysis of neuropathology at P14.

Results: Mortality increased as temperature was increased from 38°C (0%) to 39°C (50%) after HI. Hyperthermia also resulted in early behavioural deficits compared to NT animals. In adulthood, pathology scores in the thalamus, basal ganglia, cortex, and hippocampus increased as post-hypoxic temperature increased above NT. Significant global neuroprotection was seen in the TH group. However, no significant difference was seen between HI groups in the staircase test. One hour after HI, the core temperature of pups was inversely correlated with global pathology scores at P14.

Conclusion: Early temperature is a significant determinant of injury after experimental HI. Spontaneous decreases in core temperature after HI may confound neuroprotection studies.

INTRODUCTION

Temperature control after brain injury is critical to optimising recovery and functional outcome. In randomised controlled trials (RCTs) of therapeutic hypothermia (TH) for term newborns with encephalopathy of suspected hypoxic-ischaemic origin, infants in the control (normothermia, NT) groups with elevated core temperatures experienced an increased risk of adverse outcomes (1, 2). Pyrexia in adults after traumatic brain injury (TBI), stroke, or cardiac arrest is associated with greater mortality and worse neurological outcomes (3, 4, 5). Perinatal maternal fever >38°C is also the strongest single risk factor (odds ratio [OR] of 9.3) for cerebral palsy (6). Regardless of patient age and aetiology, optimising temperature after brain injury, and preventing hyperthermia, is therefore an important therapeutic goal.

In the treatment of perinatal asphyxia and resulting hypoxic-ischaemic encephalopathy (HIE), TH is the current standard of care, with better outcomes seen if cooling is commenced early within the six-hour therapeutic window (7). However, the efficacy of TH in certain settings, including the presence of severe encephalopathy or infection-induced inflammation, is still uncertain (8, 9, 10). The relationship between exposure to maternal infection and pyrexia and poor neurological outcome is well-established (6). Aseptic intrapartum pyrexia is also associated with adverse outcomes (11). However, the effect of degree and duration of hyperthermia on brain injury is unknown. In general, spontaneous temperature responses after hypoxic-ischaemic (HI) brain injury are also poorly understood, and may be an important part of the diagnostic and prognostic process for asphyxiated neonates (12). Early thermoregulation is particularly important with regards to asphyxiated infants in the developing world, where the prevalence of (maternal) infection is higher (13), as well as for the investigation of optimal temperature regulation before the initiation of active TH.

The objectives of the current study were twofold: firstly, to investigate the effects of graded increases in hyperthermia temperature after HI on long-term pathological and functional outcomes in the Vannucci rat model of unilateral HI, and secondly: to investigate whether early spontaneous temperature changes after HI are correlated with the degree of injury in this model.

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METHODS

Animals

Experimental procedures examining the effect of controlled post-HI temperature on long-term pathology and behavioural outcome were performed with postnatal-day seven (P7) Wistar rats (Charles River Laboratories, Margate, Kent, UK), carried out under Home Office license in accordance with UK regulations and approved by the University of Bristol's animal ethical review panel. Experiments investigating the effect of spontaneous post-HI temperatures on short-term pathology were performed with P7 Wistar rats (Charles River Laboratories, Sulzfeld, Germany), and reviewed and approved by the University of Oslo's animal ethics research committee. All experiments were carried out in accordance with the approved protocols as well as the ARRIVE (Animal Research: Reporting in vivo Experiments) guidelines. Pups were kept in an animal facility with their dams under a 12h:12h dark:light cycle at 21°C environmental temperature. Dams had access to food and water *ad libitum*, and pups were checked daily for health. For long-term survival and behavioural testing, pups were weaned on P28, and two to three animals were housed (split by sex) per cage until sacrifice at 11 weeks of age.

Vannucci model of unilateral hypoxia-ischaemia

The effects of post-hypoxic temperature on neonatal HI brain injury were assessed using a modified Vannucci model of unilateral HI in P7 rat pups (9, 14, 15). On P7, pups underwent ligation of the left carotid artery under anaesthesia, with 3% isoflurane in a 2:1 gas mixture of NO_2/O_2, via a nose cone. All pups within a litter were separated from their dam for the same period. After recovering under a heat lamp, pups were returned to the nest once all were alert and responsive. After a further 30-minute recovery period with the dam, pups were exposed to 8% oxygen for 90 minutes at 37°C rectal temperature in a specially-designed chamber (9, 14). During hypoxia, core temperature was continuously recorded in each chamber in "sentinel" pups carrying a rectal temperature probe (IT-21, Physitemp Instruments, Clifton,

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NJ). Rectal temperature was maintained within ± 0.2°C of the target using a water-filled mat (Tecotherm, Inspiration Healthcare Ltd Leicester, UK or CritiCool, MTRE, Yavne, Israel) inside the chamber. In P7 rats, rectal temperature correlates within 0.1°C with brain temperature (16).

Controlled post-hypoxic temperature

Prior to hypoxia, pups were randomised by litter, weight, and sex to one of five temperature treatments. In this model, normothermia (NT) refers to a rectal temperature of 37°C, with standard therapeutic hypothermia (TH) treatment occurring at 32°C (14, 15). To assess the effect of increased post-injury temperature, two other treament groups were assigned to 5h at 38°C or 39°C. Immediately after hypoxia, pups were transferred to treatment chambers at the allotted temperature. Rectal temperature was maintained within ±0.2°C of the target, as described above. After 5h of the allocated treatment, pups were removed from the treatment chambers and returned to the dams (17). Control animals without the preceding ligation or hypoxia period underwent 5h of treatment at normothermia (37°C) on P7. A total of five groups were therefore used: Control (CON), and HI followed by 5h at NT (NT37), therapeutic hypothermia (TH32), or hyperthermia at 38°C (HT38) or 39°C (HT39).

Early neurobehavioural testing

Negative geotaxis (i.e. the active movement away from the action of gravity) is an innate neurological reflex in rats that develops in the second week after birth, before their eyes open. To quantify the function of this reflex at P14, pups were placed with their head orientated downhill (14), and the time (in seconds) taken to rotate to face uphill (a rotation of 180º) was recorded. If the pup fell from the platform or did not complete the task in 60 seconds, it was returned to the starting position for a maximum of four attempts. If a pup failed in all attempts, it was given a score of 60. If, on their best attempt, a pup managed to get halfway but then fell off, they were given a score of 59. To enable the ranking of all outcomes (including death)

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for non-parametric statistical testing between groups, pups that died after hypoxia but before negative geotaxis testing at P14 were assigned a score of 61.

Long-term neurobehavioural testing of motor function

Beginning at 8 weeks of age (i.e. 7 weeks after the insult), forepaw fine motor dexterity was assessed using the staircase test (14). Briefly, sucrose pellets (three 45mg pellets per step; BioServ, Frenchtown, NJ) were placed on each of the 7 descending steps of either the right or left staircase. The narrow design of the chamber was such that a rat could only reach the pellets from the left staircase with its left paw, and from the right staircase with its right paw. Thus, by baiting one staircase at a time, each forepaw is tested independently. Rats underwent one trial with each staircase baited per day, five days per week for three weeks. During the first five days of testing, rats were allowed 7.5 mins with each baited staircase. After the first week, the testing period was reduced to 3.5 mins. As the first two steps can be reached with the tongue, retrieval of pellets from these steps was not included in the final analysis. The number of pellets (a maximum of 15 pellets beyond the first two steps per side) retrieved during this time was recorded daily, and the average for the last three days was calculated. To enable the ranking of outcomes for non-parametric statistical testing between groups, rats that died after hypoxia but prior to eight weeks of age were assigned a score of -1 in the staircase test.

Histological preparation and assessment

At 11 weeks of age, transcardiac perfusion with 10% phosphate-buffered (0.1M) formaldehyde was performed under halothane/fentanyl anaesthesia as previously described (14). Brains were immersion fixed and held in 4% formaldehyde until further processing. Coronal 3mm blocks were cut through each brain using a standardised rat brain matrix (ASI Instruments Inc., Warren, MI) and embedded in paraffin. Blocks were sectioned at 6 μm and stained with haematoxylin and eosin. The left side of the brain was examined and scored. Four areas of the brain were examined (cortex, basal ganglia, thalamus and hippocampus) by an

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investigator blinded to the treatment allocation. The severity of damage was graded from 0.0 (no injury) to 4.0 (maximum injury), with 0.5 intervals for each of the 4 regions, giving a 9-step scale of pathology (14, 16, 17). Results were analysed for each individual region, as well as an average of the scores from these regions, giving a global pathology score. Animals that died after hypoxia but prior to 11 weeks of age were assigned a pathology score of 4.5.

Spontaneous post-hypoxic temperature and short-term pathology

To investigate the connection between innate thermoregulatory responses after HI and later brain injury, a separate group of pups underwent HI as described above. Rectal temperatures of all pups were assessed after the end of hypoxia. Repeated rectal temperature measurements were taken from each pup hourly for up to six hours following hypoxia, then every 24 hours until P14. At P14, rats were sacrificed via transcardiac perfusion with saline and 10% neutral-buffered formalin under isoflurane/N_2O anaesthesia. Brains were harvested and kept in 10% neutral-buffered formalin until further processing. Six coronal 3 mm slices were cut through the brain using a standard rat brain matrix (ASI Instruments Inc., Warren, MI), and embedded in paraffin. Sections of 5 µm from the slices best representing the cortex, hippocampus, basal ganglia and thalamus were taken, and stained with hematoxylin and eosin (H&E). Regional and global pathology scores were assessed by an investigator blinded to the treatment allocation, as described above.

Statistical analysis

Statistical analyses were performed using SPSS software version 22 (SPSS Inc., Chicago, IL) and GraphPad Prism version 6.00 (GraphPad Software, La Jolla, CA). Pups used to monitor rectal and skin temperature were excluded because the stress of restraint at normothermia has previously been shown to have a neuroprotective effect in this model (18). Within groups, data was presented as median with 95% CIs for animals that performed the behavioural test or survived into adulthood for assessment of neuropathology. Between-group comparisons,

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including deaths, were carried out using the two-sided Wilcoxon-Mann-Whitney two-sample test. To assess the effect of increasing temperature above NT after HI, Spearman rank correlation analyses were performed on pathology scores and neurobehavioural scores from the NT37, HT38, and HT39 groups. A Spearman correlation analysis was also performed to investigate the effect of spontaneous temperature changes after HI with region-specific and global pathology at P14. A p-value <0.05 was generally considered to be statistically significant. If, however, multiple between-group comparisons were performed for a given outcome, the confidence intervals across treatment groups were compared, and a maximum of five comparisons were considered important. The results of these were adjusted using a Bonferroni correction. Therefore, for multiple comparisons across treatment groups, a p-value <0.05/5=0.01 was considered to be statistically significant (9).

RESULTS

Inclusions and mortality

To determine the effects of HI followed by immediate TH, NT, or hyperthermia (HT), on long-term function and brain pathology, 126 postnatal-day 7 (P7) rat pups were randomised to a control group (CON; n=27), or unilateral HI followed by 5h at one of four controlled temperatures: normothermia at 37°C (NT37; n=34), therapeutic hypothermia at 32°C (TH32; n=34), hyperthermia at 38°C (HT38; n=15) or hyperthermia at 39°C (HT39; n=16). Behavioural testing results were analysed at P14 (negative geotaxis) and 11 weeks of age (Montoya staircase test), followed by assessment of neuropathology after 11 weeks. During temperature treatment, one animal (2.9%) died in the NT37 group, and eight animals (50% mortality) died in the HT39 group.

Negative geotaxis

Compared to the CON group, step-wise increases in median time to rotate 180° at P14 were seen in the TH32, NT37, HT38, and HT39 groups (Figure 1a). For animals that performed the task (i.e. excluding deaths but including animals that performed the test but failed to complete

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it), median (95% CI) time to rotate was 4 (3-6), 9 (8-10), 11.5 (6-59), 15 (5-59), and 60 (6-60) seconds, respectively (Table 1). Including deaths and adjusting for multiple comparisons (Bonferroni correction), the CON group performed significantly better than all HI groups (p<0.001), and the HT39 group performed significantly worse compared to all other groups (p<0.001). No other between-group differences were statistically significant.

Staircase testing and fine motor control

Compared to the CON group, fewer pellets were retrieved from the lowest five steps of the staircase on the right side in the TH32, NT37, HT38, and HT39 groups (Figure 1b). For animals that survived to perform the task, median (95% CI) number of pellets retrieved across the groups was 9.3 (6.7-9.7), 5.7 (5.0-6.7), 5.7 (5-5.7), 4.7 (1.7-6.0), and 5.5 (2.7-11), respectively (Table 1). Including deaths and adjusting for multiple comparisons, the CON group performed significantly better than all HI groups (p<0.001). To determine the effect of hyperthermia on functional outcomes, the NT37 group (n=33 survivors, n=1 death) was compared to the two hyperthermia groups (n=23 survivors, n=8 deaths) in both the negative geotaxis and staircase tests. Including deaths, animals in the normothermia group were significantly faster in the negative geotaxis test at P14 (p=0.007).

Global and regional pathology scoring

Median global pathology score increased with increasing temperature after HI (Table 1). Immediate hypothermia in the TH32 group significantly reduced the global pathology score compare to the NT37 group (p=0.01; Figure 2a), as well as providing significant neuroprotection in the cortex (p=0.004), thalamus (p=0.04), and hippocampus (p=0.009; Figure 2b). Including deaths and adjusting for multiple comparisons, global pathology score increased significantly from the NT37 group to the HT38 group (p=0.003), and from the HT38 group to the HT39 group (p<0.001). Non-parametric Spearman correlation analysis showed a significant correlation between post-HI temperature in the NT37, HT38, and HT39 groups and global pathology (r=0.70, p<0.001). A similar effect of increasing temperature on injury was

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seen in regional pathology scores from the cortex, basal ganglia, thalamus, and hippocampus (p<0.001 for all; Table 1).

Spontaneous post-HI temperature and short-term pathology

Due to the significant influence of early post-hypoxic temperature on long-term outcome, further experiments were performed in the Vannucci model to determine whether spontaneous core temperature after HI could predict later injury. A total of 28 animals received HI on P7 followed by regular rectal temperature measurements and pathology analysis at P14. Two animals (7.1%) died during the survival period, or were sacrificed due to weight loss over two consecutive days. One further animal died as a result of an injury sustained during temperature measurements, and was excluded from the analysis. Immediately after HI, core temperature (median, 95% CI) was 36.6°C (36.3-37.0°C), which fell to 34.2°C (33.2-34.4°C) 1h after HI, and recovered to 35.6°C (35.2-36.1°C) over the next 24h (Figure 3). By P14, median temperature was 36.0°C (35.5-36.2°C). Core temperature 1h after HI correlated significantly with global pathology score at P14 (n=27, Spearman r = -0.55, p=0.003; Figure 4). Including deaths, animals whose temperature 1h after HI was below the 95% CI of the median (<32.2°C) had a significantly worse outcome (p=0.007) compared those with a temperature above the 95% CI of the median (>34.4°C).

DISCUSSION

In the current era of cooling therapy for infants with HIE, a search for the optimal TH protocol is still ongoing. This is exemplified by the "longer and deeper" clinical trial, which was stopped early due to a lack of benefit from longer (120 h vs 72 h) or deeper (32°C vs 33.5°C) cooling in neonates with moderate or severe HIE. These findings were subsequently supported by preclinical data in a range of animal models (9, 19, 20). In order to further elucidate some of the effects of temperature changes after HI, we investigated the effect of graded hyperthermia after HI on functional outcomes and neuropathology, as well as whether early spontaneous temperature responses after an HI insult are correlated with later injury. An

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elevation in temperature of 2°C for 5h after HI resulted in 50% mortality, and caused a significant early behavioural deficit in the negative geotaxis task. Within all studied regions (cortex, basal ganglia, thalamus, and hippocampus), increasing temperature in the range above normothermia (37°C-39°C) was correlated with significantly greater pathology scores in adulthood. In nesting animals monitored after HI, pups with larger drops in core temperature early (1h) after hypoxia had greater global pathology scores one week after the insult.

The current study extends and confirms findings in previous experimental work and clinical reports showing the detrimental effects of post-insult hyperthermia on the neonatal brain (1, 2, 21, 22). Hyperthermia is likely to exacerbate a number of the pathological processes thought to underlie the mechanism of brain injury associated with HI, many of which are potentially ameliorated by TH (23). For instance, increased metabolic rate due to post-asphyxial pyrexia, which may occur as a result of systemic inflammation, seizures, infection, or iatrogenic causes (i.e. overheating), is likely to accelerate and exacerbate the secondary energy failure associated with greater neurological injury after HI (1). In agreement with this, prevention of a +1.5°C hyperthermia after kainate-induced seizures in the Vannucci rat model of unilateral HI has previously been shown to be neuroprotective (21). Hyperthermia of 39°C has also been shown to produce a 5-fold increase in pro-apoptotic caspase-3 activation 24h after HI compared to NT (36.5-37°C) (22). Hyperthermia may cause severe oedema following ischaemia, and in an adult stroke model, hyperthermia of +2°C disrupted the integrity of the blood brain barrier (24, 25). Preventing hyperthermia early after perinatal asphyxia should therefore remain an important clinical goal.

In order to investigate spontaneous post-asphyxial temperature responses experimentally, we exposed P7 animals to an HI insult, and recorded their nesting rectal temperatures daily until P14. One hour after the end of hypoxia, median core temperature was 1.2°C lower in HI rats compared to healthy P7 rats, and increased over the next 24h (9). This is a similar response to that seen in spontaneously-breathing moderately-asphyxiated infants, who experience a drop in core temperature of around 1.5°C more than healthy controls after birth, and also recover their core temperature more slowly (12). In our model, a significant

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negative correlation between temperature 1h after hypoxia and global pathology score at P14 was seen. This was particularly evident in those with a very low temperature 1h after hypoxia (<32.2°C; n=8, 25% mortality). As hypothermia at 32°C is significantly neuroprotective in this model (9, 15), it is unlikely that the lower temperatures were damaging *per se*, and that this instead reflects a greater degree of initial injury. Similarly, animals with a temperature within the upper end of the normal range for P7 rats in our laboratory (>34.4°C; n=7) (9) had a significantly lower median global pathology compared to those with a temperature <32.2°C, suggesting that animals who experience minimal post-HI temperature changes have sustained a milder injury.

Overall, temperature responses to HI appear to be biphasic, with late and early responses corresponding with the latent (1-6h after the insult) and secondary (6-24+h after the insult) phases of HI injury (26). Our data from P7 rats, as well as historical data in asphyxiated neonates (12), suggests a greater relative early hypothermia in those with greater injury. In line with this, early post-HI reductions in cerebral blood flow and temperature production were directly linked to suppressed cerebral metabolism as a result of injury in a fetal sheep model of perinatal asphyxia (27). Similarly, in a bilateral carotid artery occlusion piglet model of neonatal HI, the severity of injury was associated with the degree of suppression of cerebral metabolism during the insult (28). Early suppression of metabolism is largely mediated by adenosine as well as the effect of hypoxia itself (27, 29), with later changes due to endogenous neurosteroid and inflammatory mediators such as allopregnanolone and platelet activating factor (30, 31, 32). However, the decrease in temperature in rats with more severe injury after HI may be due to direct hypothalamic damage and a failure to defend normal temperature, rather than a physiological response to HI. In contrast to the early response, greater injury may then result in later hyperthermia. For instance, recent data using magnetic resonance spectroscopy (MRS) thermometry around the 3rd and 7th days of life showed that those with severe HIE had higher cerebral temperatures compared to those with moderate HIE (33). This hyperthermia is likely to be associated with the hypermetabolism and cerebral hyperperfusion seen during the secondary phase of injury,

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which occurs alongside a systemic inflammatory response (26), and could also partly explain why pyrexia in the 72h after birth in infants with HIE is associated with worse outcomes (1, 2). Temperature during the secondary phase is likely to be controlled by more "traditional" cytokine mediators produced both peripherally and centrally as part of the inflammatory response to injury (31, 34). Therefore, depending on the timing of the temperature measurement, more severe injury may be associated with both relative hypothermia (early) or hyperthermia (late).

This study does have some limitations. Our primary goal was to investigate the effect of hyperthermia after HI, with a standard neuroprotective TH protocol as a control. Interestingly, neither early (negative geotaxis) nor late (staircase testing) was sensitive enough to detect the between-group differences in pathology seen in adulthood, which is a potential limitation. While it is a good test of unilateral fine motor deficits in this model, we have previously seen that performance in the staircase test does not always correlate with global pathology (35). This is why it is important to perform long-term studies when assessing neuroprotective strategies preclinically, and to include both behavioural and pathology scoring. Though inadvertent hyperthermia may increase the speed and degree of damage to the brain (1, 2), in the Western world it is unlikely that asphyxiated infants would spend many hours with significant hyperthermia without clinical intervention. However, the negative effects of hyperthermia on outcome are still relevant for treatment in developing countries, where infection and pyrexia rates are greater (13). With regards to the statistical analysis, the result of enforced hyperthermia at 39°C for 5h after HI (i.e. 50% mortality) required the assignment of behavioural test and pathology scores to animals that died so that they could be included in the analysis to represent the negative effect of hyperthermia. This is potentially analogous to the combined "death and disability" outcomes used in trials of infants with HIE (8). We also assumed that death was worse than survival with maximum injury, and assigned scores appropriately. As a result, non-parametric statistical analyses based on group ranks were used throughout in order to prevent the magnitude of the assigned score from skewing the results. Another limitation is the lack of a sham control group in study 2, and as such we are unable

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to isolate the effects of anaesthesia and surgery on temperature regulation compared to the effect of HI. We also did not directly compare the outcomes of nesting hypothermia with active TH treatment, as the regular temperature measurements taken in the nesting group constitute a stress that may alter outcomes (18). Future work will investigate how TH or hyperthermia affect later temperature regulation, including diurnal changes, as well as whether shorter periods of hyperthermia before the onset of TH negate any of the beneficial therapeutic effects of cooling. In order to optimise and personalise TH treatment, it will be important to discover whether individual temperature responses after HI could guide the most beneficial cooling temperature (9). However, based on the recent failure of the "longer and deeper" trial, as well as recent data from the rat, piglet, and fetal sheep (9, 19, 20), it is likely that deeper or longer cooling will not result in greater neuroprotection. Current cooling protocols (i.e. 33.5°C for 72h), plus controlled passive hypothermia before active TH (as practiced in many neonatal units), are likely to be close to optimal for generalised recommendations.

In conclusion, we describe early behavioural deficits and increased global neuropathological injury in adulthood after unilateral HI and hyperthermia in the neonatal rat. Additionally, in this model, early spontaneous hypothermia was correlated with later injury, which may confound studies of TH neuroprotection if not adequately controlled-for.

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FIGURE LEGENDS

Figure 1. Behavioural testing. Individual performance in the negative geotaxis test at P14 (**a**) and the staircase test at 11 weeks of age (**b**). Error bars show median (95% CI) speed of rotation (**a**) or pellets retrieved (**b**) for animals that survived to performed the task in the control (black triangles), HT32 (white circles), NT37 (light grey circles), HT38 (dark grey circles), and HT39 (hatched diamonds) groups. In the HT39 group, n=8 animals died during temperature treatment (50% mortality). *Adjusting for multiple comparisons, the control group performed significantly better than all other groups (p<0.001 for all) in both the negative geotaxis task and staircase test, and the HT39 group performed significantly worse compared to all other groups in the negative geotaxis task only (p<0.001). No other between-group differences were statistically significant.

Figure 2. Pathology scoring. a) Scatter plot of global pathology score across all five groups. Error bars show median (95% CI) of global pathology scores from survivors in the control (black triangles), HT32 (white circles), NT37 (light grey circles), HT38 (dark grey circles), and HT39 (hatched diamonds) groups. *Denotes significant difference (Wilcoxon-Mann-Whitney test) between groups (p<0.01), including deaths as an outcome and adjusted for multiple comparison. † Indicates an animal that died during temperature treatment. **b**) Floating bar plot (median with 95% CI) of regional pathology scores in survivors in the TH32 (n=34, white), NT37 (n=33, light grey), HT38 (n=15, dark grey), and HT39 (n=8, hatched) groups. Compared to normothermia, hypothermia provided significant neuroprotection in the cortex (p=0.004), thalamus (p=0.04), and hippocampus (p=0.009).

Figure 3. Spontaneous drop in core temperature after HI. Median (IQR) rectal temperature after HI (n=26-28 per time point). One hour after HI core temperature dropped significantly, and increased over the subsequent 24h. *Denotes significant difference (Wilcoxon matched pairs signed rank test) compared to preceding time point (p<0.001).

Figure 4. Post-HI temperature and global pathology. One hour after HI, core temperature of P7 rats is significantly correlated (Spearman r=-0.55, p=0.003) with global pathology score at P14. Including deaths, animals whose temperature 1h after HI was below the 95% CI of the median (<32.2°C) had a significantly worse outcome compared those with a temperature above the 95%CI of the median (>34.4°C; p=0.007). † Indicates an animal that died during the survival period.
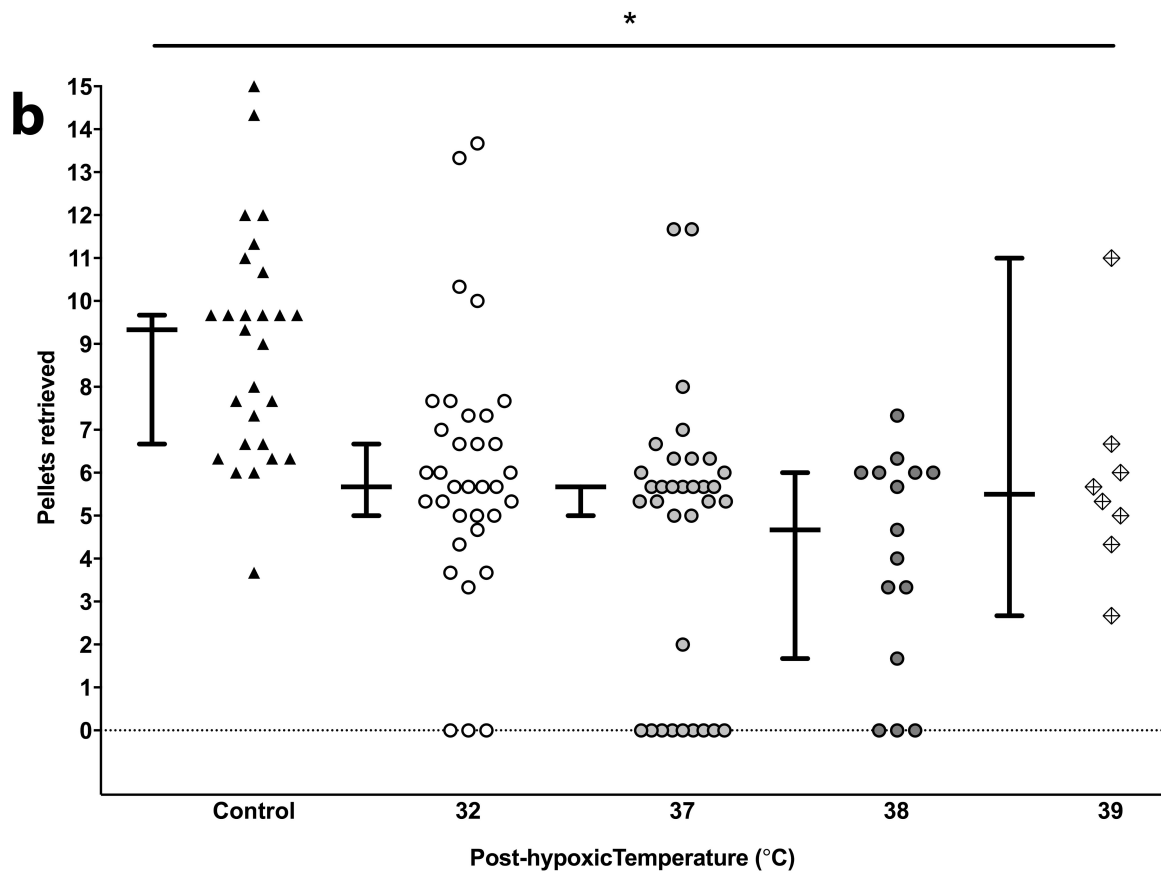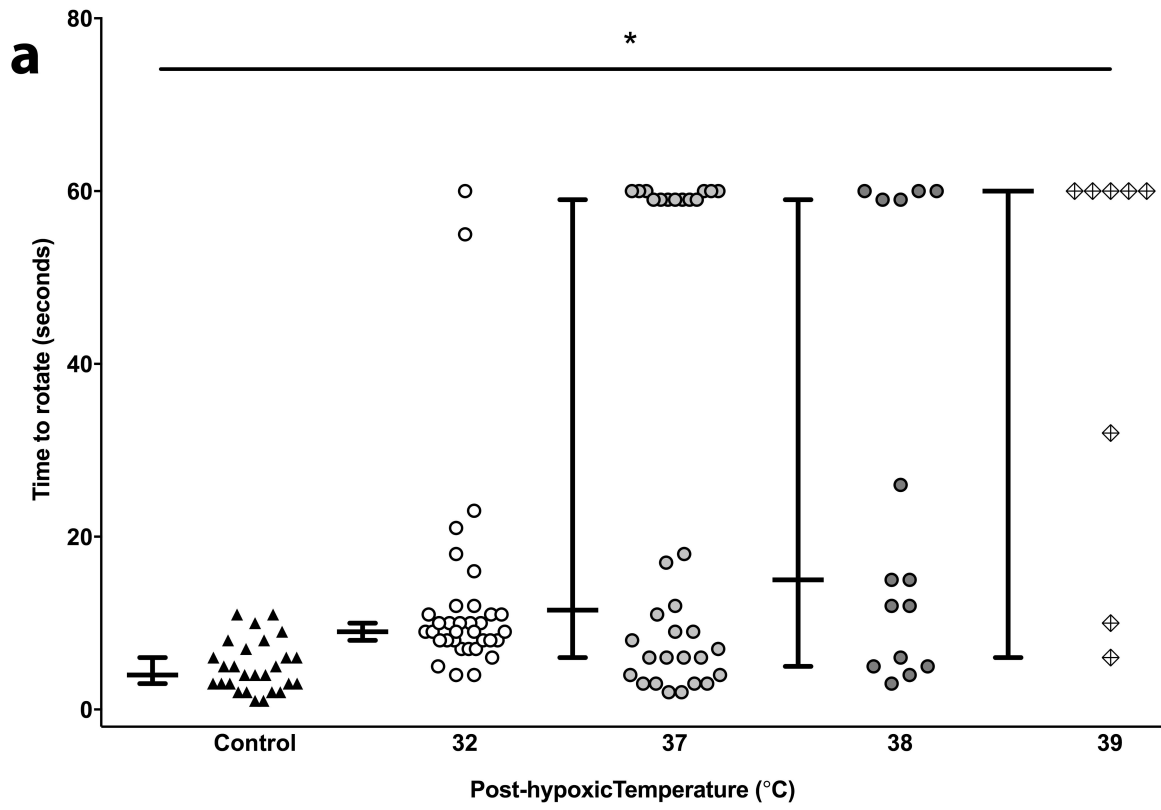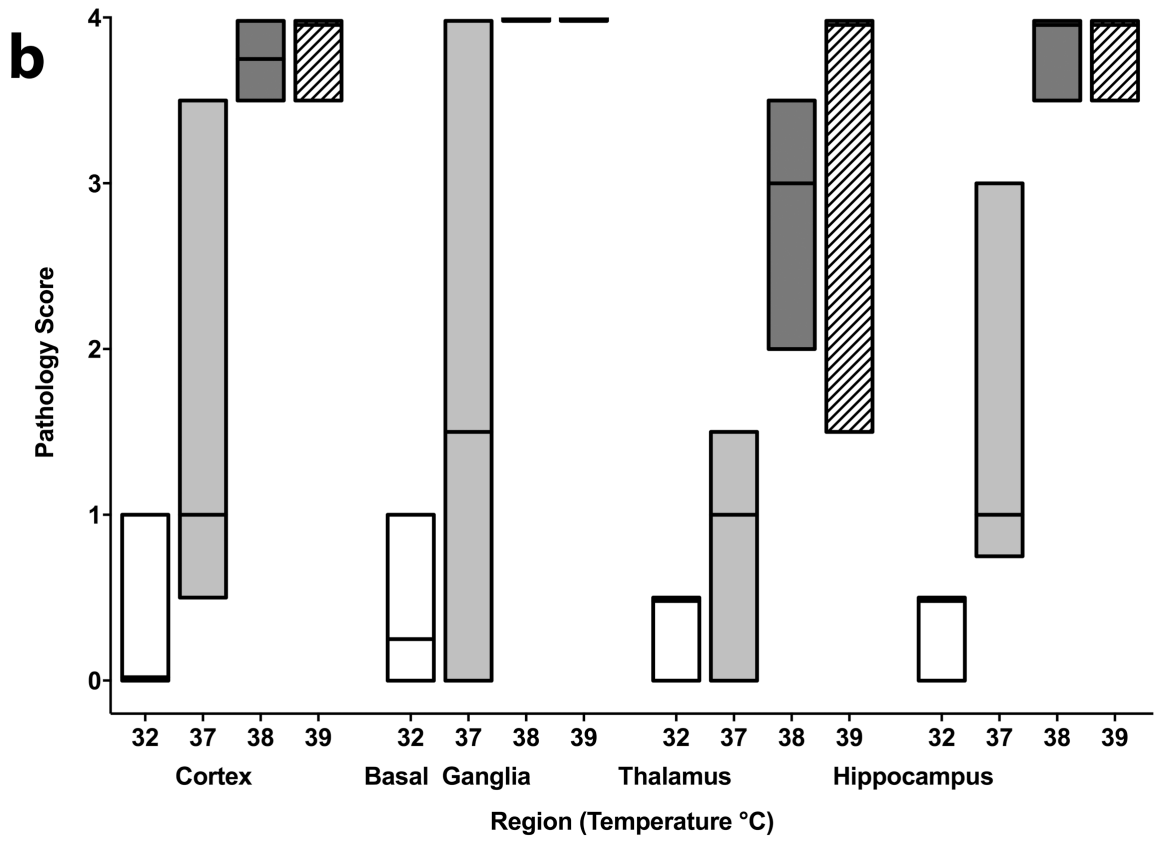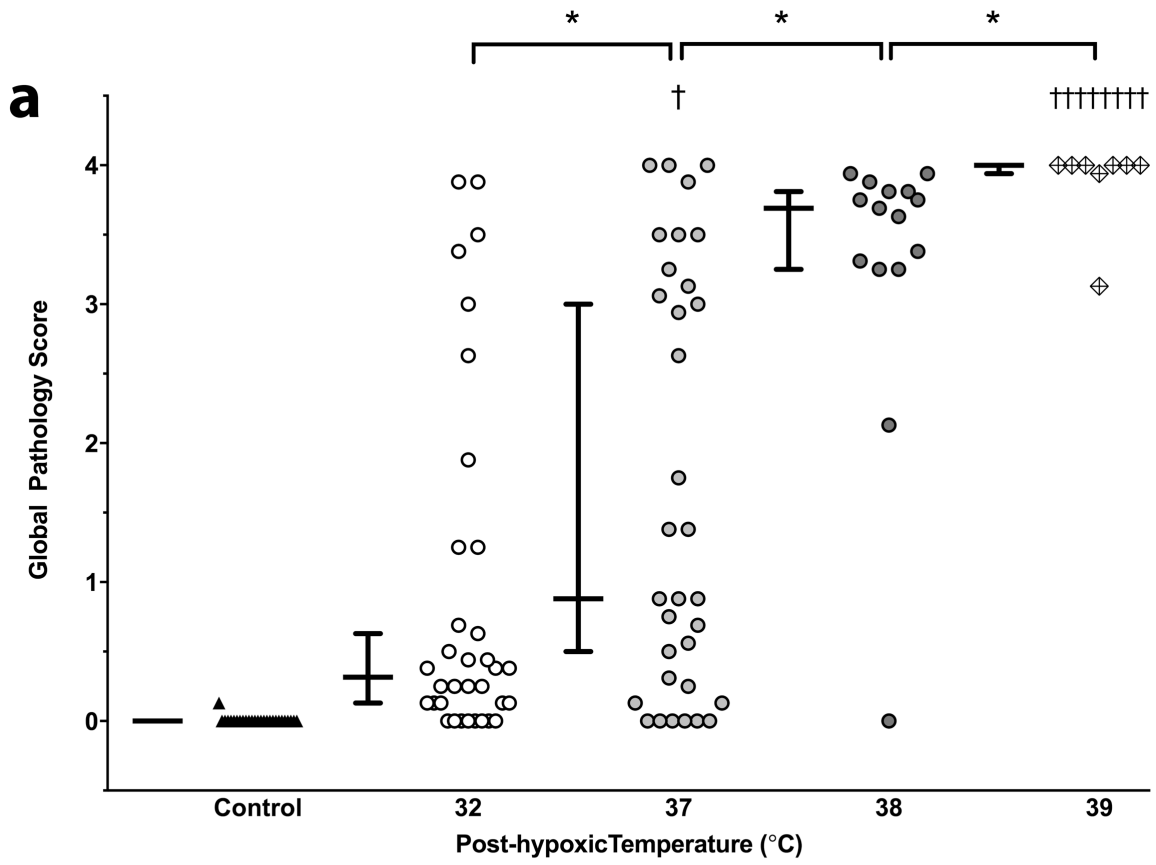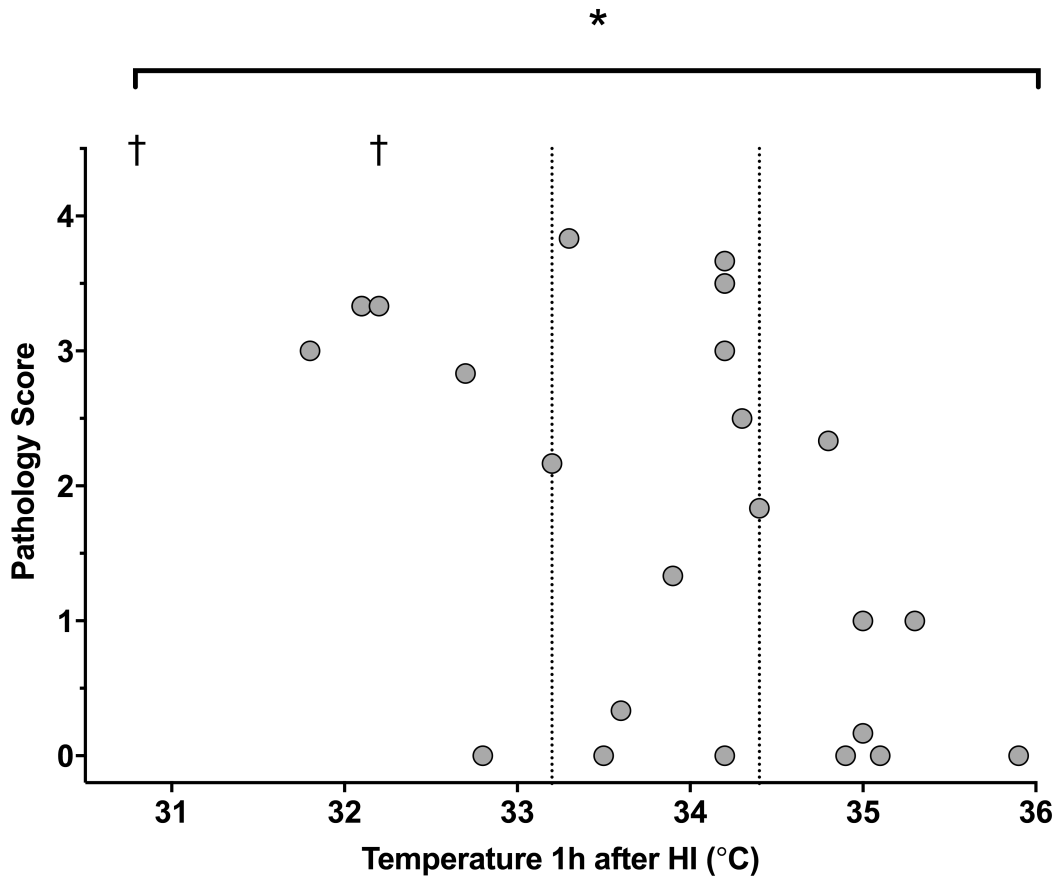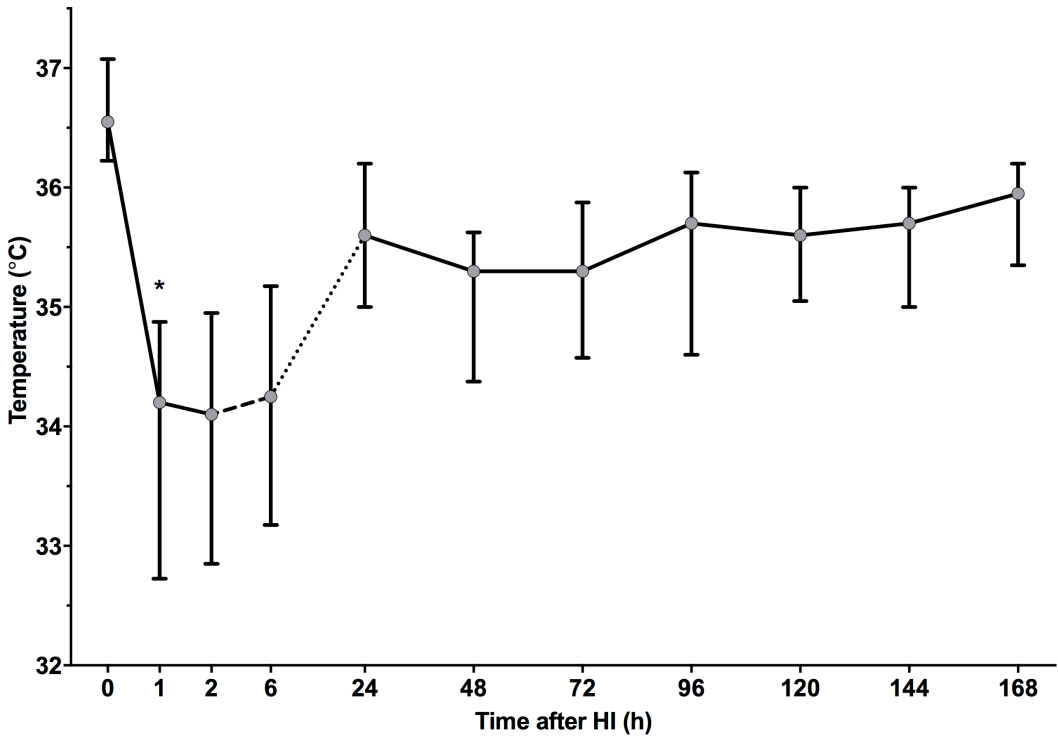
TABLES

Table 1. Effect of hypothermia and graded hyperthermia on motor function and pathology.

	Treatment group					Spearman rank regression (37°C - 39°C) [#]	
	Control (n=27)	TH32 (n=34)	NT37 (n=33)	HT38 (n=15)	HT39 (n=8)		
Negative Geotaxis (seconds)	4.0 (3.0-6.0) [‡]	9.0 (8.0-10.0)	11.5 (6.0-59.0)[a]	15 (5.0-59.0)	60.0 (6.0-60.0)[b]		
Staircase Test (pellets retrieved)	9.3 (6.7-9.7) [‡]	5.7 (5.0-6.7)	5.7 (5.0-5.7)	4.7 (1.7-6.0)	5.5 (2.7-11)		
Pathology Scores						r (95% CI)	P value
Global Score	0.0 (0.0-0.0)	0.3 (0.1-0.6)	0.9 (0.5-3.0)	3.7 (3.3-3.8)	4.0 (3.1-4.0)	0.70 (0.54-0.81)	<0.001
Cortex	0.0 (0.0-0.0)	0.0 (0.0-1.0)	1.0 (0.5-3.5)	3.8 (3.5-4.0)	4.0 (3.5-4.0)	0.71 (0.56-0.82)	<0.001
Basal Ganglia	0.0 (0.0-0.0)	0.3 (0.0-1.0)	1.5 (0.0-4.0)	4.0 (4.0-4.0)	4.0 (4.0-4.0)	0.63 (0.46-0.76)	<0.001
Thalamus	0.0 (0.0-0.0)	0.5 (0.0-0.5)	1.0 (0.0-1.5)	3.0 (2.0-3.5)	4.0 (1.5-4.0)	0.68 (0.52-0.80)	<0.001
Hippocampus	0.0 (0.0-0.0)	0.5 (0.0-0.5)	1.0 (0.8-3.0)	4.0 (3.5-4.0)	4.0 (3.5-4.0)	0.69 (0.53-0.80)	<0.001

Short- (negative geotaxis) and long-term (staircase test) motor function, and global and regional pathology scores across the five treatment groups. Data is described as the group median (with 95% CI) for animals that performed the behavioural tasks and survived for final pathology scoring. [a]Denotes significant difference between NT37 group and the combined hyperthermia (HT38+HT39) groups. [b]Denotes significant difference compared to all other groups. [#]Spearman rank regression includes animals who died during temperature treatment (n=1 in NT group, n=8 in HT39 group), who were assigned a pathology score of 4.5 to allow for ranking of outcomes.