Anesthesia and Cognitive Performance in Children: No Evidence for a Causal Relationship

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Recent findings of an association between anesthesia administration in the first three years of life and later learning disabilities have created concerns that anesthesia has neurotoxic effects on synaptogenesis, causing later learning problems. An alternative hypothesis is that those children who are likely to undergo surgery early in life have significant medical problems that are associated with a vulnerability to learning disabilities. These two hypotheses were evaluated in a monozygotic concordant–discordant twin design. Data on anesthesia administration and learning abilities and disabilities were available for 1,143 monozygotic twin pairs (56% female) from the Netherlands Twin Registry. Parents of the twins reported on anesthesia use before age 3 and again between ages 3 and 12 years. Near age 12, educational achievement and cognitive problems were assessed with standardized tests and teacher ratings. Results showed that twins who were exposed to anesthesia before age 3 had significantly lower educational achievement scores and significantly more cognitive problems than twins not exposed to anesthesia. However, there was one important exception: the unexposed co-twin from discordant pairs did not differ from their exposed co-twin. Thus, there is no evidence for a causal relationship between anesthesia administration and later learning-related outcomes in this sample. Rather, there is evidence for early anesthesia being a marker of an individual's vulnerability for later learning problems, regardless of their exposure to anesthesia.

Keywords: anesthesia, learning related problems, MZ discordant design

There has been a persistent concern about the use of anesthesia in young children — specifically those agents that have been shown in histopathological studies to be associated with neuroapoptosis and cell death in both in vivo and in vitro studies using laboratory animals (Cattano et al., 2008; Jevtovic-Todorovic et al., 2003; Johnson et al., 2008; Olney et al., 2002; Young et al., 2005). Concern about neural develop-ment has been raised because many agents used for anesthesia antagonize the N-methyl-d-aspartate gluta-mate (NMDA) receptor (such as ketamine), stimulate the gamma-aminobutyric acid A (GABA) system (such as halothane or the benzodiazepines), or both (such as nitrous oxide) (Mellon et al., 2007). The conerns revolve around the excitotoxic damage that has been demonstrated in relation to the NMDA receptor in prenatal mice and rats (Olney et al., 2002). Demonstration of damage to the regions of the brain important for learning and memory, specifically the hippocampus and dentate gyrus (Ikonomidou et al., 1999), has raised concerns about the effects of NMDA receptor antagonists and GABA agonists on later learning and memory (Jevtovic-Todorovic et al., 2003). Some (Levin et al., 1990), but not all (Li et al., 2007), studies have demonstrated that prenatal or early postnatal exposure to anesthesia is associated with later impaired performance on radial arm maze and/or water maze tasks — two tasks known to be associated with hippocampally mediated learning and memory. In non-human primates, there are some data to suggest that similar neurotoxic events can occur with early exposure to these substances (Slikker et al., 2007). There have been referential data that early exposure to anesthesia in humans might have neuro-toxic effects. For example, prenatal exposure to cocaine and ethanol, both known to induce apoptotic changes similar to anesthesia (Novikova et al., 2005; Wozniak et al., 2004) may also lead to problems with later learning. Because anesthetic agents work at the level of the synapse, examination has turned to toxic effects of anesthesia exposure during stages of life when synaptogenesis is at its greatest. Earlier case series studies have demonstrated that children who have undergone surgery may be particularly prone to

later learning problems (Wilder et al., 2009). However, these studies have been confounded by the fact that children who undergo surgery early in life often have congenital heart problems (Bellinger et al., 1999), were undergoing organ transplantation (Gilmour et al., 2009), or had some other extreme medical illness. These confounds have made it difficult to sort out the relations between later learning problems and anesthesia as opposed to the medical conditions for which an individual might undergo surgery during a critical synaptogenetic period.

Recently, however, a study was performed examining the role of anesthesia on later learning disabilities while attempting to control for these confounds. Wilder et al. (2009) performed a population-based retrospective birth cohort study and examined medical and surgical records in children with and without learning disabilities. They calculated the hazard ratio of having been exposed to one or more anesthetic agents (most often halothane and nitrous oxide) before the age of 4 in a sample of 5,357 children. They observed an increased rate of learning disabilities (LD) in those children exposed twice or more to anesthetic drugs. Further, they showed that the risk of LD increased with longer exposure to the agents. This effect held even after controlling for gestational age at birth, sex, and birth weight. Unlike previous studies, this was a population-based study, so the children studied had undergone surgery for multiple reasons, not just cardiothoracic surgery (Wilder et al., 2009).

While making a compelling case, the authors note a significant concern. They state that they are unable to determine whether there is a higher likelihood of learning disabilities in those children who underwent anesthesia simply because they had other medical problems or were, overall, at higher risk. There is clearly a higher incidence of LD in children with both psychiatric and nonpsychiatric medical comorbidity (O'Brien, 2001). Thus, an examination only of the relations between use of anesthesia in early life and subsequent learning problems fails to take into account an additional probable cause — that there are multiple, often genetically determined, reasons to have surgery (and associated anesthesia) early in life. It is difficult to control for this effect, because no one would consider either avoiding surgery in sick children or exposing healthy children to anesthetic drugs unnecessarily to control for these effects. It is possible, however, to determine whether the expression of learning-related problems is more related to the child's underlying genetics than to the exposure to anesthesia using a monozygotic concordant-discordant design.

The concordant–discordant monozygotic twin design allows for a specific analysis of environmental risk while controlling for genetic factors when the outcome variable is influenced by genetic factors, as is the case with intelligence, educational achievement, and learning disabilities. This design makes use of the fact that some monozygotic twins, while sharing essentially 100% of their DNA sequence, will be discordant on traits or exposures. Taking advantage of a very large twin register, one can search for twins concordant for exposure, concordant for no exposure, and discordant for exposure. One can then compare associated traits or, in the case of a longitudinal twin register, compare outcomes. Comparing outcomes in twins from a monozygotic pair discordant for exposure; that is, one twin has had the exposure and the other twin not, provides a direct test of whether the environmental exposure was associated with the outcome. If there is no difference in the outcome variable between monozygotic twins within a twin pair discordant for exposure evidence for a causal relationship would be substantially reduced. Comparing outcomes across twin pairs who are concordant for having an exposure to outcomes across twins who are concordant for not having an exposure provides a confirmation of the previously demonstrated association; that is, if there is no difference between these two concordant groups, there was no effect of the exposure and again causality can be questioned. If exposed twins from concordant pairs are similar to exposed twins from discordant pairs and if unexposed twins from concordant pairs are similar to unexposed twins from discordant pairs, evidence for a causal relation between exposure and outcome is suggested. If, however, unexposed twins from concordant unexposed pairs differ from unexposed twins from the discordant pairs then causality can be questioned.

In this study, we turn the power of the monozygotic concordant-discordant design to the question of anesthesia exposure and learning-related outcomes. Based on of the work of Wilder et al. (2009), a causal hypothesis predicts that exposed twins from monozygotic twin pairs discordant for exposure to anesthesia would show worse learning-related outcomes than the genetically identical twin who was not exposed. If, however, the reason for an increase in learning disabilities in exposed children is related to an underlying vulnerability for learning disabilities that happens to be related to reasons for undergoing anesthesia, there would be no difference between twins within a twin pair discordant for anesthesia exposure. Moreover, twins from pairs concordant for receiving anesthesia would have a worse outcome than twin pairs where both twins were concordant for not receiving anesthesia. These hypotheses are put forth in Table 1.

Methods

Subjects

The twin pairs in this study come from Young-Netherlands Twin Register (YNTR; Bartels et al., 2007; Boomsma et al., 2006). The YNTR contains data on twins from birth onwards. Twins are categorized by birth cohort and data collection is cohort driven. Nationwide data collection of all families is by mailed surveys. Parents of twins receive surveys when

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Table 1

Possible Outcomes Under Two Hypotheses of Causality

	Concordant unexposed twins	Discordant unexposed twins	Discordant exposed twins	Concordant exposed twins
Causal hypothesis (anesthesia causes learning problems)	Low	Low	High	High
Non-causal hypothesis (medical condition cause of learning problems)	Low	High	High	High

their twins are aged 1, 2, 3, 5, 7, 10, and 12 years of age. At ages 7, 10, and 12, teacher data are also collected, after written permission is given by the parents.

The twin pairs for this study were born between 1986 and 1995. Of the total sample of 4142 twin-families with data on anesthesia and either educational achievement and/or cognitive problems, 1569 were families with a MZ twin pair (677 MZ male or MZM and 892 MZ female or MZF). Data from 25 families were removed because one of the twins in the pair reported illness or handicap that interfered with daily functioning. To overcome interference of effects of gestational age and birthweight, both significantly related to exposure to anesthesia and educational achievement/cognitive problems, twin families were removed if gestational age was less than 32 weeks (66 families) and/or if one of the twins had a birth weight under 2000 grams (335 families). The final sample consisted of 1143 families. Zygosity was determined by blood or buccal DNA polymorphisms in 85 pairs and 259 pairs, respectively. For all other twin pairs (n = 799), zygosity was determined by questionnaire items at ages 3, 5, 7, 10, and 12. These items allow accurate determination of zygosity in 93% of same sex twin pairs (Rietveld et al., 2000).

Anesthesia

In the YNTR information on exposure to anesthesia is collected in the surveys for parents of 2-, 3-, 5-, 7-, and 10-year-old twins. For the children included in this study, parents were asked when their twins were 2 years of age if either child was exposed to anesthesia since birth (yes/no). At age 3, exposure since age 18 months was assessed. Since it has been indicated that early exposure to anesthesia might be harmful, data collected at age 2 and 3 were used to compose a variable indicating if a child was exposed to anesthesia before the age of 3. Before the age of 3 years, 23.8% of the MZM twins and 14.4 % of the MZF twins had been exposed to anesthesia. We combined information on anesthesia exposure assessed at ages 5, 7, and 10 with the exposure before the age of 3 to indicate if a twin had been exposed at all before the age when learning-related outcomes were measured. Before age 12 years, 44.8% of the MZM twins and 36% of the MZF twins had been exposed to anesthesia before age 12. Information on specific anesthetic agents of exposure was not available.

Educational Achievement and Cognitive Problems

Educational Achievement (EA) at age 12 was assessed with the Dutch CITO-elementary test (Eindtoets Basisonderwijs, 2002, www.cito.nl). The CITO test consists of 240 multiple-choice items assessing four different intellectual skills: Language, Mathematics, Information Processing, and World Orientation. Together, the performance scales result in a standardized score between 501 and 550. A higher score indicates better educational achievement. The test is administered nation-wide on three consecutive days in January or February when the children are in the final class of elementary school (grade 8), and approximately 12 years old. The CITO data were collected by mail from teachers after informed consent was obtained from the parents at age 12 and/or by self report of the twins at age 14 years or older. There was a substantial agreement among the scores from different sources (correlations in the range of .93 to .99). Bartels et al. (2002b) showed that CITO score are moderately to highly correlated to IQ (correlations of .41, .50, .60, and .63 between CITO scores assessed at age 12 and IQ performance at age 5, 7, 10, and 12, respectively).

To gain additional insight into the level of cognitive problems that children exposed to anesthesia might experience, data from the Cognitive Problems/Inattention (CP) subscale of the Conners' Teacher Rating Scale — Revised: Short Form (CTRS-R:S; Conners, 2001) were analyzed. The CTRS-R:S is a 28-item questionnaire which was sent to teachers of 12-year old twins, after written permission of the parents was obtained. Teachers were asked to answer the questions on a 4-point scale (0 = *not true at all*; 1 = *just a little true*; 2 = *pretty much true*; 3 = *very much true*). The CP subscale was derived from the addition of 5 items (forget things he/she already learns; poor in spelling; not reading up to par; lacks of interest in schoolwork; poor arithmetic). A higher score indicates more cognitive problems.

Concordant and Discordant Twin Pairs

MZ twin pairs were divided into three groups based on exposure to anesthetic drugs before age 3 years: (1) concordant non-exposed (CON-NE); (2) discordant (DIS); (3) concordant exposed (CON-E). The same was done for the information on exposure at any age before the age of 12. The sample distribution is provided in Table 2.

Table 2

Number (and Column %) of Concordant Non-Exposed, Discordant and Concordant Exposed Monozygotic Twin Pairs

Anesthesia:	Under age 3	Ever (before age 12)	Under age 3	Ever (before age 12)
Concordant — nonexposed	324 (68.6%)	225 (44.2%)	489 (80.7%)	334 (52.7%)
Discordant	71 (15.0%)	111 (21.8%)	59 (9.7%)	143 (22.6%)
Concordant — exposed	77 (16.3%)	173 (34%)	58 (9.6%)	157 (24.85%)
Total	472	509	606	634

Statistical Analyses

Within-pair analyses: Data from MZ twins pairs discordant for anesthesia exposure before the age of 3 and discordant for ever-exposed before age 12 were used in the within-pair analyses. Within pair differences between the exposed and non-exposed twin before the age of 3 years and ever were analyzed for both EA and CP with paired t-tests separately for the male and female MZ twin pairs in SPSS 16 (SPSS Inc., Chicago, Illinois).

Between individuals analyses: To test for differences between subjects, children were assigned to come from concordant non-exposed (CON-NE), concordant exposed (CON-E) or discordant pairs (DIS-NE and DIS-E). Differences between these four groups were tested by mixed-model analyses of variance in SPSS 16, with 'type twin pair by exposure' (CON-NE, DIS-NE, DIS-E, CON-E) as a fixed factor and with family

as a random factor to account for the within-family dependence of the outcome variables. Analyses were done separately for males and females and for the anesthesia status under age 3 and ever-exposed. An alpha level of .05 was chosen for all tests.

Additionally, we selected for twins who remained concordant unexposed — that is, those never exposed under age 12 and compared their EA and CP scores to scores of twins who became discordant or twins who became concordant exposed between ages 3 and 12. We considered these latter two groups to be genetically more vulnerable because they had not been exposed by age 3, but were exposed before age 12. If the association between anesthesia and LP is based on an overall vulnerability of an individual, it is expected that the twins who change status from unexposed to exposed over the 9-year period will show lower EA

Table 3

Educational Achievement (EA) and Cognitive Problems (CP): Means for the Exposed and Non-Exposed Twins From Discordant Twin Pairs

	Exposed under age 3				
EA	Exposed	Mean	Mean difference	t (df)	p
MZM ($n = 61$)	No	536.97	.443	.69 (60)	.493
	Yes	536.52			
MZF ($n = 49$)	No	534.43	.122	.163 (48)	.879
	Yes	534.31			
CP					
MZM ($n = 29$)	No	2.77	.017	.032 (28)	.970
	Yes	2.75			
MZF ($n = 27$)	No	4.24	.759	1.48 (26)	.152
	Yes	3.48			
	Exposed ever before age 12				
EA	Exposed	Mean	Mean difference	t (df)	p
MZM ($n = 98$)	No	538.68	−.143	−.318 (97)	.751
	Yes	538.83			
MZF ($n = 127$)	No	536.26	.575	1.196 (126)	.234
	Yes	535.69			
CP					
MZM ($n = 39$)	No	2.90	.154	.352 (38)	.727
	Yes	2.74			
MZF ($n = 59$)	No	2.87	.186	.582 (58)	.563
	Yes	2.68			

scores and higher CP scores than the twins who remained unexposed.

Results

Table 3 displays the mean levels of EA and CP for the exposed and non-exposed twins from the discordant pairs. As can be seen, there are no significant differences in mean scores for EA and CP between the exposed and non-exposed twins of MZ pairs for males or females ($p > .05$ for all tests). The results are the same for the analyses in which exposure is restricted to be before the age of 3 years or ever before age 12.

The absence of significant within-pair differences provides strong evidence against a causal effect of anesthesia on cognitive functioning.

Figure 1a shows the mean levels of EA for the four 'twin-type-by-exposure' groups (CON-NE, DIS-NE, DIS-E, CON-E) for males and females, as a function of anesthesia under 3 and ever-exposed before age 12. Figure 1b displays the mean levels of CP for these four groups. The means, projected in Figure 1, are also provided in Table 4. It is clear that CON-NE twins have higher scores on EA (significant for males with anesthesia exposure under age 3 and ever-exposed, and
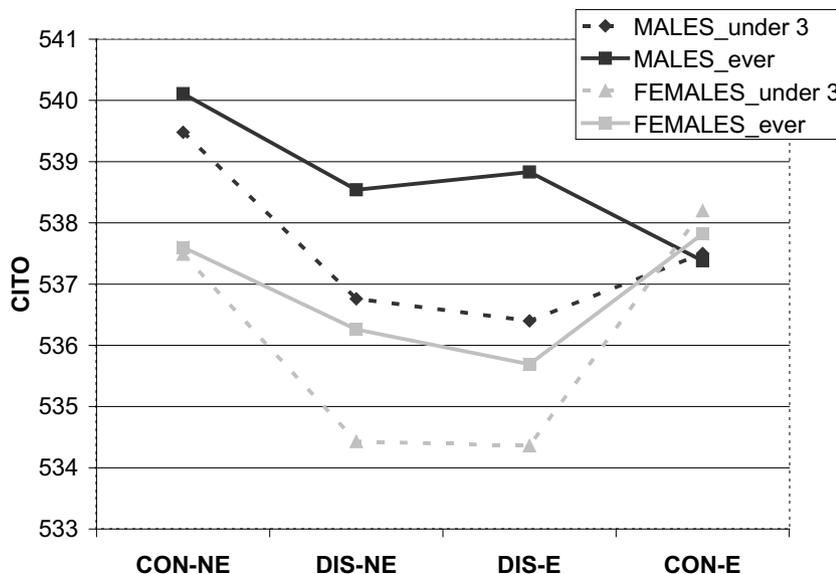

Figure 1a

Mean levels of Educational Achievement for twins exposed / non-exposed to anesthesia from Concordant and Discordant pairs; anesthesia under age 3 and ever before age 12.
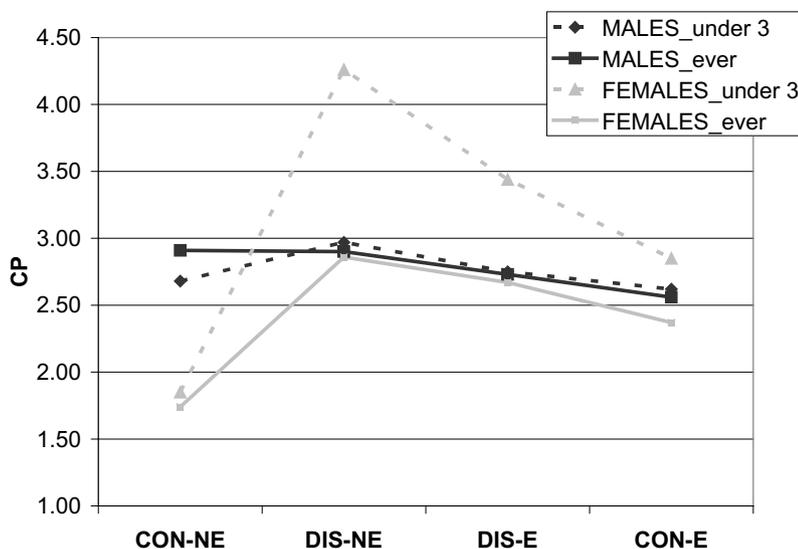

Figure 1b

Mean levels for Cognitive Problems for twins exposed / non-exposed to anesthesia from Concordant and Discordant pairs; anesthesia under age 3 and ever before age 12.

Table 4

Educational Achievement (EA) and Cognitive Problems (CP): Means for Subjects From Four Exposure Groups (Twins From Concordant or Discordant Pairs); Tests Were Done for Males and Females and for Exposure Under Age 3 and Exposure Ever Under Age 12

EA exposure	CON-NE	DIS_NE	DIS_E	CON-E
Under 3: males*	539.48	536.76	536.40	537.50
Ever: males**	540.11	538.54	538.83	537.38
Under 3: females***	537.49	534.43	534.36	538.21
Ever: females	537.60	536.26	535.69	537.82
CP	CON-NE	DIS_NE	DIS_E	CON-E
Under 3: males	2.69	2.98	2.75	2.63
Ever: males	2.92	2.90	2.73	2.57
Under 3: females*	1.85	4.26	3.44	2.85
Ever: females*	1.74	2.86	2.67	2.38

Note: * Concordant non-exposed twins score significantly higher on EA and lower on CP.

** Concordant non-exposed twins score significantly higher than concordant exposed twins on EA.

*** Concordant non-exposed and concordant exposed twins score significantly higher than discordant twins on EA.

for females with exposure under age 3). Furthermore, CON-NE female twins show significantly less CP. This indicates that the least vulnerable group (i.e. the group characterized by the fact that both members of the MZ pair were not exposed to anesthesia) performs best.

Finally, the longitudinal information on anesthesia was taken into account. We found that for males exposed at any time point, their EA score was significantly lower (537.72) (p=.004) than the EA score in the CON-NE group (539.99). For females a non-significant difference in the same direction was found. CP was not significantly different between never or ever exposed individuals.

Discussion

Monozygotic twins discordant for having received anesthesia have equivalent levels of learning-related outcomes. A child who receives anesthesia is no more likely than his genetic duplicate to have lower achievement on a standardized test for educational attainment (EA) or worse cognitive problems/inattention (CP) as rated by their teachers. Furthermore, male twins who had not received anesthesia by age 3, but whose co-twin receives anesthesia by age 12 have significantly poorer learning-related outcomes than twins from pairs where neither received anesthesia by age 12. This is the first evidence that the risk of anesthesia for 'causing' later learning disabilities is not related to the toxic effects of the anesthesia. Rather, the underlying genetic vulnerability of the individual to learning disability is likely correlated with the *reason* for the surgery for which anesthesia was administered. That is, children who are sick receive surgery. Children who receive surgery receive anesthesia. Children who are sick often also have learning disabilities. The hypothetical causal link between anesthesia and later learning problems, as appealing as it may be on the basis of laboratory work, is not supported by these data.

Classical twin studies have been informative in uncovering the underlying genetic and environmental contributions to intelligence in general and to learning disabilities specifically (Bartels et al., 2002a, 2002b; Gayan & Olson, 2001; Kovas et al., 2007; Wadsworth & DeFries, 2005). This work has demonstrated that the influences on learning disabilities overlap with general intelligence (Bartels et al., 2002b). Moreover, the genetic influences on different learning disabilities overlap, leading some researchers to argue for generalist genes among the learning disabilities (Plomin & Kovas, 2005). The classical twin design studying monozygotic and dizygotic twins, while powerful for uncovering these associations, informs little about the specific unique environmental impact of anesthesia. The classical twin design can demonstrate that there is a genetic effect on *whether* a child receives anesthesia. In fact, fitting a univariate genetic model to test for twin–twin correlations from monozygotic versus dizygotic twin pairs demonstrated a clear genetic component to whether a child received anesthesia (data not shown). In this view, it is likely that there are certain reasons why some children might undergo surgery which have a genetic risk associated with them.

A good candidate would be otolaryngological disorders such as chronic inner ear effusions and tonsilloadenoid hypertrophy. Recurrent otitis media is the most common reason for myringotomy or ventilation tube placement and is also highly heritable (Casselbrant & Mandel, 2005; Kvestad et al., 2004, 2006b). Myringotomy or placement of ventilation tubes is one of the most common reasons for undergoing surgery in childhood, at least in the United States, according to the Centers for Disease Control (Owings & Kozak, 1998). Further, the second most likely surgery is tonsillectomy with or without adenoidectomy. Tonsillitis and otitis may share a common genetic predisposition (Kvestad et al., 2006a). There has been debate as to whether chronic ear effusions,

myringotomy, or ventilation tube placement has any relation to later language problems (Lous et al., 2005). A recent study indicated the presence of hearing loss and language delays *before* the insertion of tubes (Kacmarynski et al., 2004), which suggests that there is an underlying problem prior to the anesthesia, not caused by it. Similarly, children who undergo tonsillectomy and adenoidectomy often do so because of sleep apnea. Sleep apnea has been associated with learning problems *before* surgery (Kurnatowski et al., 2006). Our data supports the hypothesis that learning problems are present before time of operation and thus may be related rather to underlying disease/vulnerability. Additionally, surgeries early in life are performed to correct congenital abnormalities such as cleft lip, cleft palate, urogenital abnormalities, strabismus, and other structural defects that can be associated with neurodevelopmental problems.

While speculative, we would suggest that using a genetically-informed design suggests a different conclusion than has been widely reported upon following the publication of the report by Wilder et al., (2009). Clearly, the decision for surgery in a young child should consider the risks and benefits of the anesthesia. One of these risks may be a later problem with learning. We would suggest, however, that screening for learning problems should be done at the time that surgery is being considered. From the data reported here, the hypothesis would be that the vulnerability for learning-related problems is already present at the time the decision for surgery is being made.

Clearly, this work is not without its own limitations. We did not directly assess for learning disabilities as in the Wilder et al. (2009) study, but rather for learning-related outcomes. Further, we did not assess for the specific types of anesthesia, although it is unclear that this would matter, given that NMDA receptor antagonists and GABA-mimetics are the most commonly used anesthetic drugs and are both associated with apoptosis. Even with these limitations, there is little evidence from these data that anesthesia exposure in early life causes later learning problems.

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