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Lack of Melatonin Secretion Induced by Moderate Hypothermia During Aortic-Arch Surgery: Possible Implications in Neurological Outcomes

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ABSTRACT

Preclinical data from the peculiar animal model of "synthetic torpor" (ST), a reversible hypothermic condition resembling natural torpor but pharmacologically induced in rats (a nonhibernating mammal), suggest that in hypothermic conditions, the neuroprotective effects of melatonin are strongly enhanced. As a protection technique, during aortic-arch surgery, patients are induced a hypothermia similar to ST. Since in ST, systemic melatonin was found particularly high, our aim was to assay serum melatonin in patients ($N = 8$) undergoing aortic surgery, either during hypothermia or in the following four recovery days. Serum markers of blood–brain barrier (BBB) integrity (the astrocytic protein S100B) and neuronal damage (neuronal-specific enolase [NSE]) were also measured. Results show that, in contrast to what observed in ST, in hypothermic patients, melatonin was dramatically reduced with respect to the preanesthesia level, slowly recovering during the postsurgery period. Also, S100B and NSE were raised during surgery, indicating a compromised BBB integrity and some ongoing neuronal damage, though both markers returned closer to preanesthesia levels within the recovery period. Together, the present results show that, in aortic-arch surgery, hypothermic patients totally lack the systemic melatonin peak that was observed in ST, the BBB was temporarily compromised, and some acute neuronal damage occurred. A main implication of this work is that, exploiting the low BBB efficiency that makes easier to reach brain parenchyma, by administering melatonin during the hypothermic stage of the surgery, the observed gap could be filled, possibly triggering the neuroprotective mechanism seen in preclinical observations and leading to better neurological outcomes for this surgical procedure.

1 | Introduction

It is well known that melatonin has neuroprotective and antioxidant effects on the nervous system, strong enough that it has been seriously considered for developing possible treatments to contrast neurodegenerative diseases and dementias [1, 2]. Unfortunately, different clinical trials conducted in recent years did not confirm the usefulness of melatonin in coping with cognitive impairments [3]. However, recent preclinical data on rodents, obtained from our laboratory [4], opened the possibility to reconsider the neuroprotective function of melatonin for

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clinical use. Specifically, by using the "synthetic torpor" (ST) animal model (e.g., a transient and reversible hypothermic condition resembling hibernation, but pharmacologically induced on rats) [5, 6], it was found that, surprisingly, melatonin is abundantly secreted during the hypothermic phase and this bulk secretion seems to act on neurons by triggering a protective molecular mechanism [4, 7]. Even though the effects of this mechanism were only partially investigated, apparently, it seems to contrast neuronal suffering by deactivating glycogen-synthase kinase 3-β (GSK3β) and the accumulation and aggregation of hyperphosphorylated Tau protein [4, 7–9], one of the major specific hallmarks observed in a broad class of neurodegenerative diseases, defined as "tauopathies" [10], and also involved in neuronal damage following ischemia [11]. It is worth noting that this protective mechanism was triggered by the ST and starts right during the hypothermic condition, not only when animals recovered euthermia [4]. The suggested interpretation by the authors was that melatonin, when acting on neurons at low body temperature, may express some peculiar functioning in a way that is not possible at physiological temperature [4, 7]. This may also explain the reason why clinical trials using melatonin as a neuroprotective agent were not as successful as expected [3].

The not obvious link between ST and cardiac surgery is based precisely on hypothermia, induced in patients with the hypothermic circulatory arrest, a strategy used as cerebral and visceral organ protection technique during aortic arch surgery [12, 13]. When it was first introduced, hypothermic circulatory arrest was performed at temperatures of about 18°C [14, 15]. The development of further cerebral protection strategies such as antegrade selective cerebral perfusion allowed a progressive shift toward higher temperatures with good results in terms of mortality and neurological complications, and mild-to-moderate hypothermia with a nasopharyngeal temperature of 25°C-26°C is now the most widespread technique [16, 17]. This, along with the development of surgical techniques such as the Elephant Trunk and, more recently, the introduction of branched grafts simplified the treatment of complex pathologies of the thoracic aorta.

Since, during ST, hypothermia by itself seems to represent a stimulus strong enough to trigger the systemic melatonin high level that was found, here we hypothesized that the same process should be observed in humans subjected to hypothermia during the aortic surgery, considering that the brain temperature is very similar in the two conditions.

Hence, to verify this hypothesis, we determined melatonin systemic levels in patients undergoing aortic-arch surgery with the use of moderate hypothermia at 25 °C of nasopharyngeal temperature as the cerebral and visceral organ protection method during aortic arch replacement. The primary endpoint of this observational study was the determination of serum melatonin levels during surgical hypothermia and in the following days, while recovering from surgery.

Moreover, to understand whether systemic melatonin may act easily on the brain parenchyma possibly inducing neuroprotection, as a secondary endpoint we also determined systemic levels of S100B, as a marker of the blood–brain barrier (BBB) integrity [18, 19], and neuronal-specific enolase 2 (NSE), as a marker of BBB integrity and neuronal damage [18, 19].

A preprint has previously been published [20].

2 | Methods

Between January 2021 and March 2022, a total of 8 patients underwent total aortic-arch replacements in the Department of Cardiovascular Surgery, S. Orsola Hospital, University of Bologna, Italy. The patients' characteristics are reported in Table 1.

All elective patients underwent routine preoperative exams, including coronary angiography (if aged 50 years or older), echocardiography, and computer tomography scans (CT scans).

2.1 | Serum Markers' Measurement Timing

The measurement timing for serum levels of melatonin, S100B, and NSE was managed by the anesthesiologist during the initial phase of the operation and the following postoperative days as listed in the following:

- ti: before anesthesia induction
- t1: target temperature of 25 °C

TABLE 1 | Pre- and intraoperative characteristics.

	***N* = 8**	***N* = 8**
Mean age (*n*, SD)	59	7.9
Male sex (*n*, %)	5	62.5%
BMI (*n*, SD)	25.9	3.4
Etiology		
Degenerative aneurysm (*n*, %)	5	62.5%
Post-dissection Aneurysm (*n*, %)	3	37.5%
Renal failure (*n*, %)	1	12.5%
Coronary disease (*n*, %)	1	12.5%
Diabetes (*n*, %)	2	25%
Smoking (*n*, %)	3	37.5%
COPD (*n*, %)	2	25%
Hypertension (*n*, %)	7	87.5%
Connective tissue disease (*n*, %)	1	12.5%
Previous cardiac surgery (*n*, %)	4	50%
Associated procedures (n, %)	4	50%
CABG (*n*, %)	1	12.5%
AVR (*n*, %)	1	12.5%
Bentall (*n*, %)	2	25%
Cannulation site		
BCT (*n*, %)	4	50%
Axillary artery (*n*, %)	3	37.5%
Ascending aorta (*n*, %)	1	12.5%
CPB time (minutes, SD)	223.6	42.4
Cross-clamp time (minutes, SD)	130	47.7
ASCP time (minutes, SD)	79.8	32.4
VIT (minutes, SD)	26.9	5.3
Preoperative ELD positioning (*n*, %)	5	62.5%

Abbreviations: AVR, aortic valve replacement; ASCP, antegrade selective cerebral perfusion; BCT, brachiocephalic trunk; BMI, Body Mass Index; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; CPB, cardiopulmonary bypass; ELD, epidural liquor drainage; SD, standard deviation; VIT, visceral ischemia time.

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- t2: chest closure;
- G1: first postoperative day
- G2: second postoperative day
- G3: third postoperative day
- G4: fourth postoperative day.

Blood samples' withdrawals were subjected to standard procedures to separate serum. Then, samples were aliquoted and stored at −80°C until assays (December 2023 for melatonin and January 2025 for S100B and NSE).

The present study was a secondary analysis, conducted on never-used aliquots of existing samples that were originally taken for a study conducted by the company Eurosets S.r.l. (see acknowledgments section, later in the text). No new blood samples were allowed to be taken specifically for this study.

2.2 | ELISA Determinations

The determinations of the planned biomarkers were conducted on different aliquots of the same samples of serum. Concentration of melatonin was measured by human melatonin ELISA Kit (E-EL-H2016-96T; CliniSciences). As reported by the producer certification, this assay is characterized by sensitivity, 9.38 pg/mL; coefficient of variation, < 10%; and detection range, 15.63–1000 pg/mL. No specific cross-reactivity of interference between human melatonin and analogs was also certified.

Concentrations of S100B and NSE were measured by Luminex, using a custom-developed panel from R&D Systems (LXSAHM-02). For all the ELISA procedures, the manufacturer instructions were precisely followed.

2.3 | Statistical Analysis

Statistical analysis was carried out using SPSS software (28.0). ELISA results for melatonin were analyzed with a 2-way ANOVA with repeated measures. Then, means of the different observational conditions were compared with the "ti" condition, taken as the baseline value, using the modified t-test (t^*) [21] and correcting the α level using Bonferroni's method. Since ELISA results for S100B and NSE were not normally distributed (Shapiro–Wilks test was significant for both), the statistical analysis for these data were conducted using a nonparametric test strategy: first, a Friedman test was conducted on the whole set of data; then, being significant, group means were compared with the baseline (i.e., ti condition) using the Wilcoxon test.

For all the comparisons, the α level was preset at $p < 0.05$.

2.4 | Surgical Technique

The induction of anesthesia started within the 8:15–9:20 AM time-window. All operations were performed through a full sternotomy. After systemic heparinization, cardiopulmonary bypass was instituted using the right axillary artery, femoral artery, innominate artery, or ascending aorta as an arterial cannulation site. Right atrial cannulation was used to ensure venous drainage. A left ventricular vent was inserted through the right superior pulmonary vein. Core cooling to 25 °C nasopharyngeal temperature was then accomplished, and circulatory arrest was instituted. Myocardial protection was achieved with infusion of crystalloid cardioplegia (Custodiol; Koehler Chemie, Alsbach-Haenlein, Germany). The aortic arch was opened, and bilateral antegrade selective cerebral perfusion was initiated by clamping the brachiocephalic trunk and cannulating the right carotid artery, in case of axillary or brachiocephalic trunk cannulation only. In order to guarantee a flow rate of 10 mL/kg/min, flow was uniformly distributed to the right and left cerebral hemispheres and adjusted to maintain the right radial pressure between 40 and 80 mmHg. Near infrared ray spectroscopy (NIRS) was used to monitor cerebral perfusion during the procedure. After the arch was completely resected and removed, the proximal thoracic descending aorta was inspected and the presence of a large intimal tear was documented at the convexity of the aortic arch at the isthmus level. The lesion was confirmed by the angioscope. The proximal descending aorta was then prepared using an external Teflon felt fixed with four internal pledgeted U-stitches. The stent–graft system was then introduced in an antegrade fashion into the descending aorta over the previously positioned stiff guidewire and then released. The side branch was then cannulated, and the lower body perfusion was reinitiated. The supraortic vessels were reimplanted, starting from the left subclavian artery. At this point, rewarming was initiated and the proximal repair was finally performed. In case of supracoronary anastomosis, or patients with previous root replacement, we perform the left carotid and innominate artery reimplantation after the proximal anastomosis at beating heart in order to reduce the myocardial ischemia time.

3 | Results

The mean age was 59 ± 7.9 years, and 5 of them (62.5%) were male. Aortic aneurysms were the main indication for surgery, with 5 (62.5%) degenerative and 3 (37.5%) postdissection aneurysms. Hypertension was the most common cardiovascular risk factor (7, 87.5%), and only one patient presented a connective tissue disorder (Loeys–Dietz syndrome). Half of the patients had previously undergone cardiac surgery procedures for previous acute Type A aortic dissection.

All patients underwent total aortic-arch replacement with the Frozen Elephant Trunk technique, with a zone 2 distal anastomosis and separate reimplantation of the supra-aortic trunks. The brachiocephalic trunk was the most common cannulation site (4, 50%) for cardiopulmonary bypass, followed by the axillary artery (3, 37.5%) and the ascending aorta (1, 12.5%). Circulatory arrest was performed at 25 degrees Celsius, and bilateral antegrade selective cerebral perfusion was always used. Associated procedures were performed in 4 (50%) patients, with the Bentall procedure being the most common (2, 25%), followed by coronary artery bypass grafting and aortic valve replacement in one case each, respectively.

All 8 patients presented an uneventful postoperative course, with no case of in-hospital mortality or major complications. In particular, there were no short-term neurological complications, including stroke and spinal cord ischemia. Postoperative outcomes are described in Table 2.

Mean hospital stay was 20.1 ± 7.4 days.

The main outcome of this observational study is shown in Figure 1; patients induced to moderate hypothermia present low levels of systemic melatonin, being significantly reduced during

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TABLE 2 | Postoperative outcomes.

	$N = 8$	$N = 8$
In-hospital mortality (n, %)	0	0
Mean ICU stay (days, SD)	3.5	1.4
Mean hospital stay (days, SD)	20.1	7.4
Neurological complications (n, %)	0	0
Dialysis (n, %)	0	0
GI complications (n, %)	0	0
Respiratory complications (n, %)	0	0
Reopening for bleeding (n, %)	0	0
Laryngeal nerve palsy (n, %)	0	0

the t1 condition ($p < 0.001$) and also in the following observational steps ($p < 0.001$ for all comparisons), excluding the G4.

Serum levels of the astrocytic marker S100B (Figure 2) present a huge and significant high peak ($p = 0.012$) in t1, slightly reducing at t3 ($p = 0.018$), and reaching a stable level around double the ti level; however, being significantly different in G1 ($p = 0.012$) and G3 ($p = 0.049$). Similarly, serum levels of NSE (Figure 2) present a dramatic and significant rise in the t1 condition ($p = 0.012$), but the higher peak value was reached in the t2 sampling ($p = 0.012$). For this marker as well, levels rapidly reduced (see Figure 2) but remaining significantly higher than those in ti in G1 ($p = 0.012$), G2 ($p = 0.025$), and G4 ($p = 0.036$). Since these measurements were made after many months from the sample withdrawal, being secondary to another study, absolute values (provided in Table 3) are lower than those reported in literature, but the observed variations are consistent between samples and also in respect to what reported by others [19, 22]. For this reason, we chose to describe and discuss the only percentage changes (Figure 2).

4 | Discussion

The very small number of patients considered, not justified by appropriate power analysis, follows mainly two reasons: (i) results are obtained from a secondary analysis of samples taken for another study, no new samples were allowed for this study; (ii) the present melatonin results are the very first to be reported in the specific conditions studied and no other preliminary data were available in literature to correctly estimate the statistical power. Hence, the present work is pivotal and all the results are preliminary and only indicative. Future dedicated clinical studies are mandatory to draw effective conclusions. However, we think that some key results obtained here are worth to be known and considered since indicative for possible important implications.

Results of the present work show that melatonin is strongly inhibited during the hypothermic condition induced in aorta surgery and both S100B and NSE peaked: at hypothermia and soon at the end of surgery, respectively.

Probably, anesthesia represents the most important factor influencing the results. Anesthesia undoubtedly has important effects on the general hormonal pattern [23] and on melatonin in particular [24]. However, to the best of our knowledge, nobody measured melatonin secretion during hypothermia, induced in patients while undergoing cardiac surgery. It is well established that the disruption of melatonin secretion represents one of the main parameters leading to common circadian disturbances in postoperative recovery [24], and this may explain the lower levels we found during the first 72 h of recovery from surgery (see Figure 1). In contrast, while Reber and colleagues [25] reported that during anesthesia, the melatonin plasma level is slightly affected, here we found the serum melatonin level dramatically lowered during the hypothermic period. There are no data describing plasma levels of melatonin in not-anesthetized hypothermic subjects since moderate hypothermia is a challenging condition to be treated in emergency or in a very controlled medical setup (cf [6]). However, considering preclinical data from the ST model (i.e., a reversible hypothermic/hypometabolic state, similar to natural torpor but pharmacologically induced), melatonin secretion resulted stimulated instead of inhibited by low brain temperature [4, 7]. From the present results, it is not possible to draw conclusion on what is the reason explaining the observed drop in melatonin. However, this drop is clearly shown by each patient included in the study, with a small between-

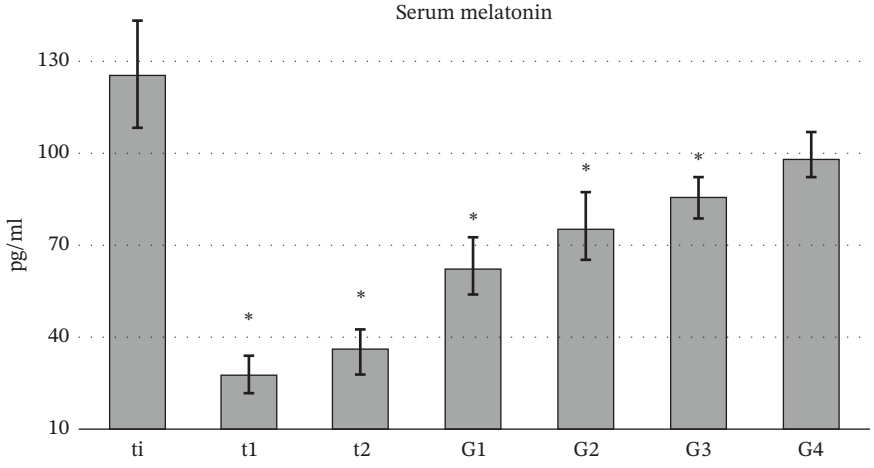

FIGURE 1 | Serum levels of melatonin. Data are expressed as means ± S.E.M., $n = 8$ (see Methods for details). *: $p < 0.05$ vs. ti. Observational conditions: ti, induction phase of general anesthesia; t1, target temperature of 25 °C; t2, chest closure; G1, first post-operative day; G2, second post-operative day; G3, third post-operative day; G4, fourth post-operative day.

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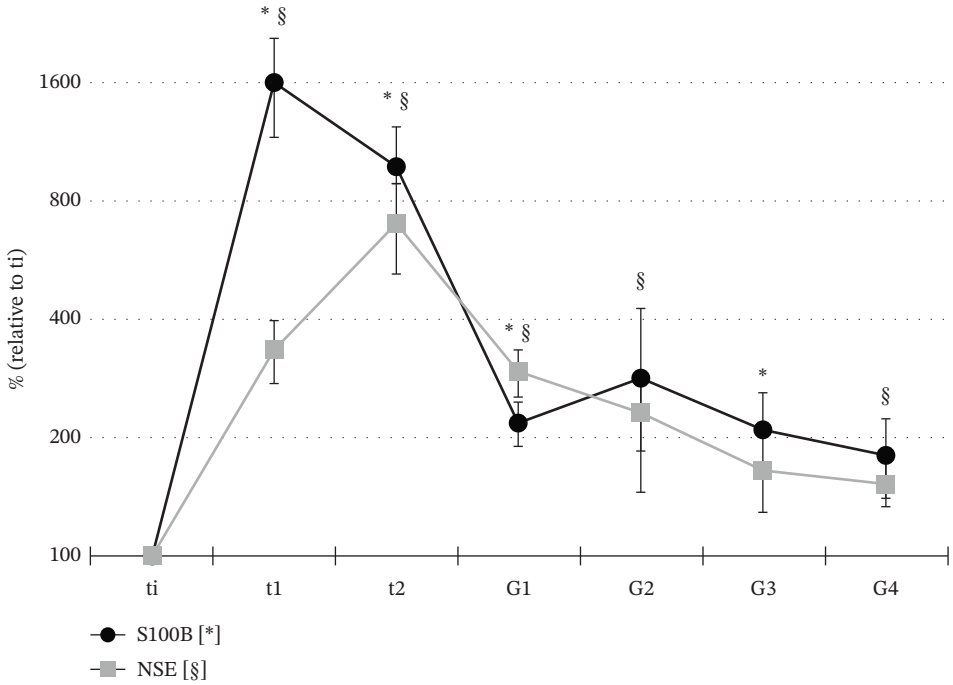

FIGURE 2 | Serum levels of S100B, marker of blood–barrier (BBB) integrity, and NSE (neuronal specific enolase), marker of BBB integrity and neuronal damage. Data are shown as percentage in respect to "ti" level and expressed as means ± S.E.M., $n = 8$ (see Methods for details). *, §: $p < 0.05$ vs. ti level for S100B and NSE, respectively. Observational conditions: ti, induction phase of general anesthesia; t1, target temperature of 25 °C; t2, chest closure; G1, first post-operative day; G2, second post-operative day; G3, third post-operative day; G4, fourth post-operative day.

subject variability. This evident result represents the main outcome of the study and could open possible new avenues to reduce neurological outcomes from this kind of surgery.

Indeed, neurological complications in cardiac surgery and aortic-arch procedures, with hypothermic circulatory arrest in particular, have long been evaluated. These complications include not only stroke and paraplegia but also more subtle post-operative cognitive disorders such as delirium, for which the pathophysiological basis is yet to be clear. Postoperative delirium is strongly linked to alteration of the circadian rhythm disruption [26–28]. Previous preclinical finding (from part of the present co-authors) showed that, in rodents, the hypothermic ST condition was able to trigger a molecular neuroprotective mechanism, apparently linked to a strong melatonin secretion [4]. Together with melatonin, a key molecular mechanism of clinical interest was the deactivation of the proapoptotic factor GSK3β, a mechanism that has been studied for a long time to pharmacologically induce neuroprotection [29–31], but with poor results [30, 31]. It is worth noting that the brain temperature reached by surgical patients during hypothermia is very close to the one reached by the brain of the ST animal model [4]. As a theoretical consequence, a possible way to stimulate neuroprotection (i.e., by the deactivation of GSK3β) and probably improve the clinical outcome of these patients is the administration of melatonin at the beginning of the hypothermia induction, during surgery.

TABLE 3 | Absolute values of serum concentration of the biomarkers S100B and NSE, in pg/mL.

Sample timing	S100B	NSE
ti	0.089 ± 0.019	8.4 ± 2.3
t1	1157 ± 0.192	19.6 ± 3.1
t2	0.828 ± 0.187	43.9 ± 11.9
G1	0.184 ± 0.039	20.6 ± 5.4
G2	0.159 ± 0.043	14.7 ± 3.4
G3	0.148 ± 0.026	10.0 ± 2.1
G4	0.141 ± 0.028	12.3 ± 4.1

Note: Explanation of sample timings is in the text, within the method section. Values are expressed as mean ± S.E.M.

As a matter of fact, melatonin is a well-known safe molecule, approved by the US Food and Drug Administration (FDA) as a "dietary supplement," diffusely used to cope with mild sleep disturbances or jet-lag circadian disruption. In addition, melatonin has numerous well-described effects as antioxidant and anti-inflammatory agents [32], also showing promising results when given in perioperatory conditions and in particular in relation to neuropsychiatric outcomes in cardiopulmonary surgery [33, 34]. Notably, the administration of melatonin was safely conducted in mild hypothermia induced on asphyctic newborns, without aversive effects [35]. Therefore, even though at physiological temperature melatonin appears to have good perioperatory protective effects, better normalizing the circadian cycles, our preclinical indications support the possibility that if the treatment is given acutely at around 25 °C of brain temperature, this may stimulate a stronger neuroprotective mechanism. This is a speculative hypothesis, supported by a small set of preliminary, but nevertheless clear, data.

The role of S100B and NSE as serum biomarkers of the BBB integrity and neuronal damage, respectively, is very well established [19, 36–38]. The rise of serum level of the astrocytic protein S100B is linked to a BBB higher leakage, while NSE,

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being a metabolic enzyme rich in neurons, when found in serum is a sign of possible neuronal damage [36]. Hypothermia by itself does not seem to modify the levels of these biomarkers [22]. Present results show that S100B peaks during hypothermia, which is also concomitant with the ongoing cardiopulmonary bypass, persisted until the end of the surgery, and then reducing but maintaining statistically significant high levels until G3. This was expected and represented a confirmation of existing literature since a disruption of BBB following cardiopulmonary bypass has been already described [39]. As specified by different other works, in extreme cases, like following serious neural insult due to cardiac arrest, serum NSE peaks at 24–72 h after the insult, and significant high levels of this biomarker can easily be found at 4-5 days and beyond [19, 22, 40]. Then, what is shown in Figure 2 may indicate that the present surgery procedure induces some acute neural suffering but rapidly solving in a very few days with respect to what is found in more significant neural injuries [22].

To summarize, considering the present results together with what was recently found in preclinical ST condition [4], we encourage to take into consideration the clinical implication may have the administration of melatonin precisely during hypothermia. Even though not widely studied at low brain temperature, exogenous melatonin represents a pharmacologically well-known molecule, having a dramatically high toxic dosage (i.e., hundreds of mg/kg [41]). Since at low temperature, melatonin could easily pass the BBB, due to the compromised integrity, it should be reasonably possible to trigger the molecular neuroprotective mechanism we observed in ST [4]. If confirmed by future clinical studies, this hypothetic treatment may represent the long-desired safe GSK3β inhibitor, instrumental to contrast neuronal suffering [29].

Nomenclature

BBB	Blood–brain barrier
G1	First postoperative day
G2	Second postoperative day
G3	Third postoperative day
G4	Fourth postoperative day
GSK3β	Glycogen-synthase kinase 3β
NIRS	Near infrared ray spectroscopy
NSE	Neuronal-specific enolase 2
S100B	Astrocytic protein, peripheral marker of BBB integrity
ST	Synthetic torpor
t1	Target temperature of 25 °C
t2	Chest closure
ti	Induction phase of general anesthesia

Author Contributions

Alessandro Leone: conceptualization, data curation, investigation, formal analysis, project administration, writing–original draft, and writing–review and editing; Alessandra Occhinegro: investigation and formal analysis; Emiliana Piscitiello: investigation and formal analysis; Paola Paterini: investigation; Simone Bugani: supervision; Carlo Mariani: investigation; Chiara Nocera: investigation; Timna Hitrec: investigation and writing–review and editing; Roberto Amici: supervision and writing–review and editing; Davide Pacini: conceptualization, project administration, supervision, and writing–review and editing; Marco Luppi: conceptualization, data curation, formal analysis, funding acquisition, project administration, supervision, visualization, writing–original draft, and writing–review and editing.

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Ethics Statement

The study was observational and conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee: *Comitato Etico-Area Vasta Emilia Centro* (CE-AVEC). Protocol approval code: 127/2022/Oss/AOUBo.

Consent

Written informed consent was obtained from all patients.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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