Potential Asphyxia and Brainstem Abnormalities in Sudden and Unexpected Death in Infants

WHAT'S KNOWN ON THIS SUBJECT: Certain characteristics of the sleep environment increase the risk for sleep-related, sudden, and unexplained infant death. These characteristics have the potential to generate asphyxia. The relationship between the deaths occurring in these environments and neurochemical abnormalities in the brainstem that may impair protective responses to asphyxia is unknown.

WHAT THIS STUDY ADDS: We report neurochemical brainstem abnormalities underlying cases of sudden infant death that are associated with and without potential asphyxial situations in the sleep environment at death. The means to detect and treat these abnormalities in infants at risk are needed.

abstract

OBJECTIVE: Sudden and unexplained death is a leading cause of infant mortality. Certain characteristics of the sleep environment increase the risk for sleep-related sudden and unexplained infant death. These characteristics have the potential to generate asphyxial conditions. We tested the hypothesis that infants may be exposed to differing degrees of asphyxia in sleep environments, such that vulnerable infants with a severe underlying brainstem deficiency in serotonergic, γ -aminobutyric acid-ergic, or 14-3-3 transduction proteins succumb even without asphyxial triggers (eg, supine), whereas infants with intermediate or borderline brainstem deficiencies require asphyxial stressors to precipitate death.

METHODS: We classified cases of sudden infant death into categories relative to a "potential asphyxia" schema in a cohort autopsied at the San Diego County Medical Examiner's Office. Controls were infants who died with known causes of death established at autopsy. Analysis of covariance tested for differences between groups.

RESULTS: Medullary neurochemical abnormalities were present in both infants dying suddenly in circumstances consistent with asphyxia and infants dying suddenly without obvious asphyxia-generating circumstances. There were no differences in the mean neurochemical measures between these 2 groups, although mean measures were both significantly lower ($P < .05$) than those of controls dying of known causes.

CONCLUSIONS: We found no direct relationship between the presence of potentially asphyxia conditions in the sleep environment and brainstem abnormalities in infants dying suddenly and unexpectedly. Brainstem abnormalities were associated with both asphyxia-generating and non-asphyxia generating conditions. Heeding safe sleep messages is essential for all infants, especially given our current inability to detect underlying vulnerabilities. *Pediatrics* 2013;132:e1616–e1625

AUTHORS: Bradley B. Randall, MD,^a David S. Paterson, PhD,^b Elisabeth A. Haas, MPH,^c Kevin G. Broadbelt, PhD,^b Jhodie R. Duncan, PhD,^b Othon J. Mena, MD,^d Henry F. Krous, MD,^c Felicia L. Trachtenberg, PhD,^e and Hannah C. Kinney, MD^b

^aDepartment of Pathology, University of South Dakota Sanford School of Medicine, Sioux Falls, South Dakota; ^bDepartment of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts; ^cDepartments of Pathology and Pediatrics, University of California, San Diego, La Jolla, California, and Rady Children's Hospital, San Diego, California; ^dSan Diego County Medical Examiner's Office, San Diego, California; and ^eNew England Research Institutes, Watertown, Massachusetts

KEY WORDS

14-3-3 proteins, bed-sharing, GABA receptors, prone sleep, serotonin, sudden infant death syndrome

ABBREVIATIONS

ARC—arcuate nucleus
DMX—dorsal motor nucleus of the vagus
GABA— γ -aminobutyric acid
GC—gigantocellularis
HG—hypoglossal nucleus
IRZ—intermediate reticular zone
NTS—nucleus of the solitary tract
PGCL—paragigantocellularis lateralis
PIO—Principal Inferior Olive
ROB—raphe obscurus
SIDS—sudden infant death syndrome
TPH2—tryptophan hydroxylase 2

Dr Randall helped conceptualize and design the study and participated in data accrual and analysis; Dr Paterson helped conceptualize and design the study and participated in data accrual and analysis of the serotonin parameters; Ms Haas participated in data accrual and analysis of the epidemiological factors of the cohort; Dr Broadbelt participated in data accrual and analysis of the GABA and 14-3-3 parameters; Dr Duncan participated in data accrual and analysis of serotonin parameters; Dr Mena participated in data accrual and analysis of autopsies and death scene investigations in the medical examiner's office; Dr Krous participated in phenotyping of the cases of sudden and unexpected death and in analyzing the data; Dr Trachtenberg participated in study design and performed all the statistical analyses; Dr Kinney helped conceptualize and design the study, participated in data accrual and analysis, and drafted the manuscript; and all authors approved the final manuscript as submitted.

www.pediatrics.org/cgi/doi/10.1542/peds.2013-0700

doi:10.1542/peds.2013-0700

Accepted for publication Sep 13, 2013

Address correspondence to Hannah C. Kinney, MD, Department of Pathology, Enders Building Room 1112, Boston Children's Hospital, 61 Binney St, Boston, MA 02115. E-mail: hannah.kinney@childrens.harvard.edu

(Continued on last page)

Sudden and unexplained death remains a leading cause of infant mortality.^{1–7} The term sudden infant death syndrome (SIDS) is currently defined as the sudden unexpected death of an infant <1 year of age, with onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including a complete autopsy and review of the circumstances of death.⁷ Unsafe sleep environments at the time of death are known to increase the risk for sudden infant death, including SIDS, $\geq$ threefold.^{3,4,6,8–10} In a study of 209 sudden unexpected infant deaths with in-depth death scene investigations, the medical examiners identified asphyxia as either the cause or potential cause of death in the majority (86%) of cases.⁶ However, only 27 of the 209 cases had reports of witnessed overlaying, wedging, or strangulation. Improved death scene investigations and the increased attribution of potential asphyxia-generating conditions to hazardous sleep environments have led to a change in death certification from SIDS to terms such as suffocation, positional asphyxia, and undetermined, suggesting that the decline in SIDS rates reflects, at least in part, a diagnostic shift.^{3,11,12}

The association of unsafe sleep environments with SIDS raises the possibility that normal infants die of asphyxia and that eliminating these dangerous sleeping conditions will in turn eradicate all SIDS deaths. Yet there is mounting evidence that at least some infants who die of SIDS are not “normal” before death but rather have underlying vulnerabilities,^{5,13–26} including genetic susceptibilities,²⁷ that put them at risk. The triple-risk model for SIDS posits that SIDS occurs when 3 factors simultaneously impinge on the infant: underlying vulnerability, critical developmental period, and exogenous stressors.²⁸ These stressors include

prone or side sleep position, found face-down, head covering, sleeping on an adult mattress or couch, soft or excessive bedding, bed-sharing, and mild upper respiratory infection.^{5,20,39} We believe that these exogenous, environmental factors share the capability to generate potentially lethal asphyxia, hypoxia, and hypercapnia (ie, homeostatic stressors), which in turn require intact brainstem defense systems to protect against death.^{5,29} Nonasphyxial homeostatic stressors include temperature imbalances and cardiovascular challenges.^{5,29} We hypothesize that at least a subset of SIDS is caused by an underlying brainstem abnormality in neural networks that mediate protective responses to asphyxia, resulting in sleep-related sudden death.^{30–35} In support of this hypothesis, we have reported deficiencies in infants who died of SIDS in interrelated neurochemical parameters in the medullary serotonergic network that plays a key role in protective respiratory or autonomic responses to homeostatic stressors.^{30–35} The parameters are related mainly to the neurotransmitters serotonin and γ -aminobutyric acid (GABA)

and the signal transduction family 14-3-3, involved in serotonin regulation.^{30–35}

Given a reduction in the overall SIDS rate associated with an increased rate of supine sleep,^{5,8–10} it is highly likely that prone sleep position plays a direct role in the chain of events leading to sudden death in some infants. The hypothesized mechanisms include ineffective protective responses to airway obstruction or rebreathing of expired gases in the face-down position (compounded by soft bedding)^{5,20} and thermal or cardiovascular stress.^{5,29} The overarching premise of this study is that infants may be exposed to degrees of asphyxia in sleep environments, such that vulnerable infants with a severe brainstem deficiency succumb even without potential asphyxia (eg, in the supine sleep position), whereas infants with intermediate brainstem deficiencies require asphyxial stressors to precipitate death (Fig 1). These borderline infants are presumably able to compensate in basal conditions but cannot mount a brainstem response to a sleep-related, asphyxial stress. In this study, we tested the hypothesis that sudden unexplained infant death,

FIGURE 1

Diagram of the potential interrelationships of sudden infant death, brainstem pathology, and asphyxia. Vulnerable infants with a severe underlying brainstem deficiency succumb even without asphyxia (Group A), whereas infants with intermediate deficiencies require asphyxial stressors to precipitate death (Group B). All infants die when the asphyxia is severe.

irrespective of the diagnosis of SIDS, is associated with potential asphyxial stressors and a range of medullary deficiencies of serotonin, GABA, or 14-3-3 parameters, between severely reduced values in infants with sudden death without obvious asphyxial stressors and normative values in control infants dying of known causes (Fig 1). According to our premise, even normal infants die of asphyxia if it is severe enough (eg, accidental head wedging), and their neurochemical parameters are likely to be in normative range. In this study, however, we did not have a sufficient sample size of infants dying of unequivocal asphyxia to examine directly this possibility.

METHODS

Patients

We studied a total of 71 cases of sudden and unexpected death that were autopsied at the San Diego County Medical Examiner's Office from 1997 to 2008, with 2 nonoverlapping data sets in which serotonin, GABA_A receptor, or 14-3-3 measures were available.^{30–35} The brainstem samples were analyzed at Boston Children's Hospital, resulting in publications in 2006³¹ and 2010,³⁰ each concerned with a separate data

set. In these publications, SIDS was defined as stated earlier; control infants also died suddenly, but a complete autopsy or death scene investigation demonstrated a pathologic explanation for the death.^{30–35} Undetermined cases were equivocal cases in which a specific diagnosis could not be made, and they were not included in the published analyses. In the current study, however, we reclassified all unexplained cases, including the undetermined or equivocal cases, according to an asphyxial grading schema developed by us^{36,37} (Tables 1–3). Although there are no established criteria for what constitutes definitive asphyxia in a sudden unexpected infant death, we devised a subjective scale of possible to probable asphyxia ranging from none (an infant sleeping supine on a hard surface without the face covered) to definitive (an infant found dead with a plastic bag over the head). Without an explicit asphyxial definition based on quantitative measurements of blood oxygen and carbon dioxide levels in the infant, we assigned each unexplained case to a group based on the potential for asphyxia (Table 1). For this study, the causes of death were assigned a group (Group A–F) according to a published asphyxia-related

scheme³⁷ after review of the reports of the death scene investigation and autopsy records by an experienced medical examiner (B.B.R.), who was blinded to the neurochemical data (Table 1). All cases and controls were obtained under the auspices of the San Diego County Medical Examiner's Office, in accordance with California law Chapter 955, Statutes of 1989 (SB1069), which permits the use of autopsy tissues from infants with sudden death for research without direct parental permission. The institutional review boards of Boston Children's Hospital and Rady Children's Hospital approved the study. All clinicopathologic information was extracted from the autopsy reports and death scene investigative reports.

Risk Factors

In the triple-risk model, we subdivided risk factors into intrinsic and extrinsic, as in previous studies.^{5,30,31,34,35,38} An intrinsic risk is defined as a genetic, environmental, or developmental factor that affects the underlying vulnerability, including male gender, African American ethnicity, prenatal exposure to maternal smoking, and prematurity (<37 gestational weeks at birth). An extrinsic risk factor is considered an exogenous stressor, as defined earlier. We assessed 3 intrinsic and 6 extrinsic risk factors (Table 4).

Neurochemical Analysis in the Medullary Serotonin System

In the 2006 data set, the medullae were analyzed for serotonin_{1A} receptor binding with tissue receptor autoradiography³¹; in the 2010 data set, medullae were analyzed for serotonin_{1A} and GABA_A receptor binding with tissue receptor autoradiography^{30,35}; levels of tryptophan hydroxylase 2 (TPH2), the key biosynthetic enzyme of serotonin, with western blotting³¹; serotonin levels with high-performance liquid

TABLE 1 Asphyxia-Related Classification Scheme³⁷

Group A: Sudden and unexplained infant death, not asphyxia-related. <i>Comment:</i> Some element of asphyxia, such as a face down on a firm mattress, is allowed as long as it is not considered potentially life-threatening.
Group B: Sudden and unexplained infant death, possibly asphyxia-related. <i>Comment:</i> Included are almost all cases of sharing any sleeping surface (except where there is clear evidence that the adult did not impinge upon the infant). Deaths occurring on certain inherently unsafe sleeping surfaces, such as sofas, beanbag chairs, waterbeds, etc., were included unless it is clear that the sleeping environment was safe (Group A), or unequivocally lethal (see Group F).
Group C: Sudden and unexpected infant death, possible life-threatening stressor that is not asphyxia-related, eg, hyperthermia. <i>Comment:</i> Included are potentially lethal extremes of temperature, high but not necessarily lethal levels of drugs, and natural diseases that could represent a possible contributory cause of death, but aren't necessarily of lethal extent.
Group D: Sudden and unexpected death, equivocal. <i>Comment:</i> This classification is used for cases where the cause of death was unclear, but for whatever reason the other classifications weren't appropriate.
Group E: Sudden and unexpected death: unclassified, no autopsy or death scene investigation.
Group F: Known cause of death (of either natural or unnatural manner).

chromatography³¹; and 14-3-3 isoform and GABA_A α ₃ levels with western blotting,^{34,35} as per methods described in the relevant publications.^{30–35} We define anatomically the medullary serotonin network in the human infant as serotonin neuronal cell bodies in the medulla that are located in the raphe nuclei (raphe obscurus [ROB], raphe magnus, and raphe pallidus), extra-raphe (gigantocellularis [GC], paragigantocellularis lateralis [PGCL], intermediate reticular zone [IRZ], lateral reticular nucleus, and subtrigeminal nucleus), and ventral surface (arcuate nucleus [ARC]) and the medullary projection sites of these serotonin source neurons (eg, hypoglossal nucleus [HG], dorsal motor nucleus of the vagus [DMX], and nucleus of the solitary tract [NTS]).^{39,40}

Statistical Analysis

The sample size for Groups A to F varied for each neurochemical measure (Table 2). Table 3 demonstrates how the Group A to F classification scheme relates to the SIDS scheme used in previous publications. As a first step toward modeling potential differences between Groups A to F, age–group interactions were tested between Group A and Group B to determine whether age had the same effect on both groups. No significant interactions were found. Therefore, all additional age–group interactions were modeled by using Groups A and B (unexplained deaths) versus Group F (known or explained cause of death) (ie, age has a common effect for Groups A and B but possibly a different effect for Group F). There was insufficient sample size to test for interactions with Groups C and D, although they were visually similar to Groups A and B.

We tested the hypothesis that the mean values of Group B (unexplained death with potential asphyxia) were intermediate

TABLE 2 Available Sample Sizes of Groups A–E

Neurochemical Measures and Data Set		Group Sample Size					
Measure	Data Set	A	B	C	D	E	F
Serotonin _{1A} receptor binding	2006 and 2010	15	35	6	6	0	9
Serotonin and TPH2 levels	2010	11	28	5	2	0	7
14-3-3	2010	10	19	2	0	0	7
GABA _A receptor binding	2010	7	16	3	0	0	7
GABA _A receptor levels	2010	8	9	0	0	0	6

The known causes of death in Group F were acute accidents; $n = 3$; congenital heart disease (1 case clinically unsuspected), $n = 3$; pneumonia, $n = 2$; and progressive malnutrition and sudden death after neonatal repair of gastroschisis.

TABLE 3 Reclassification of Cases With Sudden and Unexpected Death in Published 2006 and 2010 Cohorts

Category	Serotonin _{1A} Receptor Binding					Serotonin and TPH2 Levels				
	A	B	C	D	F	A	B	C	D	F
SIDS	15	29	5	3	0	11	22	4	0	0
Undetermined	0	6	1	2	0	0	6	1	2	0
Acute controls	0	0	0	1	7	0	0	0	0	5
Chronic controls	0	0	0	0	2	0	0	0	0	2

Category	14-3-3 Isoform Levels					GABA _A Receptor Binding					GABA _A Receptor Levels				
	A	B	C	D	F	A	B	C	D	F	A	B	C	D	F
SIDS	10	19	2	0	0	7	16	3	0	0	8	9	0	0	0
Undetermined	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acute controls	0	0	0	0	5	0	0	0	0	5	0	0	0	0	4
Chronic controls	0	0	0	0	2	0	0	0	0	2	0	0	0	0	2

between the low mean values of Group A (sudden death without potential asphyxia) and the high mean values of Group F (sudden death due to known causes of death, ie, controls) (Group A < Group B < Group F). Analysis of covariance was used to test for differences between groups, controlling for the potential effect of postconceptional age; the interaction between age and Groups A or B and F was included when statistically significant. We also used regression models to test association of the number of exogenous stressors with serotonin_{1A} binding in the medullary nuclei. $P < .05$ was considered significant.

RESULTS

Database

The study comprised a total of 71 infant deaths, with almost half of the cases in Group B (49%, 35/71) and with 20% (15/75) in Group A, 13% (9/71) in Group F,

and 8% (6/71) in each of Groups C and D (Table 2). There was no significant difference in postconceptional age between Groups A, B, and F (Table 4). Because of the small sample sizes of Groups C and D, they were not included in the analysis, although their neurochemical values were visually similar to those of Groups A and B. The known causes of death in Group F were acute accidents, $n = 3$; congenital heart disease (1 case clinically unsuspected), $n = 3$; pneumonia, $n = 2$; and progressive malnutrition and sudden death after neonatal repair of gastroschisis.

Infants in Group B were more likely than those in Group A to be discovered prone (74% vs 25%, $P = .047$) and, by definition, face down or face covered (59% vs 0%) ($P = .001$) (Table 4). The infants in Group A were more likely to have been born preterm and to have experienced a minor illness within 48 hours before

TABLE 4 Risk Factors in Groups A and B of the Asphyxia-Related Schema of Classification for Cases With Information About Serotonin_{1A} Receptor Binding in the Medullary Serotonin System

Clinical Variable	Group A, n = 15	Group B, n = 35	P ^a
Postconceptional age, wk	57.4 ± 12.1	53.3 ± 8.5	.24 ^b
Male ^c	60% (9/15)	57% (20/35)	.85
African American ^c	17% (2/12)	10% (3/29)	.57
Premature ^{c,d}	40% (6/15)	17% (6/35)	.08
0 Intrinsic risk factors	27% (4/15)	34% (12/35)	.22 ^b
1 Intrinsic risk factors	33% (5/15)	54% (19/35)	
2 Intrinsic risk factors	40% (6/15)	6% (2/35)	
Prone at discovery ^e	25% (3/12)	74% (23/31)	.047 ^f
Supine at discovery	25% (3/12)	16% (5/31)	
Side at discovery ^e	25% (3/12)	10% (3/31)	
Other position at discovery	8% (1/12)	0% (0/31)	
Discovered in car seat	17% (2/12)	0% (0/31)	
Face down or covered ^e	0% (0/10)	59% (16/27)	.001
Adult bed ^e	36% (4/11)	56% (15/27)	.28 ^g
Crib	64% (7/11)	44% (12/27)	
Bed-sharing ^e	13% (2/15)	26% (9/35)	.44
Minor illness within 48 h of death ^e	47% (7/15)	26% (9/34)	.16
0 Exogenous factors	13% (2/15)	3% (1/35)	.002 ^b
1 Exogenous factors	53% (8/15)	17% (6/35)	
2 Exogenous factors	27% (4/15)	49% (17/35)	
3 Exogenous factors	7% (1/15)	0% (0/15)	
4 Exogenous factors	0% (0/15)	6% (2/6)	

^a P value from χ^2 test, unless otherwise noted.

^b P value from *t* test. The P value for the analysis of the number of intrinsic risk factors and extrinsic risk factors is given in the first line of each analysis.

^c Known intrinsic risk factors for SIDS.

^d Premature defined as <37 gestational weeks at birth.

^e Known extrinsic risk factors for SIDS.

^f P value for sleep position (prone versus supine versus side) at discovery.

^g P value for sleep location (adult bed versus crib) at discovery.

death than Group B, but the differences were not statistically significant (Table 4). The infants in Group B were more likely to have been bed-sharing the night of death and to be discovered on an adult bed, but again the differences were not significant (Table 4). However, there was a significant difference in the number of exogenous risk factors between the 2 groups, with an average of 1.27 ± 0.80 in Group A and 2.14 ± 0.88 in Group B ($P = .002$).

Neurochemical Analysis With the Group A to F Classification Scheme

We found no significant differences in the serotonin-, GABA_A receptor-, and 14-3-3-related values between Group A ($n = 15$) and Group B ($n = 35$) in any medullary nucleus (Fig 2; Table 5), although both groups were statistically different from Group F ($n = 9$). Thus, the mean values in the different neurochemical parameters in Group B were not intermediate between those in Group A and Group F, as hypothesized. Nevertheless, there were significant differences between Group A and Group B (combined and individually) from Group F (Fig 2; Table 6).

Risk Factors and Serotonin_{1A} Receptor Binding in SIDS Cases From the 2006 and 2010 Data Sets Combined

There was a significant increase in binding in 3 of 10 medullary nuclei measured with an increase in the number of extrinsic risk factors (Fig 3; Table 7). Two of these 3 nuclei contain serotonin source neurons in the medullary serotonin network (ie, ROB and PGCL). A fourth nucleus with serotonin source neurons (ie, GC) demonstrated a marginally significant positive correlation ($P = .061$) (Table 7). The models for the effect of SIDS risk factors across all nuclei and across the nuclei that consist of serotonin source nuclei showed significantly lower serotonin_{1A}

FIGURE 2

A, Serotonin_{1A} binding (fmol/mg) in NTS is different between Groups A and B ($n = 14$, $n = 34$) and Group F ($n = 7$) ($P < .001$) but not between Groups A and B. B, 14-3-3 γ level (percentage standard) in GC is different between Groups A and B ($n = 10$, $n = 19$) and Group F ($n = 7$) ($P = .001$) but not between Groups A and B.

TABLE 5 Neurochemical Markers by Group A, B, and F Classification

Analytical Method	Nucleus in Medulla	Age-Adjusted Mean (SE)			P Value		
		A	B	F	Group	Significant Differences	Age by Group Interaction
Serotonin _{1A} receptor binding	HG	—	—	—	—	—	.025 ^a
	DMX	9.73 (1.30)	7.71 (0.78)	11.02 (1.81)	.156	None	NS
	NTS	8.59 (0.91)	7.87 (0.58)	13.68 (1.28)	<.001	A, B versus F ^b	NS
	MAO	9.16 (1.93)	12.49 (1.21)	19.58 (2.82)	.014	A, B versus F ^b	NS
	ROB	—	—	—	—	—	.009 ^a
	GC	—	—	—	—	—	.042 ^a
	PGCL	—	—	—	—	—	.005 ^a
	IRZ	—	—	—	—	—	.019 ^a
	DAO	11.46 (1.90)	13.61 (1.20)	10.08 (2.07)	.30	None	NS
	ARC	3.27 (0.89)	4.15 (0.57)	5.10 (0.98)	.39	None	NS
Serotonin and TPH2	Serotonin ROB	67.13 (7.02)	54.63 (4.31)	80.40 (8.75)	.026	B versus F ^c	NS
	Serotonin PGCL	35.68 (3.50)	28.96 (2.15)	39.15 (4.36)	.063	None	NS
	5-HIAA ROB	326.11 (42.75)	279.90 (26.26)	384.79 (53.30)	.19	None	NS
	5-HIAA PGCL	223.95 (22.17)	168.15 (13.62)	197.69 (27.65)	.10	None	NS
	TPH2	149.44 (13.32)	152.30 (7.90)	177.32 (16.02)	.34	None	NS
14-3-3 western blot	β	—	—	—	—	—	.003 ^d
	ϵ	—	—	—	—	—	.029 ^d
	η	51.01 (13.52)	56.05 (9.52)	100.31 (15.85)	.046	A, B versus F ^b	NS
	γ	52.66 (5.87)	41.71 (4.13)	74.14 (6.88)	.001	A, B versus F ^b	NS
	σ	53.90 (15.98)	64.42 (11.25)	104.17 (18.73)	.1229	None	NS
	θ	77.967 (13.91)	63.63 (9.80)	80.27 (16.31)	.5654	None	NS
	ζ	90.47 (32.39)	79.00 (22.81)	127.67 (37.98)	.5487	None	NS
GABA _A receptor binding	HG	40.31 (8.58)	47.90 (5.08)	79.59 (8.90)	.007	A, B versus F ^b	NS
	NTS	40.20 (6.53)	42.87 (3.99)	78.46 (6.75)	<.001	A, B versus F ^b	NS
	MAO	33.77 (5.88)	39.98 (3.48)	49.84 (6.10)	.18	None	NS
	ROB	27.97 (5.93)	26.17 (4.27)	45.77 (5.88)	.035	A, B versus F ^b	NS
	GC	—	—	—	—	—	.055 ^a
	PGCL	—	—	—	—	—	.039 ^a
	IRZ	—	—	—	—	—	.049 ^a
	DAO	33.42 (6.18)	35.87 (4.44)	44.94 (6.12)	.38	None	NS
	PIO	32.65 (5.29)	27.70 (3.42)	38.81 (5.20)	.21	None	NS
	ARC	26.64 (6.14)	24.07 (3.97)	26.22 (6.03)	.92	None	NS
	Western blot	58.16 (13.29)	54.58 (12.06)	114.77 (14.76)	.011	A, B versus F ^b	NS

The interaction between age and Groups A–B and F is included when significant. In the absence of a significant interaction, age-adjusted means by group are presented. 5-HIAA, 5-hydroxyindoleacetic acid; DAO, dorsal accessory olive; MAO, medial accessory olive.

^a Decrease with age in Groups A and B; no change with age in Group F; no difference in age-adjusted mean between Groups A and B.

^b Group A different from Group F, Group B different from Group F, Group A not different from Group B.

^c Group B different from Group F, Group A not different from Group B or Group F.

^d Increase with age in Group F; no change with age in Groups A–B; no difference in age-adjusted means between Groups A and B.

receptor binding levels ($P \leq .01$) in infants sleeping supine, in car seats, in cribs, and not bed-sharing (ie, less risky sleep environments). There was no association between age and number of extrinsic risks ($P = .35$).

DISCUSSION

Our major finding is that infants who die suddenly, unexpectedly, and without explanation (whether sleep environment risk factors for SIDS, with the potential for asphyxial stress, are present) demonstrate neurochemical abnormal-

ities in the medullary serotonin network compared with infants dying of known causes. These abnormalities affect multiple nuclei in the medullary homeostatic serotonin network involved in protective respiratory and autonomic responses.^{39,40} In animal models, both GABA and serotonin separately and through interactions with each other act on a shared set of neurons in the medullary homeostatic serotonin network to influence chemosensitivity, respiration, and autonomic function.³⁴ The 14-3-3 protein family is related to serotonin

regulation via its downstream effect on serotonin biosynthesis by increasing the catalytic activity of phosphorylated TPH2³⁵; 14-3-3 isoforms also inhibit regulators of G protein-coupled receptor signaling, thereby fine-tuning serotonin_{1A} receptor signaling.³⁵ A potential limitation of the study is the small sample size of Group F. Nevertheless, we have demonstrated a difference in serotonin receptor binding in medullary nuclei in 4 independent data sets in the last 2 decades, and the statistical differences have been robust and reproducible.^{30–33}

TABLE 6 Sample Size for Measurements in Groups A, B, and F in Table 5

Analytical Method		N		
		A	B	F
Serotonin _{1A} receptor binding	HG	14	33	7
	DMX	10	27	5
	NTS	14	34	7
	MAO	13	33	6
	ROB	14	32	9
	GC	14	32	9
	PGCL	14	32	9
	IRZ	14	31	9
	DAO	6	15	5
	ARC	11	27	9
Serotonin and TPH2	Serotonin ROB	11	28	7
	Serotonin PGCL	11	28	7
	5-HIAA ROB	11	28	7
	5-HIAA PGCL	11	28	7
	TPH2	10	28	7
14-3-3 western blot	β	10	19	7
	ε	10	19	7
	η	10	19	7
	γ	10	19	7
	σ	10	19	7
	θ	10	19	7
	ζ	10	19	7
	Western blot	8	9	6
GABA _A receptor binding	HG	6	16	5
	NTS	6	15	5
	MAO	6	16	5
	ROB	7	13	7
	GC	7	13	7
	PGCL	7	13	7
	IRZ	7	13	7
	DAO	7	13	7
	PIO	7	16	7
	ARC	7	16	7

5-HIAA, 5-hydroxyindoleacetic acid; DAO, dorsal accessory olive; MAO, medial accessory olive.

FIGURE 3

There is a positive association ($P = .004$) between serotonin_{1A} binding (in fmol/mg tissue) and the number of extrinsic risk factors in the dorsal accessory nucleus (component of olivary complex involved in motor control) in cases with sudden, unexpected, and unexplained death (Groups A and Group B combined [$n = 19$]). These data suggest that the higher the serotonin_{1A} binding, the less compromised the brainstem homeostatic system is, and the more exogenous triggers are needed to precipitate death.

underscoring the strength of the finding of serotonin brainstem pathology in SIDS cases.

Our data suggest that sudden death possibly associated with and putatively triggered by asphyxia (Group B) occurs in infants with an underlying (brainstem) vulnerability. Thus, asphyxia may be a key factor in the chain of terminal events leading to death in some infants with brainstem pathology. Yet we did not find that the mean values of the neurochemical parameters in the putatively asphyxia-challenged infants (Group B) as a group are intermediate between those of infants dying suddenly and without obvious asphyxial challenges (Group A) and those of infants dying of known causes (Group F). Group A represents, we believe, the category of sudden and unexplained infant deaths that today is often argued to be SIDS. Thus, this study does not support the idea that the challenged infants (Group B) have borderline values that require an asphyxial trigger to precipitate death, whereas unchallenged infants (Group A) have values that are at a lethal threshold that does not require such a trigger. We cannot rule out the possibility that differences exist between infants challenged or not challenged by asphyxia at the time of death; because there is no objective means to quantitate asphyxia in forensic scene investigations, its role in the death is probably not fully appreciated and may be underestimated by subjective observations. Furthermore, the lack of complete detail in the scene reports may result in critical missing data. Indeed, a potential limitation of the study is that the counting of the number of risk factors was compromised by missing data, and therefore the results may underestimate the correct number. Nevertheless, it is most likely that other risk factors (including potential genetic variants not tested in this study) precipitate sudden death in Group A in unknown ways. This

TABLE 7 Number of Risk Factors Versus Serotonin_{1A} Receptor Binding

Nucleus	N	Risk Factors			
		Intrinsic		Extrinsic	
		Slope (SE)	Regression P	Slope (SE)	Regression P
HG	46	−0.09 (0.55)	.87	−0.60 (0.52)	.26
DMX	32	0.64 (0.72)	.38	−0.47 (0.79)	.56
NTS	47	0.16 (0.61)	.79	−0.02 (0.58)	.97
MAO	45	1.21 (1.03)	.25	0.12 (0.94)	.90
ROB	49	1.97 (3.47)	.57	6.82 (2.79)	.018
GC	49	0.93 (1.25)	.46	1.97 (1.03)	.061
PGCL	49	0.76 (0.95)	.43	1.60 (0.78)	.045
IRZ	48	1.02 (0.73)	.17	0.78 (0.62)	.22
DAO	19	−0.23 (1.41)	.87	3.80 (1.13)	.004
ARC	38	−0.48 (0.50)	.35	0.29 (0.44)	.52

We used regression models to test association of the number of exogenous stressors with serotonin_{1A} binding in the medullary nuclei. There is a significant positive association between serotonin_{1A} binding (in fmol/mg tissue) and number of extrinsic risk factors in the ROB, PGCL, and dorsal accessory olive (DAO), such that the higher the serotonin_{1A} binding in these sites, the greater the number of risk factors are present. These data suggest that the higher the serotonin_{1A} binding, the less compromised the brainstem homeostatic system is, and the more exogenous triggers are needed to precipitate death. MAO, medial accessory olive.

possibility is supported by the dominance of the well-recognized intrinsic risk factor of prematurity in Group A compared with Group B.

Nevertheless, we found a positive correlation between the number of risk factors and serotonin_{1A} receptor binding; that is, there is increasing binding with an increasing number of exogenous (but not intrinsic) risk factors. This observation supports the hypothesis that the higher the serotonin_{1A} receptor binding (and closer to control values), the more of a trigger (ie, greater the number of exogenous risk factors) is needed to precipitate death; conversely, the lower the binding, the closer to the lethal threshold, and fewer risk factors are needed to trigger death. The affected nuclei define the serotonin network in medulla, because they contain serotonin neurons, suggesting that their function interfaces directly with exogenous risk factors. The association between higher number of extrinsic risk

factors and increased serotonin_{1A} bindings raises the question of why we did not see greater binding in Group B compared with Group A, even though Group B had higher numbers of extrinsic risk factors in certain nuclei. This lack of an association may reflect the possibility that although asphyxia may be a major risk factor, it may not necessarily act alone; rather, combinations of other factors may contribute to SIDS risk. Therefore, classifying the cases by a potential asphyxial relationship alone may not be the optimal way to analyze the data. This study emphasizes that risk factors should not be confused with causality. Moreover, there is probably a complex interplay between intrinsic and exogenous risk factors, as well as multiple risks per infant, that make it difficult to tease out a relationship between abnormal parameters in the brainstem and potential asphyxia per se. Quantitative asphyxial measures at scenes are needed.

CONCLUSIONS

In this study, we found no direct relationship between the presence of potentially asphyxial conditions in the sleep environment and the brainstem abnormalities analyzed in infants dying suddenly. The brainstem abnormalities were identified in deaths occurring with asphyxia-generating and non-asphyxia-generating conditions; infant deaths with increasing numbers of exogenous stressors were associated with intermediate brainstem abnormalities for some markers. Epidemiologic studies have shown that unsafe sleep environments increase the likelihood that an infant will die of SIDS and that SIDS rates worldwide are reduced with a change from prone to supine sleep position.^{5,8–10} Heeding safe sleep messages is essential for infants with an underlying vulnerability and for normal infants who could be exposed to severe or prolonged asphyxia in an unsafe sleep environment. Research is needed to define vulnerabilities in SIDS to develop means to identify and treat them in living infants at risk.

ACKNOWLEDGMENTS

The authors are grateful for the assistance of the medical examiners of the San Diego County Medical Examiner's Office, San Diego, California. We appreciate the critical review of the manuscript in preparation by Drs Richard G. Goldstein, Eugene E. Nattie, and Joseph J. Volpe. We thank Mr Daniel O'Connell for his assistance in manuscript preparation.

REFERENCES

1. Heron M. Deaths: leading causes for 2008. *Natl Vital Stat Rep*. 2012;60(6):1–94
2. Hauck FR, Tanabe KO, Moon RY. Racial and ethnic disparities in infant mortality. *Semin Perinatol*. 2011;35(4):209–220
3. Freemantle CJ, Read AW, de Klerk NH, Charles AK, McAullay D, Stanley FJ. In-

terpretation of recent sudden infant death syndrome rates in Western Australia. *J Paediatr Child Health*. 2005;41(12):669–670

4. Li L, Zhang Y, Zielke RH, Ping Y, Fowler DR. Observations on increased accidental asphyxia deaths in infancy while cosleeping in the state of Maryland. *Am J Forensic Med Pathol.* 2009;30(4):318–321
5. Kinney HC, Thach BT. The sudden infant death syndrome. *N Engl J Med.* 2009;361(8):795–805
6. Pasquale-Styles MA, Tackitt PL, Schmidt CJ. Infant death scene investigation and the assessment of potential risk factors for asphyxia: a review of 209 sudden unexpected infant deaths. *J Forensic Sci.* 2007;52(4):924–929
7. Krous HF, Beckwith JB, Byard RW, et al. Sudden infant death syndrome and unclassified sudden infant deaths: a definitional and diagnostic approach. *Pediatrics.* 2004;114(1):234–238
8. Willinger M, Hoffman HJ, Hartford RB. Infant sleep position and risk for sudden infant death syndrome: report of meeting held January 13 and 14, 1994, National Institutes of Health, Bethesda, MD. *Pediatrics.* 1994;93(5):814–819
9. Mitchell EA, Brunt JM, Everard C. Reduction in mortality from sudden infant death syndrome in New Zealand: 1986–92. *Arch Dis Child.* 1994;70(4):291–294
10. Beal SM. Sudden infant death syndrome in South Australia 1968–97. Part I: changes over time. *J Paediatr Child Health.* 2000;36(6):540–547
11. Malloy MH, MacDorman M. Changes in the classification of sudden unexpected infant deaths: United States, 1992–2001. *Pediatrics.* 2005;115(5):1247–1253
12. Shapiro-Mendoza CK, Tomashek KM, Anderson RN, Wingo J. Recent national trends in sudden, unexpected infant deaths: more evidence supporting a change in classification or reporting. *Am J Epidemiol.* 2006;163(8):762–769
13. Sridhar R, Thach BT, Kelly DH, Henslee JA. Characterization of successful and failed autoresuscitation in human infants, including those dying of SIDS. *Pediatr Pulmonol.* 2003;36(2):113–122
14. Meny RG, Carroll JL, Carbone MT, Kelly DH. Cardiorespiratory recordings from infants dying suddenly and unexpectedly at home. *Pediatrics.* 1994;93(1):44–49
15. Poets CF. Apparent life-threatening events and sudden infant death on a monitor. *Paediatr Respir Rev.* 2004;5(suppl A):S383–S386
16. Kinney HC, Myers MM, Belliveau RA, et al. Subtle autonomic and respiratory dysfunction in sudden infant death syndrome associated with serotonergic brainstem abnormalities: a case report. *J Neuropathol Exp Neurol.* 2005;64(8):689–694
17. Franco P, Szliwowski H, Dramaix M, Kahn A. Decreased autonomic responses to obstructive sleep events in future victims of sudden infant death syndrome. *Pediatr Res.* 1999;46(1):33–39
18. Schechtman VL, Lee MY, Wilson AJ, Harper RM. Dynamics of respiratory patterning in normal infants and infants who subsequently died of the sudden infant death syndrome. *Pediatr Res.* 1996;40(4):571–577
19. Kahn A, Groswasser J, Rebuffat E, et al. Sleep and cardiorespiratory characteristics of infant victims of sudden death: a prospective case-control study. *Sleep.* 1992;15(4):287–292
20. Thach B. Tragic and sudden death. Potential and proven mechanisms causing sudden infant death syndrome. *EMBO Rep.* 2008;9(2):114–118
21. Shannon DC, Kelly DH, O'Connell K. Abnormal regulation of ventilation in infants at risk for sudden-infant-death syndrome. *N Engl J Med.* 1977;297(14):747–750
22. Southall DP, Stevens V, Franks CI, Newcombe RG, Shinebourne EA, Wilson AJ. Sinus tachycardia in term infants preceding sudden infant death. *Eur J Pediatr.* 1988;147(1):74–78
23. Vege A, Chen Y, Opdal SH, Saugstad OD, Rognum TO. Vitreous humor hypoxanthine levels in SIDS and infectious death. *Acta Paediatr.* 1994;83(6):634–639
24. Jones KL, Krous HF, Nadeau J, Blackburn B, Zielke HR, Gozal D. Vascular endothelial growth factor in the cerebrospinal fluid of infants who died of sudden infant death syndrome: evidence for antecedent hypoxia. *Pediatrics.* 2003;111(2):358–363
25. Takashima S, Armstrong D, Becker LE, Huber J. Cerebral white matter lesions in sudden infant death syndrome. *Pediatrics.* 1978;62(2):155–159
26. Del Bigio MR, Becker LE. Microglial aggregation in the dentate gyrus: a marker of mild hypoxic-ischaemic brain insult in human infants. *Neuropathol Appl Neurobiol.* 1994;20(2):144–151
27. Opdal SH, Rognum TO. Gene variants predisposing to SIDS: current knowledge. *Forensic Sci Med Pathol.* 2011;7(1):26–36
28. Filiano JJ, Kinney HC. A perspective on neuropathologic findings in victims of the sudden infant death syndrome: the triple-risk model. *Biol Neonate.* 1994;65(3–4):194–197
29. Kinney HC, Richerson GB, Dymecki SM, Darnall RA, Nattie EE. The brainstem and serotonin in the sudden infant death syndrome. *Annu Rev Pathol.* 2009;4:517–550
30. Duncan JR, Paterson DS, Hoffman JM, et al. Brainstem serotonergic deficiency in sudden infant death syndrome. *JAMA.* 2010;303(5):430–437
31. Paterson DS, Trachtenberg FL, Thompson EG, et al. Multiple serotonergic brainstem abnormalities in sudden infant death syndrome. *JAMA.* 2006;296(17):2124–2132
32. Kinney HC, Randall LL, Sleeper LA, et al. Serotonergic brainstem abnormalities in Northern Plains Indians with the sudden infant death syndrome. *J Neuropathol Exp Neurol.* 2003;62(11):1178–1191
33. Panigrahy A, Filiano J, Sleeper LA, et al. Decreased serotonergic receptor binding in rhombic lip-derived regions of the medulla oblongata in the sudden infant death syndrome. *J Neuropathol Exp Neurol.* 2000;59(5):377–384
34. Broadbelt KG, Paterson DS, Belliveau RA, et al. Decreased GABA_A receptor binding in the medullary serotonergic system in the sudden infant death syndrome. *J Neuropathol Exp Neurol.* 2011;70(9):799–810
35. Broadbelt KG, Rivera KD, Paterson DS, et al. Brainstem deficiency of the 14-3-3 regulator of serotonin synthesis: a proteomics analysis in the sudden infant death syndrome. *Mol Cell Proteomics.* 2012;11(1):009530
36. Randall BB, Wade SA, Sens MA, et al. A practical classification schema incorporating consideration of possible asphyxia in cases of sudden unexpected infant death. *Forensic Sci Med Pathol.* 2009;5(4):254–260
37. Randall B, Donelan K, Koponen M, Sens MA, Krous HF. Application of a classification system focusing on potential asphyxia for cases of sudden unexpected infant death. *Forensic Sci Med Pathol.* 2012;8(1):34–39
38. Trachtenberg FS, Haas EA, Kinney HC, et al. Risk factor changes for sudden infant death syndrome after initiation of

- Back-to-Sleep campaign. *Pediatrics*. 2012; 129(4):630–638
39. Kinney HC, Broadbelt KG, Haynes RL, Rognum IJ, Paterson DS. The serotonergic anatomy of the developing human medulla oblongata: implications for pediatric disorders of homeostasis. *J Chem Neuroanat*. 2011;41(4):182–199
 40. Kinney HC, Belliveau RA, Trachtenberg FL, Rava LA, Paterson DS. The development of the medullary serotonergic system in early human life. *Auton Neurosci*. 2007;132(1–2):81–102

(Continued from first page)

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

Copyright © 2013 by the American Academy of Pediatrics

FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: Funded by First Candle, CJ Foundation for SIDS, Jacob Neil Boger Foundation for SIDS, Marley Jaye Cerella Foundation for SIDS, River's Gift, and Eunice Kennedy Shriver National Institute of Child Health and Development (grant R01-HD20991) (H.C.K.), NHMRC CJ Martin Fellowship (J.R.D.), and the Intellectual and Developmental Disabilities Research Center, Boston Children's Hospital (grant P30-HD18655). Funded by the National Institutes of Health (NIH).

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.