Hypothermia: Impact on plasticity following brain injury

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Abstract:

Therapeutic hypothermia (TH) is a potent neuroprotectant against multiple forms of brain injury, but in some cases, prolonged cooling is needed. Such cooling protocols raise the risk that TH will directly or indirectly impact neuroplasticity, such as after global and focal cerebral ischemia or traumatic brain injury. TH, depending on the depth and duration, has the potential to broadly affect brain plasticity, especially given the spatial, temporal, and mechanistic overlap with the injury processes that cooling is used to treat. Here, we review the current experimental and clinical evidence to evaluate whether application of TH has any adverse or positive effects on postinjury plasticity. The limited available data suggest that mild TH does not appear to have any deleterious effect on neuroplasticity; however, we emphasize the need for additional high-quality preclinical and clinical work in this area.

Keywords:

Cardiac arrest, neuroplasticity, stroke, therapeutic hypothermia, traumatic brain injury

Introduction

Therapeutic hypothermia (TH) counteracts many deleterious mechanisms of ischemic, hemorrhagic, hypoxic, and traumatic brain injuries, and there is considerable preclinical evidence that this leads to meaningful reductions in brain damage with concomitantly better recovery.[1] Although clinical evidence of efficacy is less impressive and sometimes contradictory, there is still much interest in the potential of TH.[2-4] However, several problems must be resolved before that goal is fully realized. First, mechanisms of injury vary between diseases, which may necessitate different approaches and treatment parameters, such as depth, duration, and delay in order to get optimal neuroprotection. Second, side effects have long complicated the use of TH, both by reducing the potential benefits of TH and increasing the risk of poor outcomes. For example, cooling increases the risk

of infection and can cause coagulopathy. These systemic complications have been extensively studied in an attempt to avoid them or find effective management strategies, thereby optimizing patient outcomes. For instance, using brain-selective cooling can avoid many systemic complications.

Here, we review another potential side effect of TH – the possibility that TH will adversely affect neuroplasticity, thereby delaying or weakening the neurological and functional recovery. Such deleterious effects are possible with TH, especially given its broad-spectrum effects, which could directly impact neural repair processes that spatially and temporally overlap with mechanisms of injury.[5-7] For instance, neuronal metabolism is dampened by TH, which is advantageous for mitigating brain injury; however, this same mechanism may weaken neuroplastic changes that require heightened metabolic activity.[8,9] One can assume that the duration of TH affects this risk, with days of TH potentially having the greatest chance of impeding repair compared to very brief cooling. The same concerns apply to the depth and the method

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of cooling. Nonetheless, even local application of mild TH may affect brain regions vital to neural repair (e.g., peri-infarct cortex), with likely greater impact if deeper levels of cooling are achieved.

Despite these concerns, only a few of the TH efficacy studies done in animals have considered such risks. This review explores the literature on brain repair when TH has been applied following global and focal ischemia, hypoxic-ischemic encephalopathy (HIE), intracerebral hemorrhage (ICH), and traumatic brain injury (TBI). No clinical studies have directly assessed whether TH impacts neuroplasticity, only whether cooling improves the final outcome or a specific mechanism of injury (e.g., raised intracranial pressure). Therefore, these studies do not exclude the possibility that TH may have had harmful effects on brain repair.

Method of Article Collection

Primary collection for articles reviewed here was found using the University of Alberta's Library journal article search engine and Google Scholar. Main terms were used in a variety of combinations. These included "hypothermia," "neuroplasticity," "hibernation," "neurogenesis," "brain-derived neurotrophic factor," "plasticity," "cooling," "therapeutic cooling," "therapeutic hypothermia," "angiogenesis," "synaptogenesis," "neuronal sprouting," "dendritic branching," "BrdU," "doublecortin," "golgi stain," and "targeted temperature management" in conjunction with the pathologies of interest, with English, full-text, and peer-reviewed being requirements. Following this, we explored references within these and other readings that were deemed applicable. We also identified articles from our knowledge of this research area.

Hypothermia and Neuroprotection

A review of the mechanisms of injury and the means by which cooling mitigates injury for ischemic, hemorrhagic, and traumatic injury is beyond the scope of this review. Readers are referred to excellent reviews by Yenari and Han, Kurisu and Yenari, and Wassink *et al.*.[10-12] Cooling can impact all mechanisms of injury either directly or indirectly, depending on the treatment parameters used (depth, duration, etc.). For instance, TH has been shown to mitigate excitotoxicity, oxidative stress, blood–brain barrier breakdown, and inflammation, to name a few.[10-12] Many of these are triggers and/or are essential for plasticity responses that are key to behavioral recovery. Thus, TH may directly impact repair by impeding such processes (e.g., glutamatergic neurotransmission).[13] Indirect effects are also expected. For example, a highly neuroprotective cooling protocol would likely diminish the need for

repair. Alternatively, the rescue of tissue can promote or alter the spatial pattern or timing of neuroplasticity, such as by saving enough of a circuit to enable further repair.

Plasticity Following Brain Injury

Neuroplasticity refers to molecular and anatomical modifications that are driven as a result of aging, experiences, hormones, drugs, and disease or injury to the brain. The mechanisms that enact these plastic changes, such as synaptogenesis, neurogenesis, and angiogenesis, are anatomical alterations underwritten by various molecular processes and cascades (e.g., growth factors) that are subsequently shaped and refined by internal or external factors.[5,14-17] Please refer to excellent reviews by Kolb and Gibb, Murphy and Corbett, and Carmichael for more comprehensive information on the mechanisms of plasticity.[5,18,19]

The extent of redundant connectivity within the central nervous system (CNS) provides a basis for reorganization and functional recovery following brain injury.[20] For example, imaging studies have established that corresponding contralesional brain regions are recruited to aid in behavioral recovery early after stroke. Later, as injury processes and inflammation resolves, there is a shift of recruitment to diffuse ipsilesional areas surrounding the site of injury, at least for some stroke patients, illustrating the progression of plastic processes.[21] Similarly, TBI patients demonstrate a wider cerebral activation pattern following their initial injury that gradually decreases in complexity as more efficient connections are made and recovery progresses.[22] These macroscopic changes are the result of a period of heightened synaptic malleability and neurogenesis following injury. Brain injury, especially stroke, induces peri-injury axonal sprouting through both alterations in the cellular environment, including upregulation of growth factors and genetic modifications favoring neuronal growth.[21] These new axonal connections, along with unmasked latent connections, are strengthened and refined through Hebbian processes, resulting in greater functional recovery.[22] Use of TH in a variety of pathologies promotes neuroprotection and cellular sparing, possibly reducing the need for broad-scale network recruitment while enabling restoration and retraining of salvaged tissue.

Effects of Hypothermia in Naïve Animals

There is substantial evidence that learning acquisition is heavily influenced by body temperature, with significant reductions in core temperature resulting in poor performance in serial problem-solving tasks and spatial learning tasks, such as the Morris Water Maze.[23,24]

Thus, it is natural to presume that the plastic processes underlying cognitive tasks and motor skill learning are also affected by changes in core body temperature. Klahr *et al*. recently subjected naïve rats to mild focal TH treatment (30°C–31°C) in the hemisphere contralateral to the preferred paw for 5 days, followed by 5 days of normothermia, and measured learning acquisition during a skilled reaching task throughout the 10-day treatment period.[25] They found no significant difference in learning acquisition rates between the TH and normothermic conditions, and subsequent histological analysis did not show any difference in dendritic complexity.[25] In this case, the cooling depth was mild, but clinically relevant, and it is fortunate that this dose had no obvious effects on plasticity or behavior. Presumably, by extending the duration of mild cooling, the risk to plasticity is higher. However, as demonstrated in stroke sham-operated rats, application of mild focal TH to the motor cortex for 21 days at 32°C did not negatively affect subsequent behavior, dendritic morphology, or result in cortical injury.[26] Evidence from brain slice preparations suggests that mild hypothermia (30°C–36°C) maintains ionotropic glutamate receptor function due to the reduction in metabolic demand. However, deep hypothermia (below 18°C) results in suppression of long-term potentiation (LTP), the neuroplastic process by which efficiency of synaptic transmission is upregulated via coincident bursts of high-frequency stimulation between neurons.[13,27] Taken with the behavioral data, this suggests that mild TH for long durations is not inherently harmful to natural plastic processes; however, given the risk of impairing LTP, caution should be taken when cooling to greater depths or durations. In addition, these limited studies are in naïve animals, and they do not include the study of other pharmacological agents that are often given concurrently with TH, which may potentially impact plastic processes. Finally, to fully understand the effect TH has on plasticity, we must consider brain injuries such as TBI or stroke, as TH may potentially change the ability of the brain to regulate repair processes, and must be evaluated separately.

Global Ischemia

TH is one of the few neuroprotectants that has translated from preclinical work into clinical use for global ischemia (cardiac arrest). Namely, it was previously used to prevent brain damage during cardiac surgical procedures, and more recently has been used to improve both neurological and physical outcomes following sudden cardiac arrest.[28] Although there is abundant preclinical literature demonstrating the neuroprotective capacity of TH, the bulk of outcome measures are related to behavioral functional recovery and histological protection, with little to no mention of treatment effects on plasticity.[29]

Therapeutic cooling, when properly timed and dosed, is able to rescue diverse groups of neurons, such as those in the hippocampus and cortex following a period of global ischemia. This allows for improved cognitive performance on memory tasks.[30] Those rescued cells often appear to be largely healthy when cooling is prolonged, as seen with light and electron microscopy; however, this may not be necessarily true when cooling is brief or delayed following the initial insult.[31,32] Extracellular field recordings in acute hippocampal slices following global ischemia and TH in rats show that synaptic function and LTP are functionally sustained long after the initial insult, aiding in cognitive performance.[33] Correspondingly, following systemic TH, gerbils that sustained a brief global ischemic insult had no difference in CA1 neuron resting membrane potential, input resistance, or action potential quality compared to shams.[34] Considered together, this indicates that the rescued CA1 cells spared by TH both remain healthy and retain their electrophysiological function.[34] A number of studies have confirmed that specific molecules that are critically important to plasticity are preserved by cooling. For example, 24 h of TH (32°C) following bilateral carotid occlusion in gerbils prevented CA1 downregulation of ionotropic glutamate receptor 2 (GluR2) protein, a Ca^{2+}-impermeable subunit of the α-amino-3-hydroxy-5 -methyl-4-isoxazole propionic acid (AMPAR) glutamate receptor.[35] The GluR2 subunit is important for the neuroplastic functional properties of AMPARs and can dynamically alter synaptic strength for different forms of plasticity.[36] With both electrophysiological and behavioral measures supporting efficacy of TH in maintaining homeostatic physiological conditions necessary for plasticity, it appears that TH does not impede plasticity following global ischemia. However, these studies were not measuring plasticity as their primary endpoint. In addition, factors such as cooling depth, duration, and method were not manipulated sufficiently to conclude that plasticity is unaffected regardless of treatment parameters.

Although there are few studies that directly assess the effects of TH on plasticity, the majority of studies on treatment duration following global ischemia have shown that extending cooling duration largely results in better outcomes.[32,37-39] For example, Colbourne and Corbett found that 24 h of mild systemic TH starting 1 h following global ischemia in gerbils afforded superior neuroprotection and better behavioral outcome compared to a 12 h treatment duration.[37] These neuroprotective benefits remain even after a 12 h treatment delay; Silasi and Colbourne demonstrated that delaying TH for up to 12 h following the initial global ischemic insult reduced the amount of CA1 injury and improved performance on the Morris Water Maze compared to normothermic controls.[40] Although it may be argued that this can be

simply explained by the neuroprotection afforded by TH, it also indirectly indicates that the electrophysiological properties responsible for LTP and induction of learning and memory also remain intact in the cells spared through TH treatment – a conclusion that has been shown directly in other studies.

One important moderator of neuroplasticity following brain injury is brain-derived neurotrophic factor (BDNF).[17,41,42] Notably, a study in which male rats were subjected to the two-vessel occlusion with hypotension model of global ischemia found that systemic intra-ischemic TH (33°C) resulted in increased BDNF levels within the dentate gyrus only 1 h postinsult compared to a similar BDNF increase in the normothermic group 24 h later.[43] It appears that TH shifts and attenuates the increase in BDNF levels compared to the time frame observed in normothermic animals. This is consistent with other global ischemia studies that demonstrate an early increase (within 24 h) in BDNF following both intra- and postischemic TH.[44,45] Although the early boost in BDNF levels following TH seems to hold across global ischemia animal work, this phenomenon has not been directly manipulated in a TH study to determine the direct result on postinjury plasticity.

After ischemia, neurogenesis in the dentate gyrus is upregulated for several weeks after the initial insult, but many of these cells do not survive.[46] Silasi *et al*. found that 48 h of TH initiated within an hour of ischemia increases the rate of new cell survival in the dentate gyrus by 60% at 4 weeks following the initial insult.[40] It is important to consider the possibility that continuing TH treatment beyond the first few days following the initial insult may limit both neurogenesis and cell survival in the dentate gyrus, the bulk of which occurs in the first 1 to 2 weeks following ischemia.[46] To address this concern, Silasi *et al*. focally cooled rats 1 h following global ischemia for either 1, 2, 4, or 7 days. There was no added neuroprotective benefit of cooling beyond 2 days, but there also appeared to be no harm to plasticity; there were no significant differences in immunofluorescence localization and densitometry analysis of synaptophysin and BDNF markers between groups. In addition, neurogenesis in the dentate gyrus, as measured by colocalization of Ki96 (a cell proliferation marker) and doublecortin (an immature progenitor cell marker), was not affected.[29] Based on these measures, it appears that mild cooling for both short and prolonged durations following global ischemia does not impede neurogenesis, an important aspect of plasticity.

Focal Ischemia

Most focal ischemia animal studies report positive outcomes following application of TH, with robust reductions in lesion volume and significant improvement in behavior.[10,47,48] However, clinical studies using TH following focal ischemia are limited and have mixed evidence in terms of efficacy.[11] To ensure better translational success, we must also consider that factors important to neuroprotection such as cooling duration, depth, intervention delay, and cooling/rewarming rates may ultimately harm plasticity. Although the effect of these factors on neuroprotection is well-studied preclinically, the same cannot be said for plasticity. The optimal duration of TH following focal ischemia seems to depend on treatment delay and injury severity; overall, mild cooling for longer durations results in better outcomes, even when delayed, although not everyone agrees.[32,38,39,49-56] For example, Clark *et al*. demonstrated that initiation of 48 h of mild systemic TH 1 h following permanent middle cerebral artery occlusion resulted in improved behavioral scores and lesion volume compared to 12 and 24 h durations.[48] In general, mild cooling for longer (up to a few days) appears to provide the best results; however, recent evidence suggests that this may depend upon the cell type that is assessed.[54] Mild cooling is widely used across preclinical and clinical studies due to a lower risk of adverse complications. Many have shown that cooling past approximately 32°C–34°C results in worse neuroprotection and outcome.[39,49,57,58] Therefore, caution should be taken when cooling to both a greater depth and duration, as this increases the risk of complications, interference in repair processes, and impeding plasticity.

Clinical studies typically use systemic whole-body cooling due to challenges in achieving target temperature with focal cooling methods and the longer duration required to achieve equivalent protection.[59] It is difficult to deal with the complexities of inducing hypothermia in clinical practice. For example, the recent EuroHYP-1 trial ran into several issues, such as the logistical challenge of administering such a complex treatment for a full 24 h. This trial was unfortunately discontinued due to these issues and funding expiration.[49] In addition, there is concern that systemic cooling may affect the overlapping temporal and spatial processes of neuroprotection and plasticity occurring in the ipsilesional versus contralesional hemispheres following the focal ischemic insult, and may therefore limit the extent of benefit. The neuroplastic involvement of the contralateral hemisphere is important following focal ischemia, as it is one of the sites of synaptogenesis and remapping of cortical areas, especially following severe injury.[19,60,61] Nonetheless, there is some concern of behavioral compensation and resulting contralesional plasticity limiting true recovery.[62] Thus, it is important to discern how cooling affects the contralesional hemisphere in order to best inform clinical treatment decisions. In this regard, focal cooling in rodents is advantageous, as it can be used to evaluate the effect of

cooling on a single hemisphere or area without confound. Following a motor cortex devascularization model of focal ischemic stroke, Klahr *et al*. applied focal cooling to the contralesional hemisphere and found that cooling did not affect dendritic spine complexity or skilled reaching success.[25] In addition, early cooling of the contralateral hemisphere reduced reliance on the unimpaired limb, perhaps discouraging compensatory behaviors (learned nonuse).[25] With large lesions, or in cases where cooling is less effective (e.g., insufficient depth or duration), contralateral cooling may be harmful. This is because with little tissue left in the ipsilesional hemisphere to rely upon, compensatory behaviors and contralateral reorganization may be needed, which are heightened in the days following stroke.[63] Based on this work, it appears that contralateral cooling after a focal ischemic insult does not harm plasticity and could provide benefits in certain cases by limiting compensation. However, similar work in additional focal ischemia models and clinical studies should be done to make definitive conclusions.

Hypoxic-Ischemic Encephalopathy

Numerous clinical and animal studies have shown neuroprotection after initiating mild TH following moderate or severe HIE insult in neonates.[64,65] HIE is unique among the other pathologies discussed in this review, as it occurs in infants with highly plastic brains during the intra and antepartum periods of labor. The majority of preclinical studies agree that the optimal cooling duration is 48–72 h and must be initiated within 6 h of the initial HIE insult, before the secondary metabolic failure that is common to this pathology.[12,66] However, beyond a 72 h duration, there is both clinical and preclinical evidence that cooling is harmful.[12,66-68] In fetal sheep, cooling to 31°C–33°C was initiated 3 h following 30 min of ischemia and continued for either 72 or 120 h. Compared to normothermic sheep, cooling for 72 h significantly increased neuronal cell survival, reduced the inflammatory response, and improved recovery of electrophysiological power; however, cooling past 72 h decreased neuronal survival and resulted in no improvements in any of the other parameters.[68] This corresponds to clinical data that found cooling HIE infants past 72 h to 32°C resulted in greater mortality. Due to a high mortality rate, this trial was subsequently discontinued, but interestingly, surviving infants had the lowest rate of disability upon follow-up.[67] Long-term follow-up in several HIE trials are ongoing; the Total Body Hypothermia for Neonatal Encephalopathy Trial has found an approximate 15% increase in total absence of neurological impairment and an IQ over 85 points between children who were cooled versus those who were not.[69] Similarly, the CoolCap trial, which initiated cranial cooling to 34°C within 6 h of HIE for 72 h, demonstrated that the treatment resulted

in good functional outcomes for 7–8-year-old children who had favorable assessments at 18 months of age.[70] These favorable clinical outcomes suggest that early cooling following HIE does not negatively impact the highly plastic infant brain in the long term, as long as it is within the treatment parameters that have proven to be safe and efficacious, as discussed above. Alternatively, if plasticity is affected by clinically used cooling protocols, the costs to plasticity are offset by the benefits of cooling and are no longer observable upon follow-up. If this is the case, there is potential to treat this complication by adding adjunct rehabilitation or pharmacological therapies to enhance plasticity and ultimately produce better patient outcomes. Although evidence of efficacy is promising, clinical and preclinical HIE studies on electrophysiological function during and after TH are conflicting.[68,71,72] Thus, further inquiry into the effect of TH on molecular underpinnings of plasticity and neurogenesis following HIE is recommended.

Intracerebral Hemorrhage

Most preclinical and clinical work investigating TH for ICH has assessed the effects on edema, inflammation, blood–brain barrier dysfunction, and the mitigation of functional deficits, with little focus on neuroplastic processes.[39,73,74] A recent meta-analysis of outcomes in ICH studies following the application of TH demonstrated a significant positive impact on behavioral assessment.[75] Although this benefit is concurrent with a simultaneous decrease in edema and other neuroprotective factors, it is a rough indicator that TH is not directly harming plastic processes following ICH. Supporting this conclusion, Fingas *et al*. gave rats an ICH via autologous blood infusion and focally cooled on the ipsilesional side for either 12 h, 3 days, or 6 days poststroke and found no significant group differences in behavior or lesion size.[76] If cooling does negatively affect plasticity, the expectation would be that groups who are cooled for longer would have worse behavioral performance than those cooled for a shorter duration. Although this is not a direct measure of plasticity, the behavioral improvement paired with a lack of change in histology does indicate that plastic processes are maintained regardless of focal cooling duration after ICH. Without preclinical data on plasticity following systemic cooling, it is difficult to definitively state whether TH has any adverse effects on plasticity; however, Staykov *et al*. have shown that prolonged systemic cooling (8–10 days) reduced mortality and improved patient outcome, indicating similar conclusions to preclinical data.[74] This was a small study and not a randomized control trial. Thus, further preclinical ICH studies that directly assess plasticity following TH are needed, especially if the goal of translating this therapy clinically is to become a reality.

Traumatic Brain Injury

TBI commonly results in dendritic and synaptic degeneration, cell death, edema, axonal shearing, hemorrhage, as well as a decrease in neuronal excitability, and thus relies on postinjury plastic processes to compensate for the damage.[76,77] The majority of animal studies support the use of mild-to-moderate TH in treating both focal and diffuse TBI insults, demonstrating reduced injury and improved behavior.[78] In contrast, large clinical trials have shown that TH seems to have little neuroprotective efficacy, despite some smaller studies showing benefit in patients with TBI.[79-85] This translational disconnect is likely due to a mismatch between preclinical models and clinical reality, as well as TH treatment parameters such as duration and depth of cooling, delay to treatment, and rewarming procedures.[78] In addition, individual patient differences seem to play an important role in treatment success such as age, severity and type of TBI (diffuse vs. focal), body temperature upon arrival, surgical intervention, and differing secondary injury processes between patients.[86,87] It remains to be seen which specific patient populations respond best to TH following TBI. Once these populations are identified, treatment protocols can then be optimized.

Similar to other injury pathologies, TBI induces cell proliferation in the subgranular and subventricular zones.[88,89] Bregy *et al.* demonstrated that application of TH to 30°C–33°C for 4 h following TBI boosted neurogenesis in the dentate gyrus compared to normothermic controls at 3 and 7 days postinsult, as evaluated by BrdU incorporation and doublecortin visualization.[90] In addition, 3 days following focal TBI and subsequent intravenous infusion of 4°C saline, nascent neurons colabeled with BrdU and NeuN have been demonstrated to cluster near the cortical injury site, with no colabeling observed in normothermic controls.[91] Considered together, this evidence suggests that post-TBI neurogenesis is enhanced by TH. However, there is also considerable literature that suggests both post-TH and normothermic neurogenesis following TBI are transient and may ultimately be harmful.[92-94] The sudden increase in neurogenesis that occurs following injury may expend available neural precursor cells, causing a decline in subsequent basal neurogenesis levels that are normally maintained.[94] In addition, some data suggest that this sudden burst of neurogenesis may increase the risk of developing posttraumatic seizures due to unregulated network excitability.[92,94] Due to this conflicting evidence, further preclinical study with electrophysiological and histological long-term follow-up is needed to assess whether neurons produced following TBI fully integrate into existing circuits, and whether their presence increases the incidence of posttraumatic seizures.

Neurogenesis is a vital aspect of postinjury plasticity; however, if there is a possibility that TH enhances neuronal overproliferation, maladaptive plasticity, and seizures, it warrants caution and future study.

After TBI exerts widespread injury in the CNS, GAP-43, a protein important in synapse formation and neurite outgrowth, has been shown to increase following application of TH.[95-97] Zhao *et al.* investigated the changes that immediate postinjury cooling to 33°C for 3 h had on GAP-43 7 days after TBI in rats.[7,98] They found higher levels of GAP-43 in TH-treated TBI animals compared to normothermic controls.[7] The protein was localized to the cell soma, which is associated with axonal expansion and plastic changes after injury. Comparatively, under normal conditions, the protein is localized to an axonal and presynaptic pattern of expression.[97] In addition, GAP-43 colocalized with NeuN and tau-1 (markers of mature neurons and growing axons, respectively) in neurite-like growths within the peri-contusion zone and into the core.[7] This evidence indicates that TH may enhance neurite outgrowth following TBI. Zhao *et al.* conclude that application of TH following TBI promotes axonal sprouting and plasticity.[7] Although these data are encouraging, the animals were cooled for 3 h, which is a much shorter duration than what is used clinically (e.g., Clifton *et al.* cooled for 48 h in a recent trial).[79] Similar preclinical work focusing on plasticity should be done to match clinically used durations of TH in order to observe whether the same benefits remain.

Adjunct Therapies in Therapeutic Hypothermia

The use of pharmacological intervention in conjunction with TH differs between clinical and preclinical settings. Whereas sedative and antishivering agents such as meperidine, propofol, midazolam, clonidine, and morphine are common in clinical care, preclinical studies rarely use any pharmacological intervention, despite the fact that these sedatives are crucial in mitigating the stress of cooling and improving patient recovery.[99-107] In preclinical studies, many use general anesthetics such as isoflurane, which are typically used during short durations of cooling. As clinical sedative agents are rarely used preclinically, their effect on neuroplasticity in the context of TH is unknown. Future clinical and preclinical studies should take sedative agents and their potential impact on recovery into account.

Although these pharmacological agents have not been studied in the context of TH, especially following brain injury, there has been some study on the plastic changes that these drugs induce independently. For example, one study investigating propofol demonstrated that it can decrease synapse number and alter dendritic

spine density, morphology, length, and arborization patterns.[108] This disturbance in synaptic organization resulted in atypical behavioral activity up to 6 months later. In conjunction with other work showing the effect of propofol on dendritic spine densities, this demonstrates the potential for propofol to permanently impair neuronal morphology and function, especially in developing brains.[109,110] In contrast, midazolam seems to be beneficial, as it induces changes in the signaling of sleep-induced cortical plasticity by promoting factors involved in neurogenesis and cellular sparing, and has additional neuroprotective effects.[111] Similarly, analgesics like clonidine and morphine can prevent unwanted CNS secondary neuroplastic changes like central sensitization resulting from prolonged pain stimuli.[112-114] However, some research suggests that these agents may impede memory formation and induce unhealthy changes to brain structures and neuronal connectivity when used over days.[115,116] Finally, although reduction of complications such as shivering is important, extended sedation can delay rehabilitation and mobilization, diminishing any potentially beneficial returns. Therefore, both positive and negative effects of pharmacological intervention on plasticity, rehabilitation, and recovery must be considered for optimization of treatment, especially with the concurrent use of TH.

Current Perspectives on Therapeutic Hypothermia and Plasticity

Considering the available evidence at this time, it does not appear that TH impedes plasticity or neurogenesis. However, this comes with several considerable caveats. First, it is clear that there is a substantial need for further study on the effect of TH on neuroplasticity; the bulk of the TH literature does not consider either direct or indirect effects on neuroplasticity. Second, a more holistic battery of plasticity measures and time points are needed to accurately gauge the effects of TH. Third, most of the studies discussed here used mild TH ranging from 30°C to 35°C. Experiments in which cooling depth, duration, and delay are varied are needed in order to establish which treatment parameters will optimally augment or negatively affect plasticity, especially as local cooling methods become more commonly used. Fourth, pharmacological agents that are used clinically in conjunction with TH must be assessed in preclinical models to establish their effect on plasticity following various pathologies and to ensure safety or enhance efficacy. This must be done across models and pathologies in order to fully inform clinical practice. Finally, future work must be done in both sexes, multiple species, young to aged animals, replicated in multiple laboratories, and be appropriately randomized and blinded, in accordance with STAIR guidelines.[117] Ensuring that the above aspects are considered when planning future studies on this subject will aid translational success, as plasticity is an important driving force behind optimal functional recovery and must be assessed as part of the efficacy of TH as a potential treatment.

Conflicts of interest
There are no conflicts of interest.

References

1. van der Worp HB, Sena ES, Donnan GA, Howells DW, Macleod MR. Hypothermia in animal models of acute ischaemic stroke: A systematic review and meta-analysis. Brain 2007;130:3063-74.
2. Geurts M, Petersson J, Brizzi M, Olsson-Hau S, Luijckx GJ, Algra A, *et al.* COOLIST (Cooling for ischemic stroke trial): A multicenter, open, randomized, phase II, clinical trial. Stroke 2017;48:219-21.
3. Scholefield BR, Silverstein FS, Telford R, Holubkov R, Slomine BS, Meert KL, *et al.* Therapeutic hypothermia after paediatric cardiac arrest: Pooled randomized controlled trials. Resuscitation 2018;133:101-7.
4. Mody P, Kulkarni N, Khera R, Link MS. Targeted temperature management for cardiac arrest. Prog Cardiovasc Dis 2019;62:272-8.
5. Murphy TH, Corbett D. Plasticity during stroke recovery: From synapse to behaviour. Nat Rev Neurosci 2009;10:861-72.
6. Kolb B, Teskey GC. Age, experience, injury, and the changing brain. Dev Psychobiol 2012;54:311-25.
7. Zhao CC, Wang CF, Li WP, Lin Y, Tang QL, Feng JF, *et al.* Mild hypothermia promotes pericontusion neuronal sprouting via suppressing suppressor of cytokine signaling 3 expression after moderate traumatic brain injury. J Neurotrauma 2017;34:1636-44.
8. Moser E, Mathiesen I, Andersen P. Association between brain temperature and dentate field potentials in exploring and swimming rats. Science 1993;259:1324-6.
9. Lo EH. A new penumbra: Transitioning from injury into repair after stroke. Nat Med 2008;14:497-500.
10. Yenari MA, Han HS. Neuroprotective mechanisms of hypothermia in brain ischaemia. Nat Rev Neurosci 2012;13:267-78.
11. Kurisu K, Yenari MA. Therapeutic hypothermia for ischemic stroke; pathophysiology and future promise. Neuropharmacology 2018;134:302-9.
12. Wassink G, Davidson JO, Dhillon SK, Zhou K, Bennet L, Thoresen M, *et al.* Therapeutic hypothermia in neonatal hypoxic-ischemic encephalopathy. Curr Neurol Neurosci Rep 2019;19:2.
13. Mokrushin AA, Pavlinova LI, Borovikov SE. Influence of cooling rate on activity of ionotropic glutamate receptors in brain slices at hypothermia. J Therm Biol 2014;44:5-13.

14. Carmichael ST. Emergent properties of neural repair: Elemental biology to therapeutic concepts. Ann Neurol 2016;79:895-906.

15. Carmichael ST, Kathirvelu B, Schweppe CA, Nie EH. Molecular, cellular and functional events in axonal sprouting after stroke. Exp Neurol 2017;287:384-94.

16. Overman JJ, Carmichael ST. Plasticity in the injured brain: More than molecules matter. Neuroscientist 2014;20:15-28.

17. Wieloch T, Nikolich K. Mechanisms of neural plasticity following brain injury. Curr Opin Neurobiol 2006;16:258-64.

18. Kolb B, Gibb R. Searching for the principles of brain plasticity and behavior. Corte×2014;58:251-60.

19. Carmichael ST. Plasticity of cortical projections after stroke. Neuroscientist 2003;9:64-75.

20. Warraich Z, Kleim JA. Neural plasticity: The biological substrate for neurorehabilitation. PM R 2010;2:S208-19.

21. Carmichael ST. Cellular and molecular mechanisms of neural repair after stroke: Making waves. Ann Neurol 2006;59:735-42.

22. Chen H, Epstein J, Stern E. Neural plasticity after acquired brain injury: Evidence from functional neuroimaging. PM R 2010;2:S306-12.

23. Andjus RK, Knöpfelmacher F, Russell RW, Smith AU. Some effects of severe hypothermia on learning and retention. Q J Exp Psychol (Hove) 1956;8:15-23.

24. Rauch TM, Welch DI, Gallego L. Hypothermia impairs performance in the Morris water maze. Physiol Behav 1989;46:315-20.

25. Klahr AC, Fagan K, Aziz JR, John R, Colbourne F. Mild contralesional hypothermia reduces use of the unimpaired forelimb in a skilled reaching task after motor cortex injury in rats. Ther Hypothermia Temp Manag 2018;8:90-8.

26. Auriat AM, Klahr AC, Silasi G, Maclellan CL, Penner M, Clark DL, *et al.* Prolonged hypothermia in rat: A safety study using brain-selective and systemic treatments. Ther Hypothermia Temp Manag 2012;2:37-43.

27. Bliss TV, Collingridge GL. A synaptic model of memory: Long-term potentiation in the hippocampus. Nature 1993;361:31-9.

28. Polderman KH. Application of therapeutic hypothermia in the ICU: Opportunities and pitfalls of a promising treatment modality. Part 1: Indications and evidence. Intensive Care Med 2004;30:556-75.

29. Silasi G, Klahr AC, Hackett MJ, Auriat AM, Nichol H, Colbourne F. Prolonged therapeutic hypothermia does not adversely impact neuroplasticity after global ischemia in rats. J Cereb Blood Flow Metab 2012;32:1525-34.

30. Colbourne F, Corbett D. Delayed postischemic hypothermia: A six month survival study using behavioral and histological assessments of neuroprotection. J Neurosci 1995;15:7250-60.

31. Colbourne F, Sutherland GR, Auer RN. Electron microscopic evidence against apoptosis as the mechanism of neuronal death in global ischemia. J Neurosci 1999;19:4200-10.

32. Dietrich WD, Busto R, Alonso O, Globus MY, Ginsberg MD. Intraischemic but not postischemic brain hypothermia protects chronically following global forebrain ischemia in rats. J Cereb Blood Flow Metab 1993;13:541-9.

33. Dietz RM, Deng G, Orfila JE, Hui X, Traystman RJ, Herson PS. Therapeutic hypothermia protects against ischemia-induced impairment of synaptic plasticity following juvenile cardiac arrest in sex-dependent manner. Neuroscience 2016;325:132-41.

34. Dong H, Moody-Corbett F, Colbourne F, Pittman Q, Corbett D. Electrophysiological properties of CA1 neurons protected by postischemic hypothermia in gerbils. Stroke 2001;32:788-95.

35. Colbourne F, Grooms SY, Zukin RS, Buchan AM, Bennett MV. Hypothermia rescues hippocampal CA1 neurons and attenuates down-regulation of the AMPA receptor GluR2 subunit after forebrain ischemia. Proc Natl Acad Sci U S A 2003;100:2906-10.

36. Derkach VA, Oh MC, Guire ES, Soderling TR. Regulatory mechanisms of AMPA receptors in synaptic plasticity. Nat Rev Neurosci 2007;8:101-13.

37. Colbourne F, Corbett D. Delayed and prolonged post-ischemic hypothermia is neuroprotective in the gerbil. Brain Res 1994;654:265-72.

38. Yanamoto H, Nagata I, Nakahara I, Tohnai N, Zhang Z, Kikuchi H. Combination of intraischemic and postischemic hypothermia provides potent and persistent neuroprotection against temporary focal ischemia in rats. Stroke 1999;30:2720-6.

39. MacLellan CL, Clark DL, Silasi G, Colbourne F. Use of prolonged hypothermia to treat ischemic and hemorrhagic stroke. J Neurotrauma 2009;26:313-23.

40. Silasi G, Colbourne F. Therapeutic hypothermia influences cell genesis and survival in the rat hippocampus following global ischemia. J Cereb Blood Flow Metab 2011;31:1725-35.

41. Lu B, Nagappan G, Lu Y. BDNF and synaptic plasticity, cognitive function, and dysfunction. In: Lewin GR, Carter BD, editors. Neurotrophic Factors. Vol. 220. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014. p. 223-50.

42. Chiaruttini C, Vicario A, Li Z, Baj G, Braiuca P, Wu Y, *et al.* Dendritic trafficking of BDNF mRNA is mediated by translin and blocked by the G196A (Val66Met) mutation. Proc Natl Acad Sci U S A 2009;106:16481-6.

43. Boris-Möller F, Kamme F, Wieloch T. The effect of hypothermia on the expression of neurotrophin mRNA in the hippocampus following transient cerebral ischemia in the rat. Brain Res Mol Brain Res 1998;63:163-73.

44. D'Cruz BJ, Fertig KC, Filiano AJ, Hicks SD, DeFranco DB, Callaway CW. Hypothermic reperfusion after cardiac arrest augments brain-derived neurotrophic factor activation. J Cereb Blood Flow Metab 2002;22:843-51.

45. Vosler PS, Logue ES, Repine MJ, Callaway CW. Delayed hypothermia preferentially increases expression of brain-derived neurotrophic factor exon III in rat hippocampus after asphyxial cardiac arrest. Brain Res Mol Brain Res 2005;135:21-9.

46. Liu J, Solway K, Messing RO, Sharp FR. Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. J Neurosci 1998;18:7768-78.

47. Dumitrascu OM, Lamb J, Lyden PD. Still cooling after all these years: Meta-analysis of pre-clinical trials of therapeutic hypothermia for acute ischemic stroke. J Cereb Blood Flow Metab 2016;36:1157-64.

48. Clark DL, Penner M, Orellana-Jordan IM, Colbourne F. Comparison of 12, 24 and 48 h of systemic hypothermia on outcome after permanent focal ischemia in rat. Exp Neurol 2008;212:386-92.

49. Maier CM, Ahern KV, Cheng ML, Lee JE, Yenari MA, Steinberg GK. Optimal depth and duration of mild hypothermia in a focal model of transient cerebral ischemia: Effects on neurologic outcome, infarct size, apoptosis, and inflammation. Stroke 1998;29:2171-80.

50. Carroll M, Beek O. Protection against hippocampal CA1 cell loss by post-ischemic hypothermia is dependent on delay of initiation and duration. Metab Brain Dis 1992;7:45-50.

51. Hemmen TM, Lyden PD. Hypothermia after acute ischemic stroke. J Neurotrauma 2009;26:387-91.

52. Maier CM, Sun GH, Kunis D, Yenari MA, Steinberg GK. Delayed induction and long-term effects of mild hypothermia in a focal model of transient cerebral ischemia: Neurological outcome and infarct size. J Neurosurg 2001;94:90-6.

53. Kollmar R, Schäbitz WR, Heiland S, Georgiadis D, Schellinger PD, Bardutzky J, *et al.* Neuroprotective effect of delayed moderate hypothermia after focal cerebral ischemia: An MRI study. Stroke 2002;33:1899-904.

54. Lyden PD, Lamb J, Kothari S, Toossi S, Boitano P, Rajput PS. Differential effects of hypothermia on neurovascular unit determine protective or toxic results: Toward optimized therapeutic hypothermia. J Cereb Blood Flow Metab 2019;39:1693-709.

55. Yenari MA, Hemmen TM. Therapeutic hypothermia for brain ischemia: Where have we come and where do we go? Stroke 2010;41:S72-4.

56. Han Z, Liu X, Luo Y, Ji X. Therapeutic hypothermia for stroke: Where to go? Exp Neurol 2015;272:67-77.

57. Kollmar R, Blank T, Han JL, Georgiadis D, Schwab S. Different degrees of hypothermia after experimental stroke: Short and long-term outcome. Stroke 2007;38:1585-9.

58. Huh PW, Belayev L, Zhao W, Koch S, Busto R, Ginsberg MD, *et al.* Comparative neuroprotective efficacy of prolonged moderate intraischemic and postischemic hypothermia in focal cerebral ischemia. J Neurosurg 2000;92:91-9.

59. Vieites-Prado A, Iglesias-Rey R, Fernández-Susavila H, da Silva-Candal A, Rodríguez-Castro E, Gröhn OH, *et al.* Protective effects and magnetic resonance imaging temperature mapping of systemic and focal hypothermia in cerebral ischemia. Stroke 2016;47:2386-96.

60. Biernaskie J, Szymanska A, Windle V, Corbett D. Bi-hemispheric contribution to functional motor recovery of the affected forelimb following focal ischemic brain injury in rats. Eur J Neurosci 2005;21:989-99.

61. Gharbawie OA, Karl JM, Whishaw IQ. Recovery of skilled reaching following motor cortex stroke: Do residual corticofugal fibers mediate compensatory recovery? Eur J Neurosci 2007;26:3309-27.

62. Jones TA. Motor compensation and its effects on neural reorganization after stroke. Nat Rev Neurosci 2017;18:267-80.

63. Buetefisch CM. Role of the contralesional hemisphere in post-stroke recovery of upper extremity motor function. Front Neurol 2015;6:214.

64. Galinsky R, Dean JM, Lear CA, Davidson JO, Dhillon S, Wassink G, *et al.* In the era of therapeutic hypothermia, how well do studies of perinatal neuroprotection control temperature? Dev Neurosci 2017;39:7-22.

65. Tagin MA, Woolcott CG, Vincer MJ, Whyte RK, Stinson DA. Hypothermia for neonatal hypoxic ischemic encephalopathy: An updated systematic review and meta-analysis. Arch Pediatr Adolesc Med 2012;166:558-66.

66. Davidson JO, Wassink G, van den Heuij LG, Bennet L, Gunn AJ. Therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy - Where to from here? Front Neurol 2015;outcomes.

67. Shankaran S, Laptook AR, Pappas A, McDonald SA, Das A, Tyson JE, *et al.* Effect of depth and duration of cooling on death or disability at age 18 months among neonates with hypoxic-ischemic encephalopathy: A randomized clinical trial. JAMA 2017;318:57-67.

68. Davidson JO, Draghi V, Whitham S, Dhillon SK, Wassink G, Bennet L, *et al.* How long is sufficient for optimal neuroprotection with cerebral cooling after ischemia in fetal sheep? J Cereb Blood Flow Metab 2018;38:1047-59.

69. Azzopardi D, Strohm B, Marlow N, Brocklehurst P, Deierl A, Eddama O, *et al.* Effects of hypothermia for perinatal asphyxia on childhood outcomes. N Engl J Med 2014;371:140-9.

70. Guillet R, Edwards AD, Thoresen M, Ferriero DM, Gluckman PD, Whitelaw A, *et al.* Seven- to eight-year follow-up of the CoolCap trial of head cooling for neonatal encephalopathy. Pediatr Res 2012;71:205-9.

71. McLaren J, Holmes GL, Berg MT. Functional connectivity in term neonates with hypoxic-ischemic encephalopathy undergoing therapeutic hypothermia. Pediatr Neurol 2019;94:74-9.

72. Birca A, Lortie A, Birca V, Decarie JC, Veilleux A, Gallagher A, *et al.* Rewarming affects EEG background in term newborns with hypoxic-ischemic encephalopathy undergoing therapeutic hypothermia. Clin Neurophysiol 2016;127:2087-94.

73. Kollmar R, Staykov D, Dörfler A, Schellinger PD, Schwab S, Bardutzky J. Hypothermia reduces perihemorrhagic edema after intracerebral hemorrhage. Stroke 2010;41:1684-9.

74. Staykov D, Wagner I, Volbers B, Doerfler A, Schwab S, Kollmar R.

75. Melmed KR, Lyden PD. Meta-analysis of pre-clinical trials of therapeutic hypothermia for intracerebral hemorrhage. Ther Hypothermia Temp Manag 2017;7:141-6.

76. Fingas M, Penner M, Silasi G, Colbourne F. Treatment of intracerebral hemorrhage in rats with 12 h, 3 days and 6 days of selective brain hypothermia. Exp Neurol 2009;219:156-62.

77. Andriessen TM, Jacobs B, Vos PE. Clinical characteristics and pathophysiological mechanisms of focal and diffuse traumatic brain injury. J Cell Mol Med 2010;14:2381-92.

78. Dietrich WD, Bramlett HM. Therapeutic hypothermia and targeted temperature management for traumatic brain injury: Experimental and clinical experience. Brain Circ 2017;3:186-98.

79. Clifton GL, Valadka A, Zygun D, Coffey CS, Drever P, Fourwinds S, *et al.* Very early hypothermia induction in patients with severe brain injury (the national acute brain injury study: Hypothermia II): A randomised trial. Lancet Neurol 2011;10:131-9.

80. Andrews PJ, Sinclair HL, Rodriguez A, Harris BA, Battison CG, Rhodes JK, *et al.* Hypothermia for intracranial hypertension after traumatic brain injury. N Engl J Med 2015;373:2403-12.

81. Kramer C, Freeman WD, Larson JS, Hoffman-Snyder C, Wellik KE, Demaerschalk BM, *et al.* Therapeutic hypothermia for severe traumatic brain injury: A critically appraised topic. Neurologist 2012;18:173-7.

82. Marion DW, Penrod LE, Kelsey SF, Obrist WD, Kochanek PM, Palmer AM, *et al.* Treatment of traumatic brain injury with moderate hypothermia. N Engl J Med 1997;336:540-6.

83. Jiang JY, Xu W, Li WP, Gao GY, Bao YH, Liang YM, *et al.* Effect of long-term mild hypothermia or short-term mild hypothermia on outcome of patients with severe traumatic brain injury. J Cereb Blood Flow Metab 2006;26:771-6.

84. Polderman KH, Tjong Tjin Joe R, Peerdeman SM, Vandertop WP, Girbes AR. Effects of therapeutic hypothermia on intracranial pressure and outcome in patients with severe head injury. Intensive Care Med 2002;28:1563-73.

85. Clifton GL, Miller ER, Choi SC, Levin HS, McCauley S, Smith KR Jr., *et al.* Lack of effect of induction of hypothermia after acute brain injury. N Engl J Med 2001;344:556-63.

86. Dietrich WD, Bramlett HM. Therapeutic hypothermia and targeted temperature management in traumatic brain injury: Clinical challenges for successful translation. Brain Res 2016;1640:94-103.

87. Clifton GL, Choi SC, Miller ER, Levin HS, Smith KR Jr., Muizelaar JP, *et al.* Intercenter variance in clinical trials of head trauma – Experience of the national acute brain injury study: Hypothermia. J Neurosurg 2001;95:751-5.

88. Dash PK, Mach SA, Moore AN. Enhanced neurogenesis in the rodent hippocampus following traumatic brain injury. J Neurosci Res 2001;63:313-9.

89. Kernie SG, Erwin TM, Parada LF. Brain remodeling due to neuronal and astrocytic proliferation after controlled cortical injury in mice. J Neurosci Res 2001;66:317-26.

90. Bregy A, Nixon R, Lotocki G, Alonso OF, Atkins CM, Tsoulfas P, *et al.* Posttraumatic hypothermia increases doublecortin expressing neurons in the dentate gyrus after traumatic brain injury in the rat. Exp Neurol 2012;233:821-8.

91. Kuo JR, Lo CJ, Chang CP, Lin HJ, Lin MT, Chio CC, *et al.* Brain cooling-stimulated angiogenesis and neurogenesis attenuated traumatic brain injury in rats. J Trauma 2010;69:1467-72.

92. Atkins CM, Truettner JS, Lotocki G, Sanchez-Molano J, Kang Y, Alonso OF, *et al.* Post-traumatic seizure susceptibility is attenuated by hypothermia therapy. Eur J Neurosci 2010;32:1912-20.

93. Gao X, Deng-Bryant Y, Cho W, Carrico KM, Hall ED, Chen J. Selective death of newborn neurons in hippocampal dentate gyrus following moderate experimental traumatic brain injury. J Neurosci Res 2008;86:2258-70.

Mild prolonged hypothermia for large intracerebral hemorrhage. Neurocrit Care 2013;18:178-83.

94. Neuberger EJ, Swietek B, Corrubia L, Prasanna A, Santhakumar V. Enhanced dentate neurogenesis after brain injury undermines long-term neurogenic potential and promotes seizure susceptibility. Stem Cell Reports 2017;9:972-84.

95. Aarts LH, Schotman P, Verhaagen J, Schrama LH, Gispen WH. The role of the neural growth associated protein B-50/Gap-43 in morphogenesis. In: Ehrlich YH, editor. Molecular and Cellular Mechanisms of Neuronal Plasticity. Vol. 446. Boston, MA: Springer US; 1998. p. 85-106.

96. Benowitz LI, Routtenberg A. GAP-43: An intrinsic determinant of neuronal development and plasticity. Trends Neurosci 1997;20:84-91.

97. Doster SK, Lozano AM, Aguayo AJ, Willard MB. Expression of the growth-associated protein GAP-43 in adult rat retinal ganglion cells following axon injury. Neuron 1991;6:635-47.

98. Carow B, Rottenberg ME. SOCS3, a major regulator of infection and inflammation. Front Immunol 2014;5:58.

99. Polderman KH. Mechanisms of action, physiological effects, and complications of hypothermia. Crit Care Med 2009;37:S186-202.

100. Park SM, Mangat HS, Berger K, Rosengart AJ. Efficacy spectrum of antishivering medications: Meta-analysis of randomized controlled trials. Crit Care Med 2012;40:3070-82.

101. Jain A, Gray M, Slisz S, Haymore J, Badjatia N, Kulstad E. Shivering treatments for targeted temperature management: A review. J Neurosci Nurs 2018;50:63-7.

102. Wildschut ED, van Saet A, Pokorna P, Ahsman MJ, Van den Anker JN, Tibboel D. The impact of extracorporeal life support and hypothermia on drug disposition in critically ill infants and children. Pediatr Clin North Am 2012;59:1183-204.

103. Šunjić KM, Webb AC, Šunjić I, Palà Creus M, Folse SL. Pharmacokinetic and other considerations for drug therapy during targeted temperature management. Crit Care Med 2015;43:2228-38.

104. Chamorro C, Borrallo JM, Romera MA, Silva JA, Balandín B. Anesthesia and analgesia protocol during therapeutic hypothermia after cardiac arrest: A systematic review. Anesth Analg 2010;110:1328-35.

105. Favié LM, Groenendaal F, van den Broek MP, Rademaker CM, de Haan TR, van Straaten HL, *et al.* Pharmacokinetics of morphine in encephalopathic neonates treated with therapeutic hypothermia. PLoS One 2019;14:e0211910.

106. Orban JC, Cattet F, Lefrant JY, Leone M, Jaber S, Constantin JM, *et al.* The practice of therapeutic hypothermia after cardiac arrest in France: A national survey. PLoS One 2012;7:e45284.

107. Frymoyer A, Bonifacio SL, Drover DR, Su F, Wustoff CJ, Van Meurs KP. Decreased morphine clearance in neonates with hypoxic ischemic encephalopathy receiving hypothermia. J Clin Pharmacol 2017;57:64-76.

108. Milanovic D, Pesic V, Loncarevic-Vasiljkovic N, Avramovic V, Tesic V, Jevtovic-Todorovic V, *et al.* Neonatal propofol anesthesia changes expression of synaptic plasticity proteins and increases stereotypic and anxyolitic behavior in adult rats. Neurotox Res 2017;32:247-63.

109. Briner A, Nikonenko I, De Roo M, Dayer A, Muller D, Vutskits L. Developmental stage-dependent persistent impact of propofol anesthesia on dendritic spines in the rat medial prefrontal cortex. Anesthesiology 2011;115:282-93.

110. Puskarjov M, Fiumelli H, Briner A, Bodogan T, Demeter K, Lacoh CM, *et al.* K-Cl cotransporter 2-mediated Cl-extrusion determines developmental stage-dependent impact of propofol anesthesia on dendritic spines. Anesthesiology 2017;126:855-67.

111. Álvaro-Bartolomé M, García-Sevilla JA. The neuroplastic index p-FADD/FADD and phosphoprotein PEA-15, interacting at GABAA receptor, are upregulated in brain cortex during midazolam-induced hypnosis in mice. Eur Neuropsychopharmacol 2015;25:2131-44.

112. Persec J, Persec Z, Buković D, Merc V, Pavelić J, Zupić T. Preoperative clonidine or levobupivacaine – Effect on systemic inflammatory stress response. Coll Antropol 2009;33:573-7.

113. García-Fuster MJ, Ramos-Miguel A, Miralles A, García-Sevilla JA. Opioid receptor agonists enhance the phosphorylation state of Fas-associated death domain (FADD) protein in the rat brain: Functional interactions with casein kinase ialpha, galpha (i) proteins, and ERK1/2 signaling. Neuropharmacology 2008;55:886-99.

114. Persec J, Persec Z, Husedzinovic I. Postoperative pain and systemic inflammatory stress response after preoperative analgesia with clonidine or levobupivacaine: A randomized controlled trial. Wien Klin Wochenschr 2009;121:558-63.

115. Liang D, Li X, Clark JD. Increased expression of Ca2+/calmodulin-dependent protein kinase II alpha during chronic morphine exposure. Neuroscience 2004;123:769-75.

116. Younger JW, Chu LF, D'Arcy NT, Trott KE, Jastrzab LE, Mackey SC. Prescription opioid analgesics rapidly change the human brain. Pain 2011;152:1803-10.

117. Fisher M, Feuerstein G, Howells DW, Hurn PD, Kent TA, Savitz SI, *et al.* Update of the stroke therapy academic industry roundtable preclinical recommendations. Stroke 2009;40:2244-50.