The sudden infant death syndrome (SIDS) is the sudden death of an infant under one year of age that is typically associated with sleep and that remains unexplained after a complete autopsy and death scene investigation. A leading hypothesis about its pathogenesis is that many cases result from defects in brainstem-mediated protective responses to homeostatic stressors occurring during sleep in a critical developmental period. Here we review the evidence for the brainstem hypothesis in SIDS with a focus upon abnormalities related to the neurotransmitter serotonin in the medulla oblongata, as these are the most robust pathologic findings to date. In this context, we synthesize the human autopsy data with genetic, whole-animal, and cellular data concerning the function and development of the medullary serotonergic system. These emerging data suggest an important underlying mechanism in SIDS that may help lead to identification of infants at risk and specific interventions to prevent death.
INTRODUCTION

The sudden infant death syndrome (SIDS) is the sudden and unexpected death of an infant under 12 months of age that is usually associated with a sleep period and remains unexplained after a complete autopsy, death scene investigation, and review of the clinical history (1). Typically, without warning an apparently healthy infant is found dead sometime after being placed to sleep for the night or for an afternoon nap. In the United States today, SIDS is the leading cause of death in infants between 1 and 12 months, and it is the third leading cause of all infant deaths between birth and 12 months (after congenital malformations and low birth weight) (2, 3). The overall incidence of SIDS is currently 0.57 per 1000 live births in the United States (3)—or approximately six infant deaths per day. The most significant advance in SIDS research in the past two decades has been the recognition that the prone (belly-down) sleep position more than triples the risk for SIDS (4). This seminal observation has led to vigorous national public health campaigns advocating the supine (belly-up) sleep position for infants, to a decline in the placement of infants in the prone position to sleep to fewer than 20% of parents putting infants in this position (5, 6), and to a reduction in the national SIDS rate of almost 50% (3).

Despite the dramatic public health achievement in the reduction of SIDS, this disorder remains a major problem that must not be underestimated. It is still the leading cause of postnatal infant mortality in the United States (with an overall rate higher than those of several European countries and Japan (2, 3, 7)). Moreover, the decline in rate may be due in part to diagnostic shift, in which terms such as positional asphyxia, accidental suffocation, and undetermined are used on death certificates (from which national statistics are derived) in cases previously identified as SIDS (8, 9). In addition, SIDS occurs in infants found in the supine position and in infants who were inadvertently placed in the prone position (even for the first time) or who had rolled from the supine to prone position (10). SIDS is also associated with several significant risk factors that are less modifiable than infant sleep position, e.g., maternal smoking and alcohol use during pregnancy, prematurity, and poverty (7, 11–15), as well as with genetic (immutable) factors that appear to interact with environmental factors to compound SIDS risk (16–19). Of major concern today is the substantial discrepancy in SIDS rates between white and nonwhite populations, with rates two or more times higher in certain racial and ethnic groups whose health care is commonly marginalized, e.g., African Americans (20), American Indians in the Northern Plains (102), Maori in New Zealand (15, 21), aboriginal Australians (14), and mixed-ancestry populations in Cape Town (22). In order to establish the means to eradicate all SIDS deaths—the ultimate goal of SIDS research—as well as to give biologic plausibility to the current public health messages, the underlying cause and pathogenesis of SIDS must be established.

This review summarizes two decades of brainstem research in SIDS in large part under the auspices of a National Institute of Child Health and Human Development–funded program project led by the authors, combined with a synthesis of relevant analysis reported by others. We focus primarily (but not exclusively) upon pathways in the medulla oblongata that involve the neurotransmitter serotonin (known as 5-hydroxytryptamine or 5-HT) because medullary 5-HT abnormalities are the most robust and reproducible findings in SIDS brainstem research to date (23–26). Based upon the collective work of our group and others, we offer the hypothesis that an important subset of SIDS is due to a developmental disorder of the medullary 5-HT and related neurotransmitter systems and that it is incurred prenatally but exerts its effects postnatally (Figure 1). This brainstem disorder leads in turn, we believe, to impaired protective responses to life-threatening (but common) stressors during infant sleep and to subsequent asphyxia and/or other homeostatic derangements—each stressor is potentially
nonlethal in itself, but in combination they can precipitate death (Figure 1). Given that approximately 90% of SIDS cases occur in the first six months of life, we postulate that the transition period from fetal to extrauterine life represents a critical time frame in which the brainstem circuitry for the maintenance of homeostasis undergoes rapid maturation and therefore is at greatest risk (Figure 1). In essence, we envision SIDS as a complex and heterogeneous process that involves multiple neurotransmitters and neuromodulators, multiple stressors acting simultaneously, and multiple genetic and environmental factors augmenting the intrinsic brainstem defects (Figure 1). Our objective here is to provide an up-to-date review of the rationale for brainstem research in SIDS, as well as an integrative overview of human, whole-animal, cellular, and developmental studies involving the medullary 5-HT and related neurotransmitter systems in an effort to elucidate potential mechanisms underlying sudden death in early life.

THE BRAINSTEM HYPOTHESIS

In 1987, just prior to the international recognition of the importance of the prone sleep position in SIDS, Hunt & Brouillette (27) articulated the consensus of the scientific community: “Although numerous general theories of cause are probably worthy of further clinical study, the most compelling hypothesis continues to be that SIDS is related to a brainstem abnormality in the neuroregulation of cardiorespiratory control.” This statement is, in our opinion, even more valid today than it was 20 years ago due to independent reports of subtle brainstem pathology in SIDS cases since 1987 (Table 1). The rationale for the brainstem hypothesis is based upon the following: (a) established evidence that the brainstem plays a critical role in respiratory and autonomic regulation, sleep, and arousal (28), i.e., the physiological processes considered abnormal in SIDS infants; (b) analogy to instances of sleep-related sudden death in children or adults in whom isolated or primary pathology is found by neuroimaging

and/or at autopsy in the brainstem (29–31); and (c) reports of subclinical defects in cardiorespiratory control and/or arousal that are consistent with brainstem dysfunction in infants who are studied prospectively and who subsequently die of SIDS. These defects include impaired autoresuscitation (gasping), abnormal respiratory patterning, episodic obstructive apnea during sleep, autonomic dysfunction (episodic tachycardia/bradycardia, abnormal heart rate variability), and arousal deficits (32–42).

In the decade following the review by Hunt & Brouillette, several major risk factors for SIDS in addition to prone sleep position were discovered, including face-down position, covered face in the supine position, soft bedding, bed sharing, overbundling, elevated room temperature, and minor infection at the time of death (4, 7, 10, 14, 20, 21, 42–44). The association of SIDS with these risk factors underscores the importance of the brainstem hypothesis, as these factors can be considered

Arousal: transition from sleep to wakefulness
Autoresuscitation: process by which a newborn can recover from severe asphyxia
Gasping: abrupt large breaths that occur in severe asphyxia or hypoxia
Table 1  Neurotransmitter abnormalities in the brainstem in sudden infant death syndrome infants

<table>
<thead>
<tr>
<th>Substance</th>
<th>Our laboratory</th>
<th>Other investigators</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>Widespread decreased 5-HT1A binding, increased 5-HT cells, relative decrease in 5-HTT binding (25)</td>
<td>Increase 5-HT in raphe obscurus (HPLC) (189); reduced immunostaining for 5-HT1A and 5-HT2A receptors in NTS, DMX, VLM, locus coeruleus; increased immunostaining in periaqueductal gray (26)</td>
</tr>
<tr>
<td>ChAT</td>
<td>No studies performed</td>
<td>Decreased ChAT immunostaining in HG and DMX (62)</td>
</tr>
<tr>
<td>Muscarinic receptor</td>
<td>Decreased binding in arcuate nucleus (69)</td>
<td>Decreased number of neurons immunopositive for muscarinic receptors in arcuate nucleus (190), no decrease in muscarinic receptor immunostaining in arcuate nucleus (62)</td>
</tr>
<tr>
<td>Nicotine receptor</td>
<td>No abnormal binding, except in selected mesopontine nuclei upon stratification by history of exposures (64, 65)</td>
<td>No studies performed</td>
</tr>
<tr>
<td>CA</td>
<td>No abnormal α2-adrenergic receptor binding (191)</td>
<td>Increased dendritic spines in CA neurons in VLM (68): absent PNMT immunoreactivity in NTS (66), decreased TH immunoreactivity in VLM, DMX (192), TH immunostaining not correlated with density of TH-immunostained neurons in DMX, NTS, NA, VLM (193)</td>
</tr>
<tr>
<td>α2-adrenergic receptor</td>
<td>No abnormal binding (191)</td>
<td>Reduced α-2A (but not -2B and -2C) receptors by ICC in NTS, VLM (194)</td>
</tr>
<tr>
<td>NMDA receptor</td>
<td>No studies performed</td>
<td>Increased mRNA to NR1 subunit in six nuclei in medulla, increased NR1 protein in DMX, and decreased NR1 protein in spinal trigeminal nucleus (70)</td>
</tr>
<tr>
<td>Kainate receptor</td>
<td>Reduced binding in arcuate nucleus (69)</td>
<td>No studies performed</td>
</tr>
<tr>
<td>Opioids</td>
<td>No abnormal binding to 3H-naloxone (92)</td>
<td>No changes in homogenates of medulla (195)</td>
</tr>
<tr>
<td>GABA</td>
<td>No studies performed</td>
<td>No studies performed</td>
</tr>
<tr>
<td>Substance P</td>
<td>No studies performed</td>
<td>Increased in homogenates of medulla (195), increased immunostaining in trigeminal fibers (196), increased immunostaining in NTS and spinal trigeminal nucleus (197), no difference in binding in medulla (198)</td>
</tr>
</tbody>
</table>

Abbreviations: 5-HT, 5-hydroxytryptamine; CA, catecholamines; ChAT, acetylcholine; DMX, dorsal motor nucleus of the vagus; GABA, γ-aminobutyric acid; HG, hypoglossal nucleus; HPLC, high-pressure liquid chromatography; ICC, immunocytochemistry; NA, nucleus ambiguus; NMDA, N-methyl-D-aspartic acid; NTS, nucleus of the solitary tract; PNMT, phenylethanolamine N-methyltransferase; TH, tyrosine hydroxylase; VLM, ventrolateral medulla.

Hypoxia: below-normal oxygen levels in lung, blood, or other tissues (measured at sea level)

Hypocapnia: arterial carbon dioxide levels above 40 mm Hg
collectively as exogenous stressors that lead to asphyxia, hypoxia, hypocapnia, or thermal imbalance requiring intact brainstem defense systems to protect against lethal consequences. These defense systems involve central chemosensitivity to carbon dioxide (CO₂) and peripheral and central chemosensitivity to oxygen (O₂), autoresuscitation, and arousal with head lifting and turning to escape asphyxiating microenvironments by gaining fresh air. There is now evidence, for example, that the prone position can lead to rebreathing exhaled gases, particularly in soft bedding, which in turn leads to asphyxia (combined hypocapnia and hypoxia) (43). A defect in brainstem-mediated chemosensitivity could lead to the failure of arousal to protect against lethal asphyxia in this situation. Alternatively, a defect in brainstem-mediated autoresuscitation could result in death due to an inability to respond to hypoxic gasping. The possibility of defective autoresuscitation is suggested by cardiorespiratory tracings of SIDS infants monitored at the time of death, in whom abnormal and ineffectual gasping patterns have been observed (32, 33). Hyperthermia may result from a lack
of (facial) heat loss in the face-down position, leading to heat-induced respiratory depression that is unchecked due to defective brainstem thermoregulatory mechanisms.

THE TRIPLE RISK MODEL

In 1994, as epidemiological reports emerged describing the benefit of supine sleep position in reducing SIDS rates worldwide, we proposed the Triple Risk Model as a framework for integrating the evolving epidemiological, physiological, and neuropathological data on SIDS (45). Like other models, the Triple Risk Model emphasizes the interaction of multiple factors in the pathogenesis of sudden death (46–48), but it reduces the concept to a simple Venn diagram with three interlocking circles. According to this model, SIDS results when three factors simultaneously influence the infant: (a) an underlying vulnerability in the infant, (b) a critical developmental period, and (c) an exogenous stressor, e.g., prone sleep position (45). In this model, such exogenous stressors are postulated to induce asphyxia, hypercapnia, and hypoxia. The critical period for SIDS (i.e., the first six months of life) coincides with dramatic and rapid changes in cardiorespiratory control and the sleep-wake cycle (45, 49–52). According to the Triple Risk Model, only infants with an underlying vulnerability/abnormality in the infant and an exogenous stressor die of SIDS: They do not all have the underlying vulnerability. The model also explains why SIDS rates are reduced by the change to supine sleep position: The exogenous stressor is removed, allowing the vulnerable infant to pass through the critical period unharmed. In the context of the Triple Risk Model, the risk factors for SIDS can be divided into extrinsic and intrinsic categories. Extrinsic risk factors are acquired physical stressors related to the circumstances of death, e.g., prone sleep position. Intrinsic risk factors, however, are associated with the underlying vulnerability/abnormality in the infant and increase the likelihood of SIDS by exacerbating this abnormality. The intrinsic risk factors include prematurity, male gender, African American race, poverty, adverse prenatal factors such as maternal smoking or alcohol use during pregnancy, and genetic polymorphisms (7, 11–20, 53–56).

In a recent study of sudden infant death, 85% of 209 deaths were associated with circumstances consistent with asphyxia, including prone or face-down position when found, covered head, wedging, bed sharing, and sleeping on a couch, suggesting a major role for asphyxia in the pathogenesis of sudden infant death (57). Unquestionably, normal infants can become accidentally trapped in lethal, asphyxiating situations, such as wedging and overlaying (58, 59). We postulate, however, that SIDS infants (i.e., infants with underlying brainstem vulnerability/pathology) die in asphyxia-producing circumstances from which normal infants escape. In essence, SIDS is a disorder of homeostasis in which the infant with an underlying brainstem abnormality cannot adjust to or defend against asphyxia or other metabolic challenges that result from life-threatening events during sleep. Baseline brainstem function is likely adequate (albeit with probable subclinical compromise), given that those infants who subsequently die of SIDS look normal to their caretakers when awake: A homeostatic stress during sleep, in some critical way, unmasks the brainstem defect and triggers lethal decapsulation. Indeed, very small perturbations with minor external stimuli can cause abrupt transitions in respiratory and other neural control systems in human infants (60).

BRAINSTEM PATHOLOGY IN SUDDEN INFANT DEATH SYNDROME

The fundamental paradox of the brainstem hypothesis in SIDS is that the brainstem looks normal upon review of conventionally stained sections under the light microscope. Thus, if the SIDS brainstem is normal, it is clear that quantitative methods at the cellular, subcellular, neurochemical, and/or molecular levels are needed to uncover the putative

Chemosensitivity: refers to the ventilatory response to a change in carbon dioxide/pH as sensed by tissue chemoreceptors, which are composed of neurons and/or astrocytes.
underlying pathology. The modern era of brainstem research in SIDS began with the seminal 1976 report describing subtle brainstem gliosis, which was detected and quantified with a specific astroglial fiber stain by the pediatric pathologist Dr. Richard Naeye (61). Naeye attributed this gliosis to chronic hypoxia due to sleep apnea and alveolar hypventilation prior to the lethal event. Subsequent research focused upon the hypothesis that SIDS is due to a neurotransmitter defect in brainstem cardiorespiratory–related pathways, and investigators worldwide, including our group, reported various neurotransmitter and morphological abnormalities in SIDS cases in brainstem nuclei related (and unrelated) to cardiorespiratory control using immunocytochemistry, tissue autoradiography, and cellular quantitation (Table 1) (23–26, 62–72).

The Arcuate Nucleus

The evidence for rebreathing in the prone position set forth in the early 1990s led our group to focus initially upon the protective brainstem circuits involved in chemosensitivity to CO₂ (63, 69, 73–75). A role for abnormal chemosensitivity in SIDS was also supported by the report of impaired responses to CO₂ and central hypoventilation during sleep in 11 near-miss SIDS infants, 2 of whom subsequently died of SIDS, compared to normal infants (76). We proposed that the arcuate nucleus at the ventral medullary surface is a candidate region for central chemosensitivity in humans, based upon cytological and positional homologies between arcuate neurons and neurons within the respiratory chemosensitive fields on the ventral medullary surface in cats (73, 75). Structural underdevelopment of the arcuate nucleus was subsequently observed in SIDS cases (74, 77). We also reported reduced binding to the muscarinic cholinergic and glutamate-related (kainate) receptors, as these receptors contribute to the mediation of chemosensitivity by ventral neuronal populations in experimental animals (63, 69, 75). This arcuate anomaly was similar to that reported in an infant with clinical insensitivity to CO₂ and sleep-related sudden death (31). Serotonergic neurons at the medullary ventral surface and in the midline (raphe) are now known to be preferentially chemosensitive to CO₂, and although they are not the only central chemosensitive neurons (78, 79), they appear to play a critical, potentially modulatory role (78, 80–83). A small but important population of 5-HT neurons is embedded within the human arcuate nucleus (84), suggesting that the putative dysfunction in chemosensitivity related to the arcuate anomaly specifically involves these embedded 5-HT neurons. This defect likely reflects developmental disturbance in neuron proliferation, migration, and/or differentiation because, in the majority of cases, it is characterized by an underpopulation of neurons without gliosis. Yet the report (85) of acute neuronal necrosis, apoptosis, and gliosis in the arcuate nucleus in stillborn fetuses as early as midgestation and associated with hypoxic-ischemic injury elsewhere in the brain suggests the possibility that secondary injury occurs at this site as well.

The Medullary 5-HT System

Our analysis of the medulla in SIDS led to our recognition that the ventral (arcuate) 5-HT neurons are part of a much larger 5-HT system in the caudal brainstem that, based upon extrapolations from animal data, is critical for the modulation and integration of diverse homeostatic functions (see below). This so-called medullary 5-HT system comprises the caudal domain of the entire brainstem 5-HT system (Figure 2). Notably, all of the 5-HT cell bodies in the brain are located in either rostral or caudal domains in the brainstem that differentially project to virtually all regions of the central nervous system (Figure 2) (86). These rostral and caudal domains are distinct not only in their anatomic location, but also in their developmental origins, functions, and connectivity. The rostral domain, located in the upper brainstem, projects diffusely to the cerebral cortex, thalamus, hypothalamus, basal ganglia, hippocampus, and amygdala.
To the amygdala
To the hippocampal formation
To the spinal cord
Raphé nuclei
Rostral domain
Caudal domain
a

Figure 2

(a) Sagittal view of whole human brain upon which the site of the 5-hydroxytryptamine (5-HT) neuronal cell bodies (red circles) and their projections (red lines) are superimposed. Serotonergic neurons are located in the brainstem in either the rostral or the caudal domain, each of which has different projections and functions. In the rostral domain, 5-HT neurons are present in the rostral pons and midbrain and project rostrally to the cerebral cortex, thalamus, hypothalamus, hippocampus, and basal ganglia to help modulate cognition and mood, among other functions. In the caudal domain, 5-HT neurons are located primarily in the medulla and project caudally to other brainstem regions, cerebellum, and spinal cord; this domain, known as the medullary 5-HT system, is postulated to integrate and modulate homeostatic function relative to the individual’s state. Interconnections exist between 5-HT neurons in the rostral and caudal domains but are poorly characterized. (b) Cross-sectional diagrams of the medullary 5-HT system at representative rostral and midlevels. Panel b based upon the human brainstem atlas of Olszewski & Baxter (188). The nuclei shown in red are the site of 5-HT cell bodies, positioned in the midline raphé, extra-raphé, and ventral surface. The nucleus of the solitary tract (NTS) and the hypoglossal nucleus (HG) (blue), along with the principal inferior olive (PIO) (tan), are a major target site of 5-HT projections within the medulla. Abbreviations: ARC, arcuate nucleus; IRZ, intermediate reticular zone; nGC, nucleus gigantocellularis; nPGCL, nucleus paragigantocellularis; ROB, raphé obscurus; RPA, raphé pallidus.

(Figure 2). It helps mediate arousal, cognition, mood, motor activity, and cerebral blood flow (86) and has been implicated in cognitive problems and/or derangements in autism (87), depression (88), and fetal alcohol syndrome (89). The medullary 5-HT system projects diffusely to other brainstem sites, the cerebellum, and the spinal cord (Figure 2) and is critical for respiratory and autonomic output (Figure 3). This 5-HT caudal system may be involved in other disorders of respiratory and autonomic function than SIDS, e.g., Rett syndrome (90).

Anatomically, the medullary 5-HT system comprises the 5-HT neuronal cell bodies in the medulla that are located in the raphé, extra-raphé (medial and lateral reticular formation), and ventral surface (arcuate nucleus) (Figure 2). The inhibitory or excitatory effect of 5-HT is determined largely by the pre- and postsynaptic receptor subtypes. At least 17 5-HT receptor subtypes are currently recognized and are organized into 7 groups (5-HT1–5-HT7) based upon genetic and pharmacologic features, and all but one (5-HT3) act as neuromodulators through G proteins and second-messenger systems (91). Serotonergic receptors are located both synaptically and extrasynaptically with 5-HT1A receptors in the highest abundance as autoreceptors on the soma and dendrites of 5-HT neurons; 5-HT
Figure 3
Schematic diagram of the inputs and outputs of the caudal raphé in the medulla relevant to multiple homeostatic functions. The 5-hydroxytryptamine (5-HT) neurons (red dots) innervate the specific effector systems (right). Inputs into the caudal raphé (left) include known transmitter and receptor phenotypes that are specifically on 5-HT neurons (circles) and unknown transmitter/modulator phenotypes (triangles and question marks). The caudal raphé innervates the major effector nuclei of respiration, chemosensitivity, upper airway control, and autonomic regulation, including temperature. It receives inputs from the limbic system, the hypothalamus, other brainstem regions, and the spinal cord. Abbreviations: BAT, brown adipose tissue; GABA, γ-aminobutyric acid; HG, hypoglossal nucleus; IML, intermediolateral column of the spinal cord; LHA, lateral hypothalamic area; NE, norepinephrine; NTS, nucleus of the solitary tract; PAG, periaqueductal gray; PGCL, paragigantocellularis lateralis; PreBötC, pre-Bötzinger complex; RTN, retrotrapezoid nucleus; RVLM, rostral ventrolateral medulla; VLPO, ventrolateral preoptic area.

released from axodendritic processes of the same neuron, or from adjacent neurons, may activate these autoreceptors to inhibit firing.

To date, the most robust neurochemical abnormality in SIDS involves the medullary 5-HT system (23–26), which is involved in approximately 70% of SIDS deaths (23–25) (Figure 4). For the neurochemical analysis of SIDS brainstems we use tissue receptor autoradiography, as it allows for precise neurochemical measurements in virtually all anatomic sites—even those as small as the arcuate nucleus—and it is capable of screening every nucleus for neurochemical abnormalities;
these are important features for the initial analysis of brainstem neurochemistry in SIDS, in which abnormalities are postulated but for which there may be no specific histopathologic clues. We have now published 5-HT-related brainstem abnormalities in SIDS cases in three independent data sets: (a) cases from Children's Hospital Boston and the San Diego Medical Examiner's Office, 1985–1995 (period of case accrual, prior to the onset of the national supine sleep campaign) (23, 63, 69, 92, 93); (b) American Indian infants from the Northern Plains (a major high-risk population for SIDS), 1992–1994 (35, 65); and (c) cases from the San Diego Medical Examiner's Office, 1996–2005 (in the era of safe sleep campaigns) (25).

In the first data set, we examined binding in multiple nuclei in the same brainstems (midbrain, pons, and medulla) for six neurotransmitter receptors (5-HT, muscarinic, nicotinic, opioid, α2-adrenergic, and kainate) with intentionally broad radioligands. We first observed the 5-HT receptor binding abnormality characterized by a reduction in binding or a decrease in binding with age compared to an increase in controls (age versus diagnosis interaction) with the radioligand 3H-lysergic acid diethylamide (3H-LSD), which binds to multiple 5-HT receptor subtypes, including 5-HT1A and 5-HT2A (23). This 5-HT receptor binding defect was localized almost exclusively to nuclei containing the 5-HT cell bodies in the medulla (raphé, extra-raphé, and ventral components of the medullary 5-HT system) (Figure 2) (23). In this first data set, we found no differences between SIDS and controls in binding to opioid and α2-adrenergic receptors in the same cases that had 5-HT receptor binding abnormalities (92, 93), indicating that the 5-HT binding deficits in SIDS cases are not part of a generalized receptor deficit, but rather represent a specific pattern whose cause has yet to be determined. The same pattern of 5-HT receptor binding abnormalities demonstrated by 3H-LSD in this data set was reproducible in the second independent data set of a high-risk population for SIDS, i.e., the American Indians of the Northern Plains.

In the third data set, we focused exclusively upon the medullary 5-HT system and analyzed several 5-HT-related tissue markers in SIDS and control cases. Using a radioligand specific for the 5-HT1A receptor, we found reduced binding not only in medullary nuclei containing 5-HT cell bodies, but also in nuclei receiving 5-HT projections, e.g., nucleus of the solitary tract and hypoglossal nucleus (Figure 4) (25). In addition, we found that this reduced 5-HT1A binding was associated with increased 5-HT cell number and density, morphological immaturity of 5-HT cell types, and a relative reduction in binding to the 5-HT transporter (5-HTT) compared to 5-HT cell number in the same SIDS cases (25). Notably, the 5-HTT is the key regulator of 5-HT levels at the synapse, and its upregulation can result from genetic factors, e.g., polymorphisms, and/or environmental factors, e.g., cytokines, alcohol, nicotine, and cocaine (94–96). Importantly, other groups have reported 5-HT abnormalities in the brainstem or cerebrospinal fluid of SIDS cases, including reduced 5-HT1A and 5-HT2A receptors detected by immunocytochemistry in the medulla (26), as well as genetic polymorphisms in the promoter region of the 5-HTT (56). Parenthetically, the LL (as opposed to the SS) genotype of the 5-HTT polymorphism associated with increased SIDS risk is thought to produce a mild 5-HT deficiency due to increased 5-HT uptake (97); this gene variation could potentiate an underlying medullary 5-HT abnormality when affected SIDS infants are stressed and optimal 5-HT modulation of homeostatic responses is essential.

**Risk Factors and the Medullary 5-HT System**

In the third data set we analyzed, all the SIDS cases (n = 31) had at least one intrinsic or extrinsic risk factor, 74% had at least three risk factors, and 64% were bed sharing or were found prone at the time of death (25). Medullary 5-HT abnormalities were found in SIDS infants who were (a) found dead in either...
the prone or the supine position, (b) found either face down or not face down, (c) either bed sharing or not bed sharing, and (d) born either prematurely or at term (25). Thus, although 5-HT abnormalities were common among SIDS infants irrespective of particular risk factors, they were also associated with potentially asphyxiating environments such as prone sleep position and bed sharing. The small sample of SIDS cases in this study did not uncover a significant association between the LL genotype for the 5-HTT polymorphism, 5-HTT

---

### a 5-HT\(_{1A}\) receptor binding

![Graph showing 5-HT\(_{1A}\) receptor binding densities for different regions with statistical significance marked with asterisks.](image)

**Graph Legend:**
- Controls n = 6
- SIDS n = 17

**Statistics:**
- \*P < 0.01
- \**P < 0.005

### b 5-HT neuronal cell number

![Graph showing 5-HT neuronal cell counts for different regions with statistical significance marked with asterisks.](image)

**Graph Legend:**
- SIDS n = 16
- Controls n = 7

**Statistics:**
- \*P < 0.005
- \**P < 0.0001

### 5-HT neuronal differentiation

- **Granular**
  - Image of granular cells
  - Ratio granular:combined cells
- **Multipolar**
  - Image of multipolar cells
  - Ratio multipolar:combined cells

**Graphs showing cell ratios with significance marked with asterisks.**

**Graph Legend:**
- SIDS n = 16
- Controls n = 7

**Statistics:**
- \*P < 0.005

### d Serotonin transporter binding

![Graph showing SERT binding densities with statistical significance marked with asterisks.](image)

**Graph y-axis:** SERT binding (fmol mg\(^{-1}\))/number 5-HT cells

**Graph Legend:**
- Controls
- SIDS

**Statistics:**
- \**P < 0.005
binding levels, and SIDS (25). The possibility that defects in autonomic and respiratory regulation are linked specifically to medullary 5-HT abnormalities is substantiated by our case report of a two-week-old SIDS infant who had subclinical autonomic and respiratory dysfunction at two days of age and whose autopsy revealed medullary 5-HT abnormalities (35). In the American Indians, 5-HT receptor binding in the arcuate nucleus was significantly reduced in infants of mothers who smoked during pregnancy compared with those infants whose mothers did not smoke (p = 0.010), irrespective of the cause of death (24). This association between a prenatal exposure and reduced brainstem 5-HT receptor binding in the postnatal period provides biologic plausibility as to how environmental risk factors potentiate an underlying brainstem abnormality and thereby increase the likelihood of sudden death associated with medullary 5-HT defects.

**The Relationship of Brainstem Pathology to Hypoxia-Ischemia**

Is the neurochemical brainstem pathology in SIDS infants secondary to hypoxia-ischemia, given the evidence that infants who subsequently died of SIDS had episodes of bradycardia and/or apnea days or even weeks prior to death? The possibility of chronic intermittent hypoxia (CIH) occurring prior to death is supported by reports in SIDS cases of hypoxic tissue markers, i.e., hyperplasia of pulmonary neuroendocrine cells (98), reduced hypoxanthine oxidase in the vitreous humor (99), and elevated vascular endothelial growth factor in the cerebrospinal fluid (100). The reports in SIDS of subtle, albeit nonspecific, gliosis in the brainstem (61, 67, 101, 102) are of particular interest, as the brainstem is a recognized region of vulnerability to hypoxia-ischemia in early life. Interestingly, gliosis develops within days of a brain insult, underscoring the possibility of subacute or chronic (nonagonal) injury in at least some cases. In addition, apoptosis has been reported in brainstem nuclei of SIDS cases, consistent with hypoxia-induced programmed cell death (103, 104).

Histopathologic abnormalities consistent with hypoxia-ischemia have also been reported in regions rostral to the brainstem, including reactive microglia in the hippocampus (105), cerebral white matter gliosis (106), and periventricular leukomalacia (106, 107), as well as in an increased number of copper/zinc superoxide dismutase– and glutathione peroxidase–expressing neurons in the hippocampus (108). Importantly, chronically ill infants who die from prolonged oxygenation disorders such as cyanotic congenital heart disease do not have medullary 5-HT abnormalities (or muscarinic/kainate receptor deficits) that mimic those found in SIDS (23, 69, 74); this finding counters the possibility that these neurotransmitter abnormalities are secondary to hypoxia-ischemia. Indeed, SIDS infants with medullary 5-HT abnormalities may be unable to respond to hypoxic challenges because impairment in adaptive responses to CIH is known to be regulated in part by the caudal 5-HT raphe (109). Intermittent hypoxia triggers an increase in

**Figure 4**

Multiple abnormalities in 5-hydroxytryptamine (5-HT) markers in sudden infant death syndrome (SIDS) infants compared to controls adjusted for postconceptional age in cases from the San Diego Medical Examiner’s Office, 1996–2005 (modified from Reference 25). (a) Binding to 5-HT1A receptors is significantly reduced in nuclei that contain 5-HT neurons and comprise the source neurons of the medullary 5-HT system. (b) The number of 5-HT neurons is increased in the same regions in SIDS cases compared to controls in the same data set. (c) The ratio of granular (immature) 5-HT neurons to total 5-HT neurons is significantly increased in the SIDS cases compared to controls, whereas the ratio of multipolar (mature or well-differentiated) 5-HT neurons to total 5-HT neurons is decreased, suggesting a developmental failure in 5-HT neuronal differentiation in the SIDS cases. (d) The relative binding of the 5-HT transporter (SERT) to total number of 5-HT neurons is proportionately reduced in SIDS cases compared to controls, suggesting a relative failure in 5-HT transporter regulation relative to 5-HT cell number in SIDS. Abbreviations: ARC, arcuate nucleus; GC, ganglion cells; IRN, intermediate reticular nucleus; PGCL, paragigantocellularis lateralis; ROB, raphe obscurus.
output of the phrenic nerve (which drives the diaphragm) to activate postsynaptic 5-HT1A receptors and enhance glutamatergic-mediated respiratory drive (110).

**Brainstem Abnormalities Associated with Maternal Cigarette Smoking during Pregnancy**

Although prenatal exposure to nicotine (a major toxic component of cigarette smoke) reduces utero-placental blood flow and can cause fetal brain injury secondary to hypoxia-ischemia, nicotine itself—via interactions with the brain’s endogenous nicotinic receptors—can adversely affect neural development and function (111). In the first and second data sets described above, we found no alterations in nicotinic receptor binding between SIDS and control brainstems except when the cases were stratified by a history of maternal smoking during pregnancy (64, 65). Upon stratification, we found impaired regulation of nicotinic receptor binding in exposed SIDS cases compared to nonexposed SIDS cases, as well as to exposed and nonexposed controls in the same data sets with 5-HT receptor binding abnormalities (64, 65). The affected regions were in the rostral pons and/or midbrain in the exposed SIDS cases and involved nuclei critical for arousal (e.g., locus coeruleus, raphé dorsalis, and oral pontine reticular formation) and for the coordination of defense responses (e.g., periaqueductal gray) (64, 65).

Altered nicotinic receptor regulation upon exposure to cigarette smoke in SIDS infants is of interest in light of reports of arousal deficits in living infants exposed to prenatal cigarette smoke; these deficits include reduced frequency of spontaneous arousals (112) and reduced arousal responses to auditory stimuli and mild hypoxia (113, 114). The autopsy observations in SIDS brainstems, although demonstrated in two independent studies, involve small sample sizes and thus require confirmation in larger populations. Additional brainstem findings of gliosis, apoptosis, and arcuate nucleus hypoplasia in SIDS infants associated with histories of maternal smoking during pregnancy support the possibility of harmful effects of cigarette smoke/nicotine upon brainstem development in SIDS (104, 115). The association of altered nicotinic receptor binding in SIDS infants whose mothers smoked during pregnancy with 5-HT receptor binding abnormalities suggests that certain risk factors can adversely influence non-5-HT systems, which in turn compound the primary 5-HT abnormality with further dysfunction in cardiorespiratory patterning and/or arousal. In effect, a systematic analysis of multiple transmitters/modulators that interface with the medullary 5-HT system is needed to establish the precise neurochemical pathology in all or subsets of SIDS cases. Without such information, the pathogenesis of the disorder cannot be established, and optimal interventions cannot be developed.

**The Cause and Pathogenesis of Medullary 5-HT Abnormalities**

Although the cause and pathogenesis of medullary 5-HT abnormalities in SIDS are unknown, the finding of an increased number of 5-HT neurons in SIDS cases suggests a primary developmental defect in the regulation of 5-HT cell number during gestation, when 5-HT number is mainly (but not completely) determined (84). The increased number of 5-HT neurons of simple morphology (i.e., granular) and a decreased number of neurons of complex morphology (i.e., multipolar) in SIDS cases compared to controls supports a maturation delay in 5-HT neuron differentiation (25). However, we do not know whether the extracellular 5-HT levels in the medulla in SIDS cases are higher or lower compared to controls, nor do we know the nature of the mechanistic sequence of events leading to the changes in receptors and transporter. Nevertheless, the increased number of 5-HT neurons, coupled with the reduction in 5-HT1A receptor binding and the relative reduction in 5-HTT binding in medullary sites in SIDS cases, suggests that the synthesis and availability of 5-HT (and by
extrapolation neuronal firing) are altered within 5-HT pathways.

The 5-HTT is expressed predominantly in perisynaptic sites on 5-HT neuron terminals, and is therefore a marker of 5-HT innervation in a given region. A relative reduction in 5-HTT expression may be due to reduced expression of 5-HTT protein at 5-HT neuron terminals and/or to a reduced number of 5-HT terminals and synapses. Mutations/polymorphisms in the genes coding for transcription factors and/or neurotrophins that regulate 5-HT development, along with exposure to harmful prenatal toxins, may adversely affect the development of the medullary 5-HT system. Maternal drinking and smoking during pregnancy, which are major risk factors for SIDS, impair 5-HT development in experimental animals (89, 116–118). An increased number of 5-HT neurons may lead to an excess of extracellular 5-HT and a compensatory downregulation of 5-HT1A receptors; alternatively, 5-HT synthesis and/or release may be dysfunctional in the 5-HT neurons (which become overabundant in compensation), resulting in a deficiency of extracellular 5-HT. Clearly, further research is needed to establish the cause and consequences of the medullary 5-HT defects in SIDS.

THE FUNCTION OF THE MEDULLARY 5-HT SYSTEM AND ITS RELATIONSHIP TO SUDDEN INFANT DEATH SYNDROME

How the abnormalities in the medullary 5-HT system translate into a mechanism for sudden and unexpected death in apparently healthy infants likely relates to the importance of this and related brainstem systems in multiple homeostatic processes that, if dysfunctional, could in sum promote death. These processes involve central chemoreception, modulation of inhibitory reflexes (e.g., laryngeal chemoreflex [LCR]), sleep, arousal, temperature regulation, and autorecuscitation. SIDS research depends upon the use of animal models of homeostatic dysfunction because there are no available biomarkers for identification and study of living infants at risk. Also, given that there are no known spontaneous models of SIDS in animals, we have focused upon the analysis of homeostatic function in young rats, mice, and piglets, in which abnormalities of the human medullary 5-HT and related pathology are produced by various pharmacological and genetic methods, as well as in reduced brainstem preparations and cell culture systems.

Breathing

Sympathetic projections from 5-HT neurons are present in all of the major respiratory nuclei, including (a) the phrenic nucleus in the cervical spinal cord (drive to the diaphragm), (b) the hypoglossal nucleus (upper airway patency), (c) the nucleus of the solitary tract (visceral sensory reception for baroreceptor reflex, LCR, and peripheral chemosensitivity), (d) the retrotrapezoid nucleus (additional site of chemoreceptors), and (e) the pre-Bötzinger complex (preBotC; the putative site of central rhythm generation for breathing) (81, 119, 120) (Figure 3). These terminals, many of which also contain the neuropeptides substance P (SP) and thyrotrophin-releasing hormone (TRH), arise from the raphé pallidus and the raphé obscurus (81, 121, 122). In addition, a subset of individual 5-HT axons arising in the raphé branch and send collaterals to both the preBotC and the hypoglossal nucleus (G.B. Richerson, unpublished observations; 123), suggesting a means by which raphé neurons coordinate and integrate functions between two different homeostatic-related sites. Receptors for 5-HT, SP, and TRH are located on neurons within each of the major respiratory nuclei (81); their activation in vitro induces modulatory effects on respiratory neurons that enhance excitability of respiratory neurons and respiratory network activity. For example, serotonin stimulates neurons of the preBotC via activation of 5-HT1A receptors by enhancing persistent sodium currents (124). Such 5-HT1A receptor activation also

Laryngeal chemoreflex (LCR): developmentally regulated reflex in which apnea and swallowing are triggered by the stimulation of receptors in the laryngeal lumen by a foreign liquid

Pre-Bötzinger complex (preBotC): collection of bilateral neurons in the rostral ventrolateral medulla of experimental animals; the putative site of central rhythm generation critical for breathing
converts some preBötC neurons into intrinsic “bursters” by modulation of leak channels (G.B. Richerson, unpublished observations; 123); SP also helps induce bursting activity in these neurons, and TRH induces bursting pacemaker activity in neurons within the portion of the nucleus of the solitary tract involved in respiratory control (125). Serotonin, SP, and TRH also stimulate neurons in the retrotrapezoid nucleus (83) and in the hypoglossal nucleus (126). Thus, several neurotransmitters/neuropeptides released by 5-HT neurons directly enhance the excitability of multiple subsets of neurons within the respiratory network.

Using transverse, 350–600-μm-thick slice preparations of the brainstem, the rostral portion of the respiratory network containing the preBötC can be isolated (127). Similarly, the respiratory network can be isolated in more intact preparations that contain the whole brainstem and spinal cord (128). In all of these reduced brainstem preparations, rhythmic respiratory activity continues to be generated, and motor output can be recorded in the phrenic or hypoglossal nerves. This motor output is responsive to manipulations of the 5-HT system: 5-HT2A receptor blockers, for example, reduce the frequency and amplitude of respiratory activity generated by the preBötC in rostral medullary slice preparations (G.B. Richerson, unpublished observations; 123; 124), indicating that the generation of respiratory output depends upon the release of endogenous 5-HT from 5-HT neurons in the rostral medulla. Genetic mouse models that lead to either a decrease or an increase in 5-HT neuronal number and brain 5-HT levels result in unstable respiratory output in vitro (129, 130). The Pet-1 knockout mouse with decreased 5-HT neuronal number and brain 5-HT levels, for example, generates an abnormal respiratory output with increased irregularity in rostral medullary slices (129). In contrast, mice with genetic deletion of necdin (130) and of monoamine oxidase A (an enzyme that degrades synaptic 5-HT) (131) demonstrate both increased brain 5-HT levels and substantial respiratory irregularity in the neonatal brainstem/spinal cord preparation.

Chemoreception: Response to Hypercapnia

An abnormal ventilatory response to increased CO₂ has long been postulated as a determinant of SIDS (63, 69, 74). In addition, recent observations indicate a key role for asphyxia (i.e., the combination of hypercapnia and hypoxia) in the pathogenesis of SIDS (42, 57). Certainly, reduced chemosensitivity would be detrimental in asphyxia. Serotonergic neurons are well suited to a role as central respiratory chemoreceptors, as they are closely associated with the basilar artery and its largest branches near the ventral surface of the medulla; i.e., they are in a position to directly monitor arterial PCO₂ (80). In brain slices and dissociated cell cultures in which 5-HT neurons are analyzed after isolation from synaptic inputs, 5-HT neurons respond intrinsically to increased PCO₂ with a large increase in firing rate (132, 133) (Figure 5); this response is due to a decrease in intracellular pH induced by hypercapnia (133). On average, these neurons increase their firing rate threefold in response to a decrease in pH from 7.4 to 7.2. Chemosensitivity increases during postnatal development, with a blunted response to pH before postnatal day 12 in rats (134). This physiological delay in chemosensitivity is potentially relevant to SIDS because it indicates that 5-HT neurons may be immature during the critical developmental period, throughout which all infants are susceptible to hypercapnia.

In whole animals, the role of 5-HT neurons in the response to hypercapnia is complex and depends upon the species, the anatomic locus in the medullary 5-HT system, age, gender, and the experimental paradigm. In adult rats, reduction of approximately 28% of the medullary raphé 5-HT neurons by injection of the cell-specific toxin anti-5-HT-saporin decreases the CO₂ response by 18–20% both in waking and in non–rapid eye movement (NREM) sleep (136). Inhibition of these neurons by focal dialysis of the 5-HT₁A receptor agonist 8-hydroxy-2-di-n-propylaminotetralin (DPAT), which presumably leads to inhibition of 5-HT neuronal
Figure 5

(a) Serotonergic neuronal firing in response to changes in carbon dioxide (CO2) and pH. Serotonergic neurons respond to CO2/acidosis with an increase in firing rate. Shown is the membrane potential of a 5-hydroxytryptamine (5-HT) neuron from a rat in primary cell culture recorded using a patch clamp electrode. Hypercapnic acidosis (high CO2 plus low pH) causes an increase in firing rate. This response is due to intrinsic chemosensitivity. Panel a modified from Reference 133 with permission. (b) Pharmacological "rescue" of abnormal CO2 chemosensitivity by intraventricular infusion of 5-HT in Lmx1b+/f/p mice. Central CO2 chemosensitivity is severely depressed in the absence of 5-HT neurons in these mice but can be rescued by therapeutic replacement of 5-HT. Ventilation in response to an increase in inhaled CO2 from 0% to 7% is shown for wild-type mice (blue curve), mice in which development of all 5-HT neurons is prevented by selective deletion of the gene for Lmx1b in all 5-HT neuron precursor cells (Lmx1b+/f/p) (red curve), and Lmx1b+/f/p mice (orange curve), whose lateral cerebral ventricles are infused with 5-HT via a catheter. Baseline ventilation is normal in the Lmx1b mice, but the hypercapnic ventilatory response is decreased by 50%. Exogenous 5-HT stimulates baseline ventilation, and also restores the CO2 response to normal. Panel b adapted from Reference 135 with permission.

activity via autoreceptor activation, lowers the CO2 response by approximately 20% in these states. However, daily focal treatment of the medullary raphé by microdialysis of the specific 5-HT reuptake inhibitor fluoxetine increases the CO2 response (137).

Other neurotransmitters and neuromodulators influence chemosensitivity. For example, lesioning neurons that express the neurokinin-1 receptor (NK1R), which binds to SP, are associated with a decrease in the CO2 response, an effect that is exaggerated if 5-HT and the NK1R-expressing neurons are lesioned in combination (136). Our group studied unique conditional knockout adult mice in which the transcription factor Lmx1b was genetically deleted in Pet-1-expressing cells (Lmx1b+/f/p), which resulted in a near-complete absence of brainstem 5-HT neurons. In these mice the hypercapnic ventilatory response was decreased by 50% compared to wild-type mice, whereas baseline ventilation and the hypoxic ventilatory response remained normal (82).

Of major relevance to potential pharmacological treatment of 5-HT-related deficits and defective chemosensitivity in SIDS infants is
our finding that infusion of 5-HT into the ventricle of these mice stimulated the baseline ventilation and “rescued” the blunted hypercapnic ventilatory response (82) (Figure 5). In this model, 5-HT appears to enhance the response of the remaining respiratory network to CO₂; this effect could occur, for example, by enhancing the chemosensitivity of the neurons within the retrotrapezoid nucleus (83). Taken together, these adult mouse studies show that medullary raphé 5-HT cells participate in the CO₂ response, that their effect depends on an interaction with NK1R-expressing neurons, and that there is an interaction with cells having GABA receptors, suggesting that endogenous GABA release normally modulates the CO₂ response. Moreover, microdialysis of DPAT into the medullary raphé in newborn conscious piglets inhibits the activity of 5-HT neurons and decreases the CO₂ response in an age-dependent manner, i.e., piglets younger than eight postnatal days show an increased response, whereas piglets older than eight postnatal days show a decreased response (138). When studied in early postnatal life, Pet-1 knockout mice also demonstrate irregular breathing and posthypoxic respiratory depression (129).

Of interest is the mounting evidence for sexually dimorphic features in the brainstem 5-HT system (139), given (a) that SIDS occurs twice as often in males compared to females (140), (b) our report of a greater reduction of 5-HT₁₅A binding in the medullary raphé in males compared to females dying of SIDS (75), and (c) the report that plasma levels of testosterone, but not estradiol, are significantly higher in both male and female SIDS infants compared to age-matched control infants (141). In this regard, a substantial ablation (approximately 60%) of medullary raphé neurons with a selective toxin for 5-HT decreases the CO₂ responses in NREM sleep in males only (142). In 5-HTT knockout mice, the reduction in ventilatory response is greater in males (68% reduction) compared to females (22% reduction) (142a). Notably, adult 5-HTT knockout mice demonstrate increased extracellular 5-HT (143), decreased tissue 5-HT, increased 5-HT neuronal activity, reduced 5-HT₁₅A receptor binding (144), and varying changes in postsynaptic 5-HT receptor function (145). Thus, this neurochemical phenotype mimics two of the 5-HT-related abnormalities described in the brainstem of the SIDS cases, i.e., reduced 5-HT₁₅A and relative reduction of 5-HTT binding. In sum, these data underscore the gender differences in brainstem-mediated 5-HT function, with females’ brains apparently relying less on 5-HT neurons in chemoreception and adapting more readily to the loss of 5-HT function.

Chemoreception: Response to Hypoxia

Although multiple neurotransmitters influence protective brainstem responses to hypoxia (146), there is evidence for a role of the medullary 5-HT neurons in such responses (147). In newborn piglets with a 60% reduction in medullary 5-HT neuronal number due to specific neurotoxic injections into the cisterna magna, breathing frequency is significantly increased, for example, in response to hypoxia but only during NREM sleep (142). Thus, 5-HT neuronal activity in the caudal raphé may normally suppress the ventilatory response to hypoxia in a state-dependent manner, a finding that requires verification.

The Laryngeal Chemoreflex

The LCR is elicited by water or other foreign liquids in the lumen of the larynx that stimulate mucosal receptors, which in turn project to respiratory-related medullary nuclei in the ventrolateral medulla via vagal projections to the nucleus of the solitary tract, resulting in apnea and swallowing. The LCR is developmentally regulated and occurs more commonly in newborn human infants and animals compared to adults (42, 148, 149). The LCR has long been

GABA: γ-aminobutyric acid
suspected to be a cause of or a contributing factor in some SIDS cases; investigators hypothesize that infants during the susceptible age range aspirate gastric contents during sleep and thereby trigger this reflex, which, if prolonged or unchecked, leads to lethal apnea (148–151). In anaesthetized piglets, the nonspecific inhibition of all neurons in the rostral medullary raphe (by focal dialysis of the GABA_A receptor agonist muscimol) causes a prolonged duration of apnea in the LCR (152). The LCR is also exaggerated in neonatal piglets and rats, whose body temperature rises 1–3°C above normal (150) (Figure 6). In piglets, the hyperthermic exaggeration of the LCR is most striking under postnatal day 5 and disappears by postnatal day 20 (151). Intravenous infusion of GABA_A receptor blockers reverses the enhancement of laryngeal apnea by whole-body hyperthermia, suggesting that the effect of hyperthermia on the LCR is mediated by GABAergic inhibition of respiration (151a). The specific role of medullary 5-HT neurons in modulation of the LCR in early life is under investigation.

**Autoresuscitation**

Asphyxia is strongly implicated in the sequence of events leading to SIDS (42), and is postulated to complicate rebreathing or upper airway compression in the face-down position or, alternatively, an exaggerated LCR triggered by gastric regurgitation during sleep. This asphyxia leads to death if effective life-saving mechanisms (e.g., autoresuscitation and/or arousal) do not intervene. In normal infants, autoresuscitation can terminate hypoxic gasping and thereby restore eupnea (normal breathing) (124, 153, 154); however, in vulnerable infants, autoresuscitation may fail and cause death. Tracings of infants who subsequently die of SIDS show effective gasping with large-amplitude breaths, abnormally complex breaths, and failure to increase heart rate (33). Gasping appears to involve neurons within the rostral medulla that have a bursting discharge due to pacemaker: The initiation of gasping requires severe hypoxia, a loss of inhibition, potentiation by elevated potassium levels (which often accompany hypoxia), and activation of persistent sodium channels (153). Gasping (and more specifically,
the activity of persistent sodium channels) is modulated by a variety of neurotransmitters, including 5-HT and norepinephrine via 5-HT2A and α1-adrenergic receptors, respectively (124, 154–156). Notably, neither 5-HT nor norepinephrine is required for hypoxic gasping in the isolated, perfused brainstem preparation in rats (154, 156). Yet when 5-HT1, 5-HT2, and α1 noradrenergic receptors are all blocked simultaneously, the number of gasps is significantly reduced and the restoration of eupnea is markedly impaired (154). Therefore, multiple neurotransmitter systems are likely involved in the regulation of gasping and autoresuscitation and no single neurotransmitter system appears essential.

Arousal

This protective mechanism allows for escape from asphyxiating microenvironments during sleep (42) and is postulated to be abnormal in at least some SIDS infants (39–41). Arousal comprises cortical and subcortical components and involves a progressive activation of specific subcortical and cortical brain structures. Cortical arousal results from ascending systems that originate in serotonergic, noradrenergic, dopaminergic, cholinergic, and histaminergic neurons in the rostral brainstem, basal forebrain, and hypothalamus; these systems excite neurons in the cerebral cortex, increase their firing rate, and cause electroencephalography (EEG) activation (157). Descending (subcortical) arousal, however, is mediated mainly by brainstem pathways that increase heart rate, blood pressure, respiration, and postural tone (158). Subcortical arousal involves autonomic and respiratory brainstem-mediated changes without changes in cortical (EEG) activity; in contrast, complete arousals involve cortical and autonomic activation. Positive feedback loops involving the ascending and descending arousal pathways produce brain and somatic activation (159). In light of 5-HT neurons’ preferential chemosensitivity to CO2, their diffuse and widespread ascending and descending projections, and their close association with large arteries (160), we suggest that 5-HT neurons in the rostral and caudal domain mediate in part the arousal response to hypercapnia during sleep.

A distinct feature of 5-HT neurons in both the rostral and caudal domains is that they fire differentially according to the individual’s level of arousal, with increased firing during waking, decreased firing during NREM, and almost-complete absence of firing during REM (161, 162). Based upon the relationship between arousal level and neuronal excitability, different global roles for the caudal 5-HT domain have been proposed (161, 163, 164); for example, the overall function of the caudal raphé is to act as a “gain-setter” that modulates different autonomic and somatomotor control systems at levels appropriate to arousal (164) (Figure 3). We conceptualize the medullary 5-HT system as a modulatory circuit comprising 5-HT neurons in the raphé, extra-raphé, and ventral surface of the medulla that almost completely encircle and/or intermingle with neurons within nuclei that form the final common pathways that directly produce (i.e., mediate) the specific homeostatic function (Figures 2, 3). Inputs from the limbic system, hypothalamus, spinal cord, and other brainstem sites also modulate the medullary 5-HT system (Figure 3). Although it is widely recognized that 5-HT neurons in the rostral domain are important for arousal, researchers have devoted little attention to the role of 5-HT neurons in the caudal domain in this function. Recently, however, we reported that 5-HT neurons in the medullary extra-raphé [paragigantocellularis lateralis (PGCL)] of the piglet play an important role in the sleep/wake cycle such that their inhibition results in sleep fragmentation, alternating periods of NREM and waking, and almost complete loss of REM (165) (Figure 7). Similarly, activation of 5-HT1A receptors in the piglet raphé obscurus and raphé pallidus disrupt sleep and abolish REM (166). Thus, these findings implicate the medullary (raphé and extra-raphé) in the sleep/wake cycle.
Thermoregulation

Abnormal thermoregulation in SIDS is suggested by increased risk from heavy wrapping with blankets and/or clothing (overbundling), elevated ambient temperatures at the time of death (44), and increased periadrenal brown adipose tissue (BAT) at autopsy (61). Significantly, BAT, regulated by the sympathetic nervous system, is the major source of nonshivering thermogenesis in newborn humans and animals and plays an essential role in arousal during hibernation (167). In rats and rabbits, neurons in the medullary raphé modulate the sympathetic control of BAT thermogenesis and thermoregulatory skin vasoconstriction (168, 169). Specifically, 5-HT neurons increase their firing rates during cooling in parallel with increased BAT temperature (170, 171). Moreover, local application of 5-HT into the intermediolateral column of the spinal cord (preganglionic sympathetic outflow) increases sympathetic drive to BAT (172). In newborn piglets that are chronically instrumented and conscious, activation of 5-HT1A receptors by microdialysis of DPAT directly into the raphé or PGCL reduces shivering and peripheral vasoconstriction in response to acute and chronic cold stress (166). In addition, Lmx1b−/− mice with a profound deficiency in 5-HT neurons rapidly become hypothermic when exposed to cold ambient temperatures (4°C); this thermoregulatory failure is due to impaired shivering and nonshivering thermogenesis, while thermosensation and heat conservation remain intact (82). These studies implicate 5-HT in modulation of temperature.

Cardiovascular Control

Animal studies have implicated serotonergic modulation in the control of heart rate, heart rate variability, and the baroreceptor reflex (173, 174). In the rat nucleus of the solitary tract, for example, 5-HT2A receptors facilitate modulation of the cardioaortic component in the baroreceptor reflex; this component is triggered by 5-HT released from the nodose ganglion and/or the caudal raphé (173). In rats, 5-HT2A receptors are also expressed upon the dendrites of catecholaminergic neurons, suggesting 5-HT-catecholaminergic interactions in the function of the nucleus of the solitary tract (174).
THE DEVELOPMENT OF BRAINSTEM 5-HT NEURONS

Serotonergic abnormalities in SIDS brainstems are likely to originate during prenatal development. Evidence supporting this hypothesis includes the increased risk for SIDS associated with adverse exposures during pregnancy (7, 11, 13), features of abnormal 5-HT maturation (i.e., aberrant regulation of 5-HT cell number and morphology) in the SIDS medulla (25), and altered 5-HT and nicotinic receptor binding the medulla in the postnatal period associated with adverse influences of prenatal alcohol and smoking exposure (24, 175). Given the important role of 5-HT as a growth factor in early cell division, migration, and differentiation in the brain (176, 177), 5-HT may be especially critical in the pathogenesis of the 5-HT-related abnormalities in medullary development in SIDS as well. Thus, a requisite step toward identifying underlying mechanisms in SIDS is the delineation of where, when, and how 5-HT neurons develop during embryonic and fetal life. Substantial progress has recently been made in this area of developmental neurobiology through the use of various model organisms (e.g., mouse, rat, chicken) and cutting-edge, molecular genetic approaches (e.g., gene gain- or loss-of-function studies and genetic fate mapping) (178–181). Several of these advances, which are indeed shaping contemporary SIDS research, are summarized below.

Serotonergic progenitor cells reside in the embryonic hindbrain in territories that bilaterally flank the floor plate and span much of the rostrocaudal extent of the hindbrain (182) (Figure 8). Acquiring generic 5-HT neuron identity from these progenitor cells involves turning on the expression of the transcription factor–encoding genes, which include Nkx2.2, Mash1/Ascl1, and Foxa2 (178–180, 183). This is then followed by the activation of expression of the transcription factors Perl, Lmx1b, and Gata2/3, which take descendant postmitotic precursor cells to a state of 5-HT production, the hallmark of the mature 5-HT neuron. Sonic hedgehog, a signaling molecule secreted by nearby floor plate cells, initiates this cascade of cellular and molecular events (178–180, 184). Understanding the genetic programs and signaling pathways critical to 5-HT neuron development is important because they identify avenues for exploration with respect to SIDS pathogenesis, such as alterations in various transcription factors either through genetic mutation or upon prenatal exposure to known risk factors for SIDS, such as alcohol and cigarette smoke (discussed above).

Although 5-HT production generally defines a neuron as serotonergic, there are in fact many different subtypes of 5-HT neurons that are distinguishable by their different and widespread positions in the brainstem (as constituents of the nine different types of 5-HT nuclei historically referred to as B1–B9) (185), by differences in their synaptic targets (86), and by their coexpression of different neuropeptides and neurotransmitters (e.g., GABA, SP, and/or TRH) (186), as well as by their different cellular, electrophysiological, and functional properties (e.g., some 5-HT neurons are chemosensitive and others are not; some 5-HT neurons are involved in pain, others in respiration) (183). Understanding which of these parameters come together in a single cell to define a specific functional subtype of 5-HT neuron is an area of active investigation for our group, given that different 5-HT neuronal subtypes are likely associated with different disease vulnerabilities. Of interest here is the identification of those 5-HT subtypes most relevant to SIDS; achieving this goal requires knowledge of the molecular markers of these different subtypes. In working toward this goal, we recently developed an approach that takes advantage of molecular differences among 5-HT progenitor cells, rather than relying on the identification of molecular differences among mature 5-HT neurons (187).

We are investigating the possibility that each of the molecularly distinct 5-HT progenitor cell pools gives rise to a functionally distinct subtype of 5-HT neurons. Our rationale is based upon the concept that developmental programs that define the fate and function of neurons are often
Figure 8

The organization of 5-hydroxytryptamine (5-HT) neurons as defined by embryonic origin and developmental gene expression profile. This organization differs from that based historically on anatomical architecture. (a–c) Schematics of mouse embryo at 10–12 days postcoitum (d.p.c.) illustrate the sagittal section (with the brain shown in white) (a), the transverse section (b), and the dorsal view of the hindbrain (c). The dashed lines in panels a and c represent the level of section in panel b. The 5-HT primordium is situated on either side of the floor plate and spans almost the entire length of the hindbrain. (d) Sagittal schematic of adult brainstem compressed along the mediolateral axis. Blue represents the 5-HT progenitor cells in r1 (x) or r1-derived mature 5-HT neurons (d); orange, r2 5-HT progenitors (o) or descendants (d); magenta, r3; pink, presumed r5; gray, r6–r7. B1–B9 refer to the names given to nuclei defined anatomically. The dashed lines represent coronal sections (e–h) presented rostral to caudal, left to right. r1-derived 5-HT neurons (blue) populate the B7, B6, and B4 groups in their entirety, as well as aspects of the B9, B8, and B5 nuclei. r2-derived 5-HT neurons (orange) populate the B9, B8, and B5 nuclei intermingled with both the r1-derived (blue) and the r3-derived (magenta) 5-HT neurons. Presumed r5-derived 5-HT neurons (pink) populate the B3 nucleus intermingled with more caudal (r6–r7) 5-HT neurons (gray). Figure modified from Reference 187.
set in motion by the action of factors that are differentially expressed among their antecedent progenitors.

The 5-HT progenitor territory can be subdivided along the rostrocaudal axis into groups on the basis of the broader partitioning of the hindbrain into segments (rhombomeres) with distinguishing gene expression profiles (Figure 8). Thus, aspects of mature 5-HT neuron subtype identity may be determined through the actions of rhombomere (r)-specific genetic programs on resident 5-HT progenitor and precursor cell subsets. Using the method of genetic fate mapping, we deconstructed the 5-HT neural system based on rhombomere-defined 5-HT sublineages, distinguishing those 5-HT neurons arising from the primordium as r1, r2, r3, r5, or r6–r8 (187) (Figure 8). [5-HT neurons are thought not to arise from r4 (183).] Interestingly, we found that the generated developmental and molecular map of the 5-HT neural system differs from the classically defined groupings (B1–B9) of mature 5-HT neurons (187) (Figure 8). Some anatomically defined B1–B9 nuclei receive contributions from multiple (molecularly distinct) progenitor cell pools, whereas others receive contributions from only one such pool. Are these newly identified genetic sublineages of physiological relevance? That is, do genetically defined subtypes of 5-HT neurons serve different functions, despite coexisting within a single anatomical nucleus? If so, which particular subtypes serve functions that, if defective, might render an infant vulnerable to SIDS? Answering these questions has become possible because a set of tools similar to that used to identify these genetic sublineages of 5-HT neurons can be used to selectively manipulate properties of individual sublineages, such as neurotransmission, in otherwise normal mice.

CONCLUSIONS

We postulate that SIDS results from the convergence of multiple factors primarily (but not exclusively) involving 5-HT-mediated mechanisms in the medulla (Figure 1). Given the wide array of homeostatic functions modulated by the medullary 5-HT system, we propose that an important subset of SIDS is due to a convergence of abnormal protective responses that are modulated by 5-HT and potentially by other neuromodulators and neurotransmitters (Figures 1, 3). Findings from laboratories worldwide, including ours, indicate that medullary 5-HT neurons have multiple roles in the control of breathing, blood pressure, temperature, and arousal and that their function differs by gender, age, and level of arousal. Moreover, these 5-HT neurons respond directly to CO2 as CO2 detectors, modulate the function of other CO2 receptor neurons, and affect hypercapnic and hypoxic responses in a gender-dependent manner. Neurons in the medullary raphé modulate the LCR, although we do not know whether 5-HT neurons specifically participate in this important inhibitory reflex. We speculate that primary medullary 5-HT defects cause subclinical homeostatic dysfunction days or weeks prior to the final lethal event and that they also cause repetitive hypoxia, leading in turn to secondary subtle brain injury and systemic hypoxic injury prior to death. It is also likely that particular sites of origin within the embryonic neural tube, with their unique profiles of gene expression, set in motion developmental programs that subsequently define functionally different subsets of raphé and extra-raphé 5-HT neurons (Figure 8), such that differences in origin likely determine postnatal functional differences within the 5-HT system.

Based upon the combined human and animal data presented above, we propose that SIDS results from the convergence of exogenous stressors (e.g., face-down sleep position, overbundling), a critical developmental period, sleep, and dysfunctional and/or immature cardiorespiratory and/or arousal systems (i.e., underlying vulnerability) that often involve abnormal 5-HT mechanisms (Figure 1). A lethal sequence, for example, might begin with intermittent hypoventilation, apnea, and periods of tachycardia and/or bradycardia—not uncommon events in early life. Uninterrupted
Compromised brainstem from genetic or environmental insult during embryonic development

Infancy as period of vulnerability

Sleep
Wake
Sleep
Sleep
Wake

Time

Resuscitative gasping versus prolonged apnea, inadequate arousal, no hypoxic gasping, death from lack of O₂

Death precipitated by rare events (e.g. 1–3) coinciding at a chance moment in time (e.g. 4–5)

Figure 9

Schematic diagram of the concept of sudden infant death syndrome (SIDS) as the biologic version of the perfect storm, in which the combination of multiple events results in sleep-related sudden death during a critical developmental period and in which the combination of events is far more powerful than each individual event alone. Depicted here is one possible lethal scenario, in which the vulnerable infant with an underlying abnormality in the medullary 5-HT system (influenced by adverse prenatal exposures and/or genetic susceptibilities) (1) passes through the critical postnatal period in homeostatic development (5) and experiences regurgitation of gastric contents and triggering of the laryngeal chemoreflex (LCR), i.e., a life-threatening challenge (2). The infant may be slightly febrile due to an otherwise trivial upper respiratory tract infection (3); as a consequence, the apnea component of the LCR is inordinately prolonged by mild hyperthermia (4). Further, if the infant’s ventilatory response to the progressive hypoxia and hypercapnia during the apnea is depressed, and if the hypoxic gasping and/or arousal mechanism is abnormal, oxygen lack from uninterrupted apnea results (red circle). Ultimately death occurs within minutes to hours.

The concept of a combination of events coming together to result in death extends to the cellular level as well, as exemplified by the neurotransmitter modulation of gasping. In this context, blocking 5-HT receptors (primarily 5-HT₂A) alone does not alter gasping in the isolated perfused rat brainstem preparation; however, when much smaller doses of 5-HT blockers are combined with equally small doses of noradrenergic blocker, gasping is markedly inhibited (156). Thus, gasping does not appear to depend upon a single neurotransmitter system, but it is quite vulnerable to disruption by much smaller defects in multiple neurotransmitter systems. We now conceptualize SIDS as the biologic version of the perfect storm, in which the simultaneous and chance combination of multiple events is far more powerful than any individual event alone (Figure 9). The emerging data about the role of the medullary 5-HT system in many SIDS infants may pave the way for future identification of infants at risk and specific interventions to prevent death.
SUMMARY POINTS

1. The brainstem hypothesis in SIDS states that a majority of SIDS cases are due to a defect in brainstem-mediated protective responses to homeostatic stressors during sleep in a critical developmental period.

2. The brainstem hypothesis in SIDS is supported by several lines of evidence, including independent reports of subtle brainstem findings in SIDS cases at autopsy and reports of cardiorespiratory and arousal (subclinical) deficits in infants who subsequently die of SIDS, which are consistent with brainstem dysfunction.

3. The most reproducible and robust neurochemical findings in the SIDS brainstems studied to date involve abnormalities in the medullary 5-HT system, as detected by immunocytochemical, quantitative cellular, and tissue autoradiographic methods in brainstem tissues specially prepared at the time of autopsy.

4. Extensive experimental analysis indicates that the medullary 5-HT system is critical for the modulation and integration of a variety of homeostatic functions (e.g., respiration, chemosensitivity, autoresuscitation, blood pressure, temperature) that are state and age dependent, and underscores the possibility that deficits in this system could lead to sleep-related sudden death under stress in a critical developmental period.

5. State-of-the-art genetic mapping studies indicate that different subpopulations of 5-HT neurons originate from different progenitor pools in the embryonic brainstem, underscoring the possibility that these different populations may mediate different homeostatic functions.

6. A prenatal origin of medullary 5-HT abnormalities in SIDS is suggested by (a) the finding of deficits in the regulation of 5-HT cell number and differentiation in the medullae of affected cases, (b) epidemiologic data indicating that prenatal adverse exposures (e.g., alcohol and cigarette smoke/nicotine) are major risk factors for SIDS, and (c) autopsy data showing that prenatal exposure to cigarette smoke is associated with reduced 5-HT receptor binding in the arcuate nucleus in the postnatal period, irrespective of the cause of death.

FUTURE ISSUES

1. Centralized sites for the collection and disposition of SIDS and control tissues specifically handled for research analysis need to be developed.

2. Future studies are needed to delineate the number and types of neurotransmitters and neuromodulators that may be part of the neurochemical brainstem pathology in SIDS in association with serotonin.

3. Ongoing basic science research is needed to determine the precise mechanisms whereby multiple homeostatic stressors interact with multiple brainstem neurotransmitter systems, including 5-HT, as well as with complex genetic and environmental risk factors, to cause sudden death in early life.
4. Fundamental research is needed to understand where, how, and when specific subpopulations of 5-HT neurons develop during fetal life, and if and how they are programmed to regulate specific homeostatic functions after birth.

5. Translational research is needed to develop methods to identify living infants at risk for SIDS with 5-HT-related brainstem pathology and to establish preventative strategies against sudden death during the critical period.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We would like to thank our many colleagues, medical examiners, students, and technicians for the dedicated work in SIDS research (presented above) from our laboratories. We are also grateful to the many SIDS families and organizations that have supported this research throughout the years. We appreciate the ongoing support of Dr. Holcombe E. Grier. Our work is supported by grants from the National Institutes of Health [R37-HD20991, R37-HL28066, PO1-HD36379, and Children’s Hospital Boston Developmental Disabilities Research Center (P30-HD18655)], the C.J. Murphy Foundation, the First Candle/SIDS Alliance, a Deborah Evelyn Barrett Fellowship for SIDS Research, the C.J. Foundation for SIDS, and the Scottish Crib Death Foundation.

LITERATURE CITED


145. Qu Y, Villacreses N, Murphy DL, Rapoport SL. 2005. 5-HT2A/2C receptor signaling via phospholipase A2 and arachidonic acid is attenuated in mice lacking the serotonin reuptake transporter. *Psychopharmacology (Berlin)* 180:120–20


172. Madden CJ, Morrison SF. 2006. Serotonin potentiates sympathetic responses evoked by spinal NMDA. 
   J. Physiol. 577(Pt. 2):525–37
173. Raul L. 2003. Serotonin-2 receptors in the nucleus tractus solitarius: characterization and role in the 
174. Nosjean A, Hamon M, Darmon M. 2002. 5-HT2A receptors are expressed by catecholaminergic neu-
   rons in the rat nucleus tractus solitarii. Neureport 13:2365–69
   smoking and drinking during pregnancy upon (3)H-nicotine receptor brainstem binding in infants 
   dying of the sudden infant death syndrome: initial observations in a high-risk population. Brain Pathol. 
   18:21–31
   Ann. N. Y. Acad. Sci. 600:297–313
177. Azmitia EC. 2001. Modern views on an ancient chemical: serotonin effects on cell proliferation, matu-
   ration, and apoptosis. Brain Res. Bull. 56:413–24
   Stem Cell Res. 2:5–10
180. Dahlstrom A, Fuxe K. 1964. Evidence for the existence of monoamine-containing neurons in the 
   central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. 
   neuronal changes in brain stem nuclei of forensic autopsied cases. II. Sudden infant death syndrome. 


Contents

The First Fifty Years in Research
Peter A. Ward .......................................................... 1

Graft Vascular Disease: Immune Response Meets the Vessel Wall
Richard N. Mitchell .................................................. 19

Molecular Pathology of Head and Neck Cancer: Implications for
Diagnosis, Prognosis, and Treatment
Sara I. Pai and William H. Westra .................................. 49

Mechanisms of Endothelial Dysfunction, Injury, and Death
Jordan S. Pober, Wang Min, and John R. Bradley ..................... 71

The Pathogenesis of Pituitary Tumors
Sylvia L. Asa and Shereen Ezzat ..................................... 97

PTEN and the PI3-Kinase Pathway in Cancer
Nader Chalhoub and Suzanne J. Baker ................................. 127

Pathogenesis of Classical and Lymphocyte-Predominant
Hodgkin Lymphoma
Roland Schmitz, Jens Stanelle, Martin-Leo Hansmann, and Ralf Küppers .......... 151

Molecular Genetics of Acute Lymphoblastic Leukemia
Michael A. Teitell and Pier Paolo Pandolfi ................................ 175

MicroRNAs in Cancer
Yong Sun Lee and Anindya Dutta ..................................... 199

Epigenetic Changes in Cancer
Christine A. Iacobuzio-Donahue ........................................ 229

Molecular Pathogenesis and Diagnostics of Bladder Cancer
Anirban P. Mitra and Richard J. Cote .................................. 251

Ovarian Cancer
Kathleen R. Cho and Ie-Ming Shih ..................................... 287
Drosophila Models of Neurodegenerative Diseases
Bingwei Lu and Hannes Vogel .............................................................. 315

Serrated Polyps and Colorectal Cancer: New Pathway to Malignancy
Amy E. Noffsinger .............................................................................. 343

Nod-Like Receptors: Role in Innate Immunity and Inflammatory Disease
Grace Chen, Michael H. Shaw, Yun-Gi Kim, and Gabriel Nuñez ........... 365

Tumor Suppressors, Chromosomal Instability, and Hepatitis C
Virus–Associated Liver Cancer
David R. McGivern and Stanley M. Lemon ......................................... 399

The Immunopathogenesis of Rheumatoid Arthritis
John B. Imboden .................................................................................. 417

The Pathology of Chronic Obstructive Pulmonary Disease
James C. Hogg and Wim Timens .......................................................... 435

Linking the Cellular Functions of BRCA Genes to Cancer
Pathogenesis and Treatment
Ashok R. Venkitaraman .................................................................. 461

Regulation of Hepcidin and Iron-Overload Disease
Pauline L. Lee and Ernest Beutler ....................................................... 489

The Brainstem and Serotonin in the Sudden Infant Death Syndrome
Hannah C. Kinney, George B. Richerson, Susan M. Dynecki, Robert A. Darnall,
and Eugene E. Nattie ..................................................................... 517

Molecular Pathogenesis of Cutaneous Melanocytic Neoplasms
Nageatte Ibrahim and Frank G. Haluska ............................................. 551

Indexes
Cumulative Index of Contributing Authors, Volumes 1–4 ................. 581
Cumulative Index of Chapter Titles, Volumes 1–4 .............................. 583

Errata
An online log of corrections to Annual Review of Pathology, Mechanisms of Disease articles may be found at http://pathol.annualreviews.org