PRELIMINARY COMMUNICATION

Multiple Serotonergic Brainstem Abnormalities in Sudden Infant Death Syndrome

David S. Paterson, PhD
Felicia L. Trachtenberg, PhD
Eric G. Thompson, MS
Richard A. Belliveau, BA
Alan H. Beggs, PhD
Ryan Darnall, BA
Amy E. Chadwick, BA
Henry F. Krous, MD
Hannah C. Kinney, MD

Context The serotonergic (5-hydroxytryptamine [5-HT]) neurons in the medulla oblongata project extensively to autonomic and respiratory nuclei in the brainstem and spinal cord and help regulate homeostatic function. Previously, abnormalities in 5-HT receptor binding in the medullae of infants dying from sudden infant death syndrome (SIDS) were identified, suggesting that medullary 5-HT dysfunction may be responsible for a subset of SIDS cases.

Objective To investigate cellular defects associated with altered 5-HT receptor binding in the 5-HT pathways of the medulla in SIDS cases.

Design, Setting, and Participants Frozen medullae from infants dying from SIDS (cases) or from causes other than SIDS (controls) were obtained from the San Diego Medical Examiner's office between 1997 and 2005. Markers of 5-HT function were compared between SIDS cases and controls, adjusted for postconceptional age and postmortem interval. The number of samples available for each analysis ranged from 16 to 31 for SIDS cases and 6 to 10 for controls. An exploratory analysis of the correlation between markers and 6 recognized risk factors for SIDS was performed.

Main Outcome Measures 5-HT neuron count and density, 5-HT_{1A} receptor binding density, and 5-HT transporter (5-HTT) binding density in the medullary 5-HT system; correlation between these markers and 6 recognized risk factors for SIDS.

Results Compared with controls, SIDS cases had a significantly higher 5-HT neuron count (mean [SD], 148.04 [51.96] vs 72.56 [52.36] cells, respectively; $P<.001$) and 5-HT neuron density ($P<.001$), as well as a significantly lower density of 5-HT_{1A} receptor binding sites ($P\leq.01$ for all 9 nuclei) in regions of the medulla involved in homeostatic function. The ratio of 5-HTT binding density to 5-HT neuron count in the medulla was significantly lower in SIDS cases compared with controls (mean [SD], 0.70 [0.33] vs 1.93 [1.25] fmol/mg, respectively; $P=.001$). Male SIDS cases had significantly lower 5-HT_{1A} binding density in the raphé obscurus compared with female cases (mean [SD], 16.2 [2.0] vs 29.6 [16.5] fmol/mg, respectively; $P=.04$) or with male and female controls combined (mean [SD], 53.9 [19.8] fmol/mg; $P=.005$). No association was found between 5-HT neuron count or density, 5-HT_{1A} receptor binding density, or 5-HTT receptor binding density and other risk factors.

Conclusions Medullary 5-HT pathology in SIDS is more extensive than previously delineated, potentially including abnormal 5-HT neuron firing, synthesis, release, and clearance. This study also provides preliminary neurochemical evidence that may help explain the increased vulnerability of boys to SIDS.

JAMA. 2006;296:2124-2132 www.jama.com

SUDDEN INFANT DEATH SYNdrome (SIDS) is the leading cause of postneonatal infant mortality in the United States, with an overall incidence of 0.67/1000 live births.[1-3] Despite intensive research, the causes of SIDS remain unknown. Moreover, controversies abound about the role of certain practices, eg, bed sharing[4,5] or use of pacifiers,[5-7] in SIDS, in large part due to the lack of understanding of the basic biological mechanisms. We have proposed the triple risk model,[8] which suggests that sudden death results when 3 factors impinge on the infant simultaneously: (1) an underlying vulnerability; (2) an exogenous stressor (eg, prone sleep position, bed sharing); and (3) the critical developmental period, ie, the first 6 months of postnatal life, when the infant is at greatest risk for SIDS.[8]

The serotonergic (5-hydroxytryptamine [5-HT]) system of the medulla oblongata consists of 5-HT neurons located in the midline raphé, lateral extraraphé, and ventral surface and helps regulate autonomic and respiratory function.[1] These medullary nuclei are interconnected[9] and project extensively to nuclei in the brainstem and spinal cord that influence respiratory drive,[10] blood pressure regulation,[11] thermoregulation,[12] upper airway reflexes, and arousal.[13-15] Medullary 5-HT neurons have also been proposed to be central respiratory chemosensors.[16,17] Moreover, they are involved in the induction of long-term fa-

For editorial comment see p 2143.

Author Affiliations: Departments of Pathology (Drs Paterson and Kinney and Mssrs Thompson, Darnall, and Belliveau) and Pediatrics (Genetics) (Dr Beggs), Children's Hospital Boston and Harvard Medical School, Boston, Mass; New England Research Institutes, Watertown, Mass (Dr Trachtenberg); and Rady Children's Hospital and Health Center, San Diego, Calif, and University of California, San Diego School of Medicine (Ms Chadwick and Dr Krous).
Corresponding Author: David S. Paterson, PhD, 300 Longwood Ave, Enders Bldg Room 1109, Boston, MA 02115 (david.paterson@childrens.harvard.edu).

cilitation of respiration in response to episodic hypoxia[18] and play a critical role in the generation of respiratory rhythm in vivo.[19,20]

Previously, we identified altered 5-HT receptor binding density in components of the medullary 5-HT system in SIDS cases in 2 independent data sets using a nonselective radioligand that binds to 5-HT_{1A-1D} and 5-HT_2 receptors.[21,22] Additionally, we reported a case of an infant with SIDS who displayed altered autonomic and respiratory function at birth and 5-HT receptor binding abnormalities at autopsy 2 weeks later.[1,23] Recently, polymorphisms in the promoter region (5HTTLPR)[24,25] and in intron 2[26] of *SLC6A4*, the gene for the 5-HT transporter (5-HTT), were reported in higher frequencies in populations with SIDS compared with controls. Taken together, these observations support the idea that medullary 5-HT dysfunction results in a failure of autonomic and respiratory responses to hypoxia or hypercapnia and in sudden death for at least a subset of SIDS cases.

The extent, nature, and pathogenesis of this dysfunction, however, remain to be determined. The subtype(s) (potentially up to 7) of 5-HT receptor affected and their cellular localization are unknown. It is unknown if 5-HT neuron count and the expression of 5-HTT, important markers of 5-HT function, are also altered. Moreover, the level of available 5-HT and its relationship to alterations in 5-HT receptor binding observed in SIDS is unknown. Determination of the expression and distribution of these markers of 5-HT function are necessary to fully characterize the nature and pathogenesis of 5-HT dysfunction in SIDS cases.

In this study, we determined 5-HT neuron count and density, 5-HT_{1A} receptor binding density, and 5-HTT binding density in SIDS cases compared with controls in an effort to examine in greater detail the cellular components of the 5-HT pathway and to gain greater insight into the extent and pathogenesis of the 5-HT abnormalities. We analyzed the 5-HT_{1A} receptor because it is found in high densities in regions in which binding was most severely reduced in previous studies, is recognized as a somatodendritic autoreceptor that controls 5-HT neuron firing, and plays important roles in cardiorespiratory control[12,27-30] and neural development.[31,32] We analyzed 5-HTT because it regulates synaptic 5-HT concentration[33] and because of the identification of *SLC6A4* gene polymorphisms as risk factors for SIDS.[24-26] In addition, all SIDS cases and controls in this study were genotyped for the 5HTTLPR polymorphism to allow correlation of genotype with data on neuron count and binding density.[34] We also explored the potential effects of the recognized SIDS risk factors of sex, sleep position, bed sharing, history of illness within 1 week of death, and prematurity on 5-HT neuron count, 5-HT_{1A} receptor binding density, and 5-HTT binding density to determine their potential role in the pathogenesis of SIDS.

METHODS

Clinical Database

Frozen medullae from a total of 31 infants dying from SIDS (cases) and 10 infants with acute death from causes other than SIDS (controls) were obtained from the Office of the Chief Medical Examiner, San Diego, Calif, between 1997 and 2005, representing a new data set that has not previously been published. These infants represent all infant autopsies with a postmortem interval less than 27 hours for whom a study technician was available and the brainstem collected and, for controls, for whom the death was not under investigation. All SIDS cases were diagnosed by one of the authors (H.F.K.), an expert in SIDS pathology, in conjunction with the San Diego Medical Examiner's office. The cause of death of the controls was determined at autopsy by the San Diego Medical Examiner's office, and included acute deaths without hospitalization (drowning, n=2; asphyxia secondary to a plastic bag, n=1; pneumonia with acute respiratory distress, n=2; group B streptococcal sepsis, n=1; unsuspected congenital heart disease, n=1) and 3 hospitalized deaths (suspected inborn error of metabolism, n=1; congenital heart disease, n=2).

The number of recognized SIDS risk factors in each SIDS case were divided into 2 different categories: (1) "abnormality" risks, ie, factors that might increase the probability of an infant having an underlying vulnerability (ie, the medullary 5-HT abnormality) as a result of an underlying genetic predisposition or adverse prenatal exposure; and (2) "stressors," ie, environmental or physical factors that impinge on the vulnerable infant during the critical postnatal period, possibly challenging homeostatic function.[5] The number of risk factors in each category and the total number of risk factors was determined for each SIDS case by review of the autopsy report. The race/ethnicity (defined by the parents) of each infant in the study was recorded because the SIDS rate differs significantly between different racial/ethnic populations and may influence observations.

Appropriate medullary tissue from all infants was not available for all portions of the study. For the 5-HT neuron counting portion, 16 SIDS cases and 7 controls were analyzed; for 5-HT_{1A} receptor binding, 16 SIDS cases and 6 controls were analyzed; and for 5-HTT binding, 30 SIDS cases and 7 controls were analyzed. All 31 SIDS cases and 10 controls were genotyped for the 5HTTLPR polymorphism due to the availability of nonmedullary tissue from which DNA could be extracted. All analyses were performed in a blinded fashion, with the investigator unaware of the case or control status of each infant.

This study was approved by the Committee on Clinical Investigation at Children's Hospital Boston. All tissue was obtained under the auspices of the San Diego Medical Examiner system in accordance with California law.[35] Under this statute, it is not required to obtain informed consent of individual parents for research of sudden and unexpected infant death.

Determination of Number and Density of 5-HT Neurons

Immunocytochemical testing was performed for tryptophan hydroxylase on

20-µm frozen sections of medulla using the PH8 antibody (Chemicon International, Temecula, Calif) according to a previously described protocol.[36] Sections were postfixed in 4% paraformaldehyde before being incubated in PH8 antibody (1:8000) overnight at 4°C. Staining was then developed by addition of di-amino benzamide substrate (Dako, Glostrup, Denmark) to the section before coverslipping. Tryptophan hydroxylase neurons were counted at 2 standardized levels of the mid- and rostral medulla by 1 examiner using computer-based methods with Neurolucida version 6.02.2 (Microbrightfield Inc, Williston, Vt). Medullary levels were determined by reference to the brainstem atlas of Olszewski and Baxter.[37] The mid-medulla level corresponds to Plate XII, and the rostral medulla level corresponds to Plate XIV in the atlas.[37] The perimeter of each section was traced (×2) and the distribution of immunoreactive cells within the 3 medullary regions marked using different graphic symbols and colors (×10). Immunolabeled cell bodies were counted only if they were morphologically identifiable as neurons. Immunopositive cells were identified as belonging to 1 of 5 morphological cell types: granular, fusiform, pyramidal, multipolar, or undetermined. All sections were counted twice and the mean value used for analysis.

Assessment of 5-HT$_{1A}$ Receptor and 5-HTT Binding Densities

The autoradiography procedures for determination of ^{3}H-8-OH-DPAT (^{3}H8-hydroxy-2-[di-*N*-propylamino]-tetralin) binding to 5-HT$_{1A}$ receptors and ^{125}I-RTI-55 (3 beta-[4-iodophenyl-]tropan-2 beta-carboxylic acid ester labeled with sodium iodide I 125) binding to 5-HTT were performed according to previously described protocols[38] on 20-µm sections of frozen medulla adjacent to those stained for tryptophan hydroxylase immunoreactivity. Total 5-HT$_{1A}$ receptor binding was determined by incubation of tissue sections in 4-nM ^{3}H-8-OH-DPAT (PerkinElmer Inc, Wellesley, Mass) for 60 minutes at room temperature. Nonspecific binding was determined by addition of 10-µM serotonin to the solution. Total 5-HTT binding was determined by incubation of tissue sections in 0.15-nM ^{125}I-RTI-55 (PerkinElmer) for 90 minutes at room temperature. Nonspecific binding was determined by the addition of 100-nM citalopram hydrobromide (Tocris, St Louis, Mo). Sections were then placed in cassettes and exposed to ^{3}H-sensitive film (Kodak Biomax MR; Eastman Kodak Co, Rochester, NY) for 12 weeks or to a BAS-TR2025 phosphoimaging plate (Fuji-Film Corp, Tokyo, Japan) for 4 weeks, with a set of ^{3}H standards (Amersham, Buckinghamshire, England) for determination of ^{3}H-8-OH-DPAT binding, or to film for 5 hours with a set of ^{125}I standards (Amersham) for determination of ^{125}I-RTI-55 binding. Film autoradiograms were generated according to standard laboratory procedure for development of light-sensitive film. A BAS-5000 Bioimaging Analyzer (Fuji-Film) with Image Reader version 1.8 software (FujiFilm) was used to generate digital autoradiographic images from phosphoimaging plates. For each specimen, receptor or transporter binding density was analyzed in 9 medullary nuclei (all nuclei were not available in all cases) at a defined level of the brainstem (2 autoradiograms for each nucleus) according to previously published methods.[9,22,38] Quantitative densitometry of autoradiograms was performed using an MCID 5+ imaging system (Imaging Research Inc, St Catharines, Ontario).

5HTTLPR Polymorphism Genotyping

DNA was isolated from 50 to 100 mg of brain tissue using standard methods and a Puregene reagent kit (Gentra Systems, Minneapolis, Minn) according to the manufacturer's instructions. DNA samples were saved in Tris-EDTA hydration buffer at –20°C prior to genotyping. Polymerase chain reaction (PCR) amplification was conducted in a final 20-µL volume consisting of 80 to 100 ng of genomic DNA, using an AGS Gold PCR kit (Thermo-Hybaid, Franklin, Mass) following the protocol described by Narita et al.[24] PCR products were visualized by 1% agarose gel electrophoresis with ethidium bromide staining. DNA bands were identified under UV light, and genotype assignment for each case was determined by correlation with a standard 50–base pair DNA ladder (Invitrogen Corp, Carlsbad, Calif).

Correlation of Risk Factors for SIDS With the Expression of 5-HT Markers

We compared 5-HT neuron count, 5-HT$_{1A}$ receptor binding density, and 5-HTT binding density in the raphé obscurus between the SIDS infants who were (1) male (n=15) or female (n=16); (2) born prematurely (<37 gestational weeks at birth) (n=11) or at term (n=20); (3) found dead in the prone (n=15), side (n=5), or supine (n=5) position; (4) found dead lying face down (n=9) or face up (n=10); (5) bed sharing (n=7) or not bed sharing (n=24) on the night of death; and (6) sick with minor illness within 1 week of death (n=13) or not ill (n=18). This analysis was performed to determine if different subsets of SIDS cases are characterized by differences in 5-HT neuron count, 5-HT$_{1A}$ receptor binding, and/or 5-HTT binding sites. Sufficient clinical data were not available for similar analysis concerning maternal socioeconomic class or cigarette smoking, drinking, or use of illicit drugs during pregnancy. Similarly, small sample size prohibited analysis of race.

Statistical Analysis

t Tests were used to compare the number and density of neurons in the raphé, extraraphé, and ventral regions, as well as the combined cell count by level in SIDS cases and controls. *t* Tests were also used to compare the total section area and the proportion of each type of cell, and paired *t* tests were used to compare cell counts between levels. The density of 5-HT$_{1A}$ receptor and 5-HTT binding sites in medullary nuclei in SIDS cases and controls were compared using the Wilcoxon rank sum

test. Analysis of covariance was used in all 3 marker studies to control for the effects of postconceptional age and postmortem interval on neuron counts and binding density, as well as to consider the ratio of 5-HTT binding density to 5-HT neuron count in the raphé. A *t* test was used to compare postconceptional age between SIDS cases and controls. The effects of SIDS risk factors on neuron count and binding in SIDS cases were analyzed using *t* tests. Adjustment for multiple testing was not performed due to small sample size. Dose-response regressions of genotype (indicator variables for at least one "l" allele and the "ll" genotype) on neuron count or binding density were performed to test for potential effects of 5HTTLPR genotype on 5-HT neuron count, 5-HT_{1A} receptor binding, and 5-HTT binding. In all analyses, $P<.05$ was considered statistically significant.

RESULTS

Clinicopathologic Data

Available clinicopathologic data for the 31 SIDS cases and 10 controls are presented in TABLE 1. The age for the SIDS cases ranged from 4 to 36 postnatal weeks (mean [SD], 16.4 [10.6] weeks), and in controls from 1 day to 52 postnatal weeks (12.2 [17.0] weeks) ($P=.48$). The postmortem interval was less than 27 hours in all infants and was significantly longer in SIDS cases compared with controls (mean [SD], 19.2 [5.1] hours vs 13.3 [7.5] hours, respectively; $P=.007$). Postmortem interval did not significantly affect 5-HT_{1A} receptor or 5-HTT binding density, but 5-HT neuron count decreased significantly with postmortem interval. All of the data were corrected for the effects of postmortem interval. There was no statistically significant difference between the acute and hospitalized controls in 5-HT neuron count, 5-HT_{1A} receptor binding density, or 5-HTT binding density in the raphé obscurus ($P>.20$ for all), and thus the values from acute and hospitalized controls were combined for comparison with the SIDS cases. There was no histological evidence of brainstem pathology in any of the controls.

Number and Density of Medullary 5-HT Neurons in SIDS

In the rostral medulla, the number of 5-HT neurons in the midline raphé, lateral extraraphé, and at the ventral surface was significantly higher in SIDS cases compared with controls. In the mid-medulla, the 5-HT neuron count combined for all subregions and the 5-HT neuron count in the midline raphé were significantly higher in SIDS cases compared with controls (FIGURE 1 and TABLE 2). The mean (SD) cell density of 5-HT neurons in the raphé, extraraphé, and ventral surface regions combined were significantly higher in SIDS cases vs controls in the rostral medulla (0.81 [0.40] vs 0.54 [0.26] cells/mm^2, respectively; $P<.001$) and in the mid-medulla (0.55 [0.19] vs 0.41 [0.24] cells/mm^2, respectively; $P=.003$).

Granular neurons represented a significantly greater proportion of all 5-HT cells in SIDS cases compared with controls in the rostral medulla (mean [SD] ratio of granular neurons to combined 5-HT neurons, 0.26 [0.05] vs 0.19 [0.07], respectively; $P=.04$). Multipolar cells represented a significantly smaller proportion of all 5-HT neurons counted in SIDS cases compared with controls in the mid-medulla (mean [SD] ratio of multipolar neurons to combined 5-HT neurons, 0.02 [0.01] vs 0.04 [0.02]; $P=.03$).

5-HT_{1A} Receptor Binding

5-HT_{1A} receptor binding density was significantly reduced in SIDS cases compared with controls in all nuclei analyzed, with the exception of the principal inferior olivary nucleus (FIGURE 2 and Table 2).

5-HTT Binding

No significant differences in absolute 5-HTT binding between SIDS cases and controls were observed in any of the nuclei analyzed (Table 2). The ratio of 5-HTT binding density to 5-HT neuron count in the raphé obscurus, however, was significantly lower in SIDS cases compared with controls (Table 2), suggesting a relative reduction of 5-HTT expression per 5-HT neuron count.

Risk Factors

The 31 SIDS cases in the data set comprised 15 male (48%) and 16 female (52%) infants of different races and ethnicities. Eleven (35%) were born prematurely. Fifteen SIDS cases (48%) were found in the prone sleeping position, 9 (29%) were found face down, 7 (23%) were bed sharing at the time of death, and 13 (42%) had a history of illness in the week preceding death. All 31 SIDS cases were exposed to at least 1 abnormality risk factor or stressor. Thirty (97%) were subject to at least 1 abnormality risk factor, 28 (90%) were exposed to at least 1 stressor at the time of death, and 27 (87%) were subject to at least 1 factor from both categories. The mean (SD) 5-HT_{1A} receptor binding density in the raphé obscurus was significantly lower in male (n=6) compared with female (n=10) SIDS cases (16.2 [4.8] vs 29.6 [16.5] fmol/mg, respectively; $P=.04$) and significantly higher in controls (53.9 [19.8]fmol/mg; $P=.005$) compared with male SIDS cases ($P=.02$) and female SIDS cases ($P=.05$). No significant difference in 5-HT neuron count or 5-HTT binding density, however, was observed between male and female SIDS cases. Similarly, no associations were observed for other risk factors.

5HTTLPR Genotype Analysis

Genotyping of the 31 SIDS cases revealed 6 of SS genotype, 18 of SL genotype, and 7 of LL genotype. Of the 10 controls, none were of the SS genotype, 7 were of the SL genotype, and 3 were of the LL genotype. The frequency distribution of these different genotypes was not significantly different between the SIDS cases and controls ($P=.40$). Regression analysis revealed no statistically significant relationship or consistent trends between 5HTTLPR genotype and 5-HT neuron count, 5-HT_{1A} receptor bind-

Table 1. Epidemiologic and Clinicopathologic Data for SIDS Cases and Controls*

			Abnormality Risks†					Stressors‡					
Case	PMI, h	PN Age, wk	Sex	Race/ Ethnicity§	L-allele	Premature	Prenatal Exposure	Position Found	Face Position	Bed Sharing‖	Death Site	Illness Within 1 wk of Death	Total Risks
							SIDS Cases						
1	22	36	F	Mixed	Yes†	No	No	Side‡	Side/up	No	Crib	Cold and fever‡	3
2	22	8	F	Hispanic	Yes†	No	No	Supine	NA	No	NA	Diarrhea w/vomiting‡	2
3	22	4	M†	White	Yes†	No	No	NA	NA	Yes‡	Adult bed	No	3
4	21	34	F	NA	Yes†	Yes†	No	NA	NA	No	Adult bed	Cold‡	3
5	7	14	M†	White	No	No	No	Supine	Side/up	No	Car seat	Cold‡	2
6	18	27	M†	White	Yes†	No	No	Prone‡	Side/up	No	NA	Respiratory infection‡	4
7	23	4	F	NA	Yes†	No	No	NA	NA	No	Crib	No	1
8	9	7	F	Hispanic	Yes†	No	Heroin†	NA	Side/up	No	Crib	Diarrhea and vomiting Respiratory infection‡	3
9	22	11	M†	White	No	No	No	Supine	Side/up	No	Car seat	No	1
10	18	19	F	African American†	Yes†	No	No	Prone‡	Down‡	No	Adult bed	Cold and vomiting‡	5
11	20	10	M†	White	Yes†	Yes†	No	Side‡	NA	Yes‡	NA	No	5
12	18	33	F	Asian	Yes†	No	No	Supine	Side/up	Yes‡	NA	Respiratory infection‡	3
13	22	8	F	White	No	No	No	Prone‡	NA	No	Crib	No	1
14	21	4	F	White	Yes†	Yes†	Unspecified illicit drug use†	Prone‡	Down‡	No	Crib	No	5
15	14	26	M†	Hispanic	Yes†	No	No	Prone‡	NA	No	Adult bed	No	3
16	NA	18	M†	White	Yes†	Yes†	No	Side‡	Down‡	Yes‡	NA	No	6
17	19	15	F	White	No	Yes†	No	Prone‡	Down‡	No	Crib	Cold‡	4
18	7	10	F	White	Yes†	No	No	Prone‡	Down‡	No	Crib	Respiratory infection‡	4
19	20	9	M†	NA	Yes†	No	No	Prone‡	NA	No	Crib	Cold‡	4
20	26	13	M†	Hispanic	Yes†	No	No	Prone‡	Down‡	Yes‡	NA	Cold‡	6
21	9	4	M†	White	Yes†	No	No	NA	NA	Yes‡	NA	No	3
22	15	10	F	White	Yes†	Yes†	No	Supine	Side/up	No	Car seat	No	2
23	23	6	M†	African American†	Yes†	Yes†	No	Side‡	Side/up	No	Sofa	No	5
24	23	9	M†	White	Yes†	Yes†	No	Side‡	Side/up	No	Crib	Fever‡	5
25	22	20	M†	Hispanic	Yes†	No	No	Prone‡	Down‡	No	Adult bed	No	4
26	19	36	M†	African American†	No	No	No	Prone‡	NA	No	Crib	No	3
27	22	21	M†	White	Yes†	Yes†	No	Prone‡	NA	No	Crib	No	4
28	25	23	F	Hispanic	No	Yes†	No	NA	NA	Yes‡	NA	No	2
29	20	10	F	White	Yes†	Yes†	No	Prone‡	Down‡	No	Crib	No	4
30	21	16	F	White	Yes†	No	No	Prone‡	Down‡	No	Crib	No	3
31	22	35	F	White	Yes†	No	No	Prone‡	Side/up	No	Crib	No	2
							Controls						
32	22	36	F	White	Yes†	No	No	NA	NA	No	Water bucket	NR	NR
33	13	52	M†	White	Yes†	No	No	NA	NA	No	Hospital	NR	NR
34	24	6	M†	Hispanic	Yes†	No	No	NA	NA	No	Hospital	NR	NR
35	21	34	M†	White	Yes†	No	No	NA	NA	NA	Bathtub	NR	NR
36	10	9	M†	African American†	Yes†	No	No	NA	NA	No	Adult bed	NR	NR
37	11	7	F	Hispanic	Yes†	No	No	NA	NA	No	Hospital	NR	NR
38	10	2	M†	White	Yes†	No	No	NA	NA	No	NA	NR	NR
39	1	1 d	M†	Pacific Islander	Yes†	No	No	Supine	Side/up	NA	Hospital	NR	NR
40	17	10	M†	White	Yes†	No	No	NA	NA	NA	Hospital	NR	NR
41	5	1	M†	African American†	Yes†	Yes†	No	Supine	Side/up	Yes	Couch	NR	NR

Abbreviations: NA, not available (no information available on parameter); NR, not relevant (parameter does not apply); PMI, postmortem interval; PN, postnatal; SIDS, sudden infant death syndrome.

*Available clinicopathologic and epidemiologic data for all 31 SIDS cases and 10 controls (infants with acute death from causes other than SIDS) used in the described studies. The total number of risk factors in the history of each SIDS case was determined by review of autopsy report.

†Factors that might increase the probability of an infant having a medullary 5-HT abnormality (male sex, African American race, presence of the SL or LL 5HTTLPR genotype [L-allele], prematurity, or prenatal toxin exposure [eg, cigarette smoking, use of alcohol and/or narcotics]).[5]

‡Environmental or physical factors that impinge on the vulnerable infant during the critical postnatal period, possibly challenging homeostatic function (prone or side sleeping position, face-down sleeping, bed sharing, death site, and history of minor illness within 1 week before death).[5]

§Race/ethnicity of each infant was defined by the parents, and was assessed in the study because the SIDS rate differs significantly between different ethnic populations.

‖Bed sharing is defined as an environment where the infant shares a sleep surface with 1 or more parent and/or other adult or children.[5]

ing density, or 5-HTT binding density in the raphé obscurus in SIDS cases, or in the combined data set of both SIDS cases and controls.

COMMENT

We found that the medullary 5-HT abnormalities in SIDS are more extensive than previously suggested and that they involve multiple elements of 5-HT function, including 5-HT neuron count, 5-HT_{1A} receptor expression, and relative 5-HTT binding in the same cases. This study strengthens the hypothesis that medullary 5-HT dysfunction is associated with SIDS and may lead to death by a failure of respiratory and autonomic responses to homeostatic stressors during sleep. This study also found, in an explanatory analysis, reduced 5-HT_{1A} receptor binding density in male compared with female SIDS cases, an observation that may help explain why males are more vulnerable to SIDS.[5] These 5-HT abnormalities were documented during the era of stringent public messages on risk reduction, including that for supine sleeping position. The majority (65%) of the SIDS cases in this data set, however, were sleeping prone or on their side at the time of death, indicating the need for continued public health messages on safe sleeping practices.

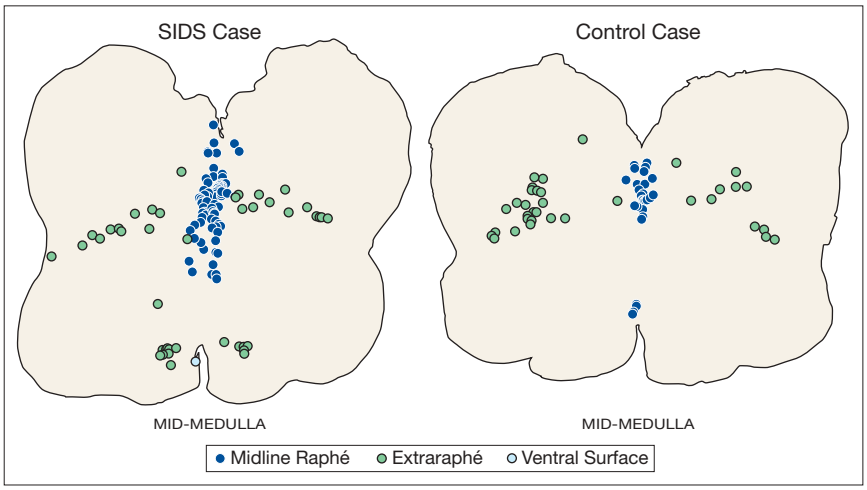
The increased number of 5-HT neurons in medullary sites in SIDS cases, coupled with the reduction in 5-HT_{1A} receptor binding and relative reduction in 5-HTT binding in these sites, suggest that the synthesis and availability of 5-HT (and by extrapolation, neuron firing) is altered within 5-HT pathways. It is unclear, however, specifically how these functions are altered. It is possible that an increased number of 5-HT neurons may lead to an excess of extracellular 5-HT and a compensatory downregulation of 5-HT_{1A} receptors. Alter-

Table 2. Medullary 5-HT Neuron Count, 5-HT_{1A} Receptor Binding Density, and 5-HTT Binding Density in SIDS Cases and Controls

	Mean (SD)		*P* Value*
	SIDS Cases	Controls	
5-HT neuron count†	n = 16	n = 7	
Rostral medulla			
Combined	148.04 (51.96)	72.56 (52.36)	<.001
Raphé	78.84 (34.87)	34.09 (35.13)	<.001
Extraraphé	63.21 (19.93)	37.80 (20.08)	.002
Ventral surface	5.99 (7.44)	0.67 (7.50)	.001
Mid-medulla			
Combined	94.94 (34.71)	54.36 (34.97)	<.001
Raphé	49.04 (20.21)	19.90 (20.36)	<.001
Extraraphé	44.19 (17.24)	32.77 (17.37)	.23
Ventral surface	1.76 (2.93)	1.68 (2.95)	.15
5-HT_{1A} receptor binding density, fmol/mg‡	n = 16	n = 6	
Medullary 5-HT source nuclei			
Raphé obscurus	24.28 (16.44)	54.68 (16.57)	.001
Gigantocellularis nucleus	11.35 (5.26)	19.28 (5.30)	.006
Paragigantocellularis lateralis nucleus	8.69 (4.09)	15.80 (4.12)	.002
Intermediate reticular nucleus	7.78 (3.03)	13.38 (3.06)	.001
Arcuate nucleus	4.13 (2.54)	9.33 (2.55)	.002
Medullary 5-HT projection nuclei			
Medial accessory olive	9.64 (7.07)	28.85 (7.14)	<.001
Nucleus of the solitary tract	7.09 (3.72)	14.67 (3.76)	<.001
Hypoglossal nucleus	5.62 (3.00)	9.01 (3.03)	.03
Principal inferior olive	4.86 (1.69)	4.63 (1.79)	.86
5-HTT binding density, fmol/mg§	n = 30	n = 7	
Raphé obscurus	44.10 (8.66)	46.74 (8.66)	.47
Gigantocellularis nucleus	25.43 (7.27)	30.18 (7.27)	.13
Paragigantocellularis lateralis nucleus	21.75 (5.50)	23.20 (5.50)	.54
Intermediate reticular nucleus	18.37 (5.03)	20.09 (5.03)	.43
Arcuate nucleus	4.56 (2.06)	4.62 (2.06)	.94
Ratio of 5-HTT binding density (fmol/mg) to 5-HT neuron count (16 cases, 4 controls)‖	0.70 (0.33)	1.93 (1.25)	.001

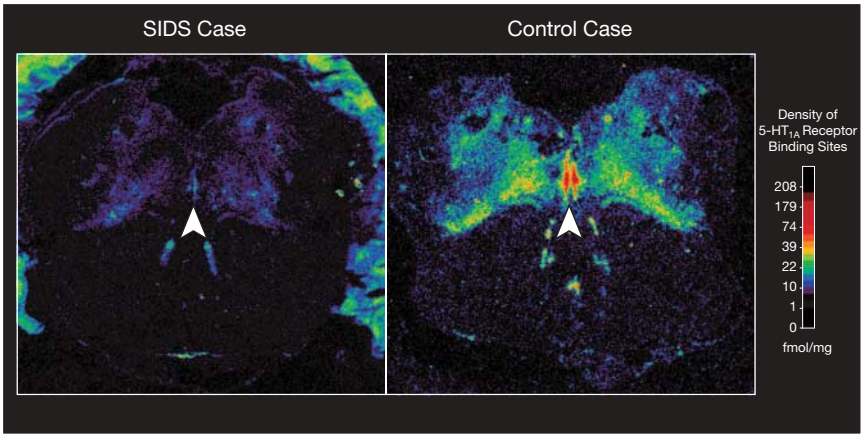
Abbreviation: SIDS, sudden infant death syndrome.
*By analysis of covariance.
†Number of 5-HT neurons is significantly greater in SIDS medullae in all regions analyzed, except for the extraraphé and the ventral surface at the mid-medulla level.
‡Density of ^{3}H-8-OH-DPAT binding to 5-HT_{1A} receptors in medullary 5-HT source nuclei (nuclei containing 5-HT cells) and in medullary 5-HT projection nuclei (nuclei that do not express 5-HT neurons but receive substantial inputs from them). 5-HT_{1A} receptor binding density is significantly lower in SIDS cases in all regions analyzed, with the exception of the principal inferior olive.
§Density of ^{125}I-RTI-55 binding to 5-HTT sites in medullary nuclei containing 5-HT cells.
‖Ratio in the raphé obscurus. Significantly lower ratio in SIDS cases compared with controls suggests a lower level of 5-HTT expression by each 5-HT neuron.

Figure 1. Medullary 5-HT Neuron Count in a SIDS Case and a Control



Distribution of mid-medullary serotonergic (5-hydroxytryptamine [5-HT]) neurons in an infant dying from sudden infant death syndrome (SIDS case) and an infant with acute death from a cause other than SIDS (control), plotted using Neurolucida version 6.02.2 (Microbrightfield Inc, Williston, Vt).There are qualitatively more 5-HT neurons in the plot from the SIDS case, particularly in the midline raphé nucleus, than in the control.

Figure 2. 5-HT_{1A} Receptor Binding Density in a SIDS Case and a Control



Illustrative autoradiogram displaying mean ^{3}H-8-OH-DPAT (^{3}H8-hydroxy-2-[di-*N*-propylamino]-tetralin) binding to 5-hydroxytryptamine 1A (5-HT_{1A}) receptors in a tissue section at the mid-medulla level from an infant dying from sudden infant death syndrome (SIDS case) and an infant with acute death from a cause other than SIDS (control). The density of 5-HT_{1A} receptor binding sites, including in the raphé obscurus (arrowheads), is visually lower in the SIDS case compared with the control. The "halo" observable around the outside of the image from the SIDS case represents binding to 5-HT_{1A} receptors in tissue from the cerebellum that was artifactually collected during sectioning of the medulla.

natively, 5-HT synthesis, release, or both may be dysfunctional in the 5-HT neurons (which are overabundant in compensation), resulting in a deficiency of extracellular 5-HT. The level of available 5-HT in the medullae of SIDS cases has yet to be determined, and the molecular and cellular regulatory mechanisms between neuron count and receptor and transporter expression are incompletely understood. It is difficult, therefore, to predict with any certainty which, if either, of these proposed conditions exist in SIDS. Determination of medullary 5-HT level in SIDS and the elucidation of the pathways and mechanisms regulating the expression of the 5-HT markers analyzed in this study will therefore be necessary before the nature and pathogenesis of the medullary 5-HT dysfunction in SIDS can be determined. The increased number of 5-HT neurons, the greater proportion of neurons of "simple" (ie, granular) morphology, and the decreased number of neurons of "complex" (ie, multipolar) morphology in SIDS cases compared with controls, however, supports the concept of an underlying developmental disorder involving abnormal regulation of 5-HT neuron count and delayed neuronal differentiation and maturation. This idea is supported by the relative reduction in 5-HTT expression observed in the medullae of SIDS cases. The 5-HTT is expressed predominantly in perisynaptic sites on 5-HT neuron terminals. Reduced 5-HTT expression may be due to reduced expression of 5-HTT protein at 5-HT neuron terminals, a reduced number of 5-HT terminals and synapses, or both, consistent with abnormal or delayed 5-HT synapse formation and neuron development. Of note, 5-HT neuron migration appears to be relatively unaffected in SIDS cases, as we observed 5-HT neurons in the same anatomical positions in the component nuclei of the medullary 5-HT system as in the controls.

In this study, we described known risk factors for SIDS either as "abnormality" risk factors or as "stressors" in an effort to distinguish between the 2 types of risks we propose are involved in the pathogenesis of SIDS. Eighty-seven percent of the SIDS cases in this study were observed to be both at risk from having medullary 5-HT abnormalities and exposed to an exogenous stressor at the time of death. These data support the triple risk model.[8] The prone or side sleeping position, face-down sleeping, and bed sharing are recognized as important risk factors or stressors for SIDS.[5,39] We identified that 77% of the SIDS cases in this study slept prone or on their side, shared a bed, or both, indicating that these sleep practices remain major risk factors for SIDS. We found that abnormalities of 5-HT markers, specifically in 5-HT_{1A} recep-

tor binding, occurred in the medullae of SIDS cases regardless of sleeping position or whether they were bed sharing at the time of death. These observations suggest that the SIDS cases within this study shared a common underlying vulnerability, ie, an intrinsic medullary 5-HT abnormality. The increased risk of SIDS in the prone or face-down position may reflect the infants' inability to respond to the asphyxial or hypercarbic challenge in the face-down position, due to the abnormalities in the medullary 5-HT system that compromise protective reflexes, including arousal and head turning.

The identification of a significantly lower density of 5-HT_{1A} receptor binding in male compared with female SIDS cases in this study provides neurochemical evidence that may help explain the increased risk of SIDS in males (with a 2:1 ratio) compared with females.[5] A recent study involving chemical ablation of approximately 60% of medullary 5-HT neurons in neonatal piglets reported that males exhibited a blunted response to inspired carbon dioxide during sleep, whereas females responded normally.[40] Of note, this carbon dioxide response is modulated in part by 5-HT_{1A} receptors in the medullary raphé.[17] This experimental finding raises the possibility that in humans male infants may similarly be less sensitive to carbon dioxide than female infants, and that loss of medullary 5-HT_{1A} receptors, as observed in the SIDS cases in this study, may attenuate respiratory responses to hypercapnia to a greater extent in male compared with female infants, thus placing them at greater risk for SIDS.

Interestingly, experimental evidence indicates that 5-HT_{1A} receptor expression in the forebrain is normally significantly lower in the human male brain compared with the human female brain.[41-43] Deficits in postnatal brain levels of 5-HT_{1A} receptor expression following in utero cocaine exposure persist for a greater length of time in male compared with female rats,[44] suggesting that the neonatal male infant brain is less resilient to exposure to at least some pharmacologically active toxins affecting 5-HT function in the maternal circulation than the neonatal female brain. Taking these observations together, intrinsic differences between male and female infants in baseline brain 5-HT_{1A} receptor expression, 5-HT neuronal plasticity, and carbon dioxide sensitivity during sleep (which may be modulated by medullary 5-HT_{1A} receptors in humans) provide evidence that may explain, at least in part, the greater risk of SIDS in male infants. Given that this observation was based on a sample size of 6 males and 10 females with a *P* value of .04 in an exploratory multiple testing environment, it is imperative to repeat it in an independent data set.

We found no significant difference in 5HTTLPR genotype frequency between SIDS cases and controls and no relationship between genotype frequency and 5-HT neuron count, 5-HT_{1A} receptor binding density, or 5-HTT binding density in SIDS cases or controls. We caution, however, that the sample size of SIDS cases and controls was small and may be insufficient to establish a link between SIDS, genotype, and 5-HT brainstem abnormalities. Interpretation of these data is also complicated by the multiple race/ethnicity categories in the data set, because the frequency of the 5HTTLPR genotype varies between racial and ethnic populations. It is also noteworthy that none of the controls were of the "SS" genotype. This is most likely a consequence of the small number of controls, but it is also possible that it may reflect a genetic bias in the control population in this study.

Study Limitations

A potential limitation of this study is the relatively small number of SIDS cases and controls in the data set due to the difficulty in accruing specimens over a reasonable time frame. Controls are particularly difficult to obtain due to rarity of acute deaths in the age group from causes other than SIDS. Due to the tissue limitations, we were not able to examine all three 5-HT markers in all cases. Despite the relatively small sample size, however, we found highly significant differences, both qualitative and quantitative, between the SIDS cases and controls in more than one 5-HT marker. Moreover, the finding of reduced 5-HT_{1A} receptor binding replicates the finding of 5-HT receptor defects in SIDS cases in the previous data sets.[21-23] The small sample size is especially problematic for the subset (risk-factor) analysis, in which multiple statistical comparisons were performed. Thus, while we observed a significant difference in 5-HT_{1A} receptor binding between male and female SIDS cases, this observation should be regarded as preliminary and will need to be confirmed in a larger data set. Other limiting factors that should be taken into consideration include the (statistically nonsignificant) difference in age between SIDS cases and controls; race/ethnicity; and exposure of the fetus to potentially harmful substances (eg, nicotine, alcohol) during gestation. However, we found that age was not a significant influence in any of our analyses, while the small sample size and the unavailability of data on maternal smoking and alcohol use for the majority of cases prevented us from assessing the effect of race/ethnicity and prenatal exposures, respectively, on our data. Future analysis of a larger data set, with complete information on all potential risk factors, is therefore needed.

CONCLUSIONS

Multiple, interrelated markers of 5-HT function are abnormally expressed in the medulla in the same SIDS cases, suggesting that basic elements of 5-HT neurotransmission, including neuron firing, synthesis, release, and clearance of 5-HT, are dysfunctional in SIDS. The finding that 5-HT neuron count is increased in the SIDS cases associated with increased morphologic immaturity suggests the possibility that the 5-HT abnormalities are developmental in origin. This study provides biological plausibility for certain risk-reduction strategies in SIDS, as well as

for the triple risk model for SIDS. Moreover, it generates new hypotheses for testing about 5-HT–related brainstem pathology underlying sudden death in early life in future SIDS autopsies and in experimental mechanistic models.

Author Contributions: Dr Paterson had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Paterson, Belliveau, Beggs, Kinney.
Acquisition of data: Paterson, Thompson, Belliveau, Darnall, Chadwick, Krous, Kinney.
Analysis and interpretation of data: Paterson, Trachtenberg, Thompson, Belliveau, Darnall, Kinney.
Drafting of the manuscript: Paterson, Kinney.
Critical revision of the manuscript for important intellectual content: Paterson, Trachtenberg, Thompson, Belliveau, Beggs, Darnall, Chadwick, Krous, Kinney.
Statistical analysis: Trachtenberg.
Obtained funding: Paterson, Kinney.
Administrative, technical, or material support: Thompson, Belliveau, Darnall, Chadwick, Kinney.
Study supervision: Paterson, Krous, Kinney.
Financial Disclosures: None reported.
Funding/Support: This work was supported by grants from the Scottish Cot Death Trust, Evelyn Deborah Barrett Fellowship, CJ Foundation for SIDS, First Candle/SIDS Alliance, CJ Murphy Foundation, National Institute of Child Health and Human Development (R37-HD20991and PO1-HD36379), and Children's Hospital Mental Retardation Core Grant (P30-HD18655).
Role of the Sponsors: None of funding organizations had any role in the design and conduct of the study; the collection, management, analysis, and interpretation of the data; or the preparation, review, and approval of the manuscript.
Acknowledgment: We thank the San Diego Medical Examiner system for help in the accrual of tissue specimens.

REFERENCES

1. Kinney HC, Paterson DS. Sudden infant death syndrome. In: Golden J, Harding B, eds. *Pathology and Genetics: Developmental Neuropathology*. Basel, Switzerland: Neuropath Press; 2004.
2. Krous HF, Beckwith JB, Byard RW, et al. Sudden infant death syndrome and unclassified sudden infant deaths: a definitional and diagnostic approach. *Pediatrics*. 2004;114:234-238.
3. Willinger M, James LS, Catz C. Defining the sudden infant death syndrome (SIDS): deliberations of an expert panel convened by the National Institute of Child Health and Human Development. *Pediatr Pathol*. 1991; 11:677-684.
4. Tappin D, Brooke H, Ecob R. Bedsharing and sudden infant death syndrome (SIDS) in Scotland, UK. *Lancet*. 2004;363:994.
5. American Academy of Pediatrics Task Force on Sudden Infant Death Syndrome. The changing concept of sudden infant death syndrome: diagnostic coding shifts, controversies regarding the sleeping environment, and new variables to consider in reducing risk. *Pediatrics*. 2005;116:1245-1255.
6. Blair PS, Fleming PJ. Dummies and SIDS: causality has not been established. *BMJ*. 2006;332:178.
7. Pacifiers for SIDS prevention: the latest study. *Child Health Alert*. 2006;24:3.
8. Filiano JJ, Kinney HC. A perspective on neuropathologic findings in victims of the sudden infant death syndrome: the triple-risk model. *Biol Neonate*. 1994; 65:194-197.
9. Kinney HC, Filiano JJ, White WF. Medullary serotonergic network deficiency in the sudden infant death syndrome: review of a 15-year study of a single dataset. *J Neuropathol Exp Neurol*. 2001;60:228-247.
10. Bou-Flores C, Lajard AM, Monteau R, et al. Abnormal phrenic motoneuron activity and morphology in neonatal monoamine oxidase A-deficient transgenic mice: possible role of a serotonin excess. *J Neurosci*. 2000;20:4646-4656.
11. Henderson LA. Caudal midline medulla mediates behaviourally-coupled but not baroreceptor-mediated vasodepression. *Neuroscience*. 2000;98:779-792.
12. Berner NJ, Grahn DA, Heller HC. 8-OH-DPAT-sensitive neurons in the nucleus raphe magnus modulate thermoregulatory output in rats. *Brain Res*. 1999; 831:155-164.
13. Krammer EB, Rath T, Lischka MF. Somatotopic organization of the hypoglossal nucleus: a HRP study in the rat. *Brain Res*. 1979;170:533-537.
14. Bartlett D Jr, Leiter JC, Knuth SL. Control and actions of the genioglossus muscle. *Prog Clin Biol Res*. 1990;345:99-107.
15. Darnall RA, Harris MB, Gill WH, Hoffman JM, Brown JW, Niblock MM. Inhibition of serotonergic neurons in the nucleus paragigantocellularis lateralis fragments sleep and decreases rapid eye movement sleep in the piglet: implications for sudden infant death syndrome. *J Neurosci*. 2005;25:8322-8332.
16. Richerson GB. Serotonergic neurons as carbon dioxide sensors that maintain pH homeostasis. *Nat Rev Neurosci*. 2004;5:449-461.
17. Messier ML, Li A, Nattie EE. Inhibition of medullary raphe serotonergic neurons has age-dependent effects on the CO2 response in newborn piglets. *J Appl Physiol*. 2004;96:1909-1919.
18. Baker-Herman TL, Fuller DD, Bavis RW, et al. BDNF is necessary and sufficient for spinal respiratory plasticity following intermittent hypoxia. *Nat Neurosci*. 2004;7:48-55.
19. Pena F, Ramirez JM. Endogenous activation of serotonin-2A receptors is required for respiratory rhythm generation in vitro. *J Neurosci*. 2002;22:11055-11064.
20. Tryba AK, Pena F, Ramirez JM. Gasping activity in vitro: a rhythm dependent on 5-HT2A receptors. *J Neurosci*. 2006;26:2623-2634.
21. 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;62:1178-1191.
22. 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;59:377-384.
23. 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;64:689-694.
24. Narita N, Narita M, Takashima S, Nakayama M, Nagai T, Okado N. Serotonin transporter gene variation is a risk factor for sudden infant death syndrome in the Japanese population. *Pediatrics*. 2001;107:690-692.
25. Weese-Mayer DE, Berry-Kravis EM, Maher BS, Silvestri JM, Curran ME, Marazita ML. Sudden infant death syndrome: association with a promoter polymorphism of the serotonin transporter gene. *Am J Med Genet*. 2003;117:268-274.
26. Weese-Mayer DE, Zhou L, Berry-Kravis EM, Maher BS, Silvestri JM, Marazita ML. Association of the serotonin transporter gene with sudden infant death syndrome: a haplotype analysis. *Am J Med Genet*. 2003;122:238-245.
27. Lalley PM, Bischoff AM, Richter DW. 5-HT-1A receptor-mediated modulation of medullary expiratory neurones in the cat. *J Physiol*. 1994;476:117-130.
28. Lalley PM. The excitability and rhythm of medullary respiratory neurons in the cat are altered by the serotonin receptor agonist 5-methoxy-N,N, dimethyltryptamine. *Brain Res*. 1994;648:87-98.
29. Gillis RA, Hill KJ, Kirby JS, et al. Effect of activation of central nervous system serotonin 1A receptors on cardiorespiratory function. *J Pharmacol Exp Ther*. 1989;248:851-857.
30. King KA, McCall RB. The effects of 8-OH-DPAT on medullary 5-HT neurons and sympathetic activity in baroreceptor-denervated animals. *Eur J Pharmacol*. 1991;200:357-360.
31. Eaton MJ, Staley JK, Globus MY, Whittemore SR. Developmental regulation of early serotonergic neuronal differentiation: the role of brain-derived neurotrophic factor and membrane depolarization. *Dev Biol*. 1995;170:169-182.
32. Emerit MB, Riad M, Hamon M. Trophic effects of neurotransmitters during brain maturation. *Biol Neonate*. 1992;62:193-201.
33. Blakely RD, Defelice LJ, Galli A. Biogenic amine neurotransmitter transporters: just when you thought you knew them. *Physiology (Bethesda)*. 2005;20:225-231.
34. Heils A, Mossner R, Lesch KP. The human serotonin transporter gene polymorphism—basic research and clinical implications. *J Neural Transm*. 1997; 104:1005-1014.
35. California Law Chapter 955, Statutes of 1989 (SB 1069).
36. Paterson DS, Thompson EG, Kinney HC. Serotonergic and glutamatergic neurons at the ventral medullary surface of the human infant: observations relevant to central chemosensitivity in early human life. *Auton Neurosci*. 2006;124:112-124.
37. Olszewski J, Baxter D. *Cytoarchitechture of the Human Brain Stem*. 2nd ed. Basel, Switzerland: S Karger; 1954.
38. Paterson DS, Belliveau RA, Trachtenberg F, Kinney HC. Differential development of 5-HT receptor and the serotonin transporter binding in the human infant medulla. *J Comp Neurol*. 2004;472:221-231.
39. Raydo LJ, Reu-Donlon CM. Putting babies "back to sleep": can we do better? *Neonatal Netw*. 2005;24: 9-16.
40. Penatti EM, Berniker AV, Kereshi B, et al. Ventilatory response to hypercapnia and hypoxia after extensive lesion of medullary serotonergic neurons in newborn conscious piglets. *J Appl Physiol*. 2006;101: 1177-1188.
41. Parsey RV, Oquendo MA, Simpson NR, et al. Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT1A receptor binding potential measured by PET using [C-11]WAY-100635. *Brain Res*. 2002;954:173-182.
42. Arango V, Underwood MD, Gubbi AV, Mann JJ. Localized alterations in pre- and postsynaptic serotonin binding sites in the ventrolateral prefrontal cortex of suicide victims. *Brain Res*. 1995;688:121-133.
43. Dillon KA, Gross-Isseroff R, Israeli M, Biegon A. Autoradiographic analysis of serotonin 5-HT1A receptor binding in the human brain postmortem: effects of age and alcohol. *Brain Res*. 1991;554: 56-64.
44. Johns JM, Lubin DA, Lieberman JA, Lauder JM. Developmental effects of prenatal cocaine exposure on 5-HT1A receptors in male and female rat offspring. *Dev Neurosci*. 2002;24:522-530.

