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SUDDEN INFANT DEATH SYNDROME: A QUALITATIVE AND QUANTITATIVE EXAMINATION OF IMMATURE IN THE BRAIN STEM

The Ohio State University

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SUDDEN INFANT DEATH SYNDROME: A QUALITATIVE AND QUANTITATIVE
EXAMINATION OF IMMATURET IN THE BRAIN STEM

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
James J. Quattrochi, B.S., M.Sc.

* * * * *

The Ohio State University
1982

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(1932-1981)

A talisman of genius
and inspiration for me.
ACKNOWLEDGMENTS

I am particularly indebted to Terry McBride who devoted endless hours towards the illustrations and the entire development of this project. My gratitude is extended to Dr. Allan Yates, my adviser, for his concern on the understanding of this syndrome and for the critical reading of the manuscript in its formative stages. Without Dr. Yates I would have found it extremely difficult to complete this task. Dr. Donald Senhauser merits particular thanks for his patience, support, and expressed interest in this research. The photographic expertise of Gil Millard and Arthur Weeks has helped me enormously in meeting innumerable deadlines. I am in special appreciation to Karen Shields and Carol Huffman for their consistent good nature in the typing and retyping of the drafts of this manuscript. I wish to thank the members of my committee: Dr. Burry, Dr. King, Dr. Mendell, Dr. Senhauser, and Dr. Yates who took time from their busy schedules to share information and generate ideas. I also wish to thank Dr. Leopold Liss for succeeding in introducing me to the field of neuropathology and who formed the cornerstone for my research commitment to SIDS. My gratitude is extended to Dr. Adrion for his support and to Dr. Fardal, the Franklin County Coroner's Office, and Dr. Reiner for their cooperation. I sincerely appreciate the support received from the National SIDS Foundation, the Central Ohio SIDS Parents Organization and the cooperation from the State of Ohio Department of Health. I value the help I received from Dr. Larry Sachs for his statistical analysis and the Tissue Lab personnel. I hope that some of what all these individuals have taught me is reflected in this dissertation.
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LIST OF ABBREVIATIONS

Å, angstrom
B₁, vitamin B₁
B₆, vitamin B₆
BAEP, brain stem auditory evoked potential
BRAC, basic rest-activity cycle
CD, cleft density
CNS, central nervous system
DDGJ, dendrodendritic gap junction
DP, dense projection
EEG, electroencephalogram
EM, electron microscopy
EPTA, ethanol-phosphotungstic acid
GJ, gap junction
GMF, glial maturation factor
GT, gigantocellular tegmentum
H&E, hematoxylin-eosin
LL, lateral lemniscus
LT, long-thin
μm, micrometer
MLF, medial longitudinal fasciculus
MS, mushroom-shaped

x
n., nucleus
NA, n. ambiguus
NREM, non-rapid eye movement
NRF, non-reticular formation
NS, n. solitarius
PSN, ponto-subicular necrosis
PT, postsynaptic thickening
PVL, periventricular leukomalacia
REM, rapid eye movement
RF, reticular formation
SEM, scanning electron microscopy
SIDS, sudden infant death syndrome
SL, subcortical leukomalacia
ST, short-stubby
T3, thyroid hormone
TEM, transmission electron microscopy
UL, uranyl acetate - lead citrate
URTI, upper respiratory tract infection
ZO, zona occludens
V, n. spinal trigeminal
XII, n. hypoglossus
INTRODUCTION

Sudden infant death syndrome (SIDS) has been defined as the sudden death of any infant which is unexpected by history and in which a thorough postmortem examination fails to demonstrate an adequate cause of death. Typically, an apparently thriving baby is found dead while asleep in bed, most commonly at night. Pathological evidence suggests asphyxia during sleep. The incidence of SIDS is approximately 1 to 3 per 1000 live births. Recently, the Health Service Administration has estimated that 6,500 to 8,000 cases occur annually in the United States. SIDS is the largest cause of death between the ages of two weeks and one year, accounting for nearly 40% of the total infant mortality. SIDS has a distinct age distribution, with the peak incidence between 2 and 4 months of age. Almost 90% of the cases observed in a Seattle, Washington study were less than 6 months of age. In our own experience in Columbus, Ohio, the peak incidence is between the ages of 5 weeks and 4 months of age. Although there are reports that indicate a seasonal incidence variation, we have not confirmed this trend in Franklin County. Higher incidences in the American Indian population and lower socioeconomic groups, and a lower incidence in the Oriental communities of the U.S. have also been reported. Many epidemiological studies suggest male dominance in SIDS. Risk for SIDS is higher in low birth weight newborn babies. Familial occurrence of SIDS has been reviewed by Beckwith, and according to Froggott,
risk of SIDS among the siblings of a SIDS infant is 4-7 times the random risk. A strikingly high incidence of sleep disorders in the families of SIDS infants has been found in Columbus, Ohio. Frequent familial incidence of SIDS was also noted in Cleveland, Ohio.

Clinical Observations

Clinical observations of SIDS and near-miss SIDS infants have implicated a respiratory control dysfunction in these infants. Infants observed to be apneic for 20 seconds or longer, possibly requiring resuscitation, are termed near-miss for SIDS. Studies of these infants have examined such parameters as prolonged apnea, periodic breathing, sleep states, arousal threshold, and ventilatory responses to hypoxia and hypercarbia. A number of pathological investigations have demonstrated tissue alterations in the lungs and the central nervous system (CNS), particularly the brain stem, suggesting chronic intermittent hypoxia, presumably from recurrent sleep apnea. For the past decade, the SIDS literature has implicated the central respiratory mechanism in the brain stem as the site of this respiratory dysfunction. It is believed that the maturational state of the neurons in the brain stem may be of major importance in the pathogenesis of SIDS.

SIDS research, indeed, includes a large amount of clinical and morphological data correlations, as well as restrained and unrestrained conclusions. The problem is to use this maze of clinical and basic scientific information to understand better the enormous complexity of the integrative capability of the brain stem and, hopefully, to determine more accurately if a brain stem structural abnormality is associated with SIDS. Specifically, how do we attempt to elucidate the anatomical substrate responsible for a brain stem dysfunction? Our approach is to perform a
qualitative and quantitative analysis of the dendrite-spine system and of synaptic profile maturity in SIDS babies compared with controls.

The evidence cited for an asphyxial mode of death is left ventricular blood PO2 values lower than controls,19 and intrathoracic distribution of petechiae.20,21 Chronic hypoxia has been suggested on the basis of several findings: pulmonary arteriolar hypertrophy and hyperplasia, right ventricular hypertrophy, a high proportion of brown fat in the periaortic region, abnormal proliferation of glial cells in the brain stem, and hyperplasia of the carotid bodies.22 Although these data suggest the possibility of hypoxia in the antemortem period, it has been suggested that many of these observations could be interpreted as delayed postnatal maturation, not necessarily due to arterial hypoxemia.

The classic report by Steinschneider in 1972 first suggested that prolonged sleep apnea may be related to the pathogenesis of SIDS.11 Sleep apnea as such is a common phenomenon seen in infants and adults. When apnea is prolonged, it is associated with hypoxia, cyanosis, disturbances of cardiac rhythm, and is seen frequently in near-miss SIDS infants.27 Currently, there appear to be three main types of sleep apnea related to SIDS.28 One is central apnea with absence of respiratory muscular activity, occurring often in active, rapid eye movement (REM) sleep. The second type is obstructive apnea, with hypotonia of the upper respiratory tract muscles causing airway obstruction, while thoracic inspiratory efforts continue, often in quiet, non-rapid eye movement (NREM) sleep. Obstructive sleep apnea in infants has been studied using impedance monitoring. Infants react with no visible signs of struggle against an obstruction, and even a mild upper respiratory tract infection (URTI) may provide such an obstruction. It seems possible that infection could be a precipitating factor in SIDS by
increasing the chances of airway obstruction. It is generally agreed that there is a higher incidence of URTI among SIDS victims than in the general population. McGinty has speculated that a URTI also may cause a degree of sleep deprivation with restlessness, irritability, and coughing. Sleep deprivation also has been shown to increase the frequency of apneic episodes and to depress the brain stem arousal mechanism. These data are consistent with the concept that stress or sleep loss may alter respiration during sleep.

In a study of familial obstructive apnea during NREM sleep, genioglossus muscle lost its tonic activity. During inspiration, increased activity of the genioglossus muscle pulls the tongue forward, but lack of tone during inspiration permits backward movement of the tongue by the negative intrathoracic pressure and upper airway obstruction. Others have speculated that loss of tone in the pharyngeal abductors (geniohyoid and stylopharyngeus) may be the cause of an upper airway obstruction.

There is relatively little agreement between sleep research centers in this country as to the mechanism of this obstructive episode. Weitzman contends that obstructive apnea is an active contraction, whereas Guilleminault regards obstructive apnea as due to a passive collapse of the airway. A Stanford study has shown that obstructive apnea may exist where no detectable cause of obstruction is found during wakefulness. A CNS defect is thought to be the prime determinant when obstructive apnea involves no such peripheral factor. Therefore, a central brain stem mechanism has been proposed for obstructive as well as central apnea.

The third type is mixed apnea, usually central apnea followed by obstructive apnea. It is thought that different mechanisms may cause mixed apnea occurring in REM sleep as compared to that occurring in NREM sleep.
Furthermore, oxygen desaturation has been found to be more rapid during mixed and obstructive apneas than in the central type. Thus, the frequency of apneic episodes recorded during a sleep period requires a careful differentiation of the type of apnea to assess properly its association with the multifactorial pathogenesis of SIDS.

In 1979, using matched controls and statistical analysis, Guilleminault questioned the specificity of sleep apnea as a predictor of risk for SIDS. Nevertheless, sleep apnea is generally considered and accepted to be an important mechanism for SIDS. The mechanism to produce death, according to Guntheroth, is the inability to overcome sleep apnea. However, during the first month of life, he has found that infants reinitiate breathing by an effective gasping response to apnea. This may explain the relative infrequency of SIDS during the first month of postnatal life.

Since 1963, the clinical concept of near-miss for SIDS has been accepted by most pediatricians, although specific criteria for diagnosis are still being evaluated. The near-miss event implies no detectable cause; however, it may represent a failure of respiratory control during specific stages of sleep, and, in that respect, be related to SIDS. The clinical monitoring of the near-miss SIDS infant has lead to a host of reports in the pediatric literature. One study demonstrated that the number of sleep apneas (mixed and obstructive) longer than three seconds in the near-miss infants was greater than that in the age-matched controls between 3 weeks and 18 weeks of age. Interestingly, the number of apneic episodes was highest at 6 weeks of age in both groups. Kelly and Shannon noted a higher incidence of periodic breathing - three or more apneic pauses of 3 or more seconds duration within periods of breathing of 20 seconds or less - during sleep in near-miss SIDS infants as compared with
12 control infants. These findings suggest a CNS dysfunction - a transient anatomic or functional defect. Abnormalities in the O₂-sensitive carotid bodies have included reduced numbers of glomic cells and neurosecretory granules. Since the glomic cells are considered to be in the afferent system of the carotid, this may simply reflect a brain stem abnormality. Chronic intermittent alveolar hypoventilation and hypoxia, which are consistent with impaired ventilatory responses to CO₂ described in near-miss infants during sleep, are suggested also by the appearance in some SIDS infants of hypertrophy of small pulmonary arteries.

Brain stem auditory evoked potential (BAEP) has been reported also to be abnormal in the near-miss infants studied. Such measurements of BAEP have added further sophistication to the study of the implication in SIDS of a brain stem abnormality. The response is characterized by distinct peaks which represent a particular level along the auditory pathway - the eighth nerve, the pons, and the midbrain. BAEP results show abnormalities in the form of wave shape, amplitude, latency, and response stability. BAEP testing may have an important role in detecting brain stem abnormality; however, conflicting reports are indicative of inconsistencies in identifying infants at risk for SIDS with BAEP testing.

Developmental Aspects of Sleep and Breathing

The role of the brain stem in the mediation of sleep and waking states represents one of the most intriguing areas of brain physiology. The classic report of Moruzzi and Magoun brought into focus the central role of the brain stem reticular formation in modulating levels of cortical activity during sleep. Cellular evidence has supported the concept of an ascending reticular mediation of electroencephalogram (EEG) desynchronization. From these data, the concept emerged of a structural substrate for
arousal to reside in the complexity of the brain stem.

During development, not only respiratory responses, but also other homeostatic functions attributable to the brain stem change. The most notable one, and perhaps closely related to SIDS, is the change in the sleep-wakeful cycle. Intrauterine recordings have provided only limited knowledge concerning the structures which mediate sleep and wakefulness. Prechtl has shown that sleep patterns and the integration of such sleep states in the newborn can be correlated with definitive behaviors and physiological data. The basic rest-activity cycle (BRAC) is a continuous 24 hour EEG characteristic of such neuronal integration. This process is subjected to cortical control and environmental influences. It has been noted that maturation of sleep is a function of postconceptional age rather than extra-uterine experience, although environmental factors may influence maturation to a degree. Kleitman termed BRAC as the recurrent active phase of sleep which influences the CNS during wakefulness. Thus, BRAC is observed in sleep as the active phase and in wakefulness as a similar oscillation of activity in the CNS. The BRAC in the fetus reflects the maternal BRAC by a yet uncertain factor which is communicated to the uterine environment. Kleitman's data on the human infant show BRAC to range from 40 to 60 minutes. With the eventual appearance of sleep-wakeful states, BRAC increases to 60 to 70 minutes in children and 80 to 90 minutes in the adult. The cycle becomes increasingly associated with sleep. With the development of higher CNS influences and maturing of lower brain stem central control, the BRAC becomes the REM-NREM cycle.

Between 33 and 35 weeks of gestation, sleep states and a waking state can be identified. With the development of REM and NREM states, REM appears to be organized in the rostral brain stem and NREM appears integrated
at several levels of the neuroaxis involving forebrain as well as the rostral brain stem, and is considered a more mature state. It has been established that the duration of REM is greatest in premature and newborn infants and decreases progressively with maturation. Another feature of the REM state is its episodic recurrence – its periodicity. The rhythmic alteration of REM and NREM sleep states suggests that the brain stem be thought of as the regulator of the REM-NREM cycle for the entire brain. Within the complex meshwork of the brain stem, it is believed that there is a temporal functioning program which consists of a flow of activity between neuronal aggregates. Between 2 and 3 months of postnatal age, the percent of sleep time in REM decreases and the percent in NREM increases. It has been noted that the magnitude of this maturational change is less in near-miss infants when compared with controls. The integration of sleep states requires a complex interaction of the brain stem and higher centers for effective control. This implies that such maturation of function produces both the need and capacity for a more integrative process. Thus, as age increases, definite patterns of sleep and wakefulness are seen in the infant. This maturational scheme allows the infant to sustain longer periods of sleep, particularly between two and four months of age.

Maturational changes in sleep states is a vulnerable period. In REM, the vulnerability is further accentuated by the overall collapse of both the rib cage and lungs when intercostal muscle activity is depressed. This decrease in lung volume reduces the O₂ gas stores available in obstructive or central apnea. Even before the lung deflates, the infant has a very small O₂ store and a high metabolic demand for O₂ compared with later in life. Consequently, during apnea in the infant there is a rapid fall of arterial O₂ saturation which is accelerated when apnea occurs in the REM.
state. With the onset of asphyxia, the chemoreceptors of both carotid and aortic bodies and of the brain stem excite reflex respiratory and circulatory reflexes. Defective chemoreceptor drive to breathing in hypoxia is being recognized in a variety of clinical situations which might provide clues for further research into the mechanisms involved in SIDS. Defective responses to hypoxia occur in familial dysautonomia and in an adrenergic blockage.59,60

It has been shown that different neural mechanisms regulate breathing in wakefulness and in REM and NREM sleep. If breathing is regulated by certain neural mechanisms in a particular phase of sleep, a defect in such mechanism might be expected to be most apparent in this sleep phase. In healthy subjects, breathing rhythm is interrupted intermittently for brief periods in sleep, often with swallowing or after a deep breath. These apneic periods can become abnormally prolonged, suggesting a pacemaker defect in the respiratory centers or failure of chemoreceptors to re-initiate breathing in response to asphyxia.38

During this critical period of postnatal CNS functional maturation, a developmental need for the NREM state is suggested as the infant spends twice as much time in NREM as in REM.55 A minor stress such as a URTI may compromise not yet adequate regulatory mechanisms. The decline in tolerance to hypoxia may also compound this vulnerability. A recent study by Harper suggests an altered sequence in the periodicity of sleep and wakefulness in infants at risk for SIDS.13 This inability to modulate sleep states properly with the waking state might result in a failure to awaken from sleep during a critical event such as apnea. Thus, the maturation of the brain stem, necessary for the integration of respiration and sleep states, may also correlate with such a critical period of functional
organization. The fact that the peak incidence of SIDS is between 2 and 4 months of age suggests that this critical period may be related to an immaturity of the control mechanisms of sleep and respiration in SIDS infants. In view of the available evidence, we believe our most urgent task is to search for a morphological clue which might identify factors responsible for defective neural regulation of breathing during sleep.

**Anatomical Considerations in Central Respiratory Control**

Involuntary rhythmic breathing movements are initiated by networks of nerve cells in the brain stem. The automatic activity of the brain stem is modified by nervous and chemical stimuli and is regulated in the framework of a complex control system. The periodic motor output of the brain stem centers, which leads to regular rhythmic breathing, is conducted via bulbospinal neurons to multiple segments in the spinal cord. The ventilatory drive must be adjusted to meet the metabolic demands for gas exchange. For this adjustment, the brain stem uses information on the arterial partial pressures of O₂ and CO₂ and on the H⁺ ion concentrations in the blood — information provided by chemoreceptor cells within the brain stem as well as in the carotid and aortic bodies. The appropriate modification of this motor output requires processing, storage, and integration of this information with other afferent signals received by the brain stem. The control of breathing must also be integrated with the control of other motor functions.

The brain stem contains an intricate network of neurons that maintain coordinated rhythmic breathing even in the absence of afferent inputs. The two main groups of respiratory neurons in the medulla are the dorsal and ventral respiratory groups. The dorsal group, near the nucleus of the tractus solitarius, contains neurons that discharge before and during
inspiration. Some of the neurons in this area are excited while others are inhibited with inflation of the lungs, and coordination of these responses result in the respiratory rhythm. The respiratory drive is transmitted to a second group of respiratory neurons located in the more caudal ventral and lateral aspects of the medulla. This ventral group, part of the nucleus ambiguus, contains approximately equal numbers of inspiratory and expiratory neurons and is primarily concerned with the vagal motor activity of respiration involving throat muscles through the recurrent laryngeal nerves. Caudally and laterally to the nucleus ambiguus, the cells in the nucleus retroambiguus are medium-sized and control the spinal motor function of the respiratory muscles including the thoracic wall and diaphragm. The similarity of firing patterns in the inspiratory neurons of the ventral group indicates that these neurons are activated by highly synchronized input signals. Hence, the rhythm of the recurring inspiratory stimuli must be generated elsewhere in the brain stem. Nevertheless, how the rhythm of breathing is established is far from clear. There is no evidence for the existence of pacemaker cells in the brain stem yet reported. Different models of neural networks have been proposed, and in all these models reciprocal inhibition and synchronization between discharging neurons are the key features in explaining rhythmicity.

How the pneumotaxic and apneustic centers in the pons and pontomedullary junction relate to the medullary centers remains unclear. The pneumotaxic center, located in the nucleus parabrachialis, may modulate the rhythmic centers in the medulla. Generally, the function of the pneumotaxic center is considered to be the fine tuning of the inspiratory cutoff. Caudal to the pneumotaxic center is the apneustic center located at the level of the cerebellar peduncles in the nucleus reticularis.
gigantocellularis and the rostral portion of the nucleus reticularis ventralis. Stimulation of the apneustic center causes tonic inspiratory muscle contraction. The main function of this center is in the tonic excitatory action on the inspiratory neurons.

The central chemoreceptors are located near the ventrolateral surface of the medulla. Stimulation of these cells depends upon the composition of the brain's extracellular fluid, specifically its pH. In turn, the pH depends on the local PCO₂ and HCO₃⁻ ion concentrations. The PCO₂ of the brain's extracellular fluid depends on the PCO₂ of the blood perfusing the medulla, on local CO₂ production, and on tissue blood flow. The central chemoreceptors are connected to respiratory neurons in the medulla and pons in order to signal local tissue pH changes which lead to increased or decreased ventilation. The peripheral chemoreceptors discharge in response to a decrease in arterial PO₂ and an increase in arterial PCO₂. The carotid bodies also respond to a decrease in arterial pH; the aortic bodies do not. The stimuli from these cells reach the brain stem via the glossopharyngeal nerve from the carotid bodies, and via the vagus from the aortic bodies. Stimuli via these nerves first reach the dorsal respiratory group. The resultant change in the brain stem ventilatory drive activates inspiratory muscles. CO₂ affects ventilation via the central as well as the peripheral chemoreceptors. Its effect on the peripheral chemoreceptors is direct and rapid, but it contributes to only a small proportion of the total ventilatory drive. The indirect effect of CO₂ on the central chemoreceptors via changes in tissue pH is slower, however it provides about 80% of the total ventilatory drive.
The centers for sleep are located in the more rostral portion of the pons. REM sleep is generated by neurons in the gigantocellular tegmental field, and NREM sleep is generated by the neurons in the median raphe. The former group secretes acetylcholine and the latter, serotonin. Arousal pathways are separate from the sleep pathways and appear to reside in nonadrenergic neurons in the rostral locus ceruleus. However, neural interconnections leading to sleep apnea have been poorly understood.

Breathing begins before birth, as early as 13 weeks of gestation in the fetus. During the first weeks of extrauterine life, responses to CO₂ decrease while responses of tidal volume to 100% O₂ increase. The role of vagal afferents also appears to change during development. The newborn's most important need is to maintain lung inflation. Thus, it is not surprising that ventilatory responses to changes in lung volume are well developed at birth. In the newborn, especially the premature infant, the Hering-Breuer reflex is dominant and acts to increase the rate of breathing. This high rate of breathing keeps the lung volume at a level well above the residual volume. During sleep, ventilatory drive is normally reduced, resulting in an increase in arterial PCO₂. Similar increases occur with airway obstruction and are accompanied by decreases in arterial PO₂.

Neuropathological and Hypoxic Considerations in SIDS

Although many mechanisms of sudden death have been proposed, SIDS research attempts have failed to elucidate a final common pathway. Currently, emphasis is being placed on a multifactorial approach, with investigative studies aimed at detection of chronic abnormalities. Several investigators have documented irregular breathing and periodic apneic episodes as a component of sleep in the preterm infant. This hypoventilation
syndrome is thought to be related to maturational inadequacy of the central control of respiration. In spite of such clinical studies in infants and correlations with near-miss and SIDS infants, investigative efforts into brain stem morphology have been limited. Neuropathological reports have described brain stem and cerebral lesion suggesting antecedent hypoxic injury. Glial proliferation, delayed myelination, ischemic cell change, and scattered perivascular lipid laden cells in the brain stem have been described. Gliosis in the brain stem of SIDS infants has been attributed to chronic hypoxia. However, glial proliferation is known to accompany normal myelination and synaptogenesis during the postnatal developmental period. Leukomalacia also has been reported in the cerebral white matter in 21% of SIDS infants, compared with 24% in infants with congenital heart disease, and 4% in infants who died from known causes.

It has been reported that SIDS infants also exhibit numerous neurons with ischemic cell changes in the brain stem. However, in another study, only 1 of 34 SIDS infants examined demonstrated similar cell change. In view of these findings, ischemic cell change and its pertinence to the pathophysiology of SIDS must be questioned more closely. Guntheroth has suggested that moderate hypoxia may be associated with apnea in SIDS. The increased vascularization in the brain stem often found in patients with chronic hypoxia, has not been observed in studies of CNS morphology in SIDS.

The human brain stem has been considered to be less vulnerable to anoxia than other areas of the brain. However, some reports have stated that brain stem damage is common in infants with anoxic insults. The concordance between diminished hypercarbic and hypoxic responses and between diminished ventilatory responses and decreased frequency of arousal
responses suggest that both deficits may be related to a common pathological lesion within the CNS. Hence, when we confront the relation of hypoxia and brain stem lesions to death, investigators are in a difficult position as regards to cause or effect, for it is possible that near-miss SIDS infants also could acquire a degree of brain stem damage.

Developmental Considerations for a Structure/Function Correlation

Our intention in this study is to construct an image of the infant brain stem - an area which has become progressively more crucial to the formulation of the pathogenesis of SIDS. Although information is more readily available concerning the postnatal development of the human cerebral cortex, reports are lacking concerning the postnatal development of the human brain stem. Knowledge of the development of reticular structures during the course of postnatal ontogenesis is a sine qua non for the application of a structure-function relationship in the brain stem. This investigation reports observations made in the study of the postnatal ontogenesis of the brain stem in both control and SIDS infants. Although this study is confined to the dendrites and synaptic profiles of the brain stem and does not represent a complete developmental series, it should be considered as representative of the general sequential development of the microstructure of the infant brain stem.

Approximately 25 years have passed since the first discussion of structure and function of the brain stem. The characteristic rostro-caudal compression of dendritic systems stimulated notions concerning input segregation in an area known for convergence of heterogenous afferent patterns. Concepts about the dendritic surface itself have shown considerable evolution. The classic spine-covered dendrites which are familiar from Cajal's drawings have proven to be limited to the perinatal
period in the kitten brain stem. With the maturation of the kitten brain stem, dendritic spines are reported to be progressively lost leaving shafts appearing smooth under light microscopy. These spines, considered to be the postsynaptic structure in chemical synapses, disappear at 2 to 3 months postnatally, and no observable replacements occur. Concurrently, reticular dendrites are seen to organize into interwoven bundles which are thought to serve as repositories of electrophysiological outputs. Such dendo-dendritic interaction is thought to coincide with the rapid postnatal development and control of complex brain stem functions.

Although the postnatal period in the kitten may correspond to well over a year of age in the human, it is quite possible that a similar maturational scheme may take place in the human brain stem. Pathology of dendritic spines in the cortex of both animals and humans has been reported in association with neuronal CNS dysfunction. It is conceivable that clinically documented sleep-respiratory problems and the micropathology of brain stem dendritic spines also may reflect an integrative neuronal dysfunction. In view of these observations, a preliminary study of the infant brain stem had demonstrated neurons to have retained more dendritic spines in cases of SIDS than in controls. These qualitative data might be interpreted as a delayed maturation, and one may then postulate that such delay leads to dysfunction of neuronal respiratory control.

Since maturity of the brain stem may correlate with the decrease in the density of spinous synapses, it is necessary to have detailed knowledge of the normal developmental pattern of these structures before one may critically evaluate a persistence of dendritic spines in SIDS. It is reasonable to suspect that spine persistence and/or alterations in spine geometry exert significant effects on integrative operations of the dendritic system.
as the receptor surface for synaptic inputs. However, in order to correlate brain stem microstructure with functional abnormality, evaluation of the dendrite-spine system requires this quantitative investigation of the normal developmental sequence of the infant brain stem using control age-matched infants compared with infants who died of SIDS. Perhaps, brain stem microstructure will provide a structural substrate through the selective quantitative analysis of its neuronal complexity.

The brain stem is a central receptive pool of neuronal aggregates with the convergence of afferent systems, both collateral and terminal. Innumerable longitudinal axons of reticular origin also add an increment of core-processed information within the brain stem. Within a neuronal aggregate, neurons give rise to dendrites with many varied lengths and branching patterns which are characteristic of the neurons in each specialized region of the brain stem. Electron microscopy reveals junctions that connect neurons—the synapses. At these junctions, information is transferred from one neuron to another, usually by means of chemical transmission. For decades, it was assumed that axosomatic and axodendritic synapses were the only possible interconnections. However, it appears that the development of neuronal connections is a subtle interplay involving a hierarchy of potential plasticity from which certain changes are realized in response to the state of the growing neuropil.

Neurons are not independent units but constitute an integral part of a continuous network. An important goal in understanding organization in the brain stem is to establish principles which govern organization. Variability in parameters of neurons and their connectivity appear to result from a potential for plasticity. This suggests a tremendous complexity of circuitry. Studies show that dendritic arborization is
modified by subtle environmental changes. Knowledge of the mechanism of formation of synaptic connections is at the crux of our understanding of the development of the brain stem as a functioning system. From these considerations, it is evident that the CNS is built up of functional units of increasing scope and complexity.

The traditional concept of a single neuron, receiving information by way of its dendrites and sending it out through its axon, can now be seen to represent only one type of functional unit. At the level of the brain stem, such functions as respiration, sleep-wakefulness states, and chemoreceptor activity call for coordination of many neuronal aggregates. In this coordination, the common focus is the synaptic circuit through the dendrites. Given this enormous complexity of the brain stem, it might be expected that the degree of variation in connections would be proportionally that much greater. The difficulty in demonstrating the degrees of variation are also greater. This study will focus on several aspects of postnatal brain stem development:

(1) the developmental pattern of dendritic spines,
(2) dendro-dendritic relationships,
(3) the maturational scheme and ultrastructure of chemical synaptic organization.

Quantitation of interneuronal connectivity has been attempted in the past with such approaches relying upon indirect indices, including the density of axons, dendrites, and spines. In such light microscopic studies there is no actual observation of interneuronal junctions, but only an estimate of the likelihood that such junctions may be present. These approaches may be misleading since the rate and extent of synaptic formation may be regulated by factors other than merely the number and
proximity of neural elements. With the advent of ethanol phosphotungstic acid (EPTA) staining, quantitation of the density and maturational state of synaptic densities is now more practical. The formation of junctions is a critical process in brain development and as such must be taken into account in relating morphology to function. In this study, the EPTA method is applied to the detailed examination of the synaptic junctions. Such quantitative data are required in order to establish correlations between changes in the number of synapses and other developmental events such as maturation of sleep states. The matching of postconceptional and postnatal ages with synaptic and dendritic spine development is clearly necessary for a meaningful interpretation of synaptic density in the study of the SIDS brain stem.

The development of appropriate synaptic relations is the basic requirement for normal neuronal operations. The Golgi staining method is particularly suited to the study of target cell development since it permits assessment of dendritic surface area during neuronal maturation and provides information on the different developmental alterations in this morphogenetic process. The underlying assumption in Golgi studies is that significant alterations in dendritic surfaces, as a result of developmental disturbances, point towards a brain dysfunction. This expresses a central dogma in neuroscience—abnormal neuronal morphology plus aberrant synapses equals abnormal neuronal function.

The understanding of human brain development has been a primary goal of neuroembryology for over a century. More than thirty years ago, Sperry proposed the theory of neuronal specificity in order to account for the development of ordered neuronal interconnections in the CNS. Observations have since provided a working hypothesis that as integration of
structure increases, so does an increase in the functional commitment of these structures.\(^{110}\)

In 1883, Camillo Golgi exposed chromate-hardened brain tissue to silver nitrate and initiated the modern era of neuromorphology.\(^{100}\) Despite the fact that the Golgi method is generally considered one of the most unpredictable neurohistological techniques, it remains today an important method for studying the structural organization of the brain. Both chemical and electrotonic synaptic transmissions are now clearly recognized in this structural organization as fundamentally different modes of interneuronal communication.\(^{98}\) The synapse is the site at which neuronal interactions are affected and modifications occur that may have dramatic effects on the processing of information in neuronal circuits. Dendrites provide the major proportion of membrane surface area for integration of synaptic inputs.\(^{95}\) Thus, the morphogenetic events in the development of these structures are important determinants of the potential functional synaptic competency of the maturing brain. It follows that a study of dendritic development and spine differentiation may provide clues to the neural substrate underlying the ontogenesis of brain stem functions.

The early postnatal weeks have been shown by many workers to be a critical time for the development of the CNS functions.\(^{111}\) The sequence of acquisition and loss of spines and synapses may underlie the degree to which reticular neurons can be modified by environmental influences. The progressive confinement of neural connections by means of synaptic rearrangements seems to be one of the bases for the gradual decrease in the vulnerability of the developing nervous system.\(^{112}\) Whatever programming is involved in the establishment of neural connections, it must relate to the population as a whole rather than to individual aggregates within the
population.

Both dendrites and spines of the pyramidal cell type have been shown to be sensitive barometers to a wide variety of extrinsic and trophic factors which reflect alterations in the dendritic field as well as in spine density. For example, changes in spine density occur as a result of sensory restriction, deafferentation, clinical states, and the aging process. Spine density, therefore, represents a functionally meaningful parameter in the description of neuronal morphology and the differentiation of cell types.

The critical questions in this investigation are:

(1) can a developmental pattern of dendritic spines be demonstrated in the infant brain stem?

(2) are there subjective semi-quantitative differences in dendritic spines between control and SIDS infants?

(3) are there quantitative differences in the magnitude of spine density?

(4) are there quantitative differences in the maturational state of synaptic connections?

Considerable information is now available on the biochemical ontogeny of many neuronal pathways while the knowledge of their functional development is still limited. As major input and output functions converge upon and emanate from the brain stem, so do many conceptual issues of neuroscience intersect at the center of this investigation. Using the data discussed in this study of the infant brain stem, we demonstrate that alterations in microstructure can not only be detected, but also be assessed with functional considerations.
Our selection of seven specific areas in the brain stem used for dendritic spine quantitation is of neuroanatomical interest concerning central respiratory control and the pathophysiology of SIDS. In the reticular formation of the medulla, the n. ambiguus and n. solitarius have been shown to contain predominately expiratory and inspiratory neurons respectively. The n. hypoglossus in the non-reticular formation is an efferent nucleus which may be implicated in the abnormal control of the genioglossus muscle in the tongue associated with obstructive apnea during sleep. The n. spinal trigeminal is a non-reticular formation afferent nucleus with receptors in the mucous membranes of the nose and efferent connections to the expiratory center neurons. This may be of importance in the regulation of breathing during a URTI. The gigantocellular tegmentum area in the pons represents the paramedian reticular formation in the region of the pneumotaxic center. The medial longitudinal fasciculus area in the medial reticular formation of the pons also represents an area of respiratory control. The n. lateral lemniscus is in the auditory pathway of the lateral pons which has been measured with BAEP recordings in near-miss SIDS infants.

In our attempt to identify an anatomical substrate which may be associated with the multifactorial pathogenesis of SIDS, it is also our intention to demonstrate that the Golgi impregnation technique and ultrastructural analysis are capable of testing the hypothesis that the sudden infant death syndrome is associated with neuronal immaturity in the brain stem.
MATERIAL AND METHODS

The present study is based upon 95 infants out of the 168 infants who died unexpectedly and who were autopsied over the past four years in Columbus, Ohio at the Franklin County Coroner’s Office and Columbus Children's Hospital. These 95 infants ranged in age from one day to one year and included 31 premature (<37 weeks gestation) and 64 term (>38 weeks gestation) infants. Clinical histories were examined for:

1. delivery complications,
2. respiratory distress at birth,
3. apneic episodes,
4. seizure activity,
5. respiratory infections,
6. incidence of SIDS in siblings.

Of these 95 infants, 61 were diagnosed as SIDS after a thorough post-mortem examination had excluded all adequate causes of death. Morphological observations were typical of SIDS and included:

1. petechiae on pleural surfaces, thymus, and epicardium,
2. subacute inflammation involving larynx and trachea.

The control population consisted of 34 infants who were diagnosed as non-SIDS with confirmed causes of death including:

1. pneumonia,
2. congenital heart disease,
3. hyaline membrane disease,
(4) sepsis,

(5) necrotizing enterocolitis,

(6) myocarditis,

(7) necrotizing tracheobronchitis,

(8) drowning,

(9) accidental death.

Postmortem findings in both groups were further compared and found to conform with the standardized autopsy protocol compiled at a recent SIDS conference for forensic pathologists. The time from death to autopsy in all cases was less than 18 hours with a mean postmortem time of 7.9 hours. Independent double blind histological reviews were performed on selected sections including: heart, lungs, kidney, ileum, liver, pancreas, adrenal, trachea, thymus, and brain (mid-pons and cortex).

Gestational age and birth weight were obtained from obstetrical records and postconceptional age was compared with crown-heel length at autopsy. Both SIDS and control groups had a mean gestational age of 39 weeks. In the SIDS group there were 40 males and 21 females with 15 premature and 46 term infants. The mean birth weight in the term infants was 3.0 kg. The mean birth weight in the preterm infants was 2.5 kg. Two of the SIDS infants had siblings who died from SIDS.

Among the control infants there were 17 males and 17 females with 16 premature and 18 term infants. The mean birth weight in the term infants was 3.4 kg. The mean birth weight in the preterm infants was 2.7 kg. One control premature infant had a documented history of apneic episodes.

**Rapid Golgi Light Microscopy**

Immediately following the removal of the brain at autopsy, the
vertebral and basilar arteries were perfused with saline followed by 10% buffered formalin or 2% buffered glutaraldehyde. Then, eight to twelve 2.5 mm thick serial cross-sections, perpendicular to the pia surface, were cut under a dissecting microscope from the rostral pons through to the caudal end of the medulla. This orientation ensured that the tissue blocks and plane of section were cut as parallel as possible to the lateral dendrites of the brain stem. After fixation in 10% buffered formalin for not less than 60 days, a modification of the rapid Golgi stain was used:

Step 1. 1 gm/300 cc osmium tetroxide • 5% potassium dichromate (6 days)
Step 2. 3.5% potassium dichromate (2 days)
Step 3. 0.75% silver nitrate (4 days)
Step 4. 3.5% potassium dichromate (3 days)
Step 5. 0.75% silver nitrate (4 days).

From each case, 48 to 66 frozen sections were cut at 150 µm intervals, dehydrated, cleared through xylene, mounted on slides with Permount, and randomly coded. In addition, Nissl and Hematoxylin-eosin (H&E) stains were performed on selected sections of the pons and medulla in all 95 infants for examination of morphology and hypoxic-ischemic cell change characterized by eosinophilia and/or karyorrhexis.

Three well-impregnated neurons (3 or more dendrites able to be seen originating from a well-stained soma and demonstrating a minimum uninterrupted stained length of at least 250 µm parallel to the plane of section) from each area of the nucleus (n) ambiguus, n. solitarius, n. hypoglossus, n. spinal trigeminal, gigantocellular tegmental field, medial longitudinal fasciculus area, and n. lateral lemniscus were
selected from each case. After careful examination, 54% of the stained sections from all cases were suitable for quantitative study. All preparations were examined without knowledge of whether the section was a SIDS or control. With respect to the brain stem reticular formation, our data include the n. ambiguus, n. solitarius, gigantocellular tegmentum, and the medial longitudinal fasciculus area. We excluded from the reticular formation the n. hypoglossus, n. spinal trigeminal, and n. lateral lemniscus. The regional identity of the neuron was noted and dendritic spines within a segment on a lateral dendrite of each neuron were counted at 1000 X with an ocular micrometer. The dendritic segment length was 100 µm and began 50 µm from the soma. It was found that at any lower magnification, the correlation between spine counts from separate examinations was unsatisfactory. Our criteria for inclusion of a structure as a countable dendritic spine are precise. Fine processes with or without a terminal bulbous element, and lengths between 0.85 and 2.6 µm were counted as spines if they were in direct continuity with the dendritic shaft. Counts were performed only on well-impregnated dendritic segments which appeared parallel to the plane of section and whose diameters were between 1.0 and 2.8 µm. Many dendritic segments were not counted due to unacceptable obliquity or tortuosity. There was no overlap of a dendritic segment with branching points in the quantitation of the segment. A qualitative analysis also was performed within each 100 µm segment with regard to dendritic nodulation, varicosities, spine morphology, and their pattern of distribution. In order to facilitate a direct comparison of spine densities, all Golgi data has been expressed in spines per micrometer. This measurement was derived by dividing the number of visible spines counted along a dendritic segment by the 100 µm
length of the segment. Spine densities were calculated for the reticular formation, non-reticular formation, pons, medulla, and for all nuclei examined in the brain stem as the mean total densities. Counts were then compared to postconceptional and postnatal ages in both SIDS and control groups. In this study, visible spines were counted on a total of 1995 dendritic segments.

Transmission Electron Microscopy (TEM)

For TEM ultrastructural analysis, 26 infants (10 SIDS and 16 controls) were studied with EPTA. For the quantitation of synaptic profiles with EPTA, 8 blocks from the paramedian areas of the pons and medulla were fixed after mincing them in 2% 0.2M phosphate buffered glutaraldehyde at pH 7.4 and then dehydrated through ethanols. Blocks were then stained for 2 hours in 1% phosphotungstic acid (Fisher) in absolute ethanol containing a trace of water (4 drops of 95% ethanol per 100 ml of absolute ethanol). Blocks were then rinsed in absolute ethanol and embedded. All blocks were thin sectioned at 0.09 μm thickness using an LKB-III ultramicrotome. Three to ten non-serial sections from each block were placed on a 300 mesh copper grid. The sections were then examined with an Hitachi Model 12 transmission electron microscope using Dupont COS-7 film. Only one section per grid was chosen for photography. Ten randomly selected areas were photographed from the one section. The negatives were developed and printed on 8x10 Kodak kodabromide F5 paper at a final magnification of 15,000 X. A total of 2,080 prints were examined with a reading magnifier at 15,000 X in a grid square measuring an area of 130 μm² for each print. This area is relatively large as compared to an area of 90 μm² covered in micrographs in one study of the rat cortex.¹⁰⁷
EPTA is a means of assessing the maturity of synapses by dividing the stained profiles into an immature/mature continuum on the basis of the presynaptic densities. Development can then be examined by the quantitation of mature synaptic profiles. The criteria used for identification of a synaptic junction were: (1) a definite postsynaptic paramembranous thickening, (2) parallel pre- and postsynaptic densities separated by a cleft of approximately .03 to .07 μm in width, (3) the presence of cleft material in varying amounts, and (4) distinct presynaptic projections. Each of these elements can be seen from a full or partial lateral view and a junction was counted only if each of these features were evident. Mature synaptic profiles were defined as having a polarized appearance with triangular or globular paramembranous opacities along one side of the cleft with distinct separations between such presynaptic projections, a discernible amount of cleft material, and a prominent postsynaptic density. Synaptic profiles which were separated by greater than 0.2 μm from end to end were counted as two synapses. The synaptic density in mm² (N) of the mature number of synapses per case was calculated according to the formulae:

\[ N = \frac{n}{A} \times 10^6 \text{ μm}^2 \]

where n is the total number of profiles in the total area (A) sampled for each case with a mm² conversion factor of 10^6 μm².

The area per case was calculated by multiplying the number of prints for each of the 8 sections of the brain stem by 130 μm², which each print represents. The quantitation of mature synaptic density then was compared with postconceptional age as to SIDS and control groups.

A qualitative analysis was also made for each age group as to the height and width of the base of the presynaptic projections, the width
of the cleft, and the type of curvature of the profile. A positive
synaptic curvature is concave to the presynaptic membrane. A negative
curvature is convex to the presynaptic membrane. These parameters are
reported to alter with maturation, so that the degree of maturity can
also be demonstrated qualitatively. By means of these values, we
observed the ultrastructural characteristics of the maturation of
synaptic structures.

**Scanning Electron Microscopy (SEM)**

If more than 6 hours elapsed postmortem, the degree of preser-
vation was not adequate for SEM. Specific blocks of the pons and
medulla which were selected at autopsy were prepared for SEM analysis
in 2% buffered glutaraldehyde for 2 days. Sections were then placed in
1% buffered osmium tetroxide, dehydrated, critical point dried, gold
coated, and examined in an AMR scanning microscope at 30 KV. We examined
and measured reticular processes and configurations by correlating the
ultrastructure with rapid Golgi microscopy.

**Statistical Methods**

Quantitation of dendritic spine density and synaptic profile den-
sity within SIDS and control groups was compared by multiple linear
regression analyses. When a regression analysis indicated a signifi-
cant difference between the regression lines for the two groups, regions
of significance were determined by the application of the Johnson-Neyman
technique. Throughout the analyses, statistical significance was
calculated at the .05 level.
RESULTS

Golgi Qualitative Analysis

The areas of the brain stem which were examined are described in Figure 1. It is virtually impossible to describe quantitatively the interwoven pattern of fibers in these regions which are characterized by such extraordinary complexity and considerable dendritic overlap (Fig. 2). Thus, distinctions between reticular neuronal aggregates were based upon position of the cell body alone and not upon the extent of the dendritic arbor. The SEM micrographs illustrate an ultrastructural correlate with neuronal aggregation and dendritic morphology (Fig. 3).

Figure 4 shows a preservation of neuronal cytoarchitecture in the magnocellular and parvocellular regions of the medulla. Minimal hypoxic alterations in the reticular neurons of the pons and medulla in a few cases were seen which consisted of scattered eosinophilic neurons (Fig. 5). These findings were present in three SIDS infants at 6, 9, and 12 weeks of age, four control infants less than 2 weeks of age, and in two infants at 2 and 12 months of age. In this study, we do not reveal any qualitative difference in gliosis between SIDS and control groups.

During the postnatal period, parameters indicative of dendritic expansion and arborization increase markedly. In particular, the structure of the dendritic membrane undergoes a definite transition.
Two characteristics of the developing dendritic membrane are spinous processes and varicosities along the dendritic shaft itself (Fig. 6). Dendrites also may show a spine-covered tortuous pattern (Fig. 7). Between birth and 4 months of age, dendritic varicosity and tortuosity are remarkable. After this time, fewer varicosities and a less tortuous pattern are observed (Fig. 8).

Neurons of the reticular formation (RF) differ from neurons of the specific nuclei in the non-reticular formation (NRF). RF neurons have long, poorly ramified dendrites. Specific NRF neurons have numerous curled, densely ramified dendrites (Fig. 9). RF neuronal types are not confined to the RF with some found in the specific nuclei or scattered in different numbers throughout the brain stem (Fig. 10). The most commonly observed neuron is characterized by a radially organized system of dendrites emanating from a multipolar soma (Fig. 11). Both the RF and the NRF neurons appear to go through a common developmental sequence. A morphological comparison between the branching patterns of iso-, allo-, and idiodendritic neurons is shown in Figure 12.

What we interpret as the earliest observable change in reticular neurons appears in dendritic rearrangement—the dendritic silhouette becomes a dense arborization. Reticular dendritic shafts are seen in close apposition with each other, allowing for dendrodendritic interaction (Fig. 13) and the possibility of electrotonic coupling between dendrites. Dendritic arbor is minimal in the rostrocaudal dimension with neurons having long, lateral, branched dendritic arrays (Fig. 14). Figure 15 illustrates neuronal maturation to be a continuous process of change. Dendrites are at first covered with a dense population of
spines. Morphology is then gradually observed to change with a decreased number of spines. The most distinguishing feature of these dendrites is the extensive variety of dendritic spines. The heterogeneity of spine morphology is seen in short-stubby (ST), mushroom-shaped (MS), and long-thin (LT) spines (Fig. 16). The spine stem alignment is relatively perpendicular to the dendrite; however, dendritic spines also emerge from the dendrite in various directions and at different angles. This may further compound the difficulty in qualitative investigations of observable spines in Golgi preparations and in studies of spine quantitation.

Our initial semi-quantitative examination leads to the definite impression of progressive dendritic rearrangements and spine loss with increasing postnatal age affecting the entire dendritic arborization. Dendritic spine loss was observed to be predominantly of the LT geometric type with the last remaining spines being virtually all ST and MS spines (Fig. 17). However, there is no apparent pattern to this spine loss along the proximal and distal portions of the dendrite (Fig. 18). Spine-free regions do not alternate with regions of spine density. Comparison between control infants and age-matched SIDS infants demonstrates a striking morphological difference in the number of dendritic spines (Fig. 19). It is evident that there exists a greater number of spines in the SIDS group than in controls throughout the first year of life.

**Golgi Quantitative Analysis**

Interpretation of the significance of spine density requires analysis of the data for each area of the brain stem studied at progressively increasing ages (Table 1). The data show the number of spines
which are resolved at 1000 X rather than estimates of total spine number. It is important to point out that for any one of the three dendrites in a particular locus, the standard error in mean spine density is small (Table 2). In both groups of infants the mean spine densities of the seven dendritic regions exhibit a decrease in mean density of visible spines over the age range studied (Table 3). These data give a clear picture of progressive lateral dendritic spine loss with age in both SIDS and control groups. Thus, the maturational sequence of dendritic spine loss seems to progress uniformly throughout the depth of the brain stem.

In Figure 20, regression analysis illustrates that the rate of spine loss with age for both SIDS and controls is significantly different. From the actual spine quantitation, 85% of the spines are lost during the first year in the controls as compared with 55% lost in the SIDS group. This is indicative of a slower rate of spine loss in the SIDS infants. However, there is a consistently greater number of spines present throughout the first year in the SIDS group as compared with the controls (P < .0001 between 29 and 88 postconceptional weeks). It is also evident that the mean spine density in the controls decreases 78% between 34 and 40 weeks. Thus, the data clearly demonstrate that spine density decreases during the first year of life; however, the SIDS group has a greater density than the controls throughout this age range with a rate of spine loss significantly lower than that of control infants.

In Figure 21, both groups of term infants show a pattern of spine loss with SIDS infants demonstrating a higher density of spines than controls (P < .0001). There is a marked decline beginning at 3 months in controls while there is a gradual decline beginning at 4 months in
SIDS. In preterm infants (Fig. 22), a gradual decrease in spine density is shown to begin at 4 months in controls and at 3.5 months in SIDS with a greater spine density in the SIDS group ($P < .001$). These three quantitative analyses indicate a pattern of spine loss with a statistically significant higher spine density in the SIDS group as compared with controls throughout the first year. At approximately 58 postconceptional weeks and between 2 and 4 postnatal months in both SIDS and controls, the trend is that of a constant decline in spine density; however, a slower rate of decline is evident in SIDS.

**EPTA Qualitative Analysis**

The maturation of synaptic junctions in the infant brain stem is examined ultrastructurally with emphasis being placed on the paramembranous densities. In Figure 23, dense projections (DP), cleft densities (CD), and postsynaptic thickenings (PT) are clearly delineated at the synaptic junction. These are considered to be the most striking features of the mature synaptic junction. In the EPTA background, faintly opacified axons and neurotubules are evident as well as the paramembranous outlines (Fig. 24).

A constant feature throughout synaptic ontogeny is the postsynaptic thickening which is a continuous extension along the length of the junction. In the mature synapse, there are large dense projections, an intracleft linear density, and a very dense postsynaptic band (Fig. 25). The lucent lines separating these elements represent the unstained pre- and postsynaptic membranes and an asymmetrical appearance is evident in the mature form. Figure 26 demonstrates that the width of the cleft tends to decrease with maturation in both SIDS and controls, with the height and width of the dense projections increasing.
Figure 27 illustrates synaptic curvature and its overall relationship with the age of the infant. The negative and straight parameters predominate in the early perinatal period with a definite trend towards predominantly straight and positive curvature later in the postnatal year.

**EPTA Quantitative Analysis**

Our quantitation is of observable EPTA stained mature synaptic profiles. Although this staining method does not represent the absolute density of synaptic junctions, EPTA is a reproducible and reliable tool for the quantitation of synaptic junctions. In Figure 28, there is a statistically significant difference in mature synaptic densities (P < .0003 at 47.5 postconceptional weeks) between SIDS and control infants. Both groups show an increase in mature synaptic density with the SIDS group showing a slower rate of increase in density (slope = 0.017) as compared with controls (slope = 0.035). With extrapolation, both regression lines diverge at 9 postconceptional weeks. Therefore, this EPTA quantitation demonstrates that the SIDS group contains a smaller mature synaptic density and a slower rate of increase in mature profiles as compared with controls.
DISCUSSION

Dendritic Classification

It is important to understand that the brain stem is a command system which processes afferent signals through the rapid synchronous interaction of interwoven dendrites such as we have demonstrated in the infant brain stem (Fig. 2 and 13). In the brain stem, three main types of neurons have been shown in Figure 12: iso-, allo-, and idiodendritic. It was on this basis of their dendritic architecture that these neuronal types were first suggested. The term isodendritic (iso - uniform) became synonymous with generalized dendritic patterns; the terms allodendritic (allos - different) and idiodendritic (idios - peculiar) with progressively specialized dendritic configurations. The term isodendritic provides an objective criterion to aggregate most of the brain stem, which at one time was arbitrarily collected under the name of reticular formation. If one uses the term, isodendritic, the ambiguity is removed to a large extent since it is possible to determine if a particular cell group displays generalized, or specialized dendritic patterns. From the dendro-architectural point of view, the generalized, or isodendritic, neuron is characterized by radiating dendrites which follow a relatively rectilinear course as we see in our observations (Fig. 11). They branch so that the primary dendritic segments are, as a rule, shorter than the secondary ones. The most characteristic feature we have shown of the isodendritic core is the considerable overlap of the dendritic fields.
The isodendritic core is regarded as a primordial matrix which has played an analogous role in the phylogeny of the nervous system as that of the mesenchyme in the ontogeny of connective tissue.\textsuperscript{125} The more specialized allodendritic and idiodendritic centers would constitute regions of functional specialization within this pluripotent nervous system. As we observe in the n. hypoglossus, n. spinal trigeminal, and inferior olive, the dendrites of these neurons follow a wavy course (Fig. 9). The dendrites do not radiate significantly or diverge from the cell body. They seem to diverge from the main dendritic trunk rather than the soma. The isodendritic branching pattern in which the most distal segments are usually longer than the proximal ones does not hold for these specialized types as shown in our study and in those of other investigators.\textsuperscript{125} The isodendritic characteristics are consistent with the fact that their afferent connections are of a very heterogenous origin. It is also significant that there is an extensive overlapping of dendrites due to its diffuse receptivity (Fig. 2). We observed spines to be distributed in a uniform fashion along the entire extent of the dendrites which might ensure this high degree of integrative receptivity within a dendritic field. The dendritic arborization (Fig. 14) is most prominent in its lateral dimension with the majority of reticular dendrites seen to be running perpendicularly to the main longitudinal tracts as also was described by Scheibel.\textsuperscript{91}

It is important when making comparisons of different developmental stages to restrict the comparisons to corresponding regions of the nervous system. Dendrites of neurons with short axons (Golgi type II) have been found to differentiate later than dendrites of the principal neurons with long axons (Golgi type I). Since the rapid Golgi staining
method does not impregnate axons well, such neuronal types in our study are not differentiated.

After our preliminary investigation, this Golgi quantitation was conducted solely on the basis of rapid Golgi material to avoid morphological differences due to the use of different techniques. The rapid Golgi staining method produces less shrinkage of the tissue, and dendritic spine morphology is better impregnated and shows less distortion than, for example, the Golgi-Kopsch technique. The rapid Golgi method involves precipitation of silver chromate within the neurons after fixation in a solution of osmium tetroxide and potassium dichromate. It generally impregnates the entire neuron, but it is more capricious and is believed to result in more unusable sections than the Golgi-Cox method. The Golgi-Cox method consists of the precipitation of metallic mercury in the neuron after fixation in a mercury salt. This method fully impregnates the soma, impregnates the dendrites for most of their length, and shows only the initial portion of the axon. However, the metal may not fully penetrate into fine dendritic branches or into dendritic spines.

Methods for semi-automated, computerized analyses of Golgi preparations undoubtedly permit assessment of quantitative data on the length, shape, and spatial distribution of dendritic spines. The wide range of application of these computer-assisted methods for increasing the analytical power of Golgi preparations is evident in consideration of the developing CNS which exerts subtle changes in neuronal architecture. Golgi studies have included concentric ring analysis of branching patterns to supplement qualitative analysis. There is no agreed upon method for analyzing most accurately the branching patterns of dendritic arbor. The concentric ring analysis quantified intersections between dendrites and the
overlapping rings. This method gives an estimate of the total dendritic 
area, its location with respect to the cell body, and the order of 
ocurrence of dendritic branching from the soma. This analysis is 
highly sensitive to the growth of new dendritic branches. Dendritic 
analysis has also employed the use of a universal stage with four ro-
tational planes for the 3-dimensional study of neuronal morphology. This method allows the general view of the neuron in a wide range of 
observational angles and minimizes overlap of the neuronal tree. Two-
dimensional projections of the neuronal tree are obtained by rotating 
the stage, and the application of a coordinate transformation results 
in a 3-dimensional mapping.

The drawbacks and hazards of the Golgi staining method are well 
known. It is capricious and does not impregnate the entire neuronal popu-
lation actually present in the tissue. However, it is specifically advan-
tageous for quantitation of neuronal morphology and dendritic spines in 
a functionally meaningful context.

**Dendritic Bundles**

It is postulated that the neuron is not the fundamental element in 
stored information since many neurons degenerate and are not replaced. 
It is believed that neuronal information is provided by the assembly of 
interwoven dendrites into bundles which represent fundamental units in 
brain stem function. Dendritic bundling does not necessarily have a 
single function, but rather could serve in a variety of ways in different 
regions of the CNS such as processing, synchronization among neurons, a 
metabolic transfer mechanism in neuronal maintenance, and as a develop-
mental unit for brain growth. Brief allusions to bundling were made by 
Cajal at the beginning of the century in the visual cortex.
One possible function of dendritic bundles, suggested by Scheibel, in the brain stem is that of the role of programming for rhythmic repetitive output such as respiration, and the rest-activity cycle. Bundles have been found in the reticularis thalami of the cat where they are the possible sites for tuning and modulating the rhythmic slow wave processes in the thalamus and cortex. Bundles are present also in the olfactory bulb of the cat where their role as the programming sites for repetitive types of output is suggested. Bundles have been found in the ventral horn and anterior commissure of the spinal cord, neocortex, and in certain cranial nerve nuclei. In the kitten brain stem, it is of interest that these bundles are reported not to be present at birth, but become apparent later during postnatal development.

It has been hypothesized that the programs of synchronized output are "loaded" in the bundle via the presynaptic afferents terminating on the spine system which covers most of the dendrites in the postnatal period of the kitten brain stem. As spines were described to be lost, the programs are subject to modulation by perhaps another synaptic system. These events may correlate with the development of synchronized output functions such as the maturation of NREM sleep first observed in the infant after three postnatal months. This correlation with function points towards the need for a precise morphometric quantitative evaluation of bundling in the infant brain stem.

Central to this concept of the programming significance of the bundle is the extraneuronal space formed between apposed dendrites shown in Figure 13. Using the extended membrane concept of Lehninger and of Revel, bundles have been explained to be made up of an outer,
carbohydrate layer (the glycocalyx) extending towards each other through a hyaluronate-rich environment of negatively charged electrolytes. Most of the oligosaccharides are linked covalently to a glycoprotein backbone which composes part of the neuronal membranes. Some of the oligosaccharides are linked to lipid moieties and contain sialic acid (gangliosides). The close apposition of dendrites affords the opportunity for synaptic interaction between the macromolecular arrangements. Cation-mediated links between adjacent polyamines may operate under input-output restrictions which may be represented by patterns of electrotonic coupling known to develop in such specialized bundle settings. The resultant stabilization of cation-mediated links could lead to specific steric configurations. Such stabilized macromolecular patterns may incorporate the coded program which directs the output function representative of the bundle. The precise nature of the steric configurations and the mechanism by which their informational content is transcribed into output function represents a fundamental problem in neuroscience.

Reticular Dendritic Spines

Morphologically different synaptic structures are found to occur on dendrites in the brain stem. The variety of spine formations which we have observed and the great number of dendritic synaptic sites these spines represent indicate how this variety of structure may play an important part in the patterning of the reticular neuronal response. Spines which appear in our Golgi preparations (Fig. 16) are small protuberances which are characteristic of the morphological types of ST, MS, and LT spines. It is reported that the density of spine distribution may depend on such variables as distance from the
soma and the thickness of the dendrite.\textsuperscript{140} Spine quantitation, limited only to those spines which are visible, under-represent the true total number of spines on dendrites. The dendrite conforms to a reliable geometric cylindrical configuration. The domain into which the spines extend is concentric. Thus, Feldman and Peters have reported a geometrically based method to compute a true spine density, where the diameter of the dendrites and the length of the spines are expressed in terms of a 3-dimensional model.\textsuperscript{140}

Proximal apical cortical densities exhibit ST and MS spines. Basilar dendrites and distal apical segments show a preponderance of LT spines.\textsuperscript{139} In the mentally retarded child, it has been reported that there is an absence of ST and MS spines and a large number of very long LT spines.\textsuperscript{141} There was no obvious relationship reported between the degree of spine abnormality and dendritic length or branching pattern. This has been termed a dendritic spine "dysgenesis" and is believed to imply a defective development as the common feature of the micropathology of mental retardation in the cortex.\textsuperscript{141} This may affirm the importance of a synaptic dendritic spine dysfunction in developmental disorders of infancy and childhood.

What is the significance of dendritic spines? In the adult cortex they are shown to display asymmetrical contacts.\textsuperscript{142} The dendritic shaft itself displays symmetrical contacts. Thus, the presence of cortical dendritic spines reflects the presence of a particular type of synapse. What their functional significance may be is far from clear. During development, it is possible that at least all the asymmetrical synapses are on spines, and that these spines may retract into the dendrite which may be the case during the developmental loss of spines seen
in this study of the infant brain stem (Figs. 15, 19, 20).

In the brain stem, spines are reported to differ from their counterpart in the cortex. We have observed unevenly scattered spines of various shapes in the brain stem, often failing to form terminal elements. In contrast, cortical spines are more numerous and closely distributed.

Recent findings regarding the development of synapses have assumed that contact between pre- and postsynaptic elements precedes the appearance of vesicles and densities. However, it has been demonstrated with TEM that spines of Purkinje cell dendrites are found in the absence of attached presynaptic elements. The appearance then of dendritic spines without presynaptic contact implies either a degeneration of the presynaptic elements or the formation of spines in the absence of terminating afferent input. Regarding this hypothesis, the induction of spines may be triggered by a stimulation provided by synaptic contacts on other areas of the dendrite as reported in the cerebellum with contacts between parallel fibers and Purkinje cell somatic spines before the dendritic spines develop. Thus, it is believed that spines do not seem to require a one-to-one induction by a presynaptic element, rather it seems that the formation of spines may be induced by a generalized stimulus. Nevertheless, a principal concept is that dendritic and/or spine morphology is a function of presynaptic activity. Deafferentation can result in a loss of spines and/or dendritic branches early in cortical development. In the kitten, deprivation of visual input in the early weeks of life has been reported to lead to deformation and loss of visual cortex spines. Alternatively, increased environmental stimulation
has been found to be associated with an enhancement of dendritic spines and branching. In line with this concept of deafferentation and spine loss, we speculate from our observations (Fig. 15) that a rearrangement of afferents may play an integral role in dendritic arborization patterns and the decrease in spine density. Spine loss also could be due to deafferentation secondary to the degeneration of neurons or axons in the developing brain stem. However, spine loss could be an intrinsic phenomenon for the development of other types of synapses. In either case, it is conceivable that new and viable synaptic complexes may develop along the dendrite. Since synaptic loci are more accurately defined with TEM, the combined Golgi-EM technique by Farien would be an appropriate approach to resolve this question.

The Interrelationship Between Dendritic and Synaptic Development

The pattern of dendritic development may be traced to the view that the period of maximal dendritic growth and differentiation is critical for the elaboration of the receptive surface of neurons for diverse synaptic inputs. The application of the Golgi staining method has permitted the specification of the maximal phase of dendritic growth and differentiation of pyramidal neurons from the twentieth to the twenty-eighth week of fetal development. It follows from this that events which interfere with dendritic development during this critical period might result in less optimal dendritic surface area for synaptic interaction. The predominant number of LT spines in this early period of development is suggested to be an early attempt to make contact with growing axons entering the dendritic domain. It is clear that dendritic growth and synaptogenesis are interdependent processes.
Neuronal maturation has been reported to be a continuous process in the visual cortex of the monkey. At first, dendrites are covered with a large population of LT spines. Later, the dendrites bear a dense population of spines of various morphological varieties as we have shown in the infant brain stem (Fig. 16).

Although the diameter of the dendrite decreases with its increasing length, the size of the synaptic area has been observed to remain unchanged so that the number of synaptic sites per unit dendritic surface area remains constant with the distance from the soma. A possible mechanism for this dendritic lengthening is thought to be the absorption of spines which could provide the membrane necessary for extension. In the distal dendritic segments of the cerebellum, it has been suggested that a decrement in branching allows the dendrite to grow longer before it branches. These considerations allow for the future investigation of a quantitative correlation between dendritic length and branching patterns and spine density in the infant brain stem.

The general consensus from studies in the cortex of man is that dendritic spines are lost with age. Whether shrinkage of the dendritic diameter actually triggers spine loss, or whether the two processes are concomitant or independent is not known. It is thought, however, that spine loss precedes and may induce dendritic shrinkage since spine loss is a progressive and continuous process in the cortex.

It has been proposed that dendritic varicosities, as we have observed in the development of the infant brain stem (Fig. 6), may
represent a dendritic reaction to expansion and generation of branches. It is reported that dendritic varicosities first decline proximally and subsequently decline distally indicating that a shift of dendritic growth towards the distal extreme of the dendrite occurs during development. The functional activity of these varicosities may dictate future branching configurations by participating in dendritic expansion and the formation of afferent connections. Following deafferentation, varicosity formation has been related to alterations of dendritic neuroplasmic flow where varicosities have been found to demonstrate a decrease in neuronal RNA synthesis and thus protein synthesis. Varicosities have been associated with an increased amount of rough endoplasmic reticulum and thus the signal for varicosity formation appears to be the need for new protein production.

The decline in spine density does not necessarily suggest a decreasing amount of synaptic contacts as the development of the dendrite progresses. Other types of synaptic contacts which might not involve spines may henceforth appear or proliferate on the dendritic shaft such as dendrodendritic or electrotonic junctions. Whether or not the loss of spines indicates a functional impairment might depend upon the collective or individual influences of perhaps these other types of synapses in development.

Dendritic growth and branching patterns are generally considered to be essential parameters of the probability of neuronal interaction in normal and pathological situations. As we have observed in this study (Fig. 20 and 17), changes in the number and geometry of spines also
should have important consequences for neuronal operations and synaptic capability.\textsuperscript{156} The proper functioning of dendrites and spines depends upon their coming into proper contact with other neurites which are destined to form synapses between them.\textsuperscript{92} The survival of dendrites would then depend upon the formation of functional synaptic connections. Modifications of the dendritic arbor are then concomitant with the maturation of functionally specific neuronal networks. This is referred to as the "theory of natural selection of neuronal connections."\textsuperscript{92} Therefore, the orientation of dendritic bundles is believed to result from a process of remodeling concomitant with the maturation of afferents.\textsuperscript{157} It is clear that the neuron has the potential for plasticity of enhanced dendritic branching and bundling and that this plasticity may extend to dendritic spines and/or the formation of other new types of synapses as dendritic spine density is observed to decrease with age in the infant brain stem. This area is a critical dimension to be explored in this study.

**Dendrodendritic Synapses**

Analysis of the ultrastructure of the CNS has shown that dendrites contact each other at various sites.\textsuperscript{158} Dendrodendritic contacts have been reported to be recognized with chemical synapses as well as electrotonic junctions (gap junctions).\textsuperscript{158} These observations had been difficult to incorporate into the classical concept of neuronal connectivity, and forced a rethinking of the basic ideas of integrative functions of the neuron and the nature of neural processing by dendritic connections. Dendrodendritic synapses have been described in thalamic nuclei,\textsuperscript{159} the superior colliculus,\textsuperscript{160} the olfactory bulb,\textsuperscript{160} and the mesencephalic fifth nucleus.\textsuperscript{161} This synapse has been shown to be ideally suited
for meeting the spatial and functional requirements of local neuronal processing within neuronal aggregates and dendritic bundles. It is now apparent that dendrodendritic circuits are an efficient way to organize synaptic interactions in a minimum of space. Similar synaptic arrangements have been found in the retina of primates. Our Golgi (Fig. 13) and SEM observations of closely apposed dendritic surfaces are consistent with dendrodendritic synaptology; however, TEM studies are needed to further qualify this synapse between dendrites.

Normal and Aberrant Patterns of Cortical Dendritic Spine Development

Aspects of neuronal organization of the human cortex with Golgi have been studied in the analysis of the structural morphology of the cortex. Conel described the postnatal neuronal development of the cerebral cortex and others have reported Golgi studies of human prenatal development. Marin-Padilla observed that the number of apical dendritic spines in pyramidal neurons increases with prenatal age while the pattern of spine distribution remains fairly constant. A noteworthy feature of the temporal pattern of dendritic development in the cortex is the extent to which dendritic spine differentiation occurs in the gestational period. Dendritic spines appear in moderate number during the seventh fetal month and continue to increase in number through the perinatal period. TEM studies have revealed the presence of axo-dendritic synapses in human fetal cortex prior to the end of the first trimester. These observations suggest that the period spanning 20 to 28 weeks of gestation is a phase of maximum dendritic growth and differentiation of cortical neurons with synaptogenesis occurring much earlier. The normal term neonate is equipped with the basic dendritic branching patterns at birth. Dendritic spine development and synaptogenesis have
been shown to reach a peak by the sixth postnatal month. The fact that much of the increase in dendritic surface area in cortical neurons occurs during the last trimester renders cortical dendritic development particularly vulnerable to noxious influences at this time. Hence, the wide spectrum of qualitative and quantitative developmental studies showing alterations in neuronal geometry and aberrant spine development may indicate the abnormal integration of dendritic synaptic integration in the cortex during gestation.

In the 18 week old fetus, cortical LT spines have been detectable on highly irregular dendritic shafts. At 26 weeks gestation, many more LT spines with multiple terminal heads are evident. At 33 weeks, LT spines predominate with a great increase in their number. Also at this time, both ST and MS spines have been demonstrated as well. In the cortex of a 6 month old infant, LT spines have been reported to be reduced, and at the same time there was noted an increase in the number of ST and MS spines. Such dendritic spine typology seems to have a developmental basis. From these reported data, the elaboration of LT spines has been shown to proceed through a fetal phase in which they predominate. Whether LT spines are progressively transformed into ST types or replaced by the latter during synaptogenesis is not known. It is also unclear whether a single class or different types of presynaptic inputs make contact with these different types of spines. It is important to note that between 3 and 4 months in the infant brain stems we studied (Fig. 21 and 22), loss of all three types of spines was observed, with the loss of LT spines being the most remarkable (Fig. 17). Therefore, we observe that the postnatal trend in the infant brain stem is towards a changing proportion of LT and ST spines with the last
remaining predominate spines being of the ST type, as the density of both decreases with age.

The Golgi stain has established a morphological basis for several categories of neurological diseases: epilepsy, mental retardation, chromosomal aberrations, and degenerative diseases. There has been much interest in pursuing investigations of the number, distribution, and morphology of dendritic spines in cases of mental retardation since the investigations of Marin-Padilla, who utilized the rapid Golgi method for the first time in studies of spine abnormalities in immature cerebral cortex. He demonstrated that in infants with chromosomal aberrations and mental retardation, cortical dendritic spines were all very long and thin or all very short and thin. An additional example of spine pathology was observed in a 3 year old child with seizure activity and mental retardation. The proximal dendrites had displayed a loss of spines with only very few LT spines. The concept of a maturation arrest has been supported by the persistence of abnormally long LT spines in the reported cases of mental retardation. From the standpoint of synaptic capability, the presence of LT spines has been demonstrated to provide less effective postsynaptic current into the dendrites. This is explained in the concept that the length of the spine may significantly alter the efficacy of integrative capabilities as a consequence of spine stem resistance and dendritic shaft resistance.

In another study on retarded children, Purpura again found the principal deficit to be an increase in the number of LT spines which he attributed to an apparent retention of the embryonic condition. The fundamental question of whether a reduction in spine density actually means a loss of synapses still remains unanswered. These studies of
cortical dendritic spine abnormalities in mental retardation provide a firm basis for pursuing abnormalities in synaptogenesis as a major part of the developmental pathobiology of the neuron since the appearance of spines is significant in terms of the functional maturation of synaptic organization. However, the functional maturation in the infant brain stem is far from being well understood.

The density of dendritic spines which we are investigating in the infant brain stem has been considered in the rat brain stem to express the state of dendritic development following a uniform pattern of change over time. Ramon y Cajal had described a dense array of spines in the infant brain stem in 1909. In the kitten, brain stem dendrites are reported to be covered with spines which are entirely lost during the postnatal period. It is not known what happens to the synapses on the spines which disappear. Scheibel has suggested that there are temporary synaptic connections which later disappear or are displaced to other parts of the neurons. In our investigation, observations on comparable rapid Golgi stained human tissue are in general agreement with the finding of a loss of dendritic spines which Scheibel reported in the kitten. In contrast to the development of cortical dendritic spines with their peak density at 6 postnatal months, our data show a declining trend in spine density from birth with a marked decrease between 3 and 4 months in both SIDS and control infants (Fig. 21 and 22). The fundamental point to be made is that this density decreases at a slower rate in the SIDS infants as compared with controls (Figs. 20, 21, 22). However, the most remarkable parameter in the SIDS group is that significantly more spines persisted throughout the first year than were quantitatively seen in controls.
Synaptic Modification and Electrotonic Transmission

In 1952, observations were made that the spine stem provides electrical resistance which attenuates the effect of the synapse on the neuron itself. It was argued that because of this attenuation, a neuron could be fired only by a large number of inputs. However, it is now believed that spine resistance adjusts the relative potency of different synapses on the dendrite. Synaptic potency could also be affected by the geometry of dendritic arborization. As a dendritic pattern changes or as bundling occurs, there also may occur a concomitant change in the arrangement of synapses within the dendritic arborization and a change in the threshold of the entire neuron. Rall was one of the first to recognize the importance of dendrites in the synaptic integration of neuronal activity. He observed that electric currents flow throughout the dendritic tree which are set up by the synaptic contacts. Rall has pointed out that the spine stem is a critical site for the control of electrotonic current between the spine head and the dendrite. There is an impedance match between spine stem resistance and the dendritic input resistance. ST spines, then, would be more effective in passing synaptic current from the spine head than LT spines. The point to be emphasized is that even relatively minor changes in the dimensions of spines can be expected to have important neurophysiological consequences for synaptic interactions.

Since it has been demonstrated that LT spines offer greater resistance than ST spines, such synaptic plasticity may provide us with a clue on how synaptic activity is affected by spine development and the neuron's genetic program. The resultant plasticity towards ST spines
which we have observed in our study of the brain stem as spine density decreases could provide the necessary requirement for a synaptic modification to be more adaptive to this decrease in electrotonic resistance. It is for this reason that we first considered that electrotonic junctions may be present in the brain stem as the density of chemical spine synapses decreases.

The literature on spinous synapses suffers from a number of weaknesses: (a) not accounting for plasticity of connections, (b) not knowing whether there is a true loss of spines or a failure of maturation, (c) not knowing whether spine reduction indicates a reduction in the number of only certain synapses. Failure to see a particular synapse does not mean necessarily that the organism cannot generate the required structure, but it may indicate that it does not develop the necessary gene expression for that particular site or that other factors are regulating its absence.

It is now accepted that communication between neurons can be mediated by both chemical and electrical modes of transmission. Numerous electrophysiological studies have demonstrated the existence of electrotonic coupling in the mouse mesencephalic trigeminal nucleus and in the cat cerebellar cortex between basket cells. Electrical (ephaptic) interactions occur almost exclusively as electrotonic coupling via a low resistance junction with gap junctions (GJ) as their morphological correlate. In the CNS, the scarcity and strategic locations of this specialized GJ favors the concept that ephaptic transmission is in accordance with functional specialization. Electrotonic or ephaptic transmission depends upon electrical properties. Synaptic or chemical transmission is characterized by much
more elaborate functional specializations. Electrotonic transmission has been postulated to be an evolutionary remnant recalling the epithelial origin of the CNS.\textsuperscript{171} It appears to have developed in regions where a strong synchronous activity is required within neuronal interactions of a common function. The integration of many intercellular signals may develop various synaptic connections to accommodate the enormous variability in performance.

The term electrotonic is used because transmission at these junctions is similar to passive electrotonic spread down an axon.\textsuperscript{172} Electrotonic transmission is usually bidirectional; however, differences in resistance on either side of the cell can lead to unidirectional effects also. This transmission is fast and allows a close synchronization of electrical activity between coupled cells in synchronously active systems. Impulses can propagate between cells more rapidly than would be possible with dendritic spine chemical transmission which is readily fatigued by repetitive stimulation. Thus, electrotonic junctions mediate electrical signalling between neurons and, more importantly, for our consideration of central respiratory control, they have been reported to be prominent in systems which respond synchronously such as the oculomotor nucleus.\textsuperscript{173}

Electrotonic junctions exhibit two characteristics which distinguish them morphologically from chemical synapses. First, the membranes are much more closely apposed than the width of the cleft which separates the chemical synaptic membranes. The small gap between these closely apposed membranes was described by Revel and Karnovsky and named the GJ.\textsuperscript{174} Second, the area of close apposition exhibits a symmetrical appearance in that there are no specialized...
structures or inclusions in the cytoplasm that would indicate morphological polarity. Such morphological symmetry is in contrast to the asymmetry of the unidirectional mature chemical synapse (Fig. 25). Thus, the most important morphological criterion for identification of a neuronal junction as a GJ is the presence of this close apposition between membranes. Whether axosomatic, axoaxonic, dendrodendritic, or somatosomatic, electrotonic junctions play a role in directly coupling cells through synaptic synchronization.

It has been demonstrated by Bennett that the electrotonic junction has its morphological correlate in a special junction of the zona occludens (ZO). He has shown that low membrane resistivity occurs at this junctional region and described electrotonic junctions as pentalaminar structures similar to the ZO. However, it has since been observed that the outer leaflets of the apposed membranes in the electrotonic junction are not fused, rather there is a narrow gap. Consequently, the electrotonic junction is a seven-layered rather than a five-layered structure. Profiles of this close apposition have demonstrated that the gap is not a continuous empty space, rather this gap consists of a 100 Å hexagonal lattice of polygonal arranged channels which are continuous with the extracellular space. In contrast to the GJ, the ZO plays an important role as a diffusion barrier; however, it may represent stages in the formation of the GJ. The GJ has been described as a macula or spot contact between membranes. The ZO has been described as a complete band around margins of the cell and prevents leakage across the membrane. In studies on embryos, formation plaques are separated by a wide intercellular space. The GJ begins to appear on the formation plaque and the intercellular space reduces
in width. Neural tube cells have been seen to have GJ's which suggests that electrotonic junctions are a "neoteric" trait. It has been proposed that, in addition to electrical communication, the GJ plays a role in intercellular recognition and is implicated in the mechanism of synaptogenesis specificity. The GJ also may serve a role in maintaining the differentiation state in mature tissue. In some studies referring to the GJ, micrographs do not always provide an unequivocal interpretation to establish this type of junction because of the plane of section or the preservation quality of the tissue. A future investigation of whether gap junctions can be identified in the reticular formation of the infant brain stem is a critical dimension to be explored.

**Synaptic Uncoupling and the Gap Junction**

Growth of the dendritic membrane and of the neuropil emerge as important parameters in the modulation of cellular communication in development. It has been postulated that the differentiation of the GJ may involve two competing regulatory processes. There may exist a continued increase in the conductance of the synapse with age resulting from the growth of the closely apposed junctional membranes. Also, there may be a decrease in shunting of synaptic current through the extrajunctional dendritic spines as they are seen to decrease in number with age. We speculate that the conductance increase of the synapse may counterbalance this shunting with the spines becoming functionally uncoupled. Whereas anatomical uncoupling may be important for cellular differentiation, it is attractive to speculate that functional dendritic spine uncoupling may be important in allowing the reticular neurons to perform a different behavioral role during brain stem
development with the modulation of arousal and sleep states, respiratory control, and chemoreceptor activity.

This hypothesis of dendritic spine uncoupling may not only change the character of functional outputs, but allow the neurons to employ a synchronous activity through the functional maturation of the GJ and more appropriate synchronous control of functional programs. Hence, this postulate may very well provide a structure/function correlation in SIDS where the persistence we demonstrate of a high dendritic spine density may prevent a critical electrotonic capability for adequate dendritic synchronization in the brain stem.

Nor far apart from this hypothesis, is the reported observation that the cells of the chick ciliary ganglion were found to be first innervated by chemical synapses, and then to be followed by the appearance of the GJ. These cells had been innervated by fast afferents which are thought to have induced the formation of the GJ. This provides another hypothesis that it may be the velocity of presynaptic fibers which determines whether the GJ is to be formed in this region.

Schmitt has suggested that the dendritic bundle is ideally suited for the interaction of dendrites and the dendrodendritic gap junction (DDGJ). Mollgard and Moller also have suggested the possibility of the DDGJ within the bundles of neuroblast processes in the fetal visual cortex. The DDGJ has also been reported in the inferior olive, and the mesencephalic fifth nucleus of the mouse where they occurred between the smooth surface of dendrites. It is believed that the DDGJ also functions in keeping the various dendritic processes in a fixed relationship during the stages of cell migration.
The dendritic arborization of the inferior olive is an interesting case with regard to the DDGJs. Closely apposed central dendrites in this area of the brain stem were found to exhibit dendritic appendages which group together in a glomerulus and form DDGJs while chemical synapses were seen to surround this glomerulus structure. Dendritic bundling also may represent the organization of reticular neurons electrotonically coupled by DDGJs. We speculate that the development of electrotonic junctions may be regulated by a quantitative decrease in spine density. Perhaps, the consistently greater density of spines in the SIDS infants as compared with controls may be correlated with a delay in sufficient dendritic spine uncoupling and be associated with a lag in the development of electrotonic synchronous function.

**Synaptogenesis**

The development of synapses is indisputably one of the central problems in developmental neurobiology. It is also indisputable that gaps in our knowledge of synaptogenesis remain to be filled. Although ultrastructural identification of synapses is not sufficient to demonstrate the necessary conditions of transmission function, physiological data have shown that when synapses are found, there is a correlation with the onset of function. Even though the chemically transmitted synapse is most commonly found in the CNS, the electrotonically coupled junction must now be kept in mind.

A continuum of morphogenetic changes in synaptic profile maturation has been reported in EPTA stained sections suggesting a sequence of developmental stages in the morphogenesis of synaptic junctions. Presynaptic processes have been shown to contain glycogen particles, mitochondria, and clustered vesicles close to the presynaptic membrane.
The postsynaptic density contains mitochondria, smooth endoplasmic reticulum, dense bodies, micropinocytotic specialization, and a dendritic spine apparatus. Profiles exhibiting the typical features of dendrites, where polyribosomes and synaptic vesicles are also present, have been reported in the presynaptic position. The specificity of the postsynaptic membrane may be related to the molecular pattern of the membrane itself. This hypothesis has been formulated as the "mosaic concept" of neuronal membrane organization.

The problem of synapse formation invites a multidisciplinary analysis with the question being whether or not the development of receptors requires an active participation of the nerve terminal. The interaction between the two components might be provided by either a neurotransmitter molecule itself or by an unidentified neurotrophic factor(s). It is believed that a relatively low rate of transmitter release from an immature presynaptic terminal may be sufficient to trigger neurotransmission and also synaptogenesis. Attempts at quantifying synaptic development have been made by a number of workers in recent years. Cragg has investigated the rate of synaptic development in different areas of the cortex. Our quantitative study of postnatal mature synaptic development may be best complimented with the overall measure of DNA content in the brain stem. It has been reported that the DNA content in the rat brain stem, which reflects cell number, remains constant during the first month of life which indicates that a full complement of reticular cells already exists at birth. This high cellular density is believed to result in a decrease in the extraneuronal space causing a decline in dendritic growth and perhaps synaptic development.
Gray described two types of synaptic contacts. The type I synapse has a very prominent postsynaptic density. In the type II synapse, the cleft is narrower and the postsynaptic density is less pronounced. In the cortex, type I has been found on dendritic spines and type II have been established on the soma, whereas those on the dendritic shaft may be of both types.

More applicable is the classification of Colonnier, who recognized asymmetrical and symmetrical synapses depending respectively upon the presence or absence of a thick postsynaptic opacity bordering the postsynaptic membrane. These correspond with Gray's types I and II. Thus, a junction with a wide postsynaptic band is termed asymmetrical (type I) and that with a narrow postsynaptic band is termed symmetrical (type II). In general, synaptic junctions are reported to appear symmetrical at first and then asymmetrical with maturation.

We observe that the presynaptic projections add an asymmetric polarity (Fig. 25) which serves to distinguish this junction from other non-synaptic junctional complexes. Synapses have been shown to first appear as profiles lacking any distinction between pre- and postsynaptic specializations. They represent a desmosomal-like appearance. It has been suggested that the basic sequence of synaptogenesis is similar for the entire CNS, and differences result from the relative speed at which the postsynaptic density thickens compared with the development of the presynaptic projections. Indicators of synaptic maturity are the height and base of the dense projections which we found to increase through development and a decrease in cleft width which we have demonstrated (Fig. 26). The number of dense projections has not demonstrated a correlation with developmental stages. Bloom
concluded that the main ultrastructural change during maturation is the gradual increase in the density of the dense projections. In another study, it has been shown that presynaptic projections tend to enlarge with maturation. This trend to increase in size is a consistent one shown in the infants we studied (Fig. 26). The protein accumulation in the postsynaptic density is thought to increase the structural integrity of the synapse through disulfide bridges between already existing proteins. Therefore, postsynaptic binding glycoproteins are a strong candidate to control the completion of synapse stabilization. During the early stages of synapse formation, it has been reported that the appearance of the postsynaptic density precedes other paramembranous structures. Qualitative studies have been unable to demonstrate a correlation between significant synaptic length changes and maturation.

In the study of synaptic parameters, synaptic curvature also has been shown to be a reliable index during development. Curvatures are categorized into negative, straight, and positive. This is believed to be the result of the plasticity of the synapse and may provide an indication to synaptic activity. It has been argued that curvature represents function/nonfunction, excitatory/inhibitory models. Negative curvatures are thought to be non-functional, and may represent regions where the presynaptic terminal exerts little control. With maturation, straight synapses have been shown to increase in frequency with negative curvatures predominating early in development. Our qualitative analysis shows a definite trend towards a predominant pattern of straight and positive curvature through the first postnatal year (Fig. 27). These results describe a maturational shift away from
the negative curvature.

**Glycoproteins in Synaptic Development**

Neurons expose on their soma and dendritic surface a carbohydrate-binding protein which may be involved in the formation of synapses. Consequently, ingrowing axons have receptors or macromolecular configurations which are complementary to those present on the neuronal surface. Cell surface glycoproteins have been shown to play a role in cell recognition and in the establishment and maintenance of intercellular connections. They may also be involved in the establishment of the appropriate circuitry in the CNS. Synaptosomal membranes have been isolated at different stages of development and have shown a progressive enrichment in glycoproteins with the major increase being associated with the maturation of the synapse. This has suggested that they take part in the building up of the immature presynaptic membrane. It also has been demonstrated that the synaptic cleft contains a relatively high concentration of glycoproteinaceous material which may be responsible for the strong adhesive property of the synaptic membranes.

**EPTA Considerations**

Aghajanian first made use of the EPTA staining method to examine the formation of synaptic contacts in the rat cortex at relatively low magnification. The EPTA approach must be quantitative to study the aspects of synaptogenesis since only in a few instances are the changes so extreme that a qualitative change in ultrastructure will be obvious. With the EPTA method, membrane structures do not appear stained. In contrast, there is an intense staining of the paramembranous components of the synaptic junctions. Thus, this method allows
quantitation of the numerical density of junctions.

Phosphotungstic acid is a high molecular weight compound which is used as a precipitating agent in the isolation of basic amino acids.\textsuperscript{200} It has been shown that selective staining of synaptic material with EPTA implies proteinaceous macromolecules containing relatively high concentrations of basic amino acids.\textsuperscript{200} EPTA becomes the exclusive electron-staining chemical and as a result none of the plasma membranes nor intracellular organelles such as mitochondria or synaptic vesicles are electron opaque which would increase the difficulty of accurately quantitating the synaptic profiles.

Osmium tetroxide produces the general staining of cellular elements which also adds to the difficulty in synaptic quantitation by this conventional method. In the developing CNS, several investigators have reported that osmium-UL stained synapses appear prior to synapses stained with EPTA.\textsuperscript{201,202} It is believed that the demonstration of EPTA stainable material at the synapse is associated with the onset of function.\textsuperscript{203} With regard to these two staining procedures, Bloom and Aghajanian have shown that EPTA complexes are qualitatively equivalent with the osmium stained profiles.\textsuperscript{118} Vrenson showed that EPTA and osmium are also quantitatively equivalent.\textsuperscript{204}

Equidensitometric analytical techniques also have been used in the study of synaptic ultrastructure so as to arrive at possible principles underlying neurotransmission.\textsuperscript{205} The negative of the micrograph (EPTA) is copied onto lithographic film and then contact printed with Agfacontour to obtain linear equidensity. The irregular outline of the negative image of dense projections is evident with the exact position of any point on their perimeters readily seen and suitable for quantitation.
In addition to EPTA, recent reports have shown that the Maillet stain (zinc iodide - osmium tetroxide) selectively impregnates synaptic vesicles and also may be considered a most specific and reliable synaptic staining method. 206

Developmental Vulnerability

Once synapses have been established, they increase in number during the brain growth spurt; however, there may be more than one period of rapid increase over the developmental period. 207 Studies have shown that the rate of brain development is not linear, but that there are sudden increases at certain moments. 191 These are called the growth spurt periods. Cell multiplication, migration, maturation, synapse formation, and death are not directly genetically determined, but rather are the resultant of the interplay between genetic expression and epigenetic factors. In the CNS, alterations of epigenesis can have far-reaching consequences since there is a critical precision necessary in forming neuronal circuitry.

In the human, neuron formation and migration have been demonstrated to commence after the first six weeks of gestation and lead to the consideration that abnormal circumstances may affect the fetus even at this early time. 191 Cell proliferation and migration seem to be completed after the first six months and after that time it appears that only glial cells and a few neurons are formed. The vulnerability of the different brain regions depends upon the stage reached in their development. 208 Although this is a valid concept, it is difficult to give an exact interpretation because of the variability in the timing of brain development in different CNS regions and types of cells. Brain maturation begins during gestation and is not complete until two years
of postnatal life. The hypothesis of vulnerability by Dobbing places the critical period at the time of fastest growth—from the thirteenth week of gestation.\textsuperscript{209} According to this theory, periods of immaturity and maximum growth correspond to periods of maximum vulnerability.

There is a body of evidence that demonstrates when the brain goes through this growth spurt period, it is particularly vulnerable to environmental modification. In one study, the increases in brain weight per unit time in months were calculated to have one peak at 32 weeks gestation, and a second peak at 5 postnatal months.\textsuperscript{210} Results in the cortex have indicated there to be a period of slow growth between 18 and 20 weeks, and between 30 and 35 weeks of gestation.\textsuperscript{211} There is then a marked increase after 36 weeks until term.

The growth spurt in the human fetus occurs in mid-pregnancy with the vulnerable period beginning at about 30 weeks of gestation.\textsuperscript{212} It has been shown that the human brain growth spurt is largely perinatal. Dobbing reported that the growth spurt begins in the brain stem region before it appears rostrally in the forebrain or cerebellum.\textsuperscript{212} It also has been demonstrated that most structural elements of the brain stem appear relatively mature at birth compared with the cortex.\textsuperscript{91} It seems very probable that reticulo-reticular circuitry is functional at birth to support initially the integrative mechanisms of cardiorespiratory control. Therefore, quantitative studies of synaptic development might be expected to differ between the brain stem and other regions of the brain. Since our data show that dendritic spine densities decrease with increasing postconceptional (Fig. 20) and postnatal (Figs. 21 and 22) ages, and that spine density increases up to 34 postconceptional weeks and then dramatically decreases 78% at 40 weeks (Fig. 20), the
brain stem growth spurt seems to occupy the prenatal period rather than show a significant synaptogenesis in the postnatal period. Since our data indicate that the SIDS infants show a consistently lower density of mature synapses than do controls (Fig. 28), vulnerability in the postnatal period may be accentuated in SIDS. Thus, changes in sensory afferent input and in nutritional-hormonal sufficiency might potentially continue to alter the maturation of synapses and synaptic capability in SIDS.

Data have suggested that neurons acquire their full complement of synapses by one year of age in the infant cortex, and that synaptic loss occurs subsequently.\textsuperscript{213} On the basis of present knowledge, cortical synaptic density increases from birth to one year with synapses gradually taking on a mature appearance at 6 postnatal months. The development of synapses has been found to be retarded in models of mental deficiency—neonatal thyroidectomy causing cretinism in rats,\textsuperscript{214} postnatal malnutrition in rats,\textsuperscript{215} and also with visual deprivation in cats.\textsuperscript{216} However, one study of synaptic density in the mentally deficient human cortex revealed no deficit in synaptic development.\textsuperscript{190}

**Synapse Elimination and Spine Loss**

A critical point which has been made in developmental studies devoted to synaptogenesis is to determine if matching of pre- and post-synaptic membranes is achieved through recognition processes or is the result of an initial diffuse stage of connectivity with subsequent elimination of redundant terminals. Studies on animal cortex show that synaptic density in the newborn is 10\% higher than in the adult.\textsuperscript{107} Cragg, in the visual cortex of the kitten, found that synaptic density was 50\% higher in the newborn than in the adult.\textsuperscript{217} These findings
confirm that the synaptic density in the cortex declines late in development.

It has been shown that neurons become particularly vulnerable during not only the growth spurt but also during a limited time course in which they are making and receiving major synaptic connections. During development, the neuron acquires the capability to interact within the neuropil through the development of membrane proteins. These proteins may serve to guide migration and in the disposition of axons and dendrites with the matching of particular synaptic connections. As dendritic growth and synaptogenesis is evident, this is when most of the plasticity of organization becomes expressed by the rearrangement of dendrites and afferents. Hereafter, a period of consolidation is believed to ensue—enhancement of correct viable connections and elimination of others. Finally, the neuron develops a functional identity. This is believed to be in part due to the development of an ability to select a specific pattern of inputs from a wide variety of connections and to maintain this pattern beyond the critical period. This period of synaptic elimination has been suggested as the basis for a critical period in development. From this view, the critical period ends when the process of elimination has progressed to the point where few synapses are capable of competitive interaction. Thus, a major function of dendrites may be to establish distinct spatial domains and bundles to minimize competition between presynaptic afferents. Such competition may cease when synchronous outputs remain or when multiple inputs become spatially or electrotonically segregated on neuronal dendrites.

The number of connections associated with the neuron is large, and specific circuits are required for efficient functioning which might be
selected from the overabundance of connections. This may be accomplished by reinforcing certain synapses while repressing others which are still present. A possible explanation of the SIDS group having a higher number of spines (Fig. 20) and a lower number of mature synapses (Fig. 28) may be that neurons are connected into incorrect circuits during this critical period through a delay in the developmental program, or that not enough of a certain type of synapse is functioning properly. The effectiveness of synaptic interactions has been reported to depend upon the number and the strength of the synapses. The strength of a synapse may depend upon the transmitter, the number of postsynaptic channels opened, the distance of the synapse from the soma, or the type of junction itself, whether chemical or electrotonic. To the extent that the strength is also influenced by postsynaptic geometry, dendritic arborization and bundling would also affect the outcome of synapse elimination.

Loss of synaptic contacts is not an uncommon phenomenon in neuronal development. For example, muscle groups have been shown to receive more motor fibers innervating them early in development than are characteristic of the adult. Our quantitation of a decrease in spine density between 3 and 4 months in the infant brain stem (Figs. 21 and 22) does not necessarily indicate the end of synaptogenesis at this time or that the loss of synapses occurs from that point onward. Rather, there is a continuous process of forming and losing synapses during the postnatal time course. The rate of synapse formation, then, may exceed the rate of attrition. However, beyond this point the rate of removal may exceed the rate at which synapses are generated. It is important to realize that the elimination of contacts may be as selective and con-
structive towards the final function of the brain stem as the formation of specific synaptic contacts.\textsuperscript{152}

Our EPTA analysis indicates that mature synaptic density is significantly different between SIDS and controls (Fig. 28). The smaller density of mature contacts in SIDS may be a particular feature of the SIDS brain stem which underlies a greater sensitivity of this paramedian region of the brain stem during the normally critical postnatal period.

Therefore, the formation of neuronal circuitry during development is a complex process. Although relatively few regions of the nervous system have been examined so far, synapse elimination is believed to be a general feature of neuronal ontogeny.\textsuperscript{112}

Trophic Factor and Vitamin Considerations

Another developmental adjustment which appears to ensure quantitative accuracy in neuronal circuitry is the competition for and the effects of trophic factors.\textsuperscript{221} A group of peptide hormones have been reported that can stimulate proliferation of glial cells and which affect brain growth and maturation.\textsuperscript{222} Glial maturation factor (GMF) is one of these factors isolated from the brain and is distinct from nerve growth factor. One study of neuroblastoma-glioma hybrid cells has shown that synapse formation decreases with the treatment of GMF.\textsuperscript{223} This low rate of formation was believed to be due to the retardation of neuronal differentiation because of the enhancement of glial properties. There also is evidence that the metabolism of astrocytes is related to synaptogenesis and that glial cells provide a radial guidance for neuronal migration.\textsuperscript{224} Studies have described a qualitative relationship between the intensity of glial reaction and the degree of spine loss, and dendritic distortion.\textsuperscript{225} We do not observe any marked difference in glial
reaction between SIDS and controls at the qualitative level. However, GMF and glial activity may be a regulatory variable in the influence of synaptic and dendritic maturation.

 Extracellular potassium ion concentrations have been found to have measurable effects on synaptic activity.\textsuperscript{226} The frequency of endplate potentials depends on this potassium concentration. Glial cells are selectively permeable to potassium and act as potassium electrodes. Removal of glial cells from pre- and postsynaptic processes, where they separate such structures from other neural elements, has been shown to allow diffusion of the excess extracellular potassium away from neuronal processes.\textsuperscript{227} This is thought to lessen the potassium effect. Glial cells maintain and modify these potassium concentrations which, then, may have an effect on the development of electrotonic communication observed in the CNS. It is of interest to note that the pyramidal dendritic spine has been shown to have an incomplete astrocyte sheath around its stem. However, no specialized membrane contacts were noted between the spine and the glial cell.\textsuperscript{228} In contrast, Purkinje dendritic spines have been shown to be completely surrounded by astrocytic processes and close membrane contacts have been found between these spines and the glial cell. These spine stem appositions with glial membranes clearly have been described as gap junctions. Interestingly, the GJ has never been observed before to link neuronal and glial membranes.\textsuperscript{178} The absence of such a specialized junction in the pyramidal cell is thought to be related to the much longer spine stem and the presence of a spine apparatus in this cortical cell as opposed to the Purkinje cell. Since we qualitatively demonstrate that ST spines are predominant in the infant brain stem (Fig. 17) and that they also are associated with a low
electrotonic resistance, they may influence the development of the GJ at these sites on the dendritic membrane. The further investigation of this ultrastructural relationship between the spine stem and glial cell is required before we may correlate a glial-spine relationship with the appearance of the GJ in the infant brain stem.

Vitamin B₆ has been demonstrated to be an essential nutrient in the normal development of the CNS. Maternal vitamin B₆ deficiency has been shown to result in morphological changes in the dendritic growth of Purkinje cells in the developing rat brain. Reports have shown a decrease in the dendritic field area which is also consistent with other nutrient deficiencies including protein and undernutrition. Wallingford has indicated that delayed neurogenesis may be central to the reduction in the final spine density in the cerebellum of vitamin B₆ deficient animals. Vitamin B₆ participates as a coenzyme in protein synthesis; therefore, a deficiency of this vitamin may result in reduced neuronal formation and early termination of cell proliferation. This is believed to reduce synaptic induction for the growth of dendritic arborization. Clinical evaluation of maternal and postnatal vitamin B₆ levels may offer an indication to the pathogenesis of SIDS if a deficiency of this vitamin is associated with the high density of dendritic spines and a low mature synaptic density which we have demonstrated in SIDS infants (Figs. 20 and 28).

Thiamine (vitamin B₁) deficiency has been postulated to have a link with SIDS. It has been attributed to a defective conversion of thiamine pyrophosphate to thiamine triphosphate which is a high energy compound thought to have a role in the ionic mechanisms of action potential generation. In one report of two near-miss infants, an increased stability
in BAEP recordings was shown after the administration of vitamin B₁. However, BAEP testing and the role of vitamin B₁ in SIDS remain inconclusive. Further studies are warranted, but if verified, this clinical test would support a brain stem dysfunction in SIDS.

Perri has shown that vitamin B₁ affects synaptic transmission, particularly in the superior cervical ganglion. In the developing rat brain, vitamin B₁ deficiency has been associated with elevated ganglioside concentrations and reduced cerebroside concentrations. Thus, vitamin B₁ may play a role in maintaining synaptic membrane specialization and other types of neuronal development such as myelin formation.

**Hormonal and Undernutrition Considerations**

The effects of hormones on brain development depend primarily on the distribution of target cells with specific receptors in the CNS. The influence of maternal hormones on the fetus is restricted by the ability of the hormone to cross the placenta. Steroid hormones, thyroid hormones, and epinephrine all cross the placenta; however, peptide hormones do not. Hormones appear to act primarily on the neurons, although glial cells have been shown to react to subsequent neuronal changes. Hormones have been demonstrated to affect cell proliferation, cell death, differentiation, and synaptogenesis. It also is important to recognize that the effects of hormones may depend upon the dose, stage of development, and the type of neuron in question. The most marked effects on the CNS are the stimulation of differentiation in the target neuron and the stimulation of dendritic growth and synaptogenesis which offer appropriate consideration of hormones in this study of the maturation of the infant brain stem.
Thyroid hormone (T₃) has been reported to have marked effects on brain development and the development of neural connections. T₃ function in the human fetus is shown to commence towards the end of the first trimester. The effects of neonatal hyperthyroidism are less marked than those of hypothyroidism. In hypothyroidism, receptor ontogenesis is retarded throughout the brain; however, alterations caused by this T₃ deficiency have shown a tendency of returning to normal. T₃ deficiency also results in a retardation of dendritic arborization, axonal growth, and synaptic density. T₃ is not only thought to be important in the formation of normal synaptic density, but also for the development of the proper circuitry in the CNS. An increase in T₃ levels has been reported to result in the early initiation of cell differentiation which leads to an initial acceleration of synaptogenesis, but with the ultimate reduction in total synaptic density. In T₃ deficiency, however, the main effect is the general retardation of neuronal differentiation and synaptogenesis. Therefore, it appears that both the acceleration of differentiation and its retardation lead to reductions in the synaptic content of the neuropil. These investigations suggest the role of T₃ as a trigger for synaptogenesis and as a tool for studying the development of the CNS. Of greater interest is that Nicholson has shown that with both hypo- and hyperthyroidism in rat cerebellar cortex, the total synaptic density is reduced for different reasons. With T₃ deficiency, this was due to a hypoplastic neuropil, whereas with increased T₃, this reduction in density was due to the decreased area of dendritic arborization.

A recent study has shown an elevated postmortem T₃ concentration in 88% of SIDS victims. The elevated T₃ is consistent with several findings
in SIDS including short QT intervals and medial muscle hypertrophy which may result from the increased cardiopulmonary hemodynamics which accompany T₃ excess. However, an elevated T₃ concentration is inconsistent with the smaller density of mature synaptic profiles (Fig. 28) and the high density of spines in SIDS as compared with controls (Fig. 20). The report of elevated T₃ levels in SIDS, indeed, suggests a necessary experimental investigation of the effects of T₃ concentrations on brain stem microstructure. Thus, this discussion highlights the difficulty in identifying valid correlations between biochemical and structural observations and brain function.

Undernutrition manifests itself by effects on the metabolism of the neuron which may change the quality of its dendritic membrane or by affecting the neuropil into which dendrites grow. An increase in dendritic branching may reflect a lesser number of neurons. A reduction in cell density may result in additional extraneuronal space which in turn allows greater dendritic elongation and arborization. Since it has been reported by Hammer that the number of dendritic spines normally declines in the rat brain stem, abnormally numerous spines were seen in the undernourished condition pointing towards a delayed development of reticular neurons in this condition. Varicosities, which normally decrease and disappear during this postnatal period, were seen to increase. He also noted a greater dendritic arborization which is thought to implicate glial cell changes.

Numerous generalizations of the effects of undernutrition in the CNS have led to different conclusions. Either the process of structural maturation is upset, or the developmental status of the neuron is delayed. With regard to synaptic density, in the rat midbrain there has been noted an increase in density while in the cortex there was reported a
decreased density. Thus, prenatal undernutrition may partially account for the increased spine density and decreased mature synaptic density which we demonstrate in SIDS infants.

Behavioral effects of undernutrition include deficits in the attention span in infants, responsiveness to sensory stimulation in rats, and other activity. Near-miss SIDS infants have been reported to have a low psychomotor development index, problems in feeding, motor tone, and various neurological abnormal reflexes. Thus, the clinical history of some SIDS infants may be suggestive of neonatal undernutrition and its effects on CNS development.

The most important factor in determining these alterations of CNS development has been shown to be the period in which undernutrition is imposed. Critical periods of maximum effect exist due to nutritional insults. These periods coincide with a tremendous growth of neuronal processes. Winick has stated that the earlier the undernutrition, the more severe the effect. Prenatal insult in the rat appears to have a profound effect on the quality of the brain stem neuropil, both neuronal and glial. Postnatal exposure, however, has been shown not to influence these reticular components. In general, a nutritional deficit during or before the critical period in the rat brain stem was found to delay the course of maturation as indicated by the parameters of spine formation and dendritic growth. In this way, the CNS growth spurt period corresponds to the vulnerable period for undernutrition.

Dobbing has demonstrated that brain stem neurogenesis and its growth spurt are largely prenatal. Harper has suggested that the pathogenesis of SIDS may lie in a disturbance in fetal development. Statistical analysis of our data clearly indicates a significant difference in
dendritic spine density (Fig. 20) and mature synaptic density (Fig. 28) between SIDS and control infants well before forty postconceptional weeks. Therefore, undernutrition at a time during gestation cannot be overlooked in investigating the dendritic and synaptic maturation of the SIDS brain stem.

Hypoxic Considerations

It is also a consideration that a maturational loss of dendritic microstructure may be delayed as a consequence of recurrent, intermittent chronic hypoxia. Data have indicated an inverse relationship between cerebral metabolic rate and resistance of the brain to hypoxia which establishes the concept that the fetal and neonatal brain is much more resistant to hypoxia and ischemia than is the adult brain.

The term, neonatal asphyxia, indicates that the condition involves both a fall in $P_{O_2}$ and a rise in $P_{CO_2}$. However, with chronic asphyxia there is also a fall in fetal arterial pressure giving the condition of hypoxia with relative ischemia. Partial asphyxia may lead to posthypoxic seizure activity and has been reported to demonstrate lesions more in the cortex of the neonate than in the brain stem.

Subtle morphological and neuropathological alterations have been observed in SIDS which increasingly are being accepted as evidence of prior illness, or an immaturity leading to vulnerability. Neuropathological reports of SIDS cases have been difficult to evaluate. Focal cerebral lesions and gliosis have been described in SIDS and compared with alterations observed in infants with chronic hypoxia, congenital heart disease, and neonatal hypoxic-ischemic injury. The interpretation of these findings is complicated by a limited selection of suitable controls and the difficulties in distinguishing pathology from
maturational glial formation in the brain stem. The specificity of
the sites in the brain stem of gliosis is thought to reflect their
relationship to reticular blood supply. Gliosis has been reported
in the border zones of the reticular vasculature, and associated with
subcortical leukomalacia in watershed zones of the subcortical white
matter in SIDS infants. This relationship to vasculature is thought
to be associated with gliosis being caused by hypoperfusion rather than
by hypoxia per se.

There have been documented defective ventilatory responses to the
CO₂ stimulus in near-miss SIDS infants. In adult sleep apnea and
hypoventilation syndromes, the defect has been shown to involve the
response to CO₂ in some patients and the response to hypoxia in others.
The extent to which CO₂ accumulates in tissues, then, may influence
the pathological response to hypoxia. Evidence has been presented which
suggests that hypoxia, combined with a high PCO₂ and respiratory acidosis
such as tracheal obstruction or pneumonia, produces an edema and necro-
sis of the gray matter. This is in contrast to hypoxia associated with
low PCO₂ and respiratory alkalosis which tends to cause edema and necro-
sis of white matter.

In some infants, the near-miss episode is similar to a seizure epi-

sode. In the first months of life, seizures may occur without tonic or
clonic features. Seizures have been considered manifestations, rather
than the cause of acute cerebral anoxia. Since seizure activity in the
temporal lobe has been reported to result in silent apneic episodes,
the need for a thorough examination, including electrophysiological
studies, of the near-miss infant are indicated.
Functional obstruction during sleep is being widely recognized as a cause of multiple episodes of asphyxia throughout the sleep period in both adults and children, and of sudden death in some patients. Although an obstructive sleep apnea syndrome sometimes is associated with obesity or obstructive lesions, it also may occur without any anatomical abnormality. The vulnerability to asphyxia during sleep has been demonstrated predominantly during the REM stage of sleep. The onset of REM normally is associated with a persistent inactive state of intercostal muscles even during a nasal obstruction. This is in contrast to the greatly augmented activity of these muscles by proprioceptive, load-compensating reflexes at the first obstructed breath in NREM sleep.

It also has been postulated that maternal smoking habits and nicotine may produce fetal brain stem injury which may be associated with abnormalities in postnatal respiration. The mechanism of nicotine-induced cell injury is thought to be anoxic-ischemic injury secondary to reduced uteroplacental blood flow. Lewak reported that SIDS occurred twice as commonly among infants of smoking mothers than among those of nonsmoking mothers. Therefore, prenatal hypoxia from maternal smoking also may be thought to contribute to the postmortem tissue alterations described by Naeye.

It must be remembered that in the infant, a great variability exists in the patterns of damage from hypoxia and anoxia. The two most critical factors appear to be ischemia and hypotension which produce two populations in perinatally damaged infants. The ischemic group has been reported to suffer their insult in the form of an acute asphyxial episode without supervening hypotension or acidosis. This
clinical situation has demonstrated a pattern of brain stem damage. Infants who suffer prolonged asphyxia complicated with hypotension have produced a cortical injury pattern. It is interesting that these two major pathological patterns could reflect two basic physiological patterns of injury.

Data have shown that there are five main types of perinatal brain damage: 1) brain stem injury due to complete asphyxia, 2) brain swelling and damage following partial asphyxia, 3) periventricular hemorrhage, 4) white matter injury due to hypoxia and hypotension (periventricular leukomalacia), and 5) damage to the basal ganglion (status marmoratus) following a sequence of partial and complete asphyxia. The extent to which the cortex is injured together with other sites is determined by the relative prominence of anoxia, hypoxia, and hypotension as components of the asphyxia sequence. Thus, the details of distribution of damage may provide clues as to the nature of the clinical process which gave rise to the injury. It now becomes increasingly evident that the correlation with apneic episodes and hypoxic damage in the brain stem of the SIDS infant is not a clear-cut cause and effect relationship.

The prototype for neonatal hypoxia-ischemia encephalopathy has been reported to be associated with perinatal asphyxia. The two lesions associated with hypoxia are ponto–subicular necrosis and status marmoratus of the basal ganglion and thalamus. The two associated with ischemia are watershed infarction and periventricular leukomalacia. The topographic pattern of this encephalopathy has been shown to be a rostral-caudal pattern of decreasing vulnerability with the brain stem being the least sensitive.
Periventricular leukomalacia (PVL) has been shown to vary with age. It exhibits coagulative necrosis, swollen axons, and occurs primarily in the neonatal period associated with a respiratory or circulatory compromise of the cerebral circulation. Subcortical leukomalacia (SL) has been seen in the white matter of the deep sulci with rarefaction of the neuropil and minimal gliosis, and is not considered to be a lesion of the perinatal period. SL has been reported to be present in 16% of SIDS infants and 13% of infants with congenital heart disease. Since SL has been associated with congenital heart disease, it is postulated that hypoxia produces this lesion in the SIDS infant. Therefore, SIDS may exhibit morphological markers of previous altered physiology, and cerebral white matter lesions such as PVL and SL may be two of these.

Banker has termed PVL as anoxic encephalopathy with its anatomical substrate damage extending into brain stem nuclei and thalamus. Friede has pointed out that acute ponto-subicular necrosis (PSN) is the neonatal form of anoxic encephalopathy. PSN consists of neuronal necrosis, characterized by karyorrhexis and shrinkage of the cells. However, this lesion has been reported in infants only between 30 weeks gestation and 2 months of age who exhibited convulsions or status epilepticus. Another pattern of brain stem lesions is that of hypotensive brain stem necrosis which may occur as a component of diffuse anoxic encephalopathy.

In our H&E examination of brain stem sections, no significance is attached to congestion and/or minimal focal perivascular hemorrhage which also have been described in another SIDS study. However, we do find significance in the acute hypoxic change of focal eosinophilia found in
nine of the 95 infants we studied (Fig. 5). Therefore, how may acute or chronic hypoxic change affect dendritic spine density and the ultrastructure of synaptic profiles?

It has been reported experimentally that rapid Golgi sections of the cortex in neonatal rats subjected to daily hypoxia of eight hours showed a 30% increase in the number of spines on the proximal portion of the dendrites. However, the total spine density on the dendrite was not seen to increase significantly under such hypoxic conditions. A study on the effects of hypoxia and acidosis in the cat CNS has demonstrated reversible apical dendritic swellings in the cortex as the only ultrastructural deviation from the normal state. It has also been reported that synaptic profiles in EPTA sections from hypoxic rat cortex demonstrated minimal shortening of dense projections; however, this was not found to be consistent with repeated episodes of hypoxia. The investigators have been unable to conclude whether this alteration is the direct result of hypoxia.

Therefore, our observations of dendritic morphology and synaptic ultrastructure as well as our quantitation of spine and mature synaptic densities in the infant brain stem appear to be more likely associated with a retarded synaptic development and immature brain stem rather than to be the result of hypoxic insult in the SIDS infant.
CONCLUSIONS

Investigators have linked SIDS to a CNS dysfunction. In particular, clinical observations of respiratory instability and abnormal sleep parameters have implicated the brain stem as the site of dysfunction. In this study, we have attempted to elucidate an anatomical substrate of this functional brain stem abnormality by performing qualitative and quantitative analyses of the dendrite-spine system and of synaptic ultrastructure. The modulation of respiratory rhythms, the integrative capacity for REM-NREM sleep and arousal, and the synchronization of output function all represent challenging problems for a brain stem structure-function correlation. The process of CNS maturation is enormously complex; however, an immature developmental pattern in the SIDS brain stem may be concluded from an analysis of the data:

1. there is a normal loss of dendritic spines with age in the infant brain stem,
2. a maturational delay exists in the loss of dendritic spines in SIDS,
3. mature synapses are demonstrable in the brain stem with EPTA,
4. mature synaptic density normally increases with age,
5. there is an immature pattern of synaptic development in the SIDS infants.

It has been reported that with dendritic spine loss dendrites are believed to assume control of more complex CNS functioning programs.
The emergence of such functions during the postnatal period is probably mandated by the biological growth of the infant as well as changes in the interaction with the environment. This requires an intricate maturation and plasticity of structure within the complex integrative system of the neuroaxis. Cortical dendritic spine pathology has been reported in association with fibrillar-neuronal CNS dysfunction. Although there have been no reports on the control or aberrant state of reticular dendritic and synaptic maturation in man, it is conceivable that the persistence of a high density of spines in the seven areas of the brain stem we studied in SIDS infants may also reflect an integrative reticular dysfunction. Our data identify a slower rate of spine loss and an immature pattern of synaptic development in SIDS as compared with control infants. This structural immaturity may be representative of an upset dendritic interaction in the brain stem during the critical postnatal period of infant growth and suggests a particular anatomical substrate in the SIDS brain stem.

It is clear that spine density and synaptogenesis are inter-dependent developmental processes in the regulation of neuronal morphogenesis. Statistical analysis identifies the phase of maximum spine development to occur before 40 postconceptional weeks. Although this supports the theory that the growth spurt occurs prenatally in the brain stem, the postnatal continuum of a high density of dendritic spines and a lower density of mature synapses in SIDS infants accentuates the already present vulnerability of this critical postnatal period.

It has been postulated that spine loss is an intrinsic phenomenon by retracting into dendrites. The significance of the brain stem dendrite-spine system has been reported to be concerned with the
integration of basic functions such as the sleep-wakeful cycle and central respiratory rhythm. However, this neurochemical spine synapse is not the only type of synapse on a dendrite. There also exists a much different electrotonic spineless junction represented by the GJ. The decrease in dendritic spine density may regulate the organization and functional development of this other type of junction. Since the length of the spine has been shown to modify the efficacy of its integrative capability, the predominance of the ST spine which we observe in the brain stem suggests a low electrotonic resistance on the dendrite and may provide the necessary requirement for the appearance of dendrodendritic gap junctions. Dendritic spines, then, may be functionally uncoupled as electrotonic conductance is increased. Thus, the development of electrotonic function may be regulated by the decrease in dendritic spines. The emergence of electrotonic function may allow the dendrite to assume a more refined synchronous synaptic capability. Electrotonic dendrodendritic connections, then, may modulate more complex functional requirements such as the development of the periodicity of NREM-REM sleep states, the lengthening of the sleep period, sleep-respiratory rhythms, and the concomitant integration of chemoreceptor activity which coincide with the 2 to 4 months of age peak incidence of SIDS. Therefore, it is possible that an anatomical and functional imbalance between these two types of intercellular connections alters interneuronal information transfer which may be responsible for abnormal respiratory control during sleep and the inability of arousal mechanisms to restore respiration after apnea in infants at risk for SIDS. Clearly, this imbalance may represent a morphological correlate of an upset dendritic interaction during the postnatal maturation of the brain stem in
SIDS. Hence, a decline in dendritic spine density does not necessarily indicate a decrease in interneuronal contact. The high density of spinous synapses in the SIDS group may prevent the development of electrotonic junctions and compromise the synchronous capability of the reticular network.

Studies on dendritic spines and synaptic ultrastructure have established unequivocally their sensitivity to changes in the external and internal environment. Extra-neuronal factors such as glial activity, trophic and hormonal influences, and undernutrition may affect dendritic and synaptic development. Hypoxia also has been shown to produce qualitative ultrastructural alterations. However, our observations of well-impregnated neurons and EPTA stained synaptic profiles clearly demonstrate an immature pattern in the SIDS brain stem from birth which appears more likely antecedent to the prolonged apnea hypothesis generally ascribed to the SIDS infant.

The mean gestational age of SIDS and controls in our study is 39 weeks. Thus, the persistent higher density of dendritic spines in the SIDS infants is not associated with prematurity. In fact, the data indicate a significant maturational lag.

This study also demonstrates that there are changes in reticular microstructure which typify the postnatal period as observed by others elsewhere in the brain. Routine neuropathological studies generally provide no direct information on the morphological status of dendrites and synapses. Yet, as shown in this investigation, quantitative changes in dendritic spines and synaptic profiles occur in the SIDS brain stem despite ambiguous findings in routine histological studies. It is hoped that this study will encourage analysis of the normal and pathological
features of dendrites and spines in the continuing search for the morphological substrate of developmental CNS disorders.

Although several interpretations and more traditional beliefs concerning synaptic capability may belie our considerations, more attentive research in brain stem synaptology and the plasticity of its neuronal integration during maturation in the infant is required. The reproducibility of our spine and synaptic quantitation will hopefully confirm this statistically significant difference in reticular microstructure between SIDS and non-SIDS cases.

The study of SIDS and near-miss SIDS infants necessitates awareness of the multifactorial aspects of the pathogenesis of SIDS as well as the stresses placed on the infant during gestation and in the extra-uterine period. Accordingly, investigators have begun to delineate more clearly the characteristics of the newborn and the suitability of control groups with which to compare SIDS. The developmental approach to the study of the human brain stem certainly needs to be encouraged, and it is hoped that an appreciation of the complex microstructure of the infant brain stem and the immature pattern of synaptic development we demonstrate in the SIDS brain stem will amplify future investigations in SIDS.

Our qualitative and quantitative data support the hypothesis that there is neuronal immaturity in the brain stem of SIDS victims. We conclude that this immature morphogenetic pattern of dendritic spines may be a precipitating factor in the pathogenesis of SIDS and may be at least partially responsible for abnormal central respiratory control in the brain stem of the SIDS infant.
TABLES
TABLE 1. Mean Dendritic Spine Density ($\times 10^{-3}/\mu m$) in Each Area of the Brain Stem with Increasing Age.

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KEY
AGE (postconceptional weeks)
NA (n. ambiguus)
NS (n. solitarius)
XII (n. hypoglossus)
V (n. spinal trigeminal)
GT (giantscellar tegmentum)
MLF (medial longitudinal fasciculus)
LL (n. lateralis semicircularis)
TABLE 2. Dendritic Spine Density in Each Area of the Brain Stem for Infants under 1 Postnatal Year of Age.

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<th>CONTROLS (n = 34) MEAN ±S.E.M.</th>
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<td>NA 119.3 ±16.2</td>
</tr>
<tr>
<td>NS</td>
<td>194.0 ±5.9</td>
<td>NS 107.1 ±13.7</td>
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<tr>
<td>XII</td>
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<tr>
<td>V</td>
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<tr>
<td>GT</td>
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<tr>
<td>MLF</td>
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<tr>
<td>LL</td>
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<td>LL 105.0 ±14.4</td>
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</tbody>
</table>

KEY
- NA (n. ambiguus)
- NS (n. solitarius)
- XII (n. hypoglossus)
- V (n. spinal trigeminal)
- GT (gigantocellular tegmentum area)
- MLF (medial longitudinal fasciculus area)
- LL (n. lateral lemniscus)

MEAN ±S.E.M. (x 10^{-3}/μm)
### TABLE 3. Dendritic Spine Density in the Brain Stem for Each Age Group.

<table>
<thead>
<tr>
<th>SIDS</th>
<th>MEAN ±S.E.M.</th>
<th>(n)</th>
<th></th>
<th>CONTROLS</th>
<th>MEAN ±S.E.M.</th>
<th>(n)</th>
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<tbody>
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<tr>
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</table>

**KEY**

AGE (postconceptional weeks)

MEAN ±S.E.M. (x 10^-3/µm)
Figure 1. The Anatomical Regions of Dendritic Spine Quantitation in the Infant Brain Stem.

The cross-section of the medulla (A) illustrates the areas of quantitation: (1) n. ambiguus, (2) n. solitarius, (3) n. hypoglossus, (4) n. spinal trigeminal. (Weil, 21 X)²⁷⁶
The cross-section of the pons (B) illustrates the areas of quantitation: (5) gigantocellular tegmental area, (6) medial longitudinal fasciculus area, (7) n. lateral lemniscus. (Weil, 21 X)
Figure 2. Reticular Dendritic Arborization.

The interwoven pattern of reticular dendrites demonstrates the intricate neuronal network of the brain stem. (rapid Golgi, 133 X)
Figure 3. Scanning Electron Microscopy in the Brain Stem.

(A) Neuronal aggregation in the magnocellular region of the medulla illustrates neuronal ultrastructure. (SEM, 3,000 X)
Figure 3 (continued).

(B) Dendrite-like neurite with an array of spinous processes along its surface illustrating the dendrite-spine system. (SEM, 14,000 X)
Figure 4. Histology of Reticular Neurons.

Magnocellular (A) and parvocellular (B) neurons of the medulla demonstrate a normal cytoarchitecture. (Nissl, 100 X)
Figure 5. Acute Hypoxic Change.

(A) Reticular neuron with cytological changes suggestive of hypoxic damage: loss of Nissl bodies, karyolysis, and eosinophilia (not apparent in black and white photomicrograph). (H&E, 2,120 X)
Figure 5 (continued).

(B) Normal reticular neuron. (H&E, 2,120 X)
Figure 6. The Developing Dendritic Membrane.

(A) A dendritic varicosity (arrow) appears as an irregular swelling of the dendrite. (rapid Golgi, 2,000 X)
Figure 6 (continued).

(B) A dense array of dendritic spines along the dendrite characterize the developing dendrite. (rapid Golgi, 264 X)
Figure 7. Dendritic Tortuosity.

A lateral reticular dendritic segment from a 1 month old term infant illustrates a spine-covered tortuous pattern.
(rapid Golgi, 672 X)
Figure 8. Dendritic Maturation.

Lateral reticular dendrites from an 8 month old preterm control infant demonstrate a decrease in varicosities and nodulation, and a less tortuous pattern. (rapid Golgi, 672 X)
Figure 9. Anatomical Distribution of Neuronal Dendritic Patterns in the Brain Stem.

(A) Neuron in the reticular formation of the medulla illustrates a long, poorly ramified dendritic pattern. (rapid Golgi, 265 X)
Figure 9 (continued).

(B) Stellate neuron in the n. hypoglossus illustrates a densely ramified, branching dendritic pattern. (rapid Golgi, 105 X)
Figure 10. An Isodendritic Neuron in the Non-Reticular Area.

Branching neurons in the n. spinal trigeminal surround an isodendritic neuron (arrow) with its long dendritic extensions. (rapid Golgi, 105 X)
Figure 11. The Isodendritic Neuron.

The neuron demonstrates long radiating dendrites which are characteristic of the isodendritic core. (rapid Golgi, 105 X)
Figure 12. The Morphology of Reticular Neurons.

The isodendritic neuron (A) of the n. ambiguus is characterized by a long, poorly branched dendritic pattern. (rapid Golgi, 265 X)
The allodendritic neuron (B) of the n. lateral lemniscus is characterized by a relatively short, densely branched dendritic pattern. (rapid Golgi, 321 X)
Figure 12 (continued).

The idiodendritic neurons (C) of the inferior olive are characterized by a highly branched, tufted dendritic pattern. (rapid Golgi, 315 X)
Figure 13. Reticular Dendrites in Close Apposition.

(A) Two dendrites within a dendritic bundle are seen in close parallel apposition. (rapid Golgi, 384 X)
(B) Dendrodendritic contact is specifically outlined between the same two dendrites. (rapid Golgi Image Removal, 384 X)
Figure 14. Lateral Dendritic Arborization.

An extensive lateral arborization of reticular dendrites in the isodendritic core of the medulla. (rapid Golgi, 105 X)
Figure 15. A Maturational Loss of Reticular Dendritic Spines.

A lateral dendrite (A) of a 1 month old control infant demonstrates a dense array of spines (rapid Golgi, 498 X).

In contrast, a lateral dendrite (B) in a 6 month old control infant demonstrates a marked reduction in spine density. (rapid Golgi, 498 X)
Figure 16. The Heterogeneity of Reticular Dendritic Spines.

Three dendritic segments illustrate: (A) short-stubby spines, (B) mushroom-shaped spines, (C) long, thin spines.
(rapid Golgi, 1,200 X)
Figure 17. The Predominance of Short-Stubby Spines.

Long thin spines (A) are demonstrated on a lateral dendrite of a 1 month old infant. (rapid Golgi, 686 X)
As spine density is observed to decrease in the 6 month old infant, the remaining spines (B) on the lateral dendrite are predominantly short-stubby spines. (rapid Golgi, 686 X)
Figure 18. A Generalized Pattern of Decreasing Spine Density on the Dendrite.

The relative decrease in spine density in an 8 month old infant is observed to be consistent throughout the length of the dendrite without specific dendritic regions of spine loss. (rapid Golgi, 567 X)
Figure 19. The Comparison of Dendritic Spine Density Between SIDS and Control Infants.

(A) A dense population of spines is evident in the 1 month old control infant. (rapid Golgi, 454 X)
(B) A similar dense population of spines in the 1 month old SIDS infant. (rapid Golgi, 454 X)
Figure 19 (continued).

(C) A reduction in spine density is evident in the 2 month old control infant. (rapid Golgi, 672 X)
(D) A similar reduction of spines in the 2 month old SIDS infant. (rapid Golgi, 672 X)
Figure 19 (continued).

(E) A marked decline in spine density in the 3 month old control infant. (rapid Golgi, 672 X)
(F) In contrast, more spines are observed in the 3 month old SIDS infant. (rapid Golgi, 672 X)
Figure 19 (continued).

(G) A further decline in spine density is observed in the 6 month old control infant. (rapid Golgi, 672 X)
(H) In contrast, a greater density of spines persists in the 6 month old SIDS infant. (rapid Golgi, 672 X)
Figure 19 (continued).

(I) Virtually all the spines are lost in the 12 month old control infant. (rapid Golgi, 454 X)
(J) A demonstration of spines continues to persist in the 12 month old SIDS infant. (rapid Golgi, 672 X)
Figure 20. Rapid Golgi Analysis of Dendritic Spine Density in SIDS and Control Infants.

The two straight lines represent multiple linear regression analysis. There is a statistically significant difference in spine density between SIDS and controls ($P < .0001$ between 29 and 88 postconceptional weeks).
Figure 21. Rapid Golgi Analysis of Dendritic Spine Density in Term SIDS and Control Infants.

There is a statistically significant difference in spine density between SIDS and controls ($P < .0001$ between 1 and 12 months of age).
Figure 22. Rapid Golgi Analysis of Dendritic Spine Density in Preterm SIDS and Control Infants.

There is a statistically significant difference in spine density between SIDS and controls (P < .001 between 1 and 12 months of age).
Figure 23. The Ultrastructural Analysis of the EPTA Synaptic Profile.

The three characteristic elements of the synaptic profile are:
(DP) presynaptic dense projections,
(CD) cleft density,
(PT) postsynaptic thickening.
(EPTA, 60,000 X)
Figure 24. The EPTA Stained Section.

The EPTA stain identifies synaptic profiles (arrows) for quantitation. Axonal membranes (ax) and neurotubules (nt) are evident in the grid section background. (EPTA, 16,800 X)
Figure 25. The Mature Synapse.

The synaptic profile demonstrates an asymmetrical appearance with four distinct presynaptic dense projections, cleft density, and a prominent postsynaptic band. (EPTA, 90,000 X)
Figure 26. Cleft Width and Dense Projection Parameters of Synaptic Maturation.

(A) A synaptic profile in a 3 month old control infant demonstrates a wide (240 Å) synaptic cleft. (EPTA, 30,000 X)
(B) In contrast, a synaptic profile in a 10 month old control infant demonstrates a narrow (160 Å) cleft with tall distinct presynaptic projections. (EPTA, 30,000 X)
Figure 27. The Synaptic Curvature Parameter in Maturation.

(A) Synaptic curvature (arrow) in the 1 month old control infant is negative. (EPTA, 30,000 X)
(B) In contrast, synaptic curvature (arrow) in the 8 month old control infant is positive. (EPTA, 30,000 X)
Figure 28. EPTA Analysis of Mature Synaptic Density in SIDS and Control Infants.

The two straight lines represent multiple linear regression analysis. There is a statistically significant difference in mature synaptic density between SIDS and controls (P < .0003 between 47 and 88 post-conceptional weeks).
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