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Cerebral oxidative stress, inflammation and apoptosis induced by intermittent hypoxia: a systematic review and meta-analysis of rodent data

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Take home message

Our meta-analysis of rodent studies firmly establishes that intermittent hypoxia, as a model of obstructive sleep apnea, alters brain inflammation, oxidative stress and apoptosis. Severity of IH and brain localization contribute to this impact.

Questions for future research and points for clinical practice

Our work shows that for several parameters, the impact of IH varies with IH severity and brain localization. More studies are needed to gain a more comprehensive view of the IH impact, including various IH severities and durations and their specific impact on different brain regions. The vast majorities of studies are performed in male rodents, studies including female rodent are thus mandatory to understand sex-related variability of IH impact. Finally, IH is predominantly used as a model to mimic OSA in rodents, and the impact of sleep fragmentation on the rodent brains remains an under-investigated issue.

Given the important consequences of intermittent hypoxia on the brain, our work also strongly support the interest of identifying and treating sleep apnea in neurology practice.

Word count: 4186

Abstract

Obstructive sleep apnea (OSA) contributes to cerebrovascular diseases and cognitive decline. Preclinical studies support the deleterious impact on the brain of intermittent hypoxia (IH)- one of the main components of OSA-, but heterogeneity in rodent species and brain regions studied, or induced by IH paradigms, can challenge interpretation of the studies.

Hence, we conducted a systematic review and meta-analysis to evaluate the impact of IH on rodent brain oxidative stress, inflammation, apoptosis, and the expression of BDNF and HIF-1.

PubMed and Web of Science search pointed out 663 articles with IH exposure and 60 were included, outcomes were oxidative stress, inflammation, apoptosis, HIF-1 or BDNF in brains. Standardized Mean Difference (SMD) were used to compare studies. Metaregressions were performed to clarify the impact of IH exposure parameters, rodent characteristics or cerebral localization on these outcomes.

IH induced oxidative stress (increased MDA, NADPH oxidase (NOX) and decreased Superoxide Dismutase (SOD)), increased inflammation (TNF α , NFkB and iNOS), HIF-1, and apoptosis evaluated by TUNEL and cleaved caspase-3. By contrast, BCL2 and BDNF expression were not significantly modified. Metaregressions showed that MDA, NOX and BDNF were associated with determinants of IH cycles (FiO₂, duration of hypoxia) and some parameters depended on localization. Rodent characteristics had few impacts on the outcomes.

Our meta-analysis robustly establishes that IH, independently of other confounders, has a strong effect on the brain by inducing oxidative stress, inflammation and apoptosis, in rodent models. Our findings support the interest of considering and treating cerebral consequences of OSA in clinical practice.

Introduction

Obstructive Sleep apnea (OSA) is a highly prevalent condition, affecting nearly 1 billion people worldwide, with a prevalence exceeding 50% in some associated conditions [1]. OSA is characterized by the repetitive occurrence of apneas and hypopneas during sleep, due to complete or partial pharyngeal collapses [2]. The pathophysiological consequences of sleep-related respiratory disturbances in OSA are characterized by sleep fragmentation, increased respiratory efforts and intermittent hypoxia (IH), the latter being acknowledged as the main mechanism responsible for the systemic deleterious consequences of OSA. OSA is therefore associated with elevated cardiovascular morbidity and mortality, through an increased risk of hypertension, atherosclerosis, coronary heart disease and cerebrovascular disease, including an increased stroke risk [2–4]. Moreover, a wide range of functional and morphological neurological alterations have been associated with OSA, including increased risk of dementia and neurodegenerative diseases, aggravated seizure and impaired cognition [5–8]. Impaired memory, attention, and executive dysfunction could be promoted by IH-induced disruption of cellular homeostasis and neuronal survival, triggering brain inflammation and oxidative stress, leading to neuronal apoptosis and astrocyte and microglial activation, and finally to neurobehavioral alterations [6,7,9–11]. The effects of IH may be dose-dependent, as some authors hypothesized that exposure to moderate IH (i.e., conditioning) may have protective effects on the brain, by activating adaptative mechanisms such as growth factor release or by stimulating the proliferation and differentiation of neural stem cells, while severe IH could be deleterious by inducing neuroinflammation and loss of neuronal plasticity [5,12,13]. However, data supporting this hypothesis are still scarce and mostly arise from preclinical models of IH, and has never been confirmed in humans. Moreover, with respect to the cognitive impairment induced by IH, most of animal studies focused on hippocampal consequences of IH. However, the effects of IH may vary depending on the brain region. In this context, we aimed in the present meta-analysis to investigate the impact of IH on brain in rodent models, with a particular emphasis on oxidative stress, inflammation and apoptosis, together with Brain-Derived Neurotrophic Factor (BDNF) production and Hypoxia-Induced Factor-1 (HIF-1) expression. Further analyses explored IH-response differences depending on brain regions, and investigated if specific animal models (species, age, sex, body weight) or intermittent hypoxia paradigms (inspired oxygen fraction (FiO₂), cycle duration, total duration of IH exposures) could modulate the brain responses to IH.

Methods

The protocol for the meta-analysis was recorded in the PROSPERO registry under the number CRD42023437237 (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42023437237).

Search methods and study selection

We searched MEDLINE and Web of Science for articles published up to May 18th, 2023. The search terms were “Intermittent hypoxia” AND “Rodent” OR “mice” OR “rat” AND “brain” OR “central nervous system” (see PROSPERO record for exact query). We also searched for keywords and MeSH related to each search term. After the initial electronic search, we screened the titles and the abstracts to retrieve relevant articles. Eligibility was considered if they were written in English and addressed cerebral outcomes of intermittent hypoxia exposure in rodents. Then, the full manuscripts were screened for inclusion and exclusion criteria (see below). Three authors (BEL, CR, ABM) independently screened each paper for inclusion, and discrepancies were individually resolved to reach agreement between readers.

Intermittent hypoxia was defined as the repetitive exposure to several consecutive normobaric hypoxia-reoxygenation cycles during the same day. All wild-type rodent models (mouse and rat, male and female, aged ≥ 4 weeks, lean or obese) were considered. We excluded studies without any control group (normoxic and untreated mice), studies in which hypoxia was hypobaric or continuous (i.e. no hypoxia-reoxygenation cycles), or studies in which hypoxia was combined with hypercapnic conditions. We also excluded studies using IH exposure in prenatal or perinatal periods and studies using transgenic or pharmacologically treated animals.

The outcomes considered as mandatory for inclusion were cerebral markers of oxidative stress (including malondialdehyde (MDA), Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) or Superoxide dismutase (SOD)), markers of inflammation (including Tumor Necrosis Factor- α (TNF α), Nuclear Factor kappa B (NF κ B) and inducible Nitric Oxide Synthase (iNOS)), apoptosis (including Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, cleaved caspase-3 or Bcl2), Brain-Derived Neurotrophic factor (BDNF), or Hypoxia-inducible factor -1 (HIF-1). We included gene expression, protein expression, and activity assays.

For each study, we extracted data concerning the outcomes but also rodents' characteristics (species, strain, age, body weight, sex) and IH protocols (FiO₂ during the hypoxic phase, duration of the hypoxic and reoxygenation phase within each cycle, number of hours of IH exposure per day, total duration of IH exposure over days or weeks). To assess the severity of IH in one single score, we also calculated an “hypoxic severity score” as follows: for each of the 5 parameters describing the hypoxic exposure, we categorized the parameters based on quartiles of the retrieved values in the studies, and attributed a score of 1 (lowest quartile) to 4 (highest quartile) for each parameter for each study. The 5 parameters

considered are 1) FiO₂ during the hypoxic phase, 2) duration of hypoxic phase, 3) duration of reoxygenation phase, 4) duration of the hypoxic exposure per day, and 5) total duration of exposure in days. We then calculated the hypoxic severity score by combining the 5 individual scores: Hypoxic severity score = X1 + X2 - X3 + X4 + X5. Because X3 is reoxygenation duration and thus opposed to hypoxia duration, we deduced it from the score instead of adding it. The higher the score, the greater the severity of hypoxic exposure (see supplementary methods for details).

Assessment of risk of bias

The assessment of risk of bias of studies was performed using the SYRCLE tool described by Hooijmans et al.[14]. This contains several types of bias: selection bias (sequence generation, baseline characteristics, and allocation concealment), performance bias (randomized housing of animals, blinding of investigators), detection bias (random outcome assessment, blinding of outcome assessor), attrition bias (incomplete outcome data), and reporting bias (selective outcome reporting). Each risk of bias was scored as High, Low, or Unclear. Three authors (BEL, CR, ABM) were involved and every discrepancy was discussed to achieve a shared decision.

Statistical analysis

For study descriptions, we used medians and interquartile ranges (IQR). For each outcome, data were abstracted and analyzed using standardized mean difference (SMD) with 95% confidence intervals, written as SMD [95% CI]. SMD is defined as $SMD = (Me - Mc) / SD$, where Mc is the mean of the outcome measure in the control group, Me is the mean of the outcome measure in the experimental group, and SD is the pooled standard deviation of the two groups. A SMD > 0.8 was considered as large, 0.5-0.8 as moderate, and 0.2-0.5 small [16]. Given the high anticipated heterogeneity in included studies we performed random effect meta-analysis by the restricted maximum-likelihood estimator method [17]. Moreover, to account for correlation among multi-arm studies we constructed a hierarchical/mixed effect model with a random intercept for study and arm. All results are presented using orchard plots [18]. In case of a missing SD we estimated it from standard errors or imputed it using mean SD of similar outcomes [19] (supplementary figure 1). We trimmed studies with SMD greater or less than 10 to limit outlier influence on the results. We explored sources of heterogeneity through pre-specified subgroup analyses and meta-regressions according to population (species, age and body weight) and details of intermittent hypoxia protocols (FiO₂ during hypoxic phases, duration of hypoxic and normoxic phases, frequency, duration per day and total duration of exposure, and hypoxic score calculated as detailed above). We performed univariate meta-regressions on study and animal characteristics for parameters studied in at least ten studies, thus excluding NFκB and HIF-1. We

replicated the meta-regression analyses after excluding outliers and influential cases to assess the robustness of the findings; these were identified through analysis of Cook's distances (i.e. excluding studies with a Cook's distance of three times the mean values) and studentized residuals (i.e. excluding studies residuals that exceeded three standard deviations). Given the exploratory nature of these analyzes we considered all p-values <0.05 as significant.

Funnel plot asymmetry was explored for primary outcomes using Egger's regression test, as recommended by the Cochrane handbook for systemic reviews of interventions [20], with $p < 0.1$ suggesting publication bias. We also performed a Trim and Fill analysis to assess the impact of small study effects on the meta-analyses results [21].

All statistical analyses were performed using R statistical software (version 4.3.3).

Results

Our literature review retrieved 633 references among which we ultimately retained 60 studies for inclusion in the meta-analysis (Figure 1). Supplementary table 1 and supplementary figure 2 present a summary of the cerebral outcomes available across the selected studies, IH exposure protocols and experimental designs.

Among the 60 studies performed on wild-type rodents, 25 were in mice (22 in C57Bl/6 mice, 3 in other strains) and 35 in rats (28 in Sprague-Dawley, 7 in Wistar rats). At inclusion, median (interquartile range [IQR]) age was 8 (6-12) weeks. Body weight were 21.75 (20-27) grams for mice and 225 (175-275) grams for rats. Males were used in 55 studies, females in 1 study, both males and females in 2 studies, and 2 studies did not report the sex of the animals.

Concerning IH protocols (Supplementary figure 2), the median (interquartile range) of FiO_2 during hypoxic periods were 6.25 (5.7-9) %, desaturation lasted 58 (20-95) seconds followed by 80 (45-120) seconds of reoxygenation. Cycles were repeated on average for 8h/day for a median duration of 14 (7-28) days.

Various brain regions were studied: 50 % of studies investigated the specific impact of IH exposure on the hippocampus, 27% on the cortex, 20% specifically on the brainstem and 3% did not specify brain localization (Supplementary table 1).

Impact of IH on brain oxidative stress

To assess the impact of IH on oxidative stress, we chose 3 markers among the most widely used in the literature: quantification of malondialdehyde (MDA), expression or activity of NADPH oxidase (NOX), and expression or activity of superoxide dismutase (SOD).

We observed that IH exposure significantly increased MDA (SMD 2.7 [2.02;3.39]). It also increased NADPH Oxidase expression or activity (SMD 1.59 [0.34;2.85]) and decreased SOD (SMD -1.91 [-2.7; -1.12]) (Figure 2 and supplementary figure 3).

Metaregressions showed that MDA levels were significantly and positively associated with duration of the reoxygenation phase (estimate 0.01, $p=0.025$) and total duration of IH exposure (estimate 0.04, $p=0.026$); and negatively associated with age (estimate -0.27, $p=0.013$) (Figure 2A and supplementary table 2). MDA expression was also specifically associated with the brainstem, cortex and hippocampus compared to global brain tissue (Supplementary table 2). NADPH oxidase was negatively associated with FiO₂ during the hypoxic phase (estimate -0.50, $p=0.014$), suggesting that NADPH oxidase is more activated by more severe (i.e., low levels) of FiO₂ than by moderate ones (Figure 2B and supplementary table 2).

Impact of IH on brain inflammation

Inflammation was evaluated through 3 markers: gene or protein expression of TNF α , expression or activity of the transcription factor NF κ B, and expression or activity of iNOS.

We observed that these 3 markers were significantly increased in the brain after IH exposure: For TNF α , the SMD was 2.13 [1.61;2.65], for NF κ B 5.28 [3.55;7.01] and for iNOS 1.24 [0.45;2.03] (Figure 3 and supplementary figure 3). In metaregressions, iNOS was negatively associated with age (estimate -0.47, $p=0.024$) and was specifically increased in the hippocampus (estimate 3.25, $p<0.001$) (supplementary table 2). There was no significant metaregression for TNF α , and they were not calculated for NF κ B due to the low number of studies.

Impact of IH on brain apoptosis

To evaluate apoptosis, our analyses were based on the quantification of TUNEL staining, cleavage of caspase-3, and protein expression of BCL2.

TUNEL staining significantly increased after IH exposure (SMD 3.33 [2.67;3.99]) and cleaved caspase-3 increased as well (SMD 2.7 [1.84;3.57]). By contrast, results concerning BCL2 expression were heterogeneous and did not show any effect of IH exposure (SMD -1.46 [-3.33;0.41])(Figure 4 and

supplementary figure 3). Metaregressions showed that BCL2 expression was associated with age (estimate 1.70, $p=0.025$) (supplementary table 2).

Impact of IH on brain HIF-1

The expression of the transcription factor HIF-1 is related to both oxidative stress and inflammation. Our meta-analysis found an increase in gene or protein expression of HIF-1 after IH exposure (SMD 3.47 [1.27;5.67]) (Figure 5A and supplementary figure 3). Due to the low number of studies ($n=6$), metaregressions could not be performed for HIF-1.

Impact of IH on BDNF

Finally, we evaluated the impact of IH exposure on protein expression of BDNF in the brain. We found that IH exposure had a variable and non-significant impact on BDNF expression (SMD -0.53 [-2.81;1.75]) (Figure 5B and supplementary figure 3). In metaregressions BDNF expression was negatively associated with the duration of hypoxic phase (estimate -0.14, $p<0.001$) (Figure 5C), and positively associated with species (stronger effect in rat vs mice, estimate 5.15, $p=0.003$) and with body weight (estimate 0.05, $p<0.001$) (Supplementary table 2).

Estimation of the risk of bias of studies

The quality of studies was assessed by using the SYRCLE tool [14]. The results are presented in supplementary figure 4 and supplementary table 3. The risk was considered as low for sequence generation in 57% of studies, baseline characteristics for 64% of studies, incomplete outcome data for 41% of studies and selective outcome reporting for 69% of studies. On the other hand, the risk was high for 28% of studies for baseline characteristics, 47% of studies for incomplete outcome data and 36% for selective outcome reporting. For all the other SYRCLE items including allocation concealment, randomized housing, blinding of investigators, random outcome assessment and blinding of outcome measurement, the information was almost never mentioned in publications, resulting in 95% of studies scored as unclear.

Small study effect

Funnel plots are presented in supplementary figure 5. For most of the outcomes except for NADPH oxidase, NFkB and BDNF, the asymmetric distribution of studies and a significant Egger regression test were observed, indicating a clear small study effect.

Discussion

OSA is a major risk factor for cerebral functional and morphological alterations, as well as cerebrovascular complications, including an increased ischemic stroke risk [3–5,22,23]. Although heterogeneity or even inconsistencies remain, a large body of literature showed that among the main OSA features, intermittent hypoxia and sleep fragmentation are both possible triggers for cerebral and cerebrovascular complications of OSA [10,24–27]. In human studies, the specific role of IH is hard to decipher, due to the frequent comorbid associations of OSA, including obesity and hypertension, and the difficulty to measure or control, on a daily basis, the stimulus (i.e., IH) severity. Animal models allow for the specific role of IH exposure to be addressed independently of confounders, in a controlled manner. Our meta-analysis results, synthesized in figure 6, firmly show that IH alone has a significant major impact (SMD often >1) on harmful intermediary mechanisms in the brain such as oxidative stress, inflammation or apoptosis, some of them being dependent on the IH severity or duration, while showing rodent model characteristics specificity of the impact of IH exposure.

Impact of IH on brain outcomes

Oxidative stress. Our meta-analysis emphasizes that IH has a major impact on brain oxidative stress, as demonstrated by levels of the lipid peroxidation marker MDA, the pro-oxidant NADPH oxidase and the anti-oxidant SOD. We chose to specifically focus our analysis on these 3 markers as they were the most commonly reported in IH studies. However, a large body of literature consistently reports increases in other markers such as isoprostanes [24], oxidized or reduced glutathione [25], DNA oxidation marker 8-OHdG [26,27], increases in xanthine oxidase [28] or decreases in anti-oxidant catalase or glutathion peroxidase [29,30].

Although specific oxidative stress in the brain were never evaluated in patients, our results are consistent with oxidative stress markers reported in plasma or tissues of OSA patients [31] as well as with the crucial role of oxidative stress in inducing cognitive and neurological dysfunction [6]. Interestingly, metaregressions showed that MDA and NADPH oxidase were associated with IH duration and FiO₂ levels respectively, suggesting that brain oxidative stress is directly related to the severity of hypoxia and that the hypoxic dose and pattern could thus be a key element for increased risk of functional and morphological brain defects. Continuous positive airway pressure (CPAP) therapy, the first line therapy in OSA, through the correction of OSA-related IH, significantly reduces systemic oxidative stress levels in patients with severe OSAS, even after only one night of treatment [32,33]. CPAP could therefore represent an efficient neuroprotective intervention in case of a sufficient treatment adherence. As an illustration, Richards et al. found that OSAS patients with mild cognitive impairment who were adherent to CPAP therapy over 1 year had improved cognitive function and decreased rate of decline compared to non-adherent controls[34].

Inflammation. We also evaluated three brain inflammation markers, TNF α , NFkB and iNOS. These 3 outcomes were robustly associated with IH exposure (SMD >1). There was no association of inflammatory markers with the hypoxic score, suggesting that these pro-inflammatory effects are sustained under IH, whatever the IH severity or duration. Heightened inflammation biomarkers levels are found in plasma and tissues of OSA patients, particularly in the context of cardiovascular or metabolic alterations induced by OSA/IH [2,35,36]. Inflammation is also strongly associated with cognitive dysfunction in OSA patients and rodent models [7,37–39]. The transcription factor NFkB, the main transcriptional regulator of inflammatory responses, is crucial in the IH-induced inflammation and its associated cardiovascular or cerebral consequences [7,35]. Among its downstream products, TNF α is one of the most described pro-inflammatory cytokines in OSA patients and in IH rodent models [2,35,36,40] and iNOS has also been investigated in rodents in various tissues including the brain [39,41]. By demonstrating the impact of IH on these 3 markers, our meta-analysis thus confirms the activation of the NFkB signaling pathway in the brain in response to IH exposure. The activation of the NFkB pathway may harm global brain health, as it has been recently shown in humans that post-stroke untreated OSA with more severe white matter hyperintensities was associated with significantly increased plasma TNF α concentrations, along with increased neurofilament light chain and decreased concentrations of platelet-derived growth factor (PDGF-AA)[42]. Future studies should investigate the neuroprotective impact of interventions targeting this pathway in OSA patients.

Together with these 3 markers, other inflammatory markers expression have been described in the brain in response to IH exposure, such as the upregulation of pro-inflammatory cytokines IL-6 [43], IL1 β [44,45] or COX2 [25], and the downregulation of anti-inflammatory cytokines IL-4 or IL-10 [44]. Astrocyte and microglial inflammation was assessed by increases of GFAP [46] and Iba1 [45], respectively. Implication of the Toll-like receptors TLR2 and TLR4 [47,48] and NLRP3 inflammasome [45,49] pathways were also demonstrated and associated with cognitive impairments, highlighting the crucial role of neuroinflammation in brain alterations and the associated neurobehavioral dysfunctions induced by IH. However, recently, a study conducted in healthy older community-dwelling people showed an association between undiagnosed mild OSA and a small decline in global cognitive function over three years that was not reversed by daily low-dose, anti-inflammatory doses of aspirin, highlighting that targeting neuroinflammation without alleviating OSA-related IH may not be sufficient [50].

HIF-1. We observe a strong increase in HIF-1 expression in the brain after IH exposure. HIF-1 is a hypoxia-induced transcription factor well known for its role in adaptive and maladaptive responses to intermittent hypoxia in various organs [7,51,52].

Apoptosis. We selected three well-known markers for apoptosis, namely TUNEL staining, cleavage of caspase 3 and expression of BCL2. While TUNEL and caspase-3 were significantly increased by IH, the impact on BCL2 was heterogeneous and non-significant. Our results are consistent with the known hypotrophic brain changes observed in OSA patients that may represent chronic cellular impairments, including, but not restricted to, cellular death [53]. Consistent results were also observed with other apoptosis markers such as cleavage of PARP or markers of extrinsic apoptosis cascade [25,54], and with 8-OHdG, a marker of DNA oxidation associated with DNA damage and apoptosis, described in the cortex and the hippocampus after IH [27,55].

BDNF. Due to the heterogeneity of available results in the literature, our meta-analysis did not evidence any significant effect of IH exposure on BDNF expression in the brain. BDNF was negatively associated with duration of the hypoxic phase, suggesting that long hypoxic phases are required to significantly decrease BDNF levels. Our results are consistent with a recent meta-analysis that did not evidence any significant impact of sleep apnea or of continuous positive airway pressure treatment, the gold standard treatment of OSA treatment, on BDNF in patients [56]. Further work is required to unravel the mechanisms linking OSA/IH and BDNF regulation.

Central role of oxidative stress in the cerebral impact of IH

HIF-1 activation is strongly linked to oxidative stress [51]. Indeed, in the brain, inhibition of oxidative stress by apocynin or by using NOX2 knock-out mice reduced HIF-1 expression and neuropathological impairment induced by IH [26], confirming the causal role of oxidative stress in the activation of HIF-1 and functional impairment. Conversely, inhibition of HIF-1 by digoxin or using *HIF-1 +/-* mice reversed the IH-induced oxidative stress consequences, including lipid peroxidation, DNA oxidation, and expression and activity of NADPH oxidase 2 [57], suggesting a bi-directional link between HIF-1 and NOX2 than can contribute to the development of neurobehavioral alterations.

Similarly, inflammation is also closely linked with oxidative stress, and many studies suggested that ROS can induce brain damage and cognitive dysfunction through several pathways including inflammation [7,11]. Anti-oxidant strategies abolished brain inflammation [54,58] and conversely, oxidative stress is diminished in NLRP3 knock-out mice exposed to IH [45], suggesting a crosstalk between inflammation and oxidative stress in the brain under IH, a condition leading to neurobehavioral damage.

Moreover, apoptosis and DNA damage induced by IH were reversed by anti-oxidant strategies such as treatment with edaravone [29], SOD overexpression [59] or NOX knock-out mice [60], suggesting a direct causal role of oxidative stress in IH-induced apoptosis in the brain. Finally, two studies found a

decrease in BDNF after IH exposure, and also showed that anti-oxidant strategies were able to reverse this impact of IH [29,58].

Altogether, this emphasizes the central role of oxidative stress in response to IH in the brain, as a deleterious stimulus related to inflammation, apoptosis, HIF-1, and BDNF dysregulation, possibly leading to neural dysfunction and cognitive impairment. This hypothesis is summarized in Figure 6.

Differential impact of IH on brain regions

Most of the studies investigated the impact of IH in the cortex, hippocampus or brainstem specific regions (locus coeruleus, solitary tract nucleus, rostral ventrolateral medulla,..). We found that the impact of IH exposure on MDA and iNOS levels were dependent on brain region, but not for the other parameters, for which the effect was sustained in all the studied brain regions. These brain region-dependent effects can be associated with specific functional alterations such as memory impairment, autonomous control, etc. and may be dependent on the cytoarchitectonic nature of the considered brain region, neocortical regions being more susceptible to variations of oxygen supply [61]. In OSA patients, a specific pattern of regional hypertrophic and hypotrophic brain changes emerges, with exposure to hypoxia being a specific trigger of these changes [53,62,63]. This suggests that further assessment of the impact of IH on the brain should be conducted by considering regional differences.

Contribution of rodent model characteristics to the impact of IH

We aimed at investigating whether rodent characteristics, such as species / strain, age, sex, or body weight, could influence the impact of IH on the brain. We found that MDA, iNOS and BCL2 were significantly associated with rodent age, older mice showing less MDA and iNOS, but more BCL2. However, in the included studies age remains in a narrow range of 4-12 weeks of age (adolescent/young adult rodent), with only one study conducted in adult mice (12 months old). Thus, further studies are needed to investigate the impact of IH in adult or aged rodents, particularly as OSA is mainly predominant in humans aged 50 or over. We were not able to include sex in the metaregressions, due to the very low number of studies including female mice. Again, further studies are required to investigate the specific question of the impact of IH in females' brain. This question should not be neglected, considering the important number of female OSA patients in clinical practice, the specificity of the clinical presentation, comorbidities and outcomes [64]. Finally, the metaregressions showed a significant contribution of species or body weight for BDNF only. This suggests that these parameters may be an explanation for the heterogeneity of BDNF results, and that IH has a robust effect on other parameters independently of the rodent characteristics.

Risk of bias and limitations of the study

For many of the SYRCL items, the risk of bias of included studies was high or unclear, due to the absence of mention of the items in the publications, as previously described in other animal meta-analyses including ours [65,66]. The heterogeneity of risk of bias among included studies may have also participated to the residual heterogeneity found in the meta-analysis that is not explained by the reported characteristics of animals and experiments in publications. In a previous work we have found that non reported laboratory conditions and author group may explain a part of this unexplained heterogeneity [67]. This highlights the need for improvement of research transparency and publication practices in animal experimentation studies. Moreover, we performed a statistical analysis that demonstrated a small study effect due to a probable publication bias, as we previously reported in meta-analyses focusing on vessels [65] or heart [66]. A publication bias is expected in animal studies given the frequent absence of pre-registered protocol and incentive to publish negative results, yielding to selective reporting of positive results.

Another limitation of the study is that parameters such as oxidative stress or inflammation are investigated at the brain region level, but there is no distinction between neurons or glial cells, further complicating interpretation of data. Some papers are associating staining markers (*i.e.* DHE for oxidative stress or TUNEL for apoptosis) with cell-specific markers, but they remain scarce. However, molecular and functional responses could considerably vary between different cell types [68], highlighting the need for precise description of cell-specific responses .

Finally, because we focused on molecular markers altered by IH, we did not include behavioral and cognitive impact of IH in our meta-analysis. This includes a variety of functional tests assessing parameters such as working or long-term memory, anxiety, or depressive behavior, which could serve as the basis for a separate meta-analysis.

Conclusions and perspectives

This is the first meta-analysis addressing molecular impact of IH on the brain in rodents. Based on a large corpus of articles and a variety of markers, we evidence a clear negative impact of IH on the brain including increased oxidative stress, inflammation and apoptosis. For some parameters, we identified some features of IH (e.g. FiO_2 during hypoxia or duration of hypoxia exposure) that were associated with an amplified impact of IH. However, in most cases, the impact of IH was robustly reproduced whatever its precise pattern, confirming that IH – as a model mimicking sleep apnea in humans – profoundly affects cerebral function. The impact of sleep fragmentation, as another important feature of OSA affecting the brain [37], remains to be specifically investigated. We also highlight the need for future studies clarifying the impact of OSA/IH on BDNF, investigating IH impact in specific brain region

and even at the cellular level, or using more various rodent models (*i.e.* aged, female) and diverse IH patterns, in particular including long exposures.

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Competing interests

The authors declare that they have no conflict of interest

Figure legends

Figure 1 : Flow-chart of the meta-analysis

Figure 2: IH increases brain oxidative stress. A-C Orchard plots for A) Malondialdehyde (MDA), B) NADPH oxidase (NOX), C) Superoxide dismutase (SOD). D) positive association between SMD for MDA and total duration of IH exposure (in days)(estimate 0.04, $p=0.026$); E) negative association between SMD for NOX and FiO_2 during hypoxic phase of cycles (in %)(estimate -0.50, $p=0.014$).

Figure 3: IH increases brain inflammation. Orchard plots for A) $TNF\alpha$, B) NF- κ B, C) iNOS.

Figure 4: IH induces apoptosis in the brain. A-C Orchard plots for A) TUNEL staining, B) Cleaved caspase 3, C) BCL2.

Figure 5: IH increases HIF-1 but not BDNF in the brain. Orchard plots for A) HIF-1 and B) BDNF. C) Negative association between BDNF and duration of hypoxic phase (in seconds) (estimate -0.14, $p<0.001$).

Figure 6/Graphical abstract : Proposed mechanisms implicated in IH-induced brain damage. We demonstrated that IH increases oxidative stress, inflammation, apoptosis and HIF-1 in rodents' brain, particularly in the cortex, hippocampus and brainstem. Based on the literature, we propose that oxidative stress has a pivotal role in mediating inflammation, apoptosis and HIF-1 activation leading to neurobehavioral and cognitive alterations.

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