Published in final edited form as:

J Pediatr. 2016 May ; 172: 81–87.e2. doi:10.1016/j.jpeds.2015.12.024.

Smaller Cerebellar Growth and Poorer Neurodevelopmental Outcomes in Very Preterm Infants exposed to morphine

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Abstract

Objective—To examine the relationship between morphine exposure and growth of the cerebellum and cerebrum in very preterm neonates from early in life to term-equivalent age, as well as to examine morphine exposure and brain volumes in relation to neurodevelopmental outcomes at 18 months corrected age (CA).

Study design—A prospective cohort of 136 very preterm neonates (24–32 weeks gestational age) was serially scanned with MRI near birth and at term-equivalent age for volumetric measurements of the cerebellum and cerebrum. Motor outcomes were assessed with the Peabody Scales of Motor Development-2 and cognitive outcomes with the Bayley-III at 18 months CA. Generalized least squares models and linear regression models were used to assess relationships between morphine exposure, brain volumes, and neurodevelopmental outcomes.

Results—A 10-fold increase in morphine exposure was associated with a 5.5% decrease in cerebellar volume, after adjustment for multiple clinical confounders and total brain volume

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The authors declare no conflicts of interest.

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($P=0.04$). When infants exposed to glucocorticoids were excluded, the association of morphine was more pronounced, with an 8.2% decrease in cerebellar volume. Morphine exposure was not associated with cerebral volume ($P=0.30$). Greater morphine exposure also predicted poorer motor ($P<0.001$) and cognitive outcomes ($P=0.006$) at 18 months CA, an association mediated, in part, by slower brain growth.

Conclusions—Morphine exposure in very preterm neonates is independently associated with impaired cerebellar growth in the neonatal period and poorer neurodevelopmental outcomes in early childhood. Alternatives to better manage pain in preterm neonates that optimize brain development and functional outcomes are urgently needed.

Search terms

premature infants; cerebellum; MRI; morphine

Morphine is commonly used in neonatal intensive care for sedation and analgesia for preterm infants requiring mechanical ventilation;¹ however, routine use of morphine has been questioned in the preterm neonate given the uncertainty regarding its benefits and risks.² Research to date has been contradictory. Morphine exposure in rats showed no neurotoxic effects on the prefrontal cortex,³ whereas other animal models have shown morphine exposure associated with Purkinje cell death and impaired cerebellar development.^{4,5} The NEOPAIN trial found no difference in neonatal death or brain injury between placebo and morphine-treated human premature infants, but those exposed to open-label morphine had higher rates of death or brain injury compared with those with no morphine exposure.⁶ Although low doses of morphine have not been associated with differences in brain volumes by term-equivalent age,⁷ significantly smaller brain volumes were reported in a small sample of preterm-born children at age 10 years.⁸ We recently showed that morphine was associated with smaller cerebellar volume in children born very preterm at age 7 years.⁹

The cerebellum is particularly vulnerable to injury rapid growth during the preterm period, with different sensitivities compared with the cerebrum.¹⁰ For example, we previously showed that postnatal exposure to glucocorticoids was specifically associated with impaired cerebellar growth in premature newborns.¹¹ Our aim was to study the association between morphine exposure, cerebral and cerebellar development, and outcomes at 18 months corrected age (CA). Due to previous work identifying specific effects of morphine on cerebellar Purkinje cells^{4,5} and our previous work^{9,12} we hypothesize that higher morphine exposure would be associated with slowed cerebellar development, but not cerebral development. Although morphine was not associated with motor outcomes in a cohort of preterm infants born in the early 1990s,¹³ we hypothesized that higher morphine exposure would be associated with poorer motor outcome and that smaller cerebellar volumes would mediate this relationship. Given the cerebellum's role in cognition,¹⁴ we also explored the association of morphine and cerebellar volume with cognitive outcomes.

METHODS

We studied a prospective cohort of 188 infants born very preterm (24 to 32 weeks GA) admitted to the neonatal intensive care unit at the British Columbia Women's Hospital from April 2006 to September 2010, participating in a study of early brain development. Neonates with adequate quality MRI scans for volumetric analysis and detailed morphine exposure recorded were included in this study (n=136) (Figure 1; available at www.jpeds.com). Infants included in this study did not differ significantly from other infants in the larger cohort in terms of sex, birth weight, gestational age, early illness severity, or the presence of postnatal infection, white matter injury (WMI), or intraventricular hemorrhage (IVH) (all P 's > 0.05). We excluded newborns if there was either (1) clinical evidence of a congenital syndrome or major malformation; (2) congenital infection; or (3) ultrasound evidence of a large parenchymal hemorrhagic infarction (> 2cm). This study was approved by the UBC/Children's and Women's Health Centre of BC Research Ethics Board with informed parental consent.

Clinical factors previously associated with impaired cerebellar development were collected, including early illness severity [as measured by the Score for Neonatal Acute Physiology-II (SNAP-II)],¹⁵ WMI, IVH, cerebellar hemorrhage, postnatal infection, duration of intubation, hypotension, pain-related stress, exposure to general anesthesia, and cumulative exposure to dexamethasone, hydrocortisone, midazolam, fentanyl, and morphine.^{11,16–18} As previously defined,¹⁹ procedural pain/stress was operationalized as the number of invasive procedures (e.g., heel lance, intravenous or central line insertion, intramuscular injection, chest tube insertion, gastrostomy tube insertion, tape removal) and nasogastric tube insertions. Cumulative doses of dexamethasone, hydrocortisone, midazolam, and fentanyl were calculated from the average daily dose, adjusted for daily weight, and multiplied by the number of days on each medication. Cumulative morphine exposure was calculated from the average daily dose of intravenous and oral morphine (equivalence calculated), adjusted for daily weight, and multiplied by the number of days on morphine from birth to term-equivalent age.

Brain Imaging Techniques

Neonates were scanned without sedation within the first weeks of life and again at term-equivalent age. MRI scans were acquired with a 1.5-T scanner (Siemens Avanto, Siemens Medical Solutions USA, Malvern, Pennsylvania), as previously described.¹² An experienced pediatric neuroradiologist blinded to clinical history reviewed the scans and used a standard grading system to determine severity of IVH²⁰ and a clinically predictive scoring system for WMI.²¹ Cerebellar hemorrhage was defined as visible blood of any age in the cerebellum on ultrasound or MRI; only punctate hemorrhages (< 4 mm) were found.

Three individuals (EWYT, JGZ, and MLS) traced the whole cerebellum ending at the cerebellar peduncles with high intra- and inter-rater reliability (Dice co-efficients = 0.92–0.99). Cerebral tissue volumes were measured for 125 infants using a fully automated Expectation Maximization based tissue labeling algorithm specifically developed and validated for premature neonatal brain anatomy,²² with overall DICE co-efficients of 0.93 for deep gray matter, 0.90 for white matter, and 0.84 for cortical gray matter.

Neurodevelopmental Outcomes

Motor development of study participants was assessed at 18 months CA with the Peabody Developmental Motor Scales-2nd edition; the Total Motor Score was used in the analyses.²³ Cognitive outcomes were assessed with the Bayley Scales of Infant and Toddler Development-3rd edition (Bayley-III).²⁴ Both assessments have a mean of 100 and a standard deviation of 15. Assessors were experienced pediatric physical therapists blinded to MR findings and degree of morphine exposure.

Statistical Analyses

Statistical analyses were performed using Stata 12.1 for Mac (Stata Corporation, College Station, Texas) and R (R Core Team, Vienna, Austria: <http://www.R-project.org/>). The cohort was divided into three groups: no morphine exposure, less than or equal to the median morphine exposure, and greater than the median morphine exposure. Differences in clinical characteristics were analyzed using Fisher exact test for categorical variables and Kruskal-Wallis for continuous variables (Table I). Generalized least squares models were used to examine the relationship between morphine exposure (log transformed) and cerebellar volume, with an exponential temporal correlation structure to account for varying times between scans. This analysis accounts for serial scans to examine growth over time. Our basic model adjusted for birth GA, age at MRI, total brain volume, early illness severity, and number of invasive procedures, which was extended to include adjustment for factors associated with poorer cerebellar development^{11,16–18} and additional confounders (Table II). The same models were used to examine the relationship between morphine exposure and cerebral volume, except that cerebellar hemorrhage was removed from the model (Table III; available at www.jpeds.com). Secondary analyses were conducted to determine whether morphine was associated with changes in cortical gray matter, deep gray matter, or white matter volumes. Linear regression models were conducted to examine the relationship of morphine exposure and age-adjusted brain volumes to motor and cognitive outcomes at 18 months CA (Table IV).

RESULTS

Our cohort of infants was born at a median age of 27.4 weeks post-menstrual age (range 24–32; inter-quartile range [IQR] 25.8–29.8) and had serial imaging at a median age of 32.3 weeks (IQR 30.8–34.0) and at 40.3 weeks (IQR 38.9–42.0). Of the 136 infants, 91 (67%) were exposed to morphine, receiving a median cumulative dose of 1.91 mg/kg. Table I describes the clinical characteristics of our cohort by morphine exposure. Of the 136 infants, 127 (93%) were followed to 18 months CA. Median scores were 96 (IQR 86–98) on the Peabody Developmental Motor Scales-2 Total Motor Quotient and 105 (IQR 100–110) on the Bayley-III cognitive composite.

Cerebellar Volume

Adjusting for GA at birth, age at MRI, total brain volume, early illness severity, and number of invasive procedures, we found a significant association between morphine exposure and smaller cerebellar volume ($P<0.001$). This association persisted after further adjustment for factors associated with poor cerebellar development and other confounders ($P=0.04$; Table

II). A 10-fold increase in morphine predicted in a 5.5% decrease in cerebellar volume. Neonates, regardless of GA at birth, had smaller cerebellar volumes with increased morphine exposure (Figure 2). As our previous work showed that glucocorticoid exposure related to smaller cerebellar volume,¹¹ we then excluded infants exposed to dexamethasone and/or hydrocortisone (n=43) from the model; the effect size of morphine (with adjustment for all other confounders) was more pronounced with an 8.1% reduction in cerebellar volume (-0.081 cm^3 , 95% CI -0.161 , -0.0005 ; $P=0.05$).

Cerebral Volume

Morphine exposure was not associated with cerebral volume, in either the basic ($P=0.87$) or extended regression model ($P=0.30$; Table III). We conducted secondary analyses with adjustment for confounders to determine if any cerebral tissue type was smaller in association with morphine exposure; all were non-significant [white matter (0.003 cm^3 , 95% CI -0.005 , 0.010 ; $P=0.50$), cortical gray matter (-0.009 cm^3 , 95% CI -0.028 , 0.010 ; $P=0.36$), and deep gray matter (-0.022 cm^3 , 95% CI -0.048 , 0.004 ; $P=0.09$).

Neurodevelopmental Outcomes

We examined the relationship of: (1) morphine exposure to motor outcomes; (2) cerebellar and cerebral volumes to motor outcomes; and (3) whether cerebellar and cerebral volumes mediated the relationship of morphine to motor outcomes. We repeated the same analyses to explore these relationships with cognitive outcomes.

Morphine exposure was significantly associated with poorer total motor scores at 18 months CA (-4.26 , 95% CI -5.96 , -2.54 ; $P<0.001$), with a less robust relationship noted with cognitive outcomes (-3.86 , 95% CI -6.57 , -1.16 ; $P=0.006$) (Table III). Considering the relationship of brain volumes (adjusted for age at MRI) to motor outcomes, both cerebellar volume (0.94 , 95% CI 0.51 , 1.37 ; $P<0.001$) and cerebral volume (0.09 , 95% CI 0.05 , 0.13 ; $P<0.001$) were positively associated with motor outcome at 18 months CA. White matter (0.16 , 95% CI 0.08 , 0.24 ; $P<0.001$), cortical gray matter (0.18 , 95% CI 0.09 , 0.27 ; $P<0.001$), and deep gray matter volumes (1.91 , 95% CI 1.29 , 2.53 ; $P<0.001$) were each positively associated with motor outcome. Similar relationships were noted with cognitive outcomes. Cerebellar volumes were significantly associated with cognitive scores (1.09 , 95% CI 0.40 , 1.78 ; $P=0.002$). Cerebral volumes were also associated with cognitive outcomes (0.08 , 95% CI 0.02 , 0.15 ; $P=0.01$), including white matter (0.14 , 95% CI 0.01 , 0.26 ; $P=0.03$), cortical gray matter volumes (0.18 , 95% CI 0.04 , 0.31 ; $P=0.01$), and deep gray matter (2.16 , 95% CI 1.21 , 3.12 ; $P<0.001$).

We then examined whether the association of morphine and neurodevelopmental outcomes was mediated in part by cerebellar and cerebral volumes. The association of morphine with motor outcome remained significant in all models, with the effect attenuated after adjusting for cerebral or cerebellar volume, and more so after adjusting for both cerebral and cerebellar volume (Table IV). The relationship of morphine with cognitive outcomes was also attenuated after adjusting for cerebellar and cerebral volumes, suggesting that the relationship between higher morphine exposure with poorer neurodevelopmental outcomes is mediated, at least in part, by slower brain growth.

DISCUSSION

Morphine exposure was differentially associated with small cerebellar volumes but not cerebral volumes. Consistent with Steinhorn et al,⁷ morphine exposure was not related to smaller brain volume; however, higher morphine exposure in very preterm neonates was associated with reduced cerebellar volume at term-equivalent age, even after adjusting for known confounders. This result is consistent with mouse models, suggesting a critical period during development when Purkinje cells of the cerebellum are particularly vulnerable to the effects of morphine.⁴ Reduced Purkinje cell dendritic length and fewer Purkinje cell numbers were noted in 1-day old mice exposed to 7–10 days of morphine, but not in 7-day old mice with the same exposure. From 24 weeks gestation to term, the cerebellum increases 5-fold in volume.⁹ Morphine exposure during this highly proliferative time of development may alter Purkinje cell survival, resulting in smaller cerebellar growth. Other opiates have also been shown to affect cerebellar growth in infants prenatally exposed to illicit drugs²⁵ and in preterm infants exposed to fentanyl.²⁶ We did not find an association with fentanyl exposure, likely because fentanyl was not frequently used in our cohort (n=30).

The duration of morphine exposure also affects Purkinje cell survival in animal models. Prolonged exposure to morphine is thought to cause apoptosis and necrosis in Purkinje cells.^{4,5} Infants of younger gestational ages are likely to receive greater exposure to morphine, thus are at greater risk of poor cerebellar growth. Consistent with these animal models, we found neonates < 26 weeks had the smallest cerebellar volumes with higher cumulative exposure to morphine (Figure 2). However, neonates 26 to < 28 weeks and neonates > 28 weeks also had less cerebellar growth with increased exposure to morphine. The potential mechanism may be related to decreased proliferation of the external granular layer. From 20–30 weeks gestation, Purkinje cells differentiate and secrete Sonic hedgehog which stimulates proliferation of the external granular layer.¹⁰ Thus, the association of smaller cerebellar growth with exposure to morphine may be related to decreased signaling of Purkinje cells rather than decreased Purkinje cell proliferation per se.

We also found that morphine exposure was associated with poorer motor and cognitive outcomes in our cohort of 136 premature newborns. This relationship appears to be mostly explained by smaller cerebellar and cerebral volumes. Additional effects may relate to microscopic effects not measured by volume analysis, such as at the level as the dendrite or synapse, or by interfering with brain metabolism.

Few studies have examined the association of morphine with motor outcomes and results are mixed.²⁷ Grunau et al¹⁹ found an association with higher morphine exposure and poorer motor outcomes at 8 months but not at 18 months CA in a different cohort of 137 children born very preterm. Another study found no difference in motor scores at 5–6 years for 66 prematurely born children with neonatal morphine exposure for 7 days compared with 33 controls in the non-morphine group.¹³ Our results suggest that higher doses of morphine are associated with poorer motor outcomes, and that this association is mediated, at least in part, by slower brain growth. This finding is consistent with the NEOPAIN trial, where preterm infants with greater morphine exposure had lower motor scores at 36 weeks postmenstrual age.²⁸ A similar relationship was found for infants with other opioid exposure; Lammers et

al²⁹ investigated whether there was a dose relationship between fentanyl and neurodevelopmental outcome up to 2 years of age in a retrospective cohort of 147 very low birth weight infants. Although they did not find a significant association of fentanyl exposure and motor outcomes at 18 months, infants with higher opioid exposure had poorer motor scores.

The relationship of morphine to cognitive outcomes, although less robust compared with that of motor outcomes, still appears to be mediated, at least in part, by slower brain growth. In our cohort, the majority of infants scored within the normal range of cognitive scores of the Bayley-III, a finding that is not unusual with this tool.³⁰ Even though a few studies have found no relationship between morphine and cognitive outcomes at school-age,^{7,8,13,31} our neonatal imaging findings and 18 month neurodevelopmental outcomes suggest that the association of morphine to long-term cognitive outcomes requires further investigation.

This study is not without limitations. Although daily morphine doses were adjusted for daily body weight, whether morphine was given in specific response to pain was not systematically collected. Animal literature suggests that morphine may be neuroprotective if given in response to pain, but neurotoxic when given in the absence of pain.³² Morphine exposure is highly correlated with multiple clinical factors, thus it is difficult to determine if slower cerebellar growth is related to cumulative deleterious effects of several neonatal exposures. Although we found an independent association of morphine exposure and smaller cerebellar after adjustment for multiple clinical confounders, the possibility of confounding by indication cannot be eliminated. However, our findings are consistent with animal models which show a specific effect of morphine on Purkinje cells of the cerebellum^{4,5} as well as the association of other opioid exposure with smaller growth of the human cerebellum.^{25,26} Some infants in our sample were exposed to large cumulative doses of morphine due to extended exposure (> 60 days), which may not be generalizable to other neonatal intensive care units. Even though we have detailed data of the neonatal course, we did not take into account factors after hospital discharge that could affect motor outcomes and mitigate potential associations of morphine and motor function. However, previously we have found in another cohort that, unlike cognitive development, parenting factors did not predict motor outcome at 18 months CA after adjusting for neonatal risk factors.¹⁹

This study suggests that morphine, after extensive consideration of confounders, is independently associated with smaller cerebellar volumes in the neonatal period. Morphine is the most common analgesic used for preterm neonates requiring mechanical ventilation,¹ yet its effectiveness in providing adequate analgesia for acute pain of invasive procedures in premature neonates remains in question.³³ Given that invasive procedures are negatively associated with brain development,^{12, 34–36} reducing pain/stress of premature newborns is an important goal. Although there is insufficient evidence to recommend routine use of opioids to manage neonatal pain,² accumulating evidence stresses the judicious use of morphine and only in the context of pain.^{2, 33,37}

In conclusion, morphine exposure in preterm neonates is independently associated with impaired cerebellar growth in the neonatal period and poorer neurodevelopment in early childhood. Even though confounding by indication cannot be completely ruled out, the

independent association of morphine with smaller cerebellar volume and poorer neurodevelopmental outcomes with increased exposure cannot be ignored. Alternatives to better manage pain in preterm neonates and strategies that optimize brain development and functional outcomes are urgently needed.

Acknowledgments

Funded by Canadian Institutes of Health Research (MOP79262 [to S.M.] and MOP86489 [to R.G.]). J.Z. was supported by the Canadian Child Health Clinician Scientist Program, Child & Family Research Institute (CFRI), Michael Smith Foundation for Health Research, and NeuroDevNet. S.M. is currently the Bloorview Children's Hospital Chair in Pediatric Neuroscience and was supported by a Tier 2 Canada Research Chair in Neonatal Neuroscience, and Michael Smith Foundation for Health Research Scholar Award. R.G. and R.B. are supported by CFRI (Senior Scientist Awards) and *Eunice Kennedy Shriver* Institute of Child Health and Human Development (R01 HD039783). C.S.'s research program is supported by the National Institutes of Health/National Institute of Neurological Disorders and Stroke (R01 NS061957 and R01 NS055064).

We gratefully acknowledge the children and families who participated in this study.

Abbreviations

CA	corrected age
CI	confidence intervals
GA	gestational age
IQR	interquartile range
IVH	intraventricular hemorrhage
WMI	white matter injury

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Figure 1.
Flow of Participants

Figure 2.
Cerebellar volumes by morphine exposure, adjusted by age at MRI and stratified by gestational age at birth: < 26 weeks (black), 26 to < 28 weeks (red), ≥ 28 weeks (green).

Table 1

Clinical features of premature newborns by morphine exposure (n=136)

Number (%) or Median (IQR)	Morphine Exposure (mg/kg)			P
	None	1.905	> 1.905	
Number	45	47	44	
Male sex	18 (40)	26 (55)	27 (61)	0.11
Gestational age at birth (weeks)	29.7 (28.6–31.4)	27.6 (26.3–29.9)	25.6 (24.9–26.6)	< 0.001
Birth weight (grams)	1225 (1080–1430)	1065 (845–1285)	738 (651–860)	< 0.001
SNAP-II	9 (0–9)	14 (9–22)	20 (10–29)	< 0.001
Pain (# invasive procedures)	66 (48–93)	100 (71–126)	226 (172–271)	< 0.001
Minor surgical procedures ^a	0 (0)	6 (13)	24 (55)	< 0.001
Major surgical procedures ^b	0 (0)	6 (13)	27 (61)	< 0.001
NEC stage 2	0 (0)	2 (4)	9 (20)	0.001
Days of Intubation	0 (0–1)	5 (3–17)	51 (31–67)	< 0.001
Hypotension (n=130)	6 (14)	14 (31)	32 (76)	< 0.001
Postnatal infection	5 (11)	15 (32)	37 (84)	< 0.001
Any white matter injury	19 (42)	8 (17)	14 (32)	0.03
Moderate-Severe WMI	11 (24)	5 (11)	7 (16)	0.23
Cystic PVL	0 (0)	1 (2)	0 (0)	>0.99
Intraventricular hemorrhage	15 (33)	27 (57)	24 (55)	0.04
Cerebellar hemorrhage (< 4mm)	3 (7)	8 (17)	11 (25)	0.06
Dexamethasone exposure (mg/kg)	0 (0–0)	0 (0–0)	0.62 (0–1.42)	< 0.001
Hydrocortisone exposure (mg/kg)	0 (0–0)	0 (0–0)	6.02 (0–16.7)	< 0.001
Midazolam (mg/kg)	0 (0–0)	0 (0–0)	7.70 (0–30.71)	< 0.001
Fentanyl (mg/kg)	0 (0–0)	0 (0–0)	0.005 (0–0.015)	< 0.001

NEC, necrotizing enterocolitis; PVL, periventricular leukomalacia; SNAP-II, Score for Neonatal Acute Physiology-Version II; WMI, white matter injury

^a includes hernia repair, gastrostomy, G-tube placement, chest tube insertion, bronchoscopy, retinal laser treatment, central venous line placement

^b includes patent ductus arteriosus ligation, laparotomy, ileostomy, bowel resections, craniotomy, stent insertion

Table 2

Cerebellar Volume, adjusted for gestational age at birth and age at MRI

	Cerebellar Volume (cm ³)				Cerebellar Volume (cm ³) adjusted for confounders			
	Co-efficient	95% CI	P		Co-efficient	95% CI	P	
Total brain volume (log ₁₀)	0.518	0.362, 0.675	<0.001		0.600	0.408, 0.792	<0.001	
Morphine exposure (log ₁₀)	-0.085	-0.130, -0.040	<0.001		-0.055	-0.108, -0.002	0.04	
SNAP-II	0.0005	-0.001, 0.002	0.59		0.0009	-0.0008, 0.003	0.30	
Pain (# invasive procedures)	-0.00006	-0.0006, 0.0005	0.82		0.0004	-0.0002, 0.0009	0.16	
Minor surgical procedures					0.016	-0.014, 0.043	0.30	
Major surgical procedures					0.018	-0.013, 0.050	0.26	
Cerebellar hemorrhage					0.019	-0.028, 0.067	0.42	
Intraventricular hemorrhage					-0.056	-0.075, -0.037	<0.001	
White matter injury					0.011	-0.026, 0.049	0.55	
Hypotension					0.008	-0.043, 0.059	0.76	
Patent ductus arteriosus					-0.075	-0.125, -0.025	<0.01	
Postnatal infection					-0.057	-0.115, 0.001	0.05	
Necrotizing enterocolitis					0.008	-0.048, 0.064	0.78	
Intubation days					-0.0006	-0.002, 0.001	0.51	
Dexamethasone exposure (mg/kg)					-0.02	-0.062, 0.023	0.36	
Hydrocortisone exposure (mg/kg)					-0.003	-0.006, -0.0004	0.09	
Midazolam (mg/kg)					-0.0008	-0.003, 0.002	0.54	
Fentanyl exposure (mg/kg)					-0.005	-0.013, 0.004	0.25	
Weight at MRI (kg)					0.004	-0.048, 0.056	0.87	

IVH, intraventricular hemorrhage; MRI, magnetic resonance imaging; SNAP-II, Score for Neonatal Acute Physiology-Version II

Table 3

Cerebral Volume, adjusted for gestational age at birth and age at MRI

	Cerebral Volume (cm ³)			Cerebral Volume (cm ³) adjusted for confounders		
	Co-efficient	95% CI	P	Co-efficient	95% CI	P
Total brain volume (log ₁₀)	0.971	0.959, 0.984	<0.001	0.961	0.945, 0.977	<0.001
Morphine exposure (log ₁₀)	-0.0002	-0.003, 0.003	0.87	-0.002	-0.006, 0.002	0.30
SNAP-II	-0.00006	-0.0002, 0.00006	0.32	-0.0001	-0.0002, 0.00002	0.09
Pain (# invasive procedures)	0.000002	-0.00003, 0.00004	0.92	0.00003	-0.00001, 0.00007	0.16
Minor surgical procedures				-0.0002	-0.002, 0.002	0.86
Major surgical procedures				0.003	0.0003, 0.005	0.02
Intraventricular hemorrhage				-0.0002	-0.002, 0.001	0.83
White matter injury				-0.004	-0.007, -0.0004	0.03
Hypotension				0.001	-0.003, 0.005	0.54
Patent ductus arteriosus				-0.0008	-0.004, 0.003	0.66
Postnatal infection				-0.002	-0.006, 0.003	0.43
Necrotizing enterocolitis				-0.0006	-0.005, 0.003	0.79
Intubation days				-0.00002	-0.0001, 0.0001	0.80
Dexamethasone exposure (mg/kg)				-0.003	-0.006, 0.0001	0.06
Hydrocortisone exposure (mg/kg)				-0.0001	-0.0003, 0.0001	0.34
Midazolam exposure (mg/kg)				0.000007	-0.0002, 0.0002	0.93
Fentanyl exposure (mg/kg)				0.0002	-0.0004, 0.0008	0.46
Weight at MRI (kg)				0.002	-0.003, 0.006	0.49

IVH, intraventricular hemorrhage; MRI, magnetic resonance imaging; SNAP-II, Score for Neonatal Acute Physiology-Version II

Table 4
Neurodevelopmental Outcomes by Morphine Exposure and Brain Volumes, adjusted for age at MRI

	Peabody Total Motor Score			Bayley-III Cognitive Score		
	Co-efficient	95% CI	P	Co-efficient	95% CI	P
Morphine exposure ($\log_{10}$)	-4.26	-5.96, -2.54	<0.001	-3.86	-6.57, -1.16	0.006
Morphine exposure ($\log_{10}$) + cerebellar volume	-3.05	-5.50, -0.60	0.01	-1.69	-5.53, 2.16	0.39
Morphine exposure ($\log_{10}$) + cerebral volume	-3.06	-5.29, -0.83	<0.01	-2.66	-6.23, 0.91	0.14
Morphine exposure ($\log_{10}$) + cerebellar & cerebral volume (total brain volume)	-2.58	-5.15, -0.01	0.05	-2.38	-5.18, 0.42	0.10