Thomas P. Gilson,[1] *M.D.; M. Gregory Balko,*[1] *M.D.; Karen S. Blisard,*[1] *Ph.D., M.D.; Kevin L. Taylor,*[1] *B.S.*

Morphologic Variations of the External Arcuate Nucleus in Infants Dying of SIDS: A Preliminary Report

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ABSTRACT: The external arcuate nucleus (EAN), located in the ventral medulla, is studied in 24 infants dying of sudden infant death syndrome (SIDS) and 15 age-matched controls to identify differences in morphology in this region, thought to be involved in respiratory regulation. Significant differences are noted in EAN neuronal density, percentage of back-to-back neurons and volume of the EAN relative to the adjacent pyramids. These changes may be useful in evaluating sudden infant death.

KEYWORDS: pathology and biology, SIDS, external arcuate nucleus

Approximately 7000 deaths each year in the United States are attributed to the sudden infant death syndrome (SIDS), making it the leading cause of post-neonatal infant mortality. The median age for SIDS deaths is approximately three months of age with 90% of all cases occurring within the first six months of life. The typical clinical history is of an infant found dead after having been put to sleep. A history of recent respiratory or gastrointestinal infection or neonatal apnea is frequently present. Pregnancy history may reveal maternal smoking, low weight gain or urinary tract infection [*1*]. Postmortem examination characteristically demonstrates pulmonary congestion or edema with petechial hemorrhages of the thymus, pleura, and epicardium; however, these findings are non-specific and inconsistent.

In 1989, an expert panel was convened to review recent developments in SIDS research. Based on this review, the panel proposed the following update of the original 1969 definition:

> "The sudden death of an infant under one year of age which remains unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical history" [*2*].

The revised definition established an upper age limit of one year for the diagnosis of SIDS and more explicitly delineated the requirements for an adequate postmortem examination.

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[1]Resident, Assistant Professors and Graduate Student, respectively, Department of Pathology and Laboratory Medicine, University of Cincinnati Medical Center, Cincinnati, OH.

Recipient of the 1993 Best Resident Paper Award, 45th Annual American Academy of Forensic Sciences Meeting, Boston, MA.

Implicit in the original and revised definitions of SIDS is the exclusionary nature of the diagnosis. The pathogenesis of SIDS is unknown; however, the presence of subtle central nervous system (CNS) abnormalities has long been hypothesized as a possible mechanism for some cases [3]. Various studies have described numerous changes including gliosis in the brainstem nuclei, [4] global delayed CNS myelination, [5] and dendritic spine abnormalities of the neurons of the brainstem [6]. The brainstem has been a major focus of research because of its role in respiratory and sleep regulation.

We have undertaken to study the morphology of the external arcuate nucleus (EAN) in infants dying of SIDS versus age matched controls. The EAN is located in the ventral medulla, an area thought to be highly important in the regulation of respiration. We analyzed the EAN with regard to (1) neuronal density, (2) presence of back-to-back neurons (a marker of neuronal dysgenesis/immaturity), and (3) volume using the adjacent pyramidal corticospinal tract as an internal control.

TABLE 1—*Study population.*

Case Number	Age (months)	Sex	Cause of Death
1	8	M	Sepsis
2	2	F	SIDS
3	1.5	F	SIDS
4	1.5	M	SIDS
5	1	F	Bacterial pneumonia
6	7	F	SIDS
7	5	F	SIDS
8	1.5	M	SIDS
9	7	M	Positional Asphyxia
10	3	F	SIDS
11	3	M	Bacterial pneumonia
12	2	F	Trisomy 18
13	4	M	SIDS
14	1	M	Accidental asphyxia
15	4	M	SIDS
16	2.5	M	SIDS
17	3	F	SIDS
18	1	F	SIDS
19	0.5	M	Sepsis
20	1	M	Acute bronchitis
21	4	M	Bacterial pneumonia
22	1	M	Trauma
23	1	M	SIDS
24	2	F	SIDS
25	1	F	SIDS
26	1.5	M	Acute bronchiolitis
27	1	F	SIDS
28	15	M	SIDS
29	1.5	M	SIDS
30	1.5	M	SIDS
31	3	M	SIDS
32	2	F	Congenital heart disease
33	11	M	Bacterial pneumonia
34	1 Day	M	Acute bronchitis
35	3	M	Prematurity
36	1	F	SIDS
37	5	M	SIDS
38	5	F	SIDS
39	2	F	SIDS

Materials and Methods

The brainstems of 24 infants dying of SIDS and 15 age-matched control infants dying of known causes were examined (Table 1). Formalin-fixed tissue was obtained from the Coroner's Offices of Hamilton County (Cincinnati, Ohio), and Jefferson County (Louisville, Kentucky). The sections of medulla were processed in paraffin by routine histologic techniques. Serial sections were cut at 10 microns and every fifth slide was stained with hematoxylin and eosin. The EAN was defined as a ventral medullary nucleus extending from the base of the pons rostrally to the area postrema caudally. On the average, five glass slides per case were examined. All neuron counts were performed at 200X magnification using an Olympus BH-2 microscope with a gridded ocular. Assessment of back-to-back neurons was performed at 400X magnification. Area measurements of the EAN and pyramid were performed on the stained serial sections using a Bausch & Lomb video camera with a Panasonic display monitor and a Summa Graphics digitizing pad and Bioquant IV software. Volume ratio determinations were calculated by dividing the sum of EAN areas by the sum of pyramid areas.

Neuronal density is defined as the total number of neurons in a right or left heminucleus divided by the calculated volume of that heminucleus. Back-to-back neurons are expressed as a percentage of the total number of neurons.

Statistical analysis was performed by Student's T-test using standard software.

Results

A representative section of a control case brainstem is shown in Fig. 1.

Several morphologic parameters of the external arcuate nucleus in SIDS cases and controls were measured as described in the methods. During our initial evaluation of the data, we noted that the SIDS cases appeared to be segregated into two relatively discrete groups. In one group, designated SIDS-A, morphologic appearance of the EAN was quite different from the controls, and the measured parameters reflected this difference. The remaining SIDS cases appeared morphologically similar to the controls and were designated SIDS-

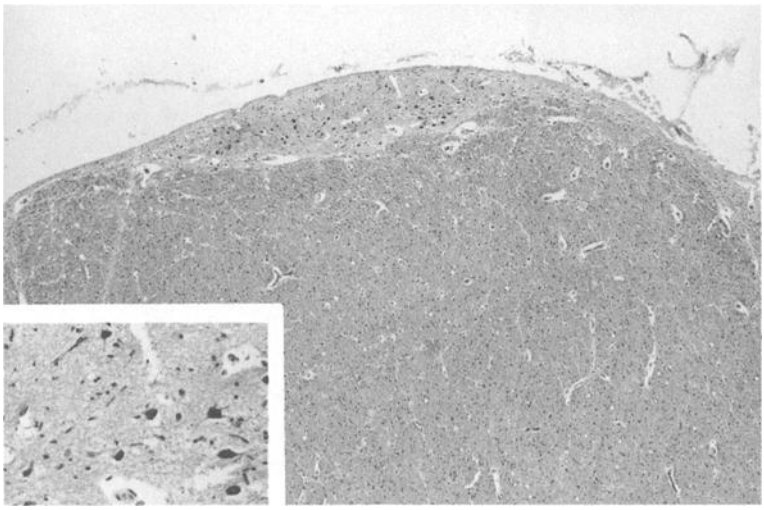

FIG. 1—*Section of brainstem from control infant showing medullary pyramid and EAN (H& E, X40). Inset shows normal cellularity of EAN (H&E, X400).*

B. There were no differences in age, clinical history, or autopsy findings in the two groups of SIDS infants. Representative sections from SIDS-A and SIDS-B cases are shown in Fig. 2.

Neuronal Density

The SIDS-A infants showed a statistically significant ($P < 0.01$) increase in neuronal density versus controls (Fig. 3). The average number of neurons/mm^2 for SIDS-A infants was 258.1 (SD = 57.8). For controls the neuronal density was 122.7 neurons/mm^2 (SD = 46.7). The SIDS-B infants showed a neuronal density of 151.0 neurons/mm^2 with a standard deviation of 32.8, which was not statistically different from controls (Fig. 3).

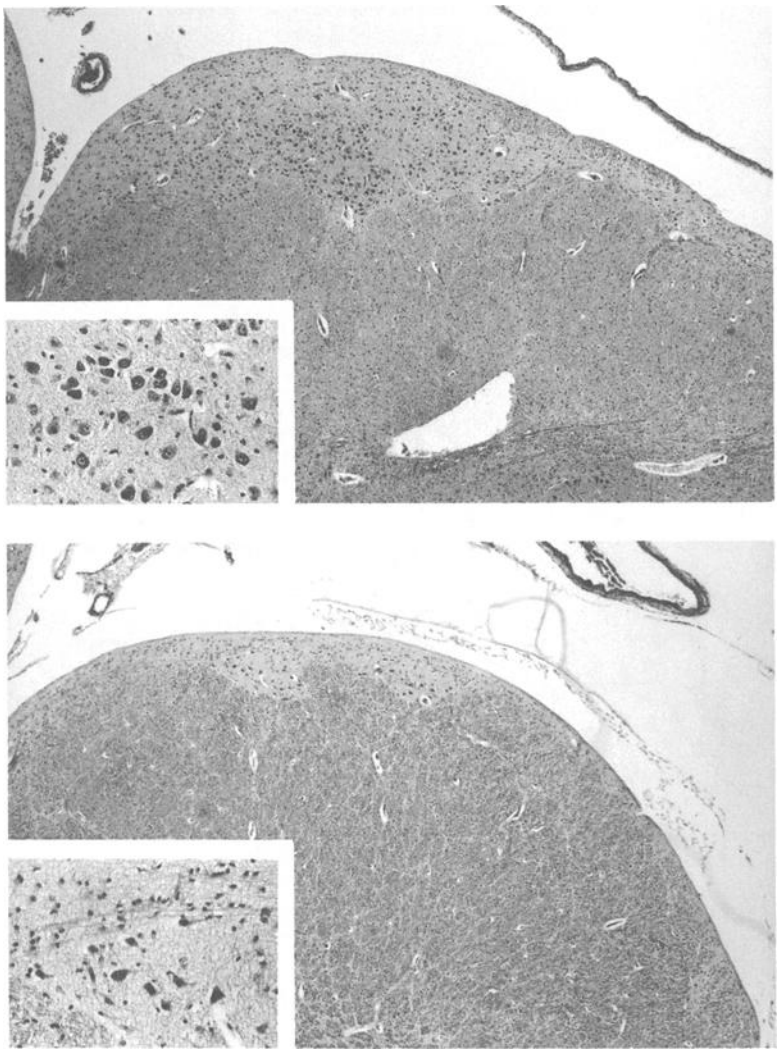

FIG. 2—A. *Section of brainstem from SIDS-A case, showing enlarged area of EAN (H&E, X40). Inset shows hypercellularity of EAN (H&E, X400).* B. *Section of brainstem from SIDS-B case is similar in appearance to control case (H&E, X40; inset X400).*

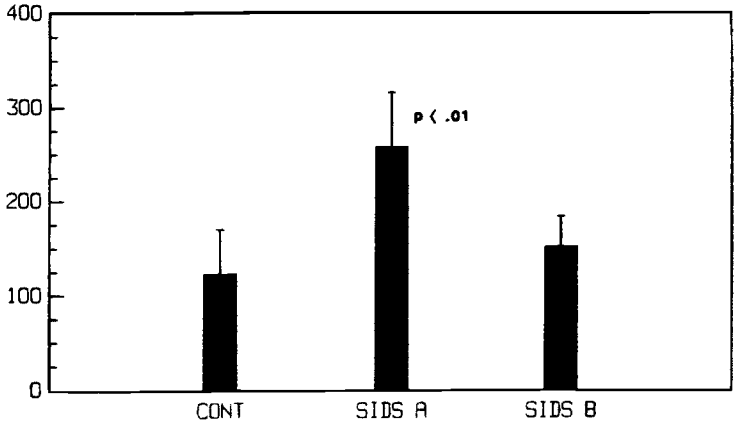

FIG. 3—*Neurons per square millimeter.*

Back-to-Back Neurons

The SIDS-A infants again showed a statistically significant increase ($P < 0.01$) versus controls with regard to back-to-back neurons expressed as a percentage of total neurons. The SIDS-B cases failed to demonstrate any significant difference from controls (Fig. 4).

Volume EAN/Pyramidal Corticospinal Tract Ratio

The volume ratio again demonstrated a statistically significant increase ($P < 0.01$) in SIDS-A infants versus controls. SIDS-B cases remained similar to controls (Fig. 5).

The results of all parameters studied are summarized in Table 2.

Two of the SIDS-A cases merit special mention. In case 30, the EAN was dramatically enlarged even in comparison to other SIDS-A cases (Fig. 6*a*). Case 38 demonstrated a bizarre architectural morphology of the EAN compared to controls and all other SIDS cases (Fig. 6*b*).

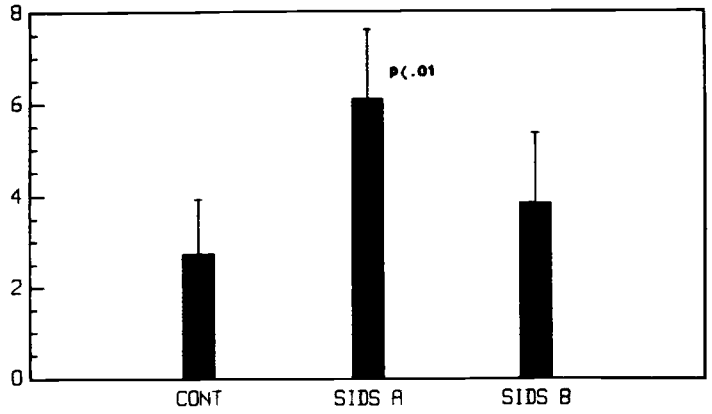

FIG. 4—*Percentage of back-to-back neurons copy for controls, SIDS-A, and SIDS-B. Cases were determined as described in the text.*

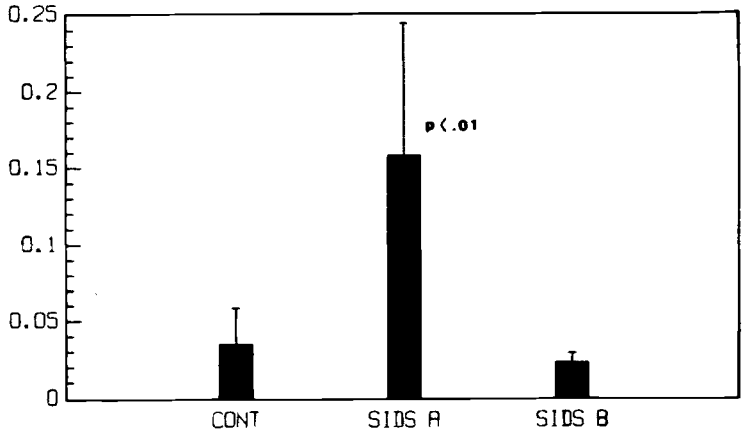

FIG. 5—*Ratio of volumes of the external arcuate nucleus to the pyramids.*

Discussion

Deaths from SIDS characteristically occur between 2 weeks and 8 months of age with a peak incidence in the second to fourth months. This time frame corresponds to a period of rapid change in the infant's autonomic, respiratory, and sleep-awake cycle status [*3*]. The possibility of a delayed or incomplete transition into a more mature status may account for SIDS deaths.

Programmed cell death is well-established as a normal event in the course of fetal CNS development. The time course of this process overlaps with the observed age distribution spectrum seen in SIDS. The increased neuronal density observed in the external arcuate nucleus of the SIDS-A infants may reflect a failure of this process and a lethal inability of the infant to progress to a more mature status. The immaturity of the EAN in SIDS-A infants is also supported by the presence of increased back-to-back neurons in higher percentages than controls. This finding suggests dysgenesis of the EAN in SIDS-A infants possibly as a reflection of immaturity.

A recent report by Kinney et al. [*7*] describes an immunohistochemical marker for immature neurons (GAP-43), and perhaps, this could provide a more definitive answer to the question of delayed neuronal development in SIDS. Their experience with two cases was inconclusive.

The increased volume ratio of the EAN to the pyramidal corticospinal tract may also be a reflection of immaturity/dysgenesis. It has been shown that the neurons of brainstem nuclei in SIDS infants have an increased number of dendritic spines [*6*]. This number declines normally during infancy possibly as autonomic and respiratory neural pathways become better defined. If this process is aberrant in SIDS, it may result in a mass effect

TABLE 2—*Data summary.*

EAN Parameter	Control	SIDS-A	SIDS-B
1) Neurons/mm^2	122.7 ± 46.7	258.1 ± 57.8[a]	151.0 ± 32.8
2) % back to back	2.7 ± 1.19	6.1 ± 1.50[a]	3.8 ± 1.52
3) Ratio of Volumes	0.035 ± 0.023	0.158 ± 0.086[a]	0.022 ± 0.006
N	15	17	7

[a] $P < 0.01$ compared to control.

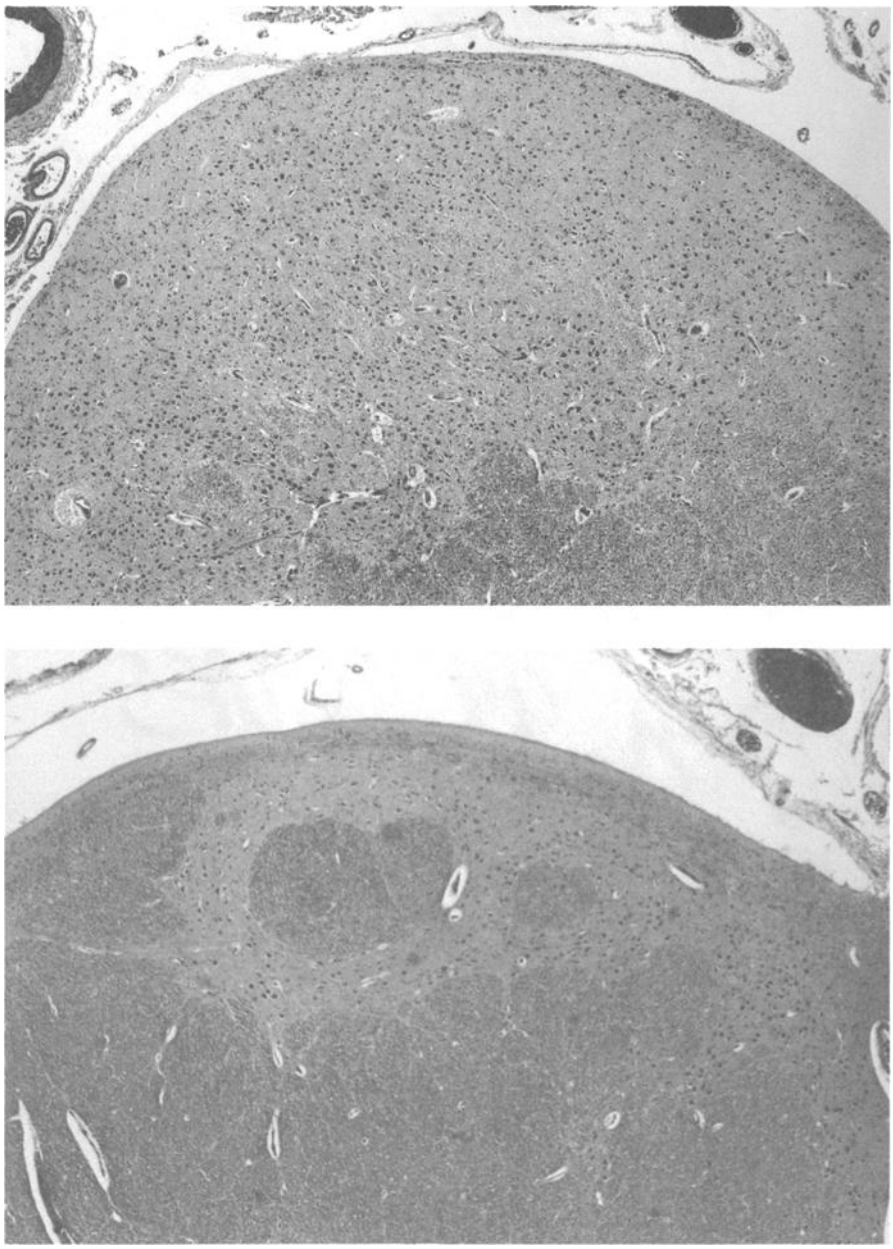

FIG. 6—*Section of brainstem from case 30, showing dramatically enlarged EAN* (A) *and from case 38, showing markedly abnormal morphology of the EAN* (B) *(both, H&E, X40).*

causing the EAN to remain larger than normal controls. In the relatively neuron-poor pyramids this mass effect may be less pronounced or absent with the result that the EAN/pyramid ratio is elevated.

The finding of a subset of SIDS infants with EAN similar to controls is not surprising. As a diagnosis of exclusion, SIDS may very likely represent a heterologous family of

disorders. Acylcarnitine profiles were not performed in our cases to rule out the possibility of medium-chain acyl-Co A dehydrogenase deficiency, which has been suggested as a possible etiology in up to five percent of SIDS cases [*8*]. This relatively common inborn error of metabolism, or others, may explain some of the SIDS-B cases. Furthermore, abnormalities in SIDS may not all be confined to the CNS and some evidence suggests all CNS abnormalities in SIDS may not be confined to the brainstem [*9*].

The two markedly abnormal SIDS-A cases (case 30 and case 38) are interesting in that they show an exaggerated increase in the neuronal density, percentage of back-to-back neurons and EAN/pyramid ratio. It would be expected that if SIDS-A represents a failure of programmed cell death in the infant brain, there would be a spectrum of microscopic changes secondary to the degree of failure of the cell-death process. Presumably these two cases represent a more substantial or near total failure of the process.

In summary, there appears to be a significant subpopulation of SIDS infants who show morphologic variations of the external arcuate nucleus (increased neuronal density, increased back-to-back neurons and increased size ratio relative to the adjacent pyramid). These findings may be a reflection of CNS dysgenesis/immaturity in SIDS. Because of the high percentage of SIDS-A cases (17/24), the EAN area may be worthwhile to sample for histology in all SIDS cases. From a practical standpoint neuronal density greater than 200 neurons/mm^2 and/or a percentage of back-to-back neurons greater than 5% and/or a volume ratio of EAN to pyramid of greater than .075 should suggest SIDS. Further studies should better define these values and may permit simplification of the procedure.

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Address requests for reprints or additional information to
Thomas P. Gilson, M.D.
Office of the Chief Medical Examiner
520 First Ave.
New York, NY 10016–6402