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Review Article

Effects of general anaesthesia during pregnancy on neurocognitive development of the fetus: a systematic review and meta-analysis

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Abstract

Background: The US Food and Drug Administration warned that exposure of pregnant women to general anaesthetics may impair fetal brain development. This review systematically evaluates the evidence underlying this warning.
Methods: PubMed, EMBASE, and Web of Science were searched from inception until April 3, 2020. Preclinical and clinical studies were eligible. Exclusion criteria included case reports, *in vitro* models, chronic exposures, and exposure only during delivery. Meta-analyses were performed on standardised mean differences. The primary outcome was overall effect on learning/memory. Secondary outcomes included markers of neuronal injury (apoptosis, synapse formation, neurone density, and proliferation) and subgroup analyses.
Results: There were 65 preclinical studies included, whereas no clinical studies could be identified. Anaesthesia during pregnancy impaired learning and memory (standardised mean difference −1.16, 95% confidence interval −1.46 to −0.85) and resulted in neuronal injury in all experimental models, irrespective of the anaesthetic drugs and timing in pregnancy. Risk of bias was high in most studies. Rodents were the most frequently used animal species, although their brain development differs significantly from that in humans. In a minority of studies, anaesthesia was combined with surgery. Monitoring and strict control of physiological homeostasis were below preclinical and clinical standards in many studies. The duration and frequency of exposure and anaesthetic doses were often much higher than in clinical routine.
Conclusion: Anaesthesia-induced neurotoxicity during pregnancy is a consistent finding in preclinical studies, but translation of these results to the clinical situation is limited by several factors. Clinical observational studies are needed.
Prospero registration number: CRD42018115194

Keywords: anaesthesia; brain development; fetus; neurocognitive development; pregnancy

Editor's key points

- The authors conducted a systematic review of the evidence underlying the warning that exposure of pregnant women to general anaesthetics may impair fetal brain development.
- There were 65 preclinical studies included, while no clinical studies were identified.
- Anaesthesia during pregnancy impaired learning and memory and resulted in neuronal injury in all experimental models, irrespective of the anaesthetic drugs and timing in pregnancy.
- However, monitoring and strict control of physiological homeostasis were below standards in many studies and the duration and frequency of exposure and anaesthetic doses were often higher than used clinically, which limits translation to humans.

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In the early 2000s, Ikonomidou and colleagues[1] and Jevtovic-Todorovic and colleagues[2] reported that exposure of the developing brain of rats to general anaesthesia evoked widespread apoptotic neurodegeneration resulting in persisting learning and memory impairments. These studies opened a new field of research. Soon, accumulating preclinical evidence showed that virtually all commonly used general anaesthetics impair brain development in multiple animal species, both for exposure before and after birth.[2–11] Therefore, in 2016, the US Food and Drug Administration (FDA) issued a warning that repeated or lengthy use (>3 h) of general anaesthetics in children younger than 3 yr or in pregnant women during the third trimester may result in impaired neurodevelopmental outcome of the exposed children.[12,13] Research investigating the hazards of anaesthetic drugs for children is encouraged by the US SmartTots initiative (https://smarttots.org/), which aims to improve the safety of paediatric anaesthesia.[14,15] In Europe, the Safetots initiative (https://www.safetots.org/) emphasises that inappropriate conduct of anaesthesia could be much more harmful to the developing brain than the anaesthetic drugs themselves.[16–18]

Most of the research focused on the effects of anaesthesia performed shortly after birth. However, although exposure of the developing brain to anaesthesia before birth occurs much less frequently, it is not uncommon. A first occasion is for non-obstetric surgery on the mother, with the fetus being an innocent bystander, for which an incidence of 0.48–0.73% of all pregnancies has been reported.[19,20] A second situation is when the fetus itself needs surgery for fetal indications and in which maternal general anaesthesia is required (e.g. for open or fetoscopic myelomeningocele repair).[21]

To date, only narrative reviews on the effects of anaesthesia *in utero* have been published[22–24]; no recent systematic reviews including meta-analysis critically analysing the studies forming the basis of the FDA warning are available. Here, we systematically review and quantify the effects of exposure to general anaesthesia on fetal brain development and its clinical relevance.

Methods

The study protocol was registered and published in the International Prospective Register of Systematic Reviews of the National Institute for Health Research (CRD42018115194). The guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[25] were followed.

Eligibility criteria

Studies investigating the effects of general anaesthesia during pregnancy on fetal brain development of humans or other mammals were eligible, irrespective of the anaesthetic, as long as it was approved for use in humans. Studies investigating neuroprotective strategies were also eligible. The comparator was a group without anaesthesia (for assessing neurotoxicity) or a group with anaesthesia (neuroprotective strategies). (Pre) clinical interventional and clinical observational studies were eligible. Articles from onset of the databases until April 3, 2020 could be included.

Exclusion criteria were *in vitro* models, chronic exposure, exposure only during delivery, case reports, abstracts, and languages other than English, French, Dutch, or German. Studies were excluded when there were confounding factors (e.g. intentional hypoxia) or when outcome parameters were not directly related to brain development.

Information sources and search strategy

PubMed, EMBASE, and Web of Science were searched with a search string comprising four concepts: anaesthesia, during pregnancy, embryo/fetus/newborn, and neurological outcome (Supplementary material). For every concept, relevant synonyms and subcategories were included in collaboration with an experienced biomedical librarian.

Study selection

After removal of duplicates, screening for eligible articles (abstract and full text) was done independently by two authors. Discrepancies were resolved by a third author.

Data extraction

A standardised table was used for the data extraction. Extracted items included animal species, anaesthesia exposure (drug, dose, duration, frequency), gestational age at the time of exposure, presence of surgical stimulation, monitoring, and outcome parameters.

Data synthesis

Five important outcome parameters were analysed in separate meta-analyses: learning and memory, apoptosis (e.g. assessed by quantification of caspase), synapse formation (e.g. assessed by quantification of synaptophysin), neurone density, and proliferation. Studies were only included in meta-analyses when quantitative data could be extracted for one or more of these outcome parameters. Outcome parameters were summarised as standardised mean differences (SMDs),[26,27] with negative values representing a worse outcome in the anaesthesia group. SMDs of different studies were combined using a random-effects meta-analysis.[28] A random article effect is added to take into account that multiple SMDs from the same article might be present. Heterogeneity was assessed using Cochran's χ^2 test (significant: $P<0.1$) and the I^2 statistic (considerable heterogeneity: $I^2>75\%$).[29–32] Funnel plots were constructed to assess publication bias. All calculations were performed using SAS software (SAS System for Windows version 9.4, SAS Institute Inc., Cary, NC, USA) (details: Supplementary material).

In addition to these meta-analyses reported in the main body of this article, a narrative synthesis including all eligible studies and all outcome parameters is reported separately in Supplementary material.

Outcome

Primary outcome was the overall effect on learning and memory. Secondary outcomes were the effects on four markers of neuronal injury (apoptosis, synapse formation, neurone density, and proliferation). In addition, we performed subgroup analyses for species, anaesthetic drugs, surgical stimulation, pregnancy trimester, magnitude of exposure, and monitoring (Supplementary material). Two exposure categories were defined, based on the FDA warning: an exposure of a single time to a maximum of 1 MAC (minimum alveolar concentration) for 3 h or less (low exposure) *vs* exposure to >1 MAC or lasting >3 h or occurring more than one time (high exposure).[12,13] For monitoring, we distinguished two

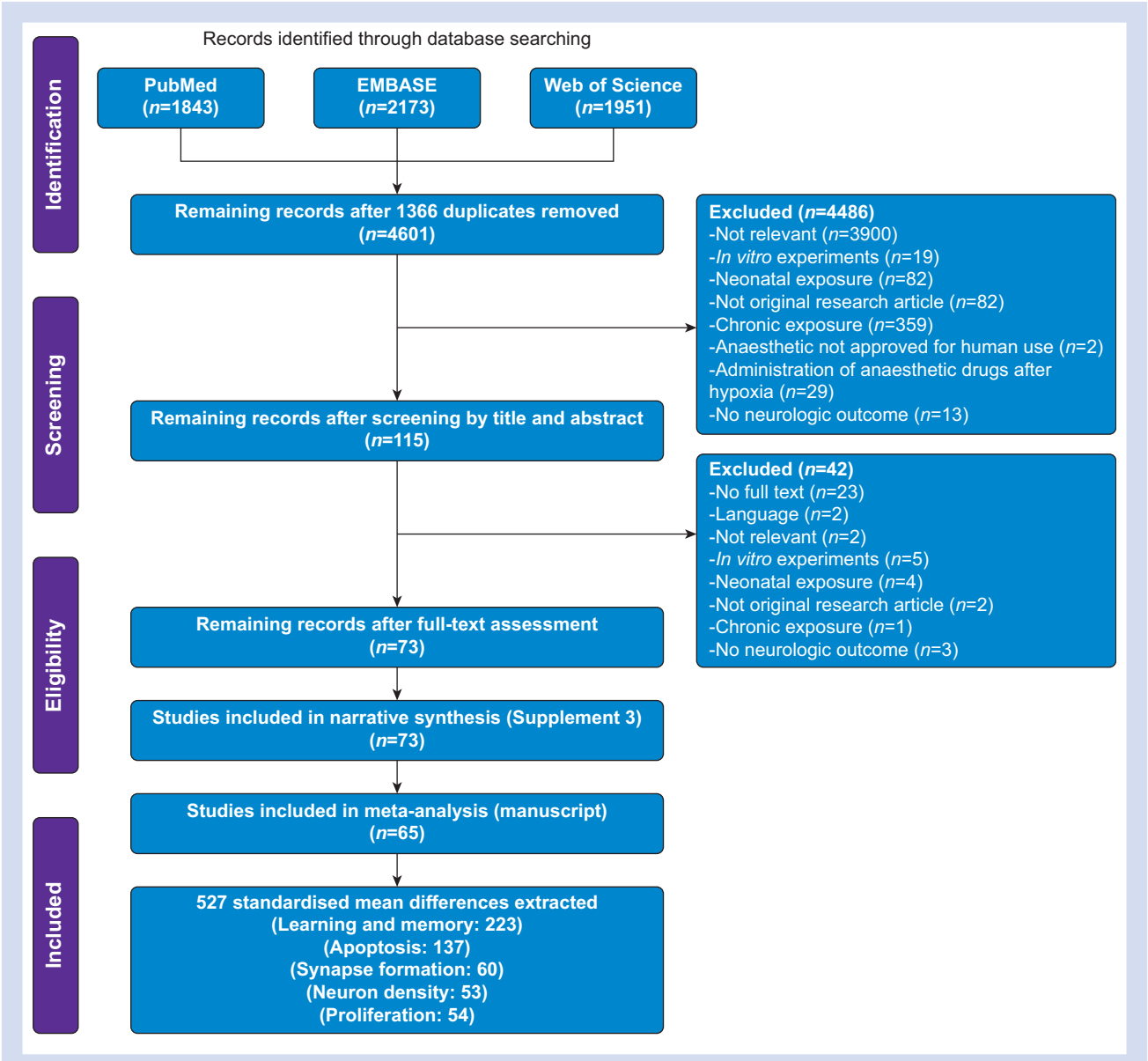

Fig 1. Flow diagram of study selection. Articles were identified in PubMed, Embase, and Web of Science. Seventy-three articles were included in the narrative synthesis described in the Supplementary material (Methodology meta-analysis). For use in meta-analysis of the manuscript, 527 standardised mean differences were extracted from 65 articles.

categories: use of blood gas analysis plus measurement of BP (BGBP) *vs* less monitoring (no-BGBP).

Study characteristics

Study characteristics are described using the subgroups mentioned above (e.g. the number of studies using rats). Additionally, for every study the type of monitoring which was applied is indicated in detail using a standardised table (Supplementary Table S4.1). For every study, it was checked if the monitoring was in accordance with the standards for preclinical research in developmental anaesthetic neurotoxicity,[14] adhering to the standards of the ASA for clinical anaesthesia,[33] or both.

Extensive physiological monitoring and tracheal intubation are practically difficult in rodents, but they are feasible in larger animals such as guinea pigs, pigs, sheep, and non-human primates.[14] The concentration of volatile anaesthetics and the composition of the carrier gas should be reported.[14] Body temperature should be measured several times an hour.[14] Pulse oximetry should be used in larger animals.[14] End-tidal CO_2 should be measured in larger animals via the tracheal tube and in smaller animals using anaesthetic chambers.[14] In the absence of continuous monitoring of oxygenation and ventilation, the impact of the anaesthesia protocol on blood gas values needs to be reported at least once for each anaesthesia protocol used.[14]

Table 1 The study characteristics of the 65 included studies.

	Number of publications
Animal species	
Rat	41
Mouse	14
Non-human primate	5
Sheep	3
Rabbit	1
Guinea pig	1
Human	0
Anaesthetic drug	
Sevoflurane	19
Isoflurane	18
Ketamine	15
Propofol	10
Halothane	2
N_2O	2
Enflurane	2
Desflurane	1
Neuroprotection: dexmedetomidine	4
Magnitude of exposure	
Low exposure: ≤3 h and ≤1 MAC and 1 time	31
High exposure: >3 h or >1 MAC or >1 time	46

According to the guidelines of the ASA for anaesthesia in humans, minimal monitoring includes the measurement of inspiratory oxygen and expiratory CO_2 concentrations, arterial BP, and the use of ECG and pulse oximetry.[33]

Risk of bias

The SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk of bias tool was used to assess the risk of bias.[34] This tool is an adapted version of the Cochrane Risk of Bias tool modified for animal studies. This instrument assesses selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases. The SYRCLE's risk of bias tool provides signalling questions to evaluate each of these domains.[34]

Sensitivity analysis

The studies in which physiological homeostasis was maintained using methods also applied in clinical anaesthesia were selected for sensitivity analysis.

Results

Study selection and data extraction

Figure 1 displays the PRISMA flow chart, with 65 articles included in the quantitative analysis. Study characteristics are displayed in Table 1, Supplementary Table S2, and Supplementary Figure S1. Supplementary Table S3 shows the summary of extracted data for individual articles.

Primary outcome: learning and memory

Learning and memory was assessed in 44 (68%) studies. Most frequently used tests were the Morris water maze test, followed by the radial arm maze test (Supplementary Table S2). Meta-analysis showed a highly significant impairment (−1.16 [−1.46 to −0.85]) (combined SMD [95% confidence interval]) after prenatal exposure to anaesthesia (Fig 2).

Secondary outcomes

Neuronal injury was evaluated in 48 (74%) studies. Most commonly used techniques to assess neuronal injury were histology and immunoblotting of biomarkers (Supplementary Table S2). Meta-analyses showed significantly more apoptosis (−1.99 [−2.84 to −1.14], Fig 3), impairment in synapse formation (−1.06 [−1.62 to −0.51], Supplementary Fig 2), decreased neurone density (−1.25 [−1.98 to −0.53], Supplementary Fig 3), and decreased proliferation (−2.04 [−3.01 to −1.07], Supplementary Fig 4).

Subgroup analysis and clinical characteristics

Species

We could not identify eligible human studies, hence we only report on animal studies. Most studies were performed in rats, followed by mice, non-human primates, sheep, rabbits, and guinea pigs (Table 1). For rats, mice, and non-human primates, meta-analyses found significant impairments for at least one outcome parameter per species (Figs 2 and 3, Supplementary Figs S2–S4, e.g. neurone density for rats: −1.07 [−1.82 to −0.31], learning and memory for mice: −1.03 [−1.86 to −0.19], and apoptosis for non-human primates: −2.20 [−2.93 to −1.46]). No impairments were observed in the meta-analysis of three included sheep studies (apoptosis: 0.04 [−0.77 to 0.84], Fig 3). There was only one study using rabbits and one using guinea pigs, therefore no meta-analysis could be performed for these species.

Anaesthetic drugs

Sevoflurane was the most commonly investigated anaesthetic, followed by isoflurane, ketamine, propofol, halothane, nitrous oxide, enflurane, and desflurane (Table 1). Meta-analyses found significant impairments for at least one outcome parameter for sevoflurane, isoflurane, ketamine, and propofol (Figs 2 and 3, Supplementary Figs S2–S4, e.g. proliferation for sevoflurane: −2.86 [−4.59 to −1.12], learning and memory for isoflurane: −0.96 [−1.63 to −0.30], neurone density for ketamine −1.28 [−2.49 to −0.07], and apoptosis for propofol: −2.94 [−3.99 to −1.88]). No meta-analyses could be performed for halothane, nitrous oxide, enflurane, and desflurane because of the limited number of studies.

Surgery

In 62 (95%) articles, animals were exposed to anaesthesia without surgical stimulation. Meta-analyses demonstrated significant neurotoxic effects for exposure to anaesthesia alone for all outcome parameters (learning and memory: −1.16 [−1.48 to −0.84], apoptosis: −2.06 [−2.90 to −for all outcome parameters (learning and memory: −1.16 [−1.48 to −0.84], apoptosis: −2.06 [−2.90 to l, halothane, nitrous oxide, enflurane, and desflurane (Table 1). Meta-analyses found significant imp[35–37] surgery was performed during anaesthesia, allowing only apoptosis to be included in a meta-analysis which showed no negative effects (−1.31 [−11.46 to 8.84], Fig 3).

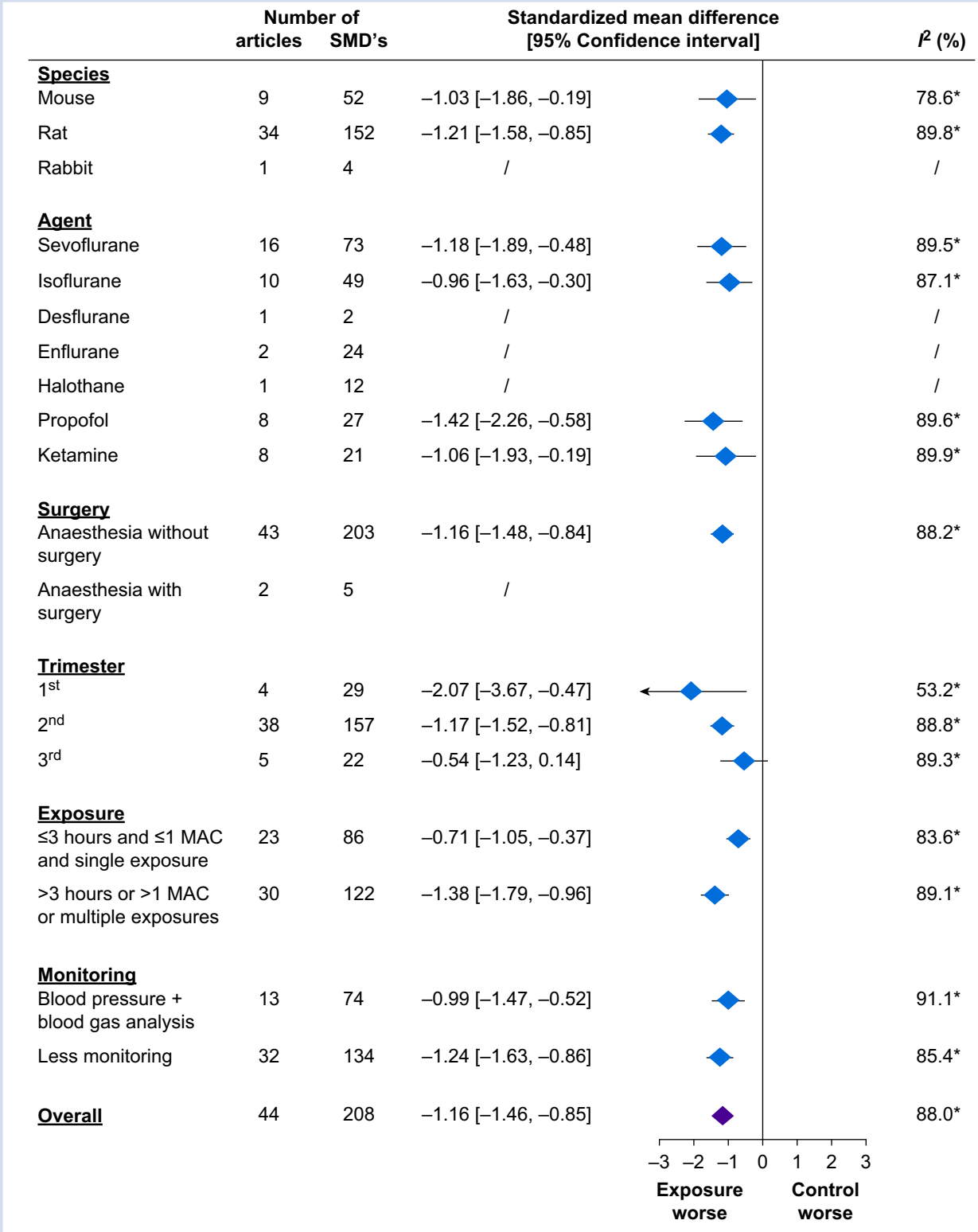

Fig 2. Meta-analysis of all neurobehavioural tests assessing learning and memory. Meta-analysis was performed only when ≥3 studies were available for each subgroup. SMD, standardised mean difference. *χ^2 test of Cochran Q: $P<0.1$.

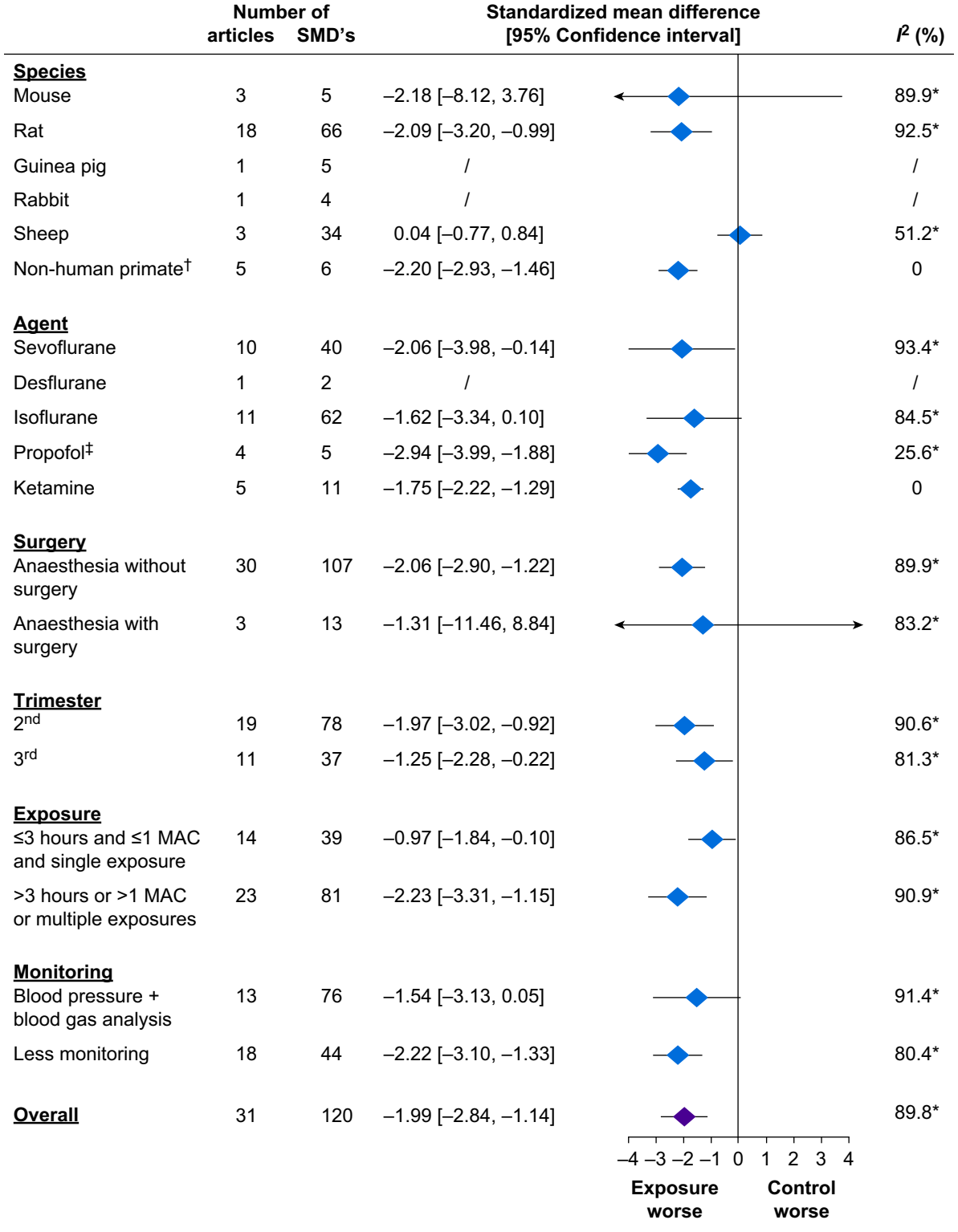

Fig 3. Meta-analysis of apoptosis in the brain. Meta-analysis was performed only when ≥3 studies were available for each subgroup. SMD, standardised mean difference. *χ^2 test of Cochran Q: $P<0.1$. †Fixed-effects model. ‡One random effect.

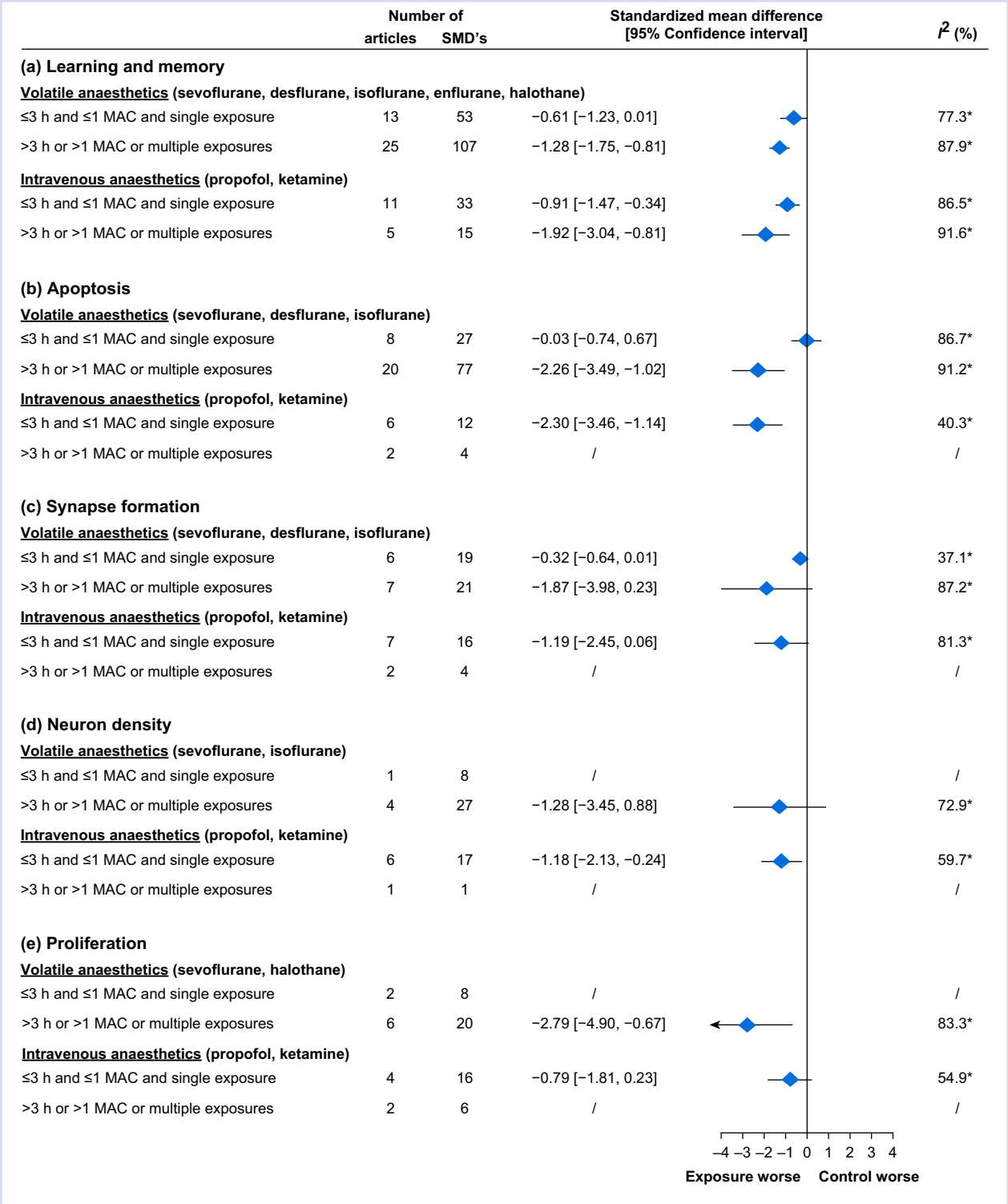

Fig 4. Meta-analysis of (a) learning and memory and (b) apoptosis for the exposure categories for different anaesthetics. Meta-analysis was performed only when ≥3 studies were available for each subgroup. SMD, standardised mean difference. *χ^2 test of Cochran Q: P<0.1. ‡One random effect.

Gestational age at exposure

In most studies, pregnant animals were exposed to anaesthesia during the second trimester of pregnancy (49 studies), followed by the third (12 studies), and first (four studies) trimester equivalent. Meta-analyses showed significant impairments for all outcome parameters in all trimesters, with learning and memory in the third trimester being the only exception (e.g. learning and memory during the first trimester: −2.07 [−3.67 to −0.47], synapse formation during the second trimester: −1.00 [−1.55 to −0.45], or apoptosis during the third trimester: −1.25 [−2.28 to −0.22], Figs 2 and 3 and Supplementary Figs S2–S4).

Magnitude of exposure

The effects of high exposure were investigated more frequently than those of low exposure (Table 1). Meta-analyses showed that the average combined SMDs were more negative in the high exposure group for all outcome parameters (e.g. for learning and memory −1.38 *vs* −0.71, respectively, Figs 2 and 3, Supplementary Figs S2–S4), suggesting dose-dependent effects. High exposure resulted in significant impairments for all outcome parameters (e.g. apoptosis: −2.23 [−3.31 to −1.15]), except for neurone density (−1.60 [−3.41 to 0.21]). By contrast, low exposure did not result in significant impairments of synapse formation (−0.55 [−1.10 to 0.01], Supplementary Fig. S2) and proliferation (−0.93 [−1.87 to 0.01], Supplementary Fig. S4). Figure 4 shows the results for volatile and i. v. anaesthetics separately. For volatile anaesthetics, low exposure did not result in significant impairments for any outcome parameter (learning and memory: −0.61 [−1.23 to 0.01], apoptosis: −0.03 [−0.74 to 0.67], synapse formation: −0.32 [−0.64 to 0.01]), whereas after high exposure significant impairments were observed for learning and memory (−1.28 [−1.75 to −0.81]), apoptosis (−2.26 [−3.49 to −1.02]) and proliferation (−2.79 [−4.90 to −0.67]). For i. v. anaesthetics, impairments were observed both after low exposure (learning and memory: −0.91 [−1.47 to −0.34], apoptosis: −2.30 [−3.46 to −1.14], neurone density: −1.18 [−2.13 to −0.24]), and high exposure (learning and memory: −1.92 [−3.04 to −0.81]).

Monitoring and anaesthesia

Monitoring in accordance with the standards for preclinical research[14] was used in 29 of the 65 (45%) studies. Monitoring adhering to the guidelines of the ASA for human anaesthesia was used in only five (8%) studies. Standard ASA monitoring *without* ECG was used in four (6%) additional studies. All other studies used less monitoring (Supplementary Table S4).

Tracheal intubation and controlled positive pressure ventilation were used in only 10 (15%) studies. Nine of these studies were performed in larger animals and one in rats[38] (Supplementary Table S4.2). In the remaining 55 (85%) studies, animals were breathing spontaneously; 54 of these were performed in rats and mice. Attempts to confirm adequate minute ventilation were done by analysing arterial (17 studies) and venous (six studies) blood gas samples. Three studies claimed to have achieved adequate ventilation by referring to previous studies that had demonstrated normal blood gas values when following a specific anaesthesia protocol.[3,39–41] In the remaining 29 (45%) studies, no attempt was made to confirm adequate ventilation. Pulse oximetry was used in 20 (31%) studies. In six (9%) studies, pregnant animals were excluded when oxygen saturation was <95% for >5 min. Inspiratory oxygen concentration ranging from 21% to 100% was used (Supplementary Table S4).

Arterial BP was measured in 26 (40%) studies. One study claimed to have achieved normotension by referring to previous studies that had demonstrated adequate BPs for the specific anaesthesia technique used.[3,39–41] BP was taken into account for further decision making in 10 (15%) studies. In six studies, pregnant rats were excluded and replaced when systolic BP was <80% of baseline value for >5 min. In four studies, vasopressors were administered in case of hypotension (Supplementary Table S4).

Less monitoring was used in studies investigating the effects of i. v. anaesthetics when compared with models studying volatile anaesthetics (Supplementary Table S4).

The average of the combined SMD was less negative for the BGBP group when compared with the no-BGBP group for all outcome parameters (e.g. apoptosis: −1.54 *vs* −2.22, respectively, Figs 2 and 3, Supplementary Figs 2–4). Significant impairments were observed for all outcome parameters for the no-BGBP group (learning and memory: −1.24 [−1.63 to −0.86], apoptosis: −2.22 [−3.10 to −1.33], synapse formation: −1.08 [−1.82 to −0.34], neurone density: −1.28 [−2.49 to −0.07], and proliferation: −2.28 [−3.58 to −0.97]). In the BGBP group, significant differences were observed only for learning and memory (−0.99 [−1.47 to −0.52]), but not for all other outcome parameters (apoptosis: −1.54 [−3.13 to 0.05], synapse formation: −0.92 [−1.88 to 0.03], neurone density: −1.19 [−2.41 to 0.02], and proliferation: −1.43 [−10.64 to 7.79]). Supplementary Fig 5 shows that low exposure with BGBP monitoring does not result in significant impairments for all available outcome parameters (learning and memory: −0.49 [−0.95 to −0.02], apoptosis: −0.07 [−0.97 to 0.83]), whereas high exposure with no-BGBP monitoring results in significant impairments for all outcome parameters (learning and memory: −1.37 [−1.89 to −0.86], apoptosis: −2.27 [−3.48 to −1.05], synapse formation: −2.13 [−4.26 to −0.01], proliferation: −3.17 [-4.90 to −1.43], not enough studies could be included for neurone density).

Neuroprotection: dexmedetomidine

Four studies investigated the neuroprotective effects of dexmedetomidine in rats. Dexmedetomidine was administered i. p. 15 min before the start of ≥1 MAC sevoflurane/isoflurane/propofol anaesthesia and in three studies repeated every 2 h.[40,42–44] The anaesthesia plus dexmedetomidine group was compared with anaesthesia without dexmedetomidine. Meta-analysis showed a significant improvement in learning and memory (0.79 [0.13 to 1.45], Fig 5), but a non-significant decrease in apoptosis (1.38 [−1.53 to 4.30]). Not enough studies could be included to perform meta-analyses for synapse formation, neurone density, and proliferation.

Risk of bias

Supplementary Table S5 displays the evaluation of every item of the SYRCLE risk of bias tool. Randomisation was mentioned in 51 out of the 65 studies (78%), but in none of these studies were more details provided about the method (e.g. a random number generator) or allocation concealment. In six (9%) studies, neurocognitive assessment of the parental animals was used to ensure equal groups at baseline. Random housing

was described in none of the studies. Investigators were not blinded, but in 35 (54%) studies the outcome assessors were blinded. Animals/areas in brain regions were selected randomly for outcome assessment in 23 (35%) studies. Missing outcome data were mentioned in 11 (17%) articles. Another important source of bias was the statistical analysis of the offspring born to one mother being considered as an independent observation. To overcome this, in eight (12%) articles, clustering of fetuses per doe was taken into account.[37–39,45–49] In the funnel plots (Supplementary Fig 6), there is asymmetry for all outcome parameters, clearly suggesting the presence of publication bias.

Sensitivity analysis

After selecting studies using both positive pressure ventilation and standard ASA monitoring with or without ECG, eight (12%) studies were withheld: one in rabbits,[37] three in sheep,[36,50,51] and four in non-human primates.[6,8,10,52] In those, low exposure did not result in more apoptosis (0.32 [−0.42 to 1.06], Supplementary Fig 7), but high exposure resulted in a non-significant increase in apoptosis (−0.90 [−1.92 to 0.13]).

Discussion

Principal findings

Fetal exposure to general anaesthesia results in impaired learning and memory, neuronal injury, or both in several experimental animal species, irrespective of the anaesthetic drug or gestational age. However, most experiments were done under general anaesthesia without concomitant surgical intervention, a situation rarely encountered in clinical anaesthesia. For volatile anaesthetics, these effects were reported only after exposure to >1 MAC, lasting >3 h or when given more than once. For i. v. anaesthetics, neurotoxicity was observed at lower doses/durations/frequencies. Furthermore, conduct of anaesthesia and monitoring were often below preclinical standards and far below the standards usually applied in the clinic. Dexmedetomidine attenuated anaesthesia-induced neurotoxicity. These findings are corroborated by the narrative synthesis (Supplementary material).

Methodological issues limiting clinical translation

As no clinical studies are available, it is important to investigate whether and to what extent these preclinical observations can be translated to the clinical scenario.

It would be crucial to use animal species having a brain development comparable to humans, for which rodents are less than ideal. Rodents are often used because of the availability of validated neurobehavioural tests, similar brain histology, and an extensive knowledge of molecular pathways.[14] However, the brain growth spurt during which the brain is at its peak growth and most vulnerable to external factors (e.g. anaesthesia)[53–56] occurs *perinatally* in humans but *postnatally* in rodents. In contrast, guinea pigs, sheep, and non-human primates are *prenatal* brain developers.[14,39,53,55,57–59] Hence, to translate findings from animal experiments to the human setting, the effects of anaesthesia on fetal brain development should more appropriately be investigated in animals that develop their brain in the *perinatal* period, such as rabbits or pigs. However, we identified only one study in rabbits[37] and none in pigs.

In all except three articles, animals were exposed to a surgical level of general anaesthesia without surgical stimulation. In reality, pregnant women are exposed to anaesthesia almost exclusively to allow surgery (except situations in which sedation is needed on the ICU).[19]

Positive pressure ventilation and standard ASA monitoring are used routinely in pregnant women but were used in only a minority of studies, probably because most studies used rodents, in which these are practically difficult to perform. Likewise, the guidelines for monitoring in preclinical research were followed only in about half of the studies. Isoflurane causes respiratory depression resulting in hypoxaemia, hypercarbia, or both, both of which can induce apoptosis and impair neurocognitive outcome.[60–63] Positive pressure ventilation decreases apoptosis and improves neurocognitive outcome.[64] In many of the included studies, it is not possible to distinguish if the observed neuronal injury was a direct effect of the anaesthetic or the indirect result of respiratory depression.

For volatile anaesthetics, we found that only repeated exposures, doses of >1 MAC or durations of >3 h resulted in neurological impairments. This corroborates the FDA warning against repeated and lengthy use (defined as >3 h) of anaesthesia in young children and during pregnancy.[12,13] However, repeated exposure during pregnancy is uncommon in humans.[19] The use of doses >1 MAC has been advocated in major fetal surgery (e.g. for meningomyelocele repair, to induce uterine relaxation).[65] However, these surgeries are also feasible using lower doses of volatile anaesthetics in combination with tocolytic agents.[66] Non-obstetric surgery during pregnancy rarely lasts >3 h.[19] Furthermore, we suggest that the duration of general anaesthesia should be interpreted in relation to the duration of pregnancy and life expectancy, which are considerably shorter in almost all investigated animal species than in humans. Taking these considerations into account, a 3 h anaesthetic in rats, mice, non-human primates, sheep, rabbits, and guinea pigs would correspond to 37, 43, 5, 6, 27, and 12 h, respectively, of anaesthesia in humans.[67] Therefore, even in the low exposure groups, the animals were exposed to relatively longer durations of exposure than what is typically encountered during surgery in pregnant women. Unfortunately, no studies are available investigating the animal equivalent of 3 h in humans (15 min for rats or 13 min for mice[67]).

For i. v. anaesthetics, low exposure was found to result in neurological impairments, whereas for volatile anaesthetics, only high exposure impaired neurological outcome. Notably, i. v. anaesthetics were administered in sedative doses, whereas the doses used for volatile anaesthetics resulted in surgical levels of general anaesthesia (Supplementary Tables S1 and S3). Thus, i. v. anaesthetics could be more harmful to the brain than volatile anaesthetics. In contrast, two studies directly comparing volatile with i. v. anaesthetics (at similar depths of anaesthesia) found that propofol was less harmful than isoflurane.[8 45] The reasons for these conflicting observations are purely speculative. In fact, there might be a certain degree of 'autoregulation' of the depth of anaesthesia when volatile anaesthetics are inhaled spontaneously (a negative feedback mechanism with the animal stopping inhalation and limiting further uptake of

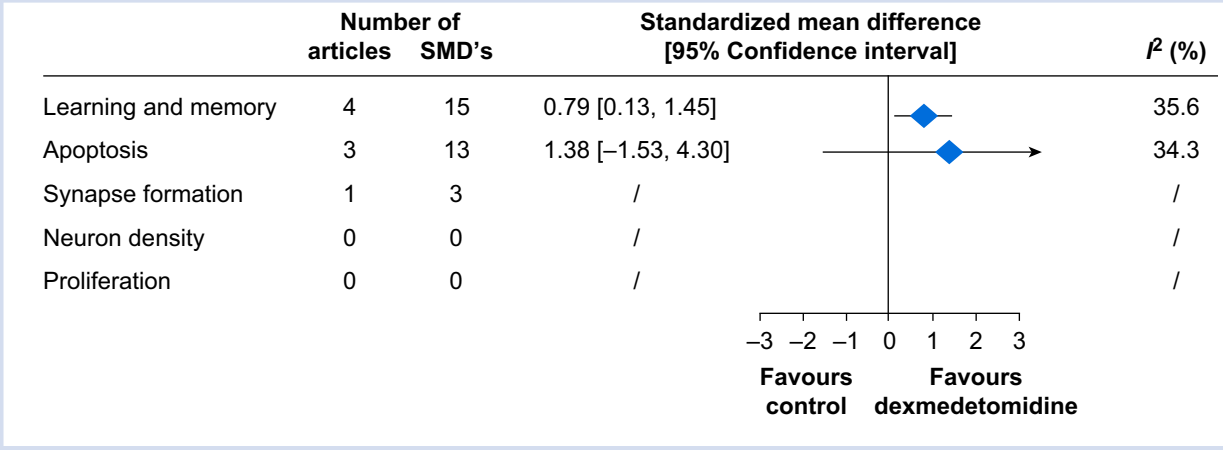

Fig 5. Meta-analysis of the neuroprotective effect of adding dexmedetomidine to general anaesthesia. Meta-analysis was performed only when ≥3 studies were available for each subgroup. SMD, standardised mean difference. *χ^2 test of Cochran Q: $P<0.1$.

anaesthetics when levels of anaesthesia become too deep).[68] This mechanism does not exist for i. v. anaesthetics. Therefore, it is possible that respiratory depression was more pronounced in the group of i. v. anaesthetics. This remained unrecognised, as in the studies using i. v. anaesthetics less monitoring was used than in the group of volatile anaesthetics.

The arguments above illustrate that the translational value of many preclinical studies is limited because they do not reflect the clinical scenario in women undergoing surgery. To estimate the effects of clinical anaesthesia on fetal brain development, only studies investigating low exposure in the selected studies of the sensitivity analysis should be considered (Supplementary Fig. S7). These three studies show that a clinically relevant exposure to general anaesthesia during pregnancy does not result in more apoptosis. Therefore, it can be concluded that for typical surgeries during pregnancy using clinically relevant exposures and standard physiological monitoring, anaesthesia-induced neurotoxicity is most probably less severe. Notably, research into anaesthetic neurotoxicity has not been stimulated by clinical observations, but by observations from the laboratory.[61,69] As a matter of concern, the animal data still warrant caution for the exceptional cases in which pregnant women need to undergo long and complex surgeries (such as fetal myelomeningocele repair or cardiac surgery) or even week long deep sedation on the ICU (e.g. when requiring extracorporeal membrane oxygenation for the treatment of adult respiratory distress syndrome caused by influenza or COVID-19).[70,71]

Risk of bias was high in most studies. A specific source of bias is that in all except eight articles, offspring born to one mother were statistically analysed as independent observations. However, offspring born to the same mother share genetics and environmental influences. Most likely, physiological disturbances in one pregnant animal during anaesthesia affect all fetuses.[72] Appropriate statistical methods (i.e. a mixed-effects model) need to be used for sample size calculation and to analyse these clustered data.[73–77] When ignoring clustering, the chance of false positive findings increases.[76]

Strengths and limitations

This review has a number of limitations. First, no clinical data were available. Second, some conclusions need to be interpreted cautiously because of the wide variety of methods used to assess learning, memory, and neuronal injury, and the resulting high statistical heterogeneity. We attempted to reduce the latter by repeating the meta-analysis and only including the latency time of the Morris water maze test and apoptosis in the hippocampus (Supplementary Figs 8 and 9). This was in vain however, potentially because a relatively high number of SMDs were included in the meta-analysis. The Cochran's χ^2 test has excessive power to detect clinically unimportant heterogeneity when many studies are included.[30,31]

The strength of this review is that it identifies a number of methodologic problems with currently available translational studies that should be addressed in future research, including analysis of more clinically relevant scenarios in appropriate models, and the need for clinical studies.

Conclusions

In laboratory animals, anaesthesia during pregnancy results in neuronal injury leading to impairments of learning and memory irrespective of animal species, type of anaesthetic, and timing during pregnancy. Notably, human data are not available. Translation of the laboratory findings to the human setting is complicated by several confounders. Rodents (the most frequently used animal species) have brain development that significantly deviates from that in humans. In most studies, anaesthesia was performed without surgical stimulation. Physiologic monitoring and control of homeostasis were below preclinical[14] and clinical standards[33] in many studies. The duration and frequency of exposure and the anaesthetic doses were often much higher than in clinical routine. In studies more closely mimicking the clinical setting, the results suggest that neurological outcome was not impaired by fetal exposure to maternal anaesthesia. This systematic review and meta-analysis suggest that

anaesthesia-induced neurotoxicity during pregnancy is a consistent finding in laboratory conditions. Neurodevelopmental effects are much smaller in models mimicking typical clinical situations and applying routine monitoring standards. Clinical observational studies are needed for confirmation. Future animal studies should focus on models appropriately reflecting typical clinical situations, applying surgical stimulation, and using physiological monitoring.

Authors' contributions

Substantially contributed to the manuscript: all authors
Wrote the protocol: TB, SR
Searched databases: TB
Screened for eligible articles: TB, LVDV
Resolved discrepancies: SR
Performed the meta-analysis: TB, SF
Wrote the first version of the manuscript: TB
Made substantial contributions to the design of the study, acquisition, analysis, interpretation of data, critically revised, and approved the final submitted manuscript: all co-authors

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Declarations of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

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