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Sudden infant death syndrome and the central nervous system: a review of the triple-risk theory

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Thesis

**SUDDEN INFANT DEATH SYNDROME AND THE
CENTRAL NERVOUS SYSTEM:
A REVIEW OF THE TRIPLE-RISK THEORY**

by

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MATTHEW C. HOGAN

ABSTRACT

Sudden infant death syndrome (SIDS) is the devastating condition in which an infant suddenly and unexplainably passes away over the course of sleeping. This is an unfortunate situation that many new parents dread every night as they lay their newborns to rest. SIDS is the leading cause of death in infants aged from one month to one year, and the medical world still does not fully understand what causes it. However, the triple-risk theory is a new model that sets out to explain the pathology of this syndrome through the combination of genetic vulnerabilities, a critical time period, and external stressors. This thesis summarizes the current research in the realm of the central nervous system (specifically the cerebellum and brainstem) as a means of evaluating the validity of this new model.

The analyzed literature concentrated on a few important topics, such as proven risk factors, evidence of homeostatic abnormalities, and significant associations with the occurrence of SIDS. It was found that there was central nervous system dysfunction on most levels, including: damaged Purkinje cells in the cerebellum, malformations of the human choroid plexus, decreased neuropeptide signaling (both orexin and brain-derived neurotrophic factors), malformations of the amino acid neurotransmitters (both excitatory

glutamate and inhibitory GABA), and finally significant reductions in the receptor density and activity of the serotonin system. These irregularities were associated, in most studies, with either the prone sleeping position or known maternal nicotine use during pregnancy.

In conclusion, the triple-risk model is currently the most accurate description of SIDS, given its reasonable three criteria and present-day research. This is because the studies, and real-life victims, were all concentrated within the critical time period of transition from intra-uterine to extra-uterine life, satisfying the first element of timing. The list of central nervous system dysfunctions found in SIDS cases was compelling enough to fulfill the second factor of inherent vulnerability. Finally, the associations between low oxygen rebreathing and the prone sleep position, or over-heating and tight swaddling displayed a strong relationship with the occurrence of SIDS and satisfied the third and final event, which was the induction of an exogenous stressor. These three factors of the triple-risk model allow for the variations in victim pathology, but still offers a compelling and coherent understanding of the sudden infant death syndrome.

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LIST OF ABBREVIATIONS

BDNF.....	Brain-Derived Neurotrophic Factor
CNS.....	Central Nervous System
CSF.....	Cerebrospinal Fluid
CVN.....	Cardio-inhibitory Vagal Neuron
GABA.....	γ -Aminobutyric acid
GFAP.....	Glial Fibrillary Acidic Protein
IHH.....	Intermittent Hypercapnic-Hypoxia
IPSC.....	Inhibitory Post-Synaptic Current
NMDA.....	N-methyl-D-aspartate
NT.....	Neurotransmitter
PC.....	Purkinje Cell
5-HT.....	Serotonin
5-HTT.....	Serotonin Transporter
SIDS.....	Sudden Infant Death Syndrome

INTRODUCTION

Sudden Infant Death Syndrome

Sudden Infant Death Syndrome (SIDS), or more commonly “Crib Death,” is when an infant suddenly and inexplicitly passes away, normally during sleep, despite appearing outwardly healthy (Krous et al., 2004). This diagnosis is accompanied by a thorough autopsy, examination of the scene of the death, and evaluation of medical history (Krous et al., 2004). After all of these investigational criteria have been satisfied, and there is still no apparent explanation, the case is attributed to Crib Death (Krous et al., 2004). SIDS is the third main cause of death for all infants in the United States from birth to one year (congenital malformations and low birth weight being first and second, respectively), and the leading cause of death for all infants between one and twelve months (Mathews et al., 2006). These statistics hold true, despite experiencing a marked decline from 1.2 cases per 1000 live births in 1992 to 0.72 cases per 1000 live births in 1998 (Corwin et al., 2003).

The exact etiology of SIDS is not understood, however a number of risk factors that have been identified. In accordance with the above statistic on SIDS decline in the United States between 1992 and 1998, this was most directly associated with the “Back to Sleep” campaign to inform families of the increased risk of infant prone sleeping (belly down) as opposed to the lower risk of supine sleeping (belly up) (Corwin et al., 2003). There is a discrepancy between different races and ethnicities, with infants of American Indian and non-Hispanic black mothers experiencing the highest occurrences of SIDS (roughly 2.3 times that of non-Hispanic white mothers) (Mathews et al., 2006). Other

known SIDS risk factors include low birth weight, maternal usage of illicit drugs, smoking during pregnancy, and lack of breastfeeding (Corwin et al., 2003).

Understanding these risk factors is important to decreasing the occurrence of the condition; however, the shift in prevalence could be associated to the ambiguous nature of the diagnosis (Malloy & MacDorman, 2005).

A syndrome that has no outwardly identifiable pathology is difficult to consistently diagnose. Pathologists have turned to studying the brainstem in hopes that SIDS could be grounded in an infant's inability to maintain homeostasis in the face of certain lethal situations during sleep (asphyxiation or hypercapnia) (Panigrahy et al., 2000). The ventral medulla, partly through the action of serotonin and its corresponding receptors, plays a pivotal role in regulating homeostasis during sleep (Panigrahy et al., 2000). These actions include chemoreception, arousal, airway reflex control, thermoregulation, respiratory drive, and blood pressure regulation (Panigrahy et al., 2000). The research that has been conducted on the brainstem of SIDS patients has shown that medullary serotonin irregularities are of the most common, and therefore of the most interest when pursuing the etiology of the syndrome (Panigrahy et al., 2000). However, current research has expanded from the brainstem to include cerebellar activity as well other chemical messenger systems in addition to serotonin.

This thesis will review the current literature on brainstem and cerebellum research as it pertains to SIDS and its possible pathological explanations. Crib Death has numerous proven risk factors, and by virtue of their diversity, are a testament to the complexity of the condition. The shift from *in utero* to *ex utero* is an intricate process in

which the brainstem, and its homeostatic functions, must properly develop as it undergoes rapid maturation (Kinney et al., 2009). Although SIDS is most likely a condition that precipitates from a combination of factors, this review will concentrate on the brainstem/cerebellum and the various chemical messaging systems in an attempt to understand possible explanations for the mysterious death of infants up to the age of twelve months.

This report will first supply background on a few major risk factors associated with SIDS, as well as explaining the most recent etiological hypothesis for the syndrome, the triple-risk theory. Next, descriptions of the anatomy and function of the nervous system will be provided with information on related neurotransmitter systems. Finally, there will be an in-depth look at evidence from recently published studies that will lead to a discussion of the involvement of the brainstem and cerebellum in the SIDS.

Known Risk Factors

There are a number of known risk factors for SIDS. However, this particular section will concentrate only on the two most prevalent and well-studied issues: maternal nicotine use and sleep position/improper swaddling. Maternal nicotine use can be considered either smoking while pregnant or postnatal exposure at home (Machaalani & Waters, 2008). Although an expectant mother's use of nicotine is arguably more damaging to the development of the child's central nervous system, an infant's body is still incredibly susceptible to smoke exposure in the critical transition from intra-uterine to extra-uterine life (Fanous et al., 2006).

Cigarette smoking is dangerous because there is a high volume of toxic substances and carcinogens released when tobacco leaves are ignited (Lavezzi et al., 2013). Nicotine is only one of these toxins, but poses a great risk to fetal brain development due to being one of the few lipid-soluble chemicals (Lavezzi et al., 2013). This means that nicotine can freely pass through the blood brain barrier, the last line of defense between one's central nervous system and the rest of the body, and elicit damaging effects (Lavezzi et al., 2013). In particular, nicotine interacts with a subgroup of acetylcholine receptors that are heavily expressed on neurons (Purkinje Cells) that are important for the integration of communication between the brainstem and the cerebellum (Lavezzi et al., 2013).

In addition to directly affecting the development of neurons that are involved with autonomic integration, nicotine can alter the environment in which the brain resides. The central nervous system (CNS) is bathed and suspended in cerebrospinal fluid, the main secretory product of the human choroid plexus in the ventricular system, and supplies the key component of the cranial environment (Lavezzi et al., 2013). Neurons of the choroid plexus are no exception to the reach of the lipophilic nicotine molecule. Significant correlations were discovered between perinatal tobacco use and apoptosis, hyper expression of Substance P (indicator of CNS insult), and a decreased number of blood capillaries that support both the choroid plexus and surrounding neurons (Lavezzi et al., 2013). This type of physical change could prove important to the pathology of SIDS.

The first nine months of an infant's life in the womb are incredibly important to the growth of their central nervous system (Duncan et al., 2010). However, the first few

transitional months after birth are a critical time of development for the cerebrum, cerebellum, and brainstem (Duncan et al., 2010). Exposure to tobacco smoke and nicotine can still elicit dangerous effects at this stage, and can occur as either a result of second-hand smoke or contaminated breast milk (Say et al., 2007). For example, there are specific chemical messengers, called brain-derived neurotrophic factors (BDNF), that are important to the normal maturity of a developing CNS (Tang et al., 2008). Nicotine can directly interfere with this signaling molecule, as well as BDNF's receptor (TkrB), which together serve to regulate the development and apoptosis of neurons, some of which are specific to the homeostatic region of the brainstem (Tang et al., 2008). Therefore, the postnatal exposure to nicotine can be significantly damaging to a developing CNS, much like prenatal usage.

A 2006 epidemiological study concentrating on known risk factors associated with SIDS revealed a surprising correlation. The cohort analysis was successful in revealing a number of trends, but one in particular stood out. Maternal smoking proved to be the single most significant factor that was associated with Crib Death and brainstem alterations (**Figure 1**) (Matturri et al., 2006). The study showed other risk factors that may have occurred concurrently with maternal smoking, but the undeniable correlation between the two is apparent. A detail to note, though, is that this correlation is weak when concerning SIDS cases without brainstem disturbances (Matturri et al., 2006). Crib Death is an ambiguous syndrome and also occurs when there is no apparent brainstem anomaly, but in accordance with the goals of this thesis, attention is being given to the association of known risk factors and CNS irregularities.

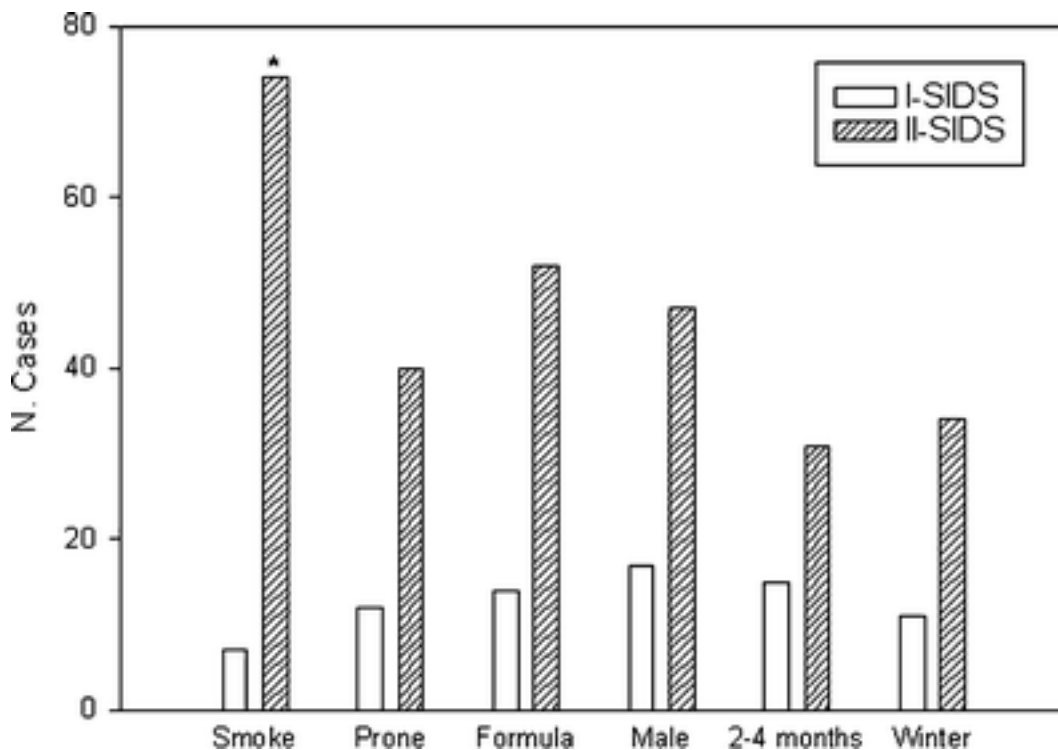


Figure 1: Risk Factors among SIDS with and without Brainstem Abnormalities

This figure shows a list of known risk factors for Sudden Infant Death Syndrome and the number of cases that fall into each category. I-SIDS (the white bar) represents the number of cases without a noticeable brainstem alteration. II-SIDS (the shaded bar) represents the number of cases with a noticeable brainstem change (adapted from Matturri et al., 2006).

Although correlation does not necessarily equate to causation, maternal nicotine use both before and after childbirth has an established association in either the cause or pathology of Crib Death. Whether nicotine targets signaling molecules, their receptors, specific neurons, or the cranial environment in general, nicotine has been a focal point for SIDS research. Specific evidence of damage or alterations in the brainstem and cerebellum will be discussed in detail later. Continuing with known risk factors, the other major contributing issue has been attributed to infant sleep position or improper

swaddling. Both of these are unique, in that they can put a sleeping infant in an environment considered potentially threatening.

Hyperthermia, or the state of high body temperature, is the first major concern of an infant that has been swaddled too tightly (Tolcos et al., 2000). This can also result from the child's immune system fighting an infection or a room being excessively heated, but the potential consequences are the same: an increased central nervous system stress-load by the way of decreased cerebral flow, edema, increased permeability of the blood-brain barrier, gliosis, and neuronal damage (Tolcos et al., 2000). An infant's CNS is particularly vulnerable to external threats because of the delicate transition to extra-uterine life, so the damaging effects of hyperthermia are not only more dangerous, but more likely to occur with improper swaddling techniques (Tolcos et al., 2000). On a molecular level, hyperthermia has been linked to changes in the proper functioning of a few neurochemical systems (Tolcos et al., 2000). Dopamine release in the striatum and hypothalamus have shown marked increases, as well as serotonin release in the hypothalamus, cerebral cortex, and striatum (Tolcos et al., 2000). However, the implications for these adjustments of the neurotransmitter systems will also be discussed later.

Improper swaddling and excessively heating a room are not the only ways to induce a threatening environment for a newborn. Something as trivial as the position in which the newborn sleeps could have significant effects on health (Corwin et al., 2003). Sleeping in an awkward and stifling position could cause asphyxia and it was recently found that 86% of 209 sudden unexpected infant deaths in a major study were associated

with asphyxia (Randall et al., 2013). Also, numerous tissue markers of chronic or intermittent hypoxia were discovered in the etiological studies of Crib Death infants (Cummings et al., 2011). It is known that bradycardia (slowing of heart rate) can be brought about by severe anoxia or hypoxia, and can further lead to hypotension and improper breathing (Cummings et al., 2011). These conditions generally lead to a process known as autoresuscitation, which is meant to reestablish a vital breathing rate, heart rate, and appropriate blood pressure, but no evidence of autoresuscitation occurs in some SIDS cases (Cummings et al., 2011). Most healthy individuals would be able to tolerate this temporary stressful environment while sleeping, but for an infant with an underdeveloped CNS, it could be life-threatening.

The sleep position that is believed to possess the highest potential for Sudden Infant Death Syndrome is the prone, or belly-down, position (Corwin et al., 2003). This is because the child is face down in their crib, and breathing could become a laborious act while promoting a gaseous environment of intermittent hypercapnic-hypoxia (IHH) (Machalani & Waters, 2006). IHH refers to the moments during sleep that an infant's mouth and nose could be in a suffocating position that would promote rebreathing of carbon dioxide, thus raising the CO₂ level, and lack fresh oxygen, thus lowering the O₂ level (Machalani & Waters, 2006). Recent studies have shown that IHH, as a result of prone sleeping, could induce neuronal cell damage and produce neurotoxic byproducts similar in the pathology of maternal nicotine usage on an infant's developing CNS (Machalani & Waters, 2006). The similarities in the manifestation of injury in the nervous system between maternal tobacco use and IHH are of particular importance.

The Triple-Risk Theory

Researchers have been attempting to locate the exact origin of SIDS for years; however, a recent theory has been gaining traction that might help explain the complexity of SIDS. This new model takes most major known risk factors into consideration as being part of the bigger diagnosis, as opposed to having tunnel vision on any one influence. It is called the Triple-Risk Theory for SIDS (Kinney et al., 2005). This hypothesis essentially describes Crib Death as a condition due to three major factors happening concurrently: an inherent (possibly genetic) vulnerability, a susceptible or critical period of development, and the presence of external stressors (**Figure 2**) (Randall et al., 2013). The Triple-Risk model implies an interesting combination of scenarios and should be dissected by each factor.

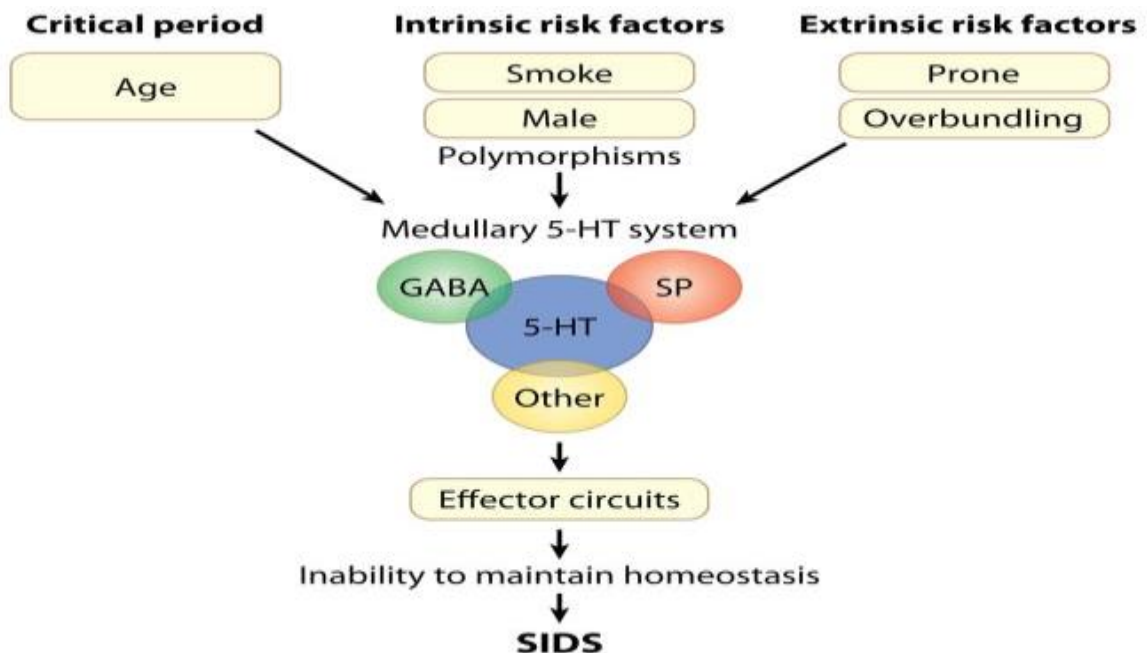


Figure 2: The Triple-Risk Model

Graphical depiction of the Triple-Risk Hypothesis. The three major threats are the critical age of the infant, any intrinsic susceptibilities, and external stressors. These converge on the infant's ability to maintain homeostasis, and with an inability to sustain life, results in SIDS. (adapted from Kinney et al., 2009)

The first factor is that the infant may have an underlying vulnerability. This could include an acquired deficiency of a particular neurotransmitter system or a developmental issue with certain homeostatic centers of the central nervous system (Kinney et al., 2005). The necessity of an inherent susceptibility would help explain why not all infants who sleep in the prone position fall victim to Crib Death (Kinney et al., 2009). The second factor is that the infant must be in a particularly critical period of development (Kinney et al., 2005). The first six months post-birth are critical as the child transitions from intra-uterine to extra-uterine life, and infants up to this age are at their greatest risk for SIDS as the majority of cases occur before six months (Paterson et al., 2006). The third and final factor is that the infant is exposed to an exogenous stressor that would challenge its ability to maintain a thriving internal environment (homeostasis) (Kinney et al., 2005). The external stressors could be a number of things: respiratory infection, excessive bedding, bed-sharing, head covering, and prone (face-down) sleeping (Randall et al., 2013). It is believed that these stressful events could induce a period of asphyxia, hypercapnia, or hypoxia (Broadbelt et al., 2011). While these situations are known to occur multiple times a night as most individuals shift in their sleep, it normally does not present a life-threatening issue. However, for an infant with limited mobility and a potentially underdeveloped brainstem/cerebellum, SIDS is a very real possibility.

The two major known risk factors discussed before play an important role in this hypothesis. Maternal nicotine use is proven to cause a disruption in the gross anatomy of the CNS, as well as throw certain chemical messenger systems out of balance, potentially giving the child an underlying vulnerability (Tang et al., 2008). Sleep position and

improper swaddling can force an infant into a stressful situation (Corwin et al., 2003). Combining these risk factors during a critical period of development could be the perfect storm necessary for SIDS to precipitate. This thesis will explore the recently published literature on anomalies of the brainstem, cerebellum, and certain neurotransmitter systems as a means of discerning the validity of the Triple-Risk hypothesis. In order to understand these irregularities though, background information will first be given on the Nervous System.

The Nervous System

All systems of the body serve an important role in the preservation of life. The musculoskeletal system provides the body with stability, protection, and movement (Rouissi et al., 2016). The cardiovascular system provides the body with nutrients, oxygen, and infection-fighting immunity (Bhave & Eagle, 2016). However, the nervous system has a slightly different responsibility. The nervous system is in charge of receiving external and internal stimuli, integrating that information at a higher level, and relaying messages to the body as to how it should handle the situation (Zaaimi et al., 2013). An external stimulus could be information about the ambient temperature or sighting a potential predator, while an internal stimulus could be information about pathogenic antigens or the gastrointestinal system relaying a message about an empty stomach (Zaaimi et al., 2013). In accordance with all other systems of the body, the nervous system has definitive macroscopic and microscopic differences.

Anatomically, the nervous system is split into the peripheral and the central nervous systems (Dauth et al., 2016). The peripheral nervous system pertains to all

nervous tissue found outside of the skull or vertebral column, and handles the job of receiving and distributing messages (Dauth et al., 2016). This division is then split into the somatic and autonomic systems, with the autonomic section being divided again into the sympathetic and para sympathetic nervous systems (Dauth et al., 2016). The somatic nervous tissue contains the tract of information exchange that is under one's conscious, or voluntary, control while the autonomic system deals with information beyond one's cognition (Uchida et al., 2016). On that note, the sympathetic and parasympathetic divisions of the autonomic nervous system are usually antagonistic of each other as they calibrate the involuntary actions taken by one's body (Uchida et al., 2016). Although humans have some degree of conscious control over the activity of several bodily systems, the central nervous system handles input from the entire body. It integrates almost all information from both the internal or external environments and dictates an appropriate response to the provided information (Dauth et al., 2016). The CNS is made up of the spinal cord and the brain, which is the combination of the cerebrum, cerebellum, and brainstem (Dauth et al., 2016).

The spinal cord is the bundle of nervous tissue that travels inside the majority of our vertebral column and branches out at particularly important locations (Ohlsson et al., 2013). It is, much like the rest of the CNS, surrounded by three protective layers of tissue (meninges): the pia mater (inner most), arachnoid mater (middle), and the dura mater (outer most) (Reinhold & Rittner, 2016). The spinal cord is mostly a highway for information, but does contain some lower level integration as it handles reflexes (Ohlsson et al., 2013). A reflex is when a stimulus is analyzed by groupings of neuronal bodies in

the spinal cord and a decision is sent to the body's periphery without further analysis at the higher levels of the brain (Kozyrev & Coolen, 2016). Reflexes are pivotal in self-preservation, as they often help prevent the body from encountering injury in a minimal amount of time. Although the spinal cord is an important part of the central nervous system, sudden infant death research has either not concentrated here or has not yet yielded valid results, and for this reason will not be discussed further.

The cerebrum is superiorly located and the most commonly recognized structure of the central nervous system (Ekinci et al., 2008). The cerebrum contains many sulci (fissures) and gyri (ridges) in its exceedingly folded appearance (Ekinci et al., 2008). These folds increase the surface area of the cerebrum, and much like other physiological processes, increase its ability to carry out the function of this particular organ (Ekinci et al., 2008). This part of the central nervous system is divided into two hemispheres (right and left) and these two hemispheres are anatomically divided into four lobes (frontal, parietal, temporal, and occipital) (Yang et al., 2014). Although the lobes of the cerebrum have some overlap in responsibilities, the centralized cortical neurons in these locations mainly serve the role of integration of specific information (Bozkurt et al., 2016). The frontal lobe is involved with judgment, planning, and movement initiation (Bozkurt et al., 2016). The parietal lobe handles the integration of a broad range of sensory information (Bozkurt et al., 2016). The temporal lobe handles auditory and speech integration, while the occipital lobe deals mostly with visual stimuli from the eyes (Bozkurt et al., 2016).

One of the major differences between humans and other mammals, or between primates and other mammals, is that the cerebrum has evolved to be a significantly larger

portion of the central nervous system (Isler & van Schaik, 2009). This is believed to be associated with humans' increased ability to reason and deal with complex sensory information (Isler & van Schaik, 2009). As humans and other mammals evolved, more multifaceted information was delegated to the higher cortical regions of the cerebrum, however, other parts of the CNS still assumed important and more basic roles for the animal (Isler & van Schaik, 2009). One of these structures exists in an inferior and posterior location to the cerebrum, and is aptly named the cerebellum (Latin for "little brain") because of its similarity to a smaller cerebrum (Ekinici et al., 2008). Due to the higher cortical functioning of the cerebrum, and its lesser role in bodily homeostatic maintenance, this review will not further discuss the potential cerebral contributions to crib death.

The cerebellum has a similar structure to the cerebrum in that it is highly folded, containing numerous gyri and sulci, and can be anatomically divided into right and left hemispheres (Ekinici et al., 2008). Conversely, it only contains two major lobes (anterior and posterior) when compared to the four lobes of the cerebrum (frontal, parietal, temporal, occipital) (Ekinici et al., 2008). The cerebellum is classically known for its contributions to movement through balance, posture, coordination of voluntary movement, and some motor learning (Cooper et al., 2012). This is largely because of its communication with the motor cortex of the cerebrum, where movement is initiated (Cooper et al., 2012). However, new studies implicate the cerebellum as taking a larger role in higher level cognition due to its interconnectedness with the cerebrum overall (Cooper et al., 2012). The cerebellum is indeed highly connected to the cerebrum, but the

majority of SIDS research is concentrated on the cerebellum's interactions with the brainstem, where it aids in more primitive bodily functioning.

In the case of the cerebellum, it is a particular class of neurons that has gained attention concerning crib death. Neurons are the main type of cell that constitute the nervous system, as they play a specialized role in message reception, integration, and propagation (Lai et al., 2016). These cells have specialized structures that aid in their quick and efficient communication. The axon is the long projection-like structure where electrical impulses are conducted away from the cell body and towards other neurons (Higuero et al., 2016). These neurons are then able to receive incoming messages through their other branch-like projections called dendrites (Lai et al., 2016). There is a synapse, or small gap, between the axon of one neuron and the dendrite of the other where the message converts from electrical to chemical and back to electrical, but details of synaptic transmission will be discussed later (Higuero et al., 2016).

The specific neuron being studied in the cerebellum is called the Purkinje cell (PC) (**Figure 3**). PCs are some of the largest brain neurons and are classified by intricate dendritic trees that allow them to interface with both the brainstem and the cerebrum (Lavezzi et al., 2013). The Purkinje cell falls under the category of an inhibitory neuron because it releases γ -aminobutyric acid (GABA) as its main neurotransmitter with the result of decreasing unnecessary neuronal firing between cells (Lavezzi et al., 2013). PCs are part of the "Purkinje-Olivo-Dentate network" where they are the intermediate messengers between excitatory synapses with the inferior olive (brainstem) and the deep cerebellar nuclei of the dentate nucleus (cerebellum) (Lavezzi et al., 2013). Malfunctions,

such as neuronal damage, in this connection between the brainstem and the cerebellum could prove to be a point of evidence as investigators attempt to identify the pathology of SIDS (Lavezzi et al., 2016).

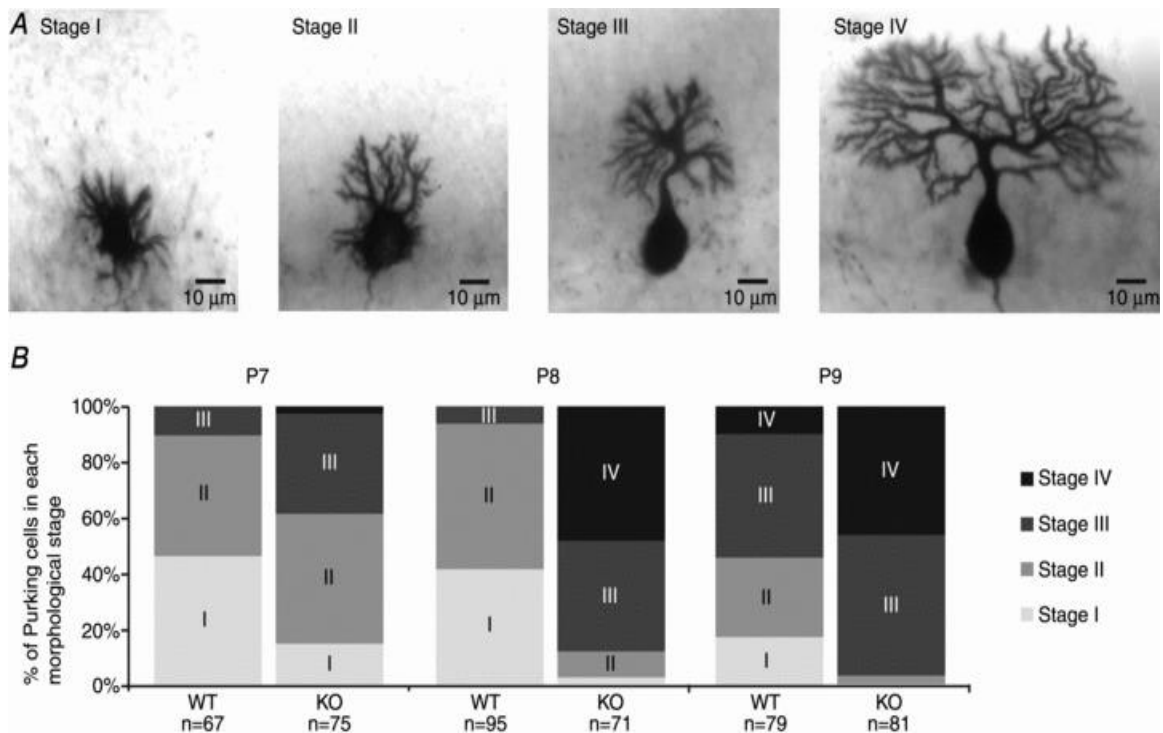


Figure 3: Morphological Development of Purkinje Cells

Note the sophisticated branching of the dendritic tree that receives a high level of neuronal input from both the cerebellum and the brainstem. The chart at the bottom depicts varying degrees of serotonin receptor knockout with dendritic growth, implying a connection with this neurotransmitter (adapted from Oostland et al., 2013).

Although the cerebellum has strong associations with primitive bodily processes, the brainstem is by far the most implicated in the homeostatic dysfunction of SIDS patients. The brainstem is inferiorly located in the upper CNS and is continuous with the spinal cord and the cerebellar/cerebral cortexes (Ekinci et al., 2008). Anatomically, the structure is split into the medulla, pons, and midbrain as one travels superiorly from the

spinal cord (Ekinci et al., 2008). The brainstem houses the primary sensory and motor innervation to both the facial and neck regions, as well as containing ten of the twelve major cranial nerves (Yagmurlu et al., 2016). Besides these major structures, the brainstem has life-sustaining responsibilities that are carried out completely autonomous of one's consciousness (Young, 2012).

These responsibilities include the maintenance of homeostasis in the body through regulation of respiratory, cardiac, and other vital functions (Young, 2012). Homeostasis refers to the ability of one's body to maintain a consistent and thriving internal environment despite any external conditions (Kong et al., 2016). This responsibility entails reliable communication between the cerebellum, the brainstem, and the rest of the body in order to react appropriately to certain life-threatening situations (Hunt et al., 2015). The primary mode of communication between neurons is through synaptic transmission, which is a type of interaction that utilizes neurotransmitters (NTs) (Higuero et al., 2016). For this reason, it is here in the realm of signaling molecules that most of the recent SIDS research has been concentrated.

Neurotransmitters are small molecules, often derived from amino acids, that are created and stored in the synaptic terminals of neurons (Zhang et al., 2016). These compounds are released into a synaptic gap due to the electrical stimulation from an outgoing message (Zhang et al., 2016). Once released, NTs travel across the synapse to the surface membrane of the neighboring neuron, where they interact with specific NT receptors (**Figure 4**) (Zhang et al., 2016). While interacting with the receptor, the NT will usually cause the opening of ion channels, which ultimately calibrate the internal ion

concentration and results in the increase or decrease of subsequent neuronal firing (Gibb, 2016). NTs are then cleared from the receptor and either metabolized by enzymes in the synaptic cleft or in the presynaptic terminal upon reabsorption (Mukunda & Narayanan, 2016). There is a diversity of both NTs and receptors that vary depending on the neuron or the location in the nervous system; however, the most widely studied NT system concerning SIDS is serotonin (Mukunda & Narayanan, 2016).

Serotonin, or 5-hydroxytryptamine (5-HT), is derived from the amino acid tryptophan, and like other signaling molecules derived from aromatic amino acids is classified as a monoamine NT (Medel-Matus et al., 2017). Interestingly, the majority of bodily 5-HT is actually found in the enteric nervous system of the gastro-intestinal tract, however, another large concentration is in the brainstem of the CNS (Medel-Matus et al., 2017). Despite gastric functionalities, the 5-HT system plays a major role in important tasks such as regulating mood, appetite, and sleep (Jafurulla et al., 2016). The Serotonin system has this wide variety of functions due to the diversity of NT receptors that are able to interact with this particular signaling molecule (Jafurulla et al., 2016). There are seven families of 5-HT receptors, three of which contain multiple subgroups (Frazer & Hensler, 1999). 5-HT receptors are mostly G-protein coupled receptors, however one family of receptors is a ligand-gated ion channel (NT binding directly opens the channel for ion flux) (Frazer & Hensler, 1999).

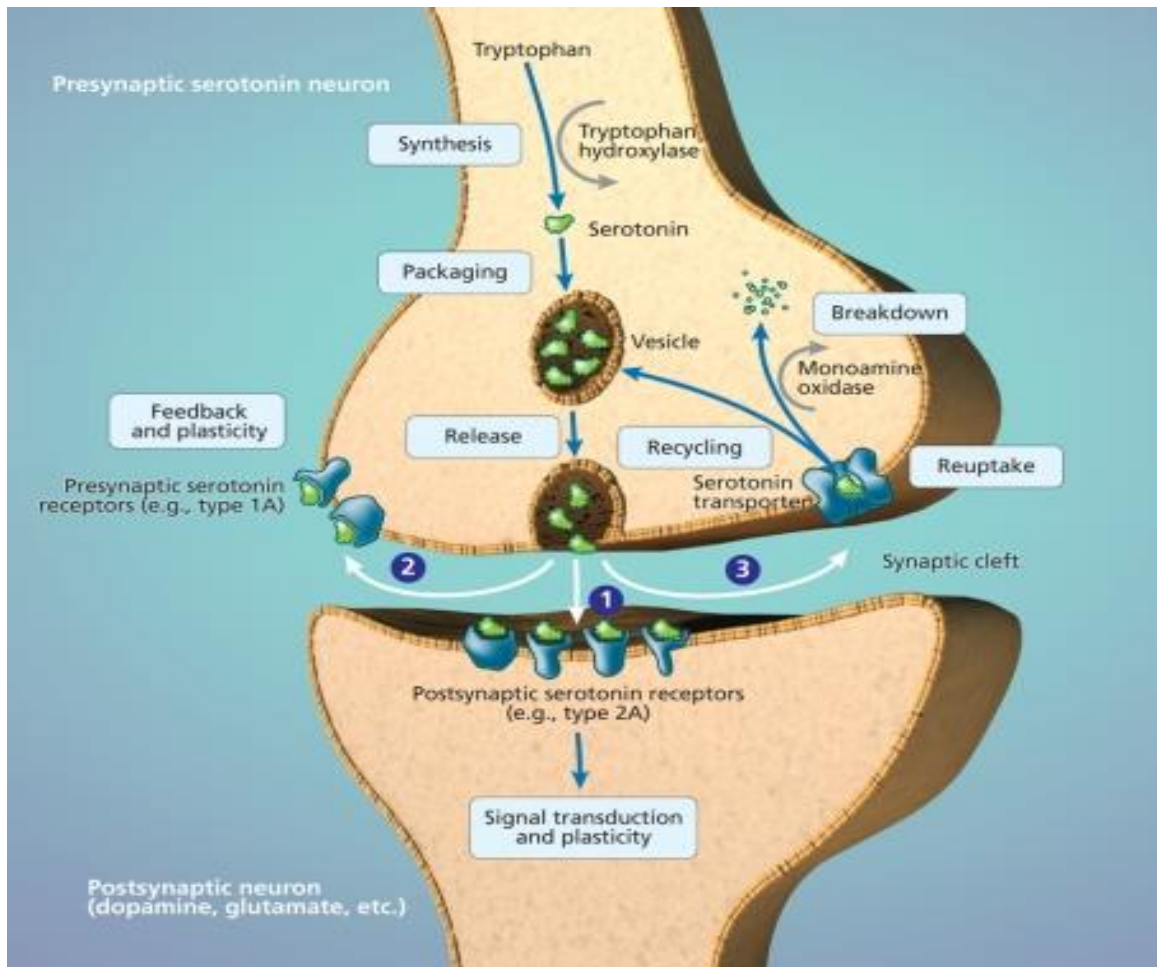


Figure 4: Serotonin Production and Synaptic Activity

Fate of serotonin in a neuron. It is first created from tryptophan, then stored in vesicles until the proper electrical stimulation causes the release of NTs into the synapse. Upon release it interacts with specific receptors on the postsynaptic neuron until it is cleared or reabsorbed by the presynaptic neuron (adapted from Rot et al., 2009).

SPECIFIC AIMS

The goal of this thesis is to evaluate the validity of the triple-risk model for the sudden infant death syndrome. It will do so by integrating the numerous published studies that have investigated an association between SIDS, known risk factors, and the central nervous system. This thesis will first look to the dysfunction of the cerebellum and later concentrate on the abnormalities of the brainstem, both in regards to their associated neurons and NT systems. Information on current research will be accompanied by a description of their statistical analysis as a means of understanding the potential influence of random error. The last section will discuss these results and conclusions will be drawn on the validity of the triple-risk hypothesis.

PUBLISHED STUDIES

The Cerebellum and Purkinje Cells

As discussed above, the cerebellum plays an interesting role in the central nervous system. It is mostly concerned with coordinated motor functions, but due to the nature of its connection with the brainstem, the cerebellum also participates in homeostasis (Kinney et al., 2002). This connection is mostly through the large Purkinje cells (PCs) that interface between nuclear centers of the brainstem and deeper regions of the cerebellum (Kinney et al., 2002). PCs are pivotal to communication and their death or dysfunction has been a point of research for investigators.

A team of pediatric researchers discovered an interesting association between PC dysfunction, maternal nicotine use, and the occurrence of SIDS. The investigators were utilizing a silver stain technique on histological slides of the cerebellum of numerous infants (both cases and controls – infants having recently passed away due to known causes) (Lavezzi et al., 2016). The silver stain has an affinity for proteins in the nucleolar organizer regions (NORs) of the nucleolus, a site in the nucleus of cells where ribosomes are produced (Lavezzi et al., 2016). Ribosomes ultimately create vital proteins for the cell and so this technique was utilized as a means of establishing a neuronal health standard and observing for potential signals of cellular damage (Lavezzi et al., 2016). What Dr. Lavezzi found is that partial or full NOR alterations were almost exclusively associated with infants

who had passed away suddenly and inexplicably between 27 gestational weeks to eight months postnatal (Lavezzi et al., 2016).

The investigators recorded neuronal health by assigning a score (1-4) that correlated with the percentage of positive argyrophilic NOR neurons, with 4 being the highest (>80%) (Lavezzi et al., 2016). The results indicated that both fetal and infant samples of the controls recorded a consistent score of 4, while only around half of the SIDS samples had the same nucleolar health level (Lavezzi et al., 2016). Overall, silver staining NOR patterns were significantly different for cases when compared to the controls (48% vs 0% respectively, $p < 0.01$) (Lavezzi et al., 2016). For further reference, a p-value that is below 0.05 means that the results are not compatible with a null hypothesis of there being no significant difference between cases and controls (Angst et al., 2016). The calculated p-value below 0.01 then equates to a statistically significant finding that these NOR alterations in the cases are not due to random chance, assuming minimal systemic bias in the study design (Lavezzi et al., 2016).

Although the investigators were looking for an observed pattern of neuronal abnormalities, they were also interested in analyzing cigarette-smoking as a potential factor. This is because nicotine contains high levels of oxidants and free radicals that could potentially induce oxidative and hypoxic stress to the CNS (Lavezzi et al., 2016). The investigators found that 75% of the smokers in the study were mothers who had lost a child to sudden death, and that 11 of the 18 recorded SIDS cases in this study displayed some degree of altered NOR expression in

purkinje cells ($p < 0.01$) (Lavezzi et al., 2016). This shows that there was a statistically significant association between maternal tobacco use and the dysfunction of neuronal nucleoli (Lavezzi et al., 2016). Dr. Lavezzi has conducted other studies to explore alternate signals of cerebellar stress that may be linked with either SIDS or maternal smoking.

Previous to the last study, Dr. Lavezzi and her team examined the histological slides of cerebellums from 65 fetuses or infants who had recently passed away. The control group was defined as the 18 cases that had explained death without cerebellar contribution upon autopsy (9 fetal and 9 infant), and the group under observation was defined as the 47 cases whose death had no explanation post-autopsy (21 fetal and 26 infant) (Lavezzi et al., 2013). The first step in this study was to develop an accurate framework of PC migration and general appearance over the course of *intra* and *extra*-uterine maturation. This was completed by identifying PCs via immunoreactivity for calbindin-D28k, and tracing their progression from samples of 25 gestational weeks through samples of 11 to 12 months post-birth (**Figure 5**) (Lavezzi et al., 2013). This gave the research team a point of reference for PC location and structure as they analyzed the cases who had succumbed to an unexplained death.

After creating the context for PCs, the results showed some interesting findings. It was found that 72% of the 47 samples from unexpected deaths displayed some level of abnormal PC developmental patterns for the age at their death (Lavezzi et al., 2013). These abnormalities included: islands of immature PCs in the

wrong cellular layer (20 cases), random hypoplasia in the correct cellular layer (11 cases), random hyperplasia in the correct cellular layer (9 cases), and overall damaged PC morphology (26 cases) (**Figure 5**) (Lavezzi et al., 2013). This is to be compared to the control group where only 3 of the 18 samples displayed infrequent PC irregularities (Lavezzi et al., 2013). These results pointed to a statistically significant ($p < 0.01$) difference of PC alteration between SIDS and non-SIDS victims (Lavezzi et al., 2013).

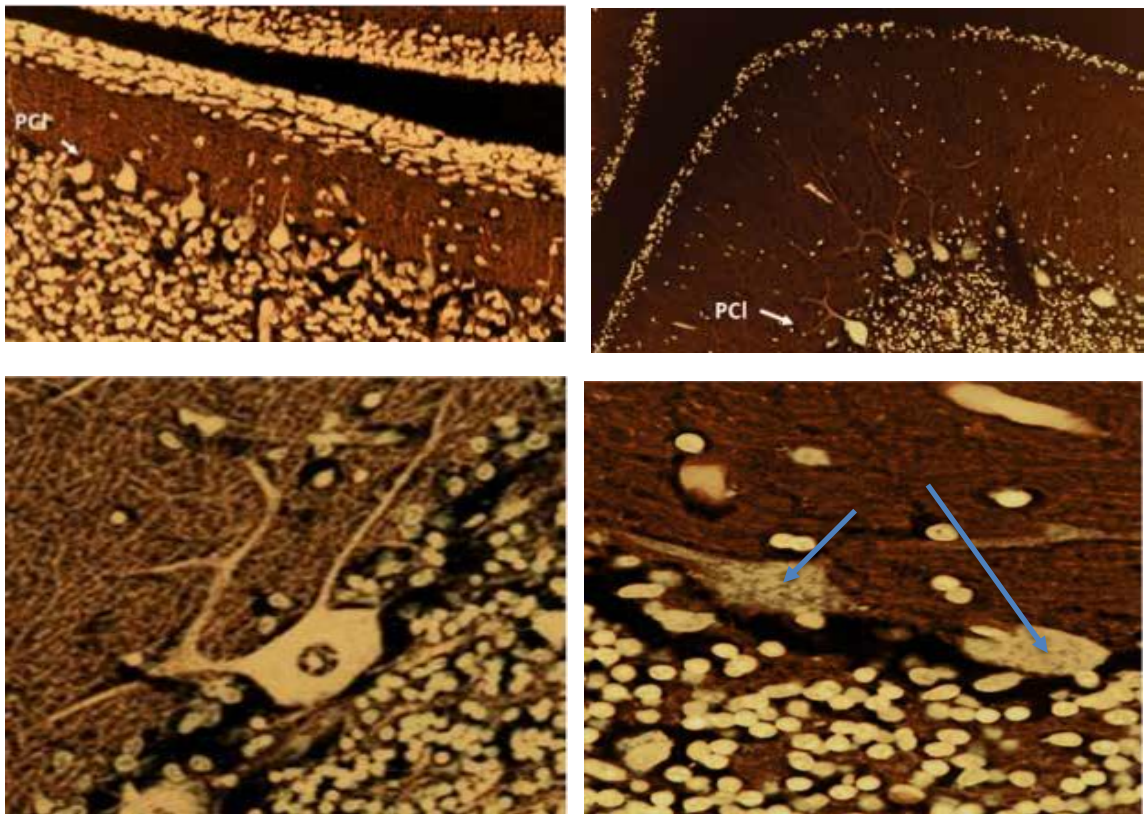


Figure 5: Purkinje Cell Appearance and Alteration

Top Left - PC development and cerebellar layering in control fetus of 34 gestational weeks. Top Right - PC development and cerebellar layering in control infant after a few days postnatal. Bottom Left – Magnified image of healthy developing PC. Bottom Right – Magnified image of altered PC morphology in SIDS victim (shrunken and without distinct nucleus – blue arrows) (adapted from Lavezzi et al., 2013).

Similar to the previously discussed study, this investigation also included maternal nicotine usage as a factor. There were 22 cases of SIDS with mothers who admitted to be smokers, and of those 22 cases, 20 samples (91%) displayed flawed maturation of the cerebellar PC layer (Lavezzi et al., 2013). In fact, there was statistically significant evidence that lends to a strong association between cigarette smoking and PC damage ($p < 0.01$), as well as between cigarette smoking and altered cellular layer development ($p < 0.01$) (Lavezzi et al., 2013). Assuming no systemic bias in the study design, these p-values point to the overall relationship between PC abnormalities and maternal nicotine use as too substantial to be caused by random error (Lavezzi et al., 2013).

In the realm of fetal and early infant CNS development, Purkinje cells share a common point of origin with brainstem neurons important to homeostasis. This location is called the rhombic lip, and it is located near the border of the pons and medulla of the brainstem (Kinney et al., 2002). This location is essential to the proper maturation and migration of both brainstem neurons and PCs, so investigators interested in early neuronal irregularities have turned their attention to this site (Kinney et al., 2002). As mentioned before, the cerebellum communicates with the brainstem for homeostatic reasons via the PCs in the “Purkinje-Olivo-Dentate network” (Lavezzi et al., 2013). Investigators researching early CNS development have found that the integrity of this communication can be effected by the delayed formation and incorrect circuitry of a stressed rhombic lip (Kinney et al., 2002). This germinal center illustrates how early CNS

insult could influence how pertinent cerebellar and brainstem neurons connect for vital bodily processes, such as homeostasis (Kinney et al., 2002). These studies offer quality evidence in regards to the potential role that cerebellar dysfunction plays in the outcome of crib death; however, the key component to homeostasis, and the leading suspect in SIDS pathology, is the brainstem.

The Brainstem

A.) Human Choroid Plexus, Cerebrospinal Fluid, and the Fourth Ventricle

Cerebrospinal fluid (CSF) bathes the entire CNS and is produced by the human choroid plexuses of the ventricular system (Lavezzi et al., 2013). The fourth ventricle is intimately connected to the brainstem, as it houses numerous neuronal centers for pivotal homeostatic functions (Lavezzi et al., 2013). The choroid plexus, and its produced CSF, is important to the development of these nuclei; however, it forms a boundary with much of the neurovascular networks, and thus is prone to injury early in its growth (Lavezzi et al., 2013). A few studies have been conducted to analyze the choroid plexus, as well as the contents of the CSF in the fourth ventricle, in hopes of understanding a relationship between SIDS and potential risk factors.

In regards to the general histologic appearance, a study confirmed that only 24% (7 out of 29) of control subjects displayed any variations of choroid plexus morphology while the SIDS group recorded 82% (45 out of 55) of subjects with evident changes (Lavezzi et al., 2013). This turned out to be a statistically significant difference ($p < 0.05$) between victims of SIDS and fetal/infant explained deaths (Lavezzi et al., 2013). These alterations can be examined in more detail if considered separately among the three

cellular compartments of the choroid plexus. The vasculature of all 29 controls, and all sudden fetal deaths, had no noticeable malformations; however, 10 of the 30 sudden infant deaths displayed a statistically significant ($p < 0.01$) desquamated endothelium of the vasculature and decreased capillary content (Lavezzi et al., 2013). The interstitial spaces of nearly 90% of the controls were clear of unhealthy cells or debris, while over 50% of the sudden fetal and infant cases had dense collagen and stromal cell build-up ($p < 0.01$) (Lavezzi et al., 2013). Finally, the choroid plexus epithelium showed statistically significant evidence of a torn appearance in the SIDS victims ($p < 0.05$), but only minor evidence in the controls (13%) and sudden fetal death cases (8%) (Lavezzi et al., 2013).

In addition to these malformations, certain staining techniques were used to ascertain markers of CNS stress and apoptosis (cell death). As described previously, substance P is a neuropeptide released as an indicator of neuronal damage (Ozawa & Takashima, 2002). Immunostaining for this peptide in the fourth ventricle of the samples exhibited a statistically significant association between 40% of the SIDS cases, but registered nonsignificant results for the two sets of controls or the sudden fetal deaths (Lavezzi et al., 2013). The most substantial observations of this study came from the TUNEL staining (Terminal deoxynucleotidyl transferase dUTP Nick End Labeling), which is a means of detecting cell death in histologic slides (Lavezzi et al., 2013). TUNEL staining revealed zero evidence of there being apoptotic neurons in the control groups, however, both sudden fetal and infant samples presented over 40% of the cases with significant TUNEL immunopositivity (**Table 1** for full results) (Lavezzi et al., 2013).

Table 1: Choroid Plexus and Fourth Ventricle Malformations

This table summarizes the full results of the study out of Milan University that set out understand any underlying associations between ventricular alterations and sudden infant or fetal death. The table highlights numerous relationships between both noticeable damage via light microscopy and CNS damage or stress via immunostaining (adapted from Lavezzi et al., 2013).

Fourth ventricle/choroid plexus (4VCP) alterations in SIDS and SIDS victims compared to controls.				
Choroid Plexus Alterations	Group 1 fetal control	Group 2 SIDS	Group 3 infant control	Group 4 SIDS
Vasculature				
Capillary impoverishment	0/14 (0%)	0/25 (0%)	0/15 (0%)	10/30 (33%)
Interstitial				
Presence of cystic cells	1/14 (7%)	14/25 (56%)	2/15 (13%)	17/30 (57%)
Epithelium				
Stripping from underlying matrix	3/14 (21%)	2/25 (8%)	1/15 (6%)	12/30 (40%)
Prevalence of dark cells	0/14 (0%)	0/25 (0%)	0/15 (0%)	20/30 (66%)
Substance P immunopositivity (Class 3 of PI)	3/14 (21%)	9/25 (36%)	1/15 (6%)	12/30 (40%)
Apoptosis (TUNEL) immunopositivity (Class 3 of AI)	0/14 (0%)	11/25 (44%)	0/15 (0%)	13/30 (43%)

Aside from the relationship between unexplainable sudden fetal/infant death to choroid plexus alterations, there were also results that described a connection with maternal nicotine use. It was found that of the 22 sudden death victims with morphological changes, 19 had mothers who were smokers (86%) (Lavezzi et al., 2013). In addition to the SIDS cases, only 5 controls showed poor choroid plexus development, but 4 (80%) of these also had cigarette-smoking mothers (Lavezzi et al., 2013). Both of these pathologic findings were statistically significant as well ($p < 0.05$), which means the results are not compatible with a null hypothesis of there being no real association between nicotine use and choroid plexus damage (Lavezzi et al., 2013).

The choroid plexus is not the only point of concentration for investigators interested in the contributions of the fourth ventricle. A different study has analyzed the contents of the fourth ventricle as a marker of infection and possible association with SIDS. This marker is Interleukin-6 (IL-6), which is a cytokine (or chemical messenger) released by immune cells in response to foreign pathogens (Rognum et al., 2009). It was found that CSF in the fourth ventricle contained a larger amount of IL-6 for victims of SIDS when compared to controls, and that there was a statistically significant ($p < 0.05$) increase of expression in IL-6 receptors of the arcuate nucleus in the brainstem (Rognum et al., 2009). Arcuate neurons do not normally express IL-6 receptors and are pivotal in the regulation of sleep and cardiorespiratory functions, therefore the IL-6 and neuronal interaction could suggest negative implications for sudden infant death (Rognum et al., 2009). Investigators believe that infection, as seen through elevated IL-6 levels in the CSF, could provide an exogenous stress that challenges the homeostatic centers of the brainstem (Rognum et al., 2009). This stress is also believed to be cumulative depending on other major risk factors such as sleep position (Rognum et al., 2009). Evidence shows that infants with a mild infection have 1.7 times the incidence of SIDS and that infants in a prone sleep position have 10.4 times the incidence; however, if an infant has both a mild infection and is in an improper sleep position, then the incidence of SIDS could be as high as 29 times that of a control (Rognum et al., 2009).

These studies illuminate one possible way in which brainstem anomalies and major known risk factors could play a causal part in the pathology of SIDS. Other key studies point to the involvement, and subsequent dysfunction, of chemical messenger

systems in the brainstem. Communication between neurons and the rest of the body is vital in the proper functioning of homeostasis, and should be considered on a class by class basis.

B.) Neuropeptides

Maintaining a consistent and stable internal environment during sleep requires proper coordination. One of the major signaling molecules of the brainstem involved in this task is orexin, also known as hypocretin, which can be functionally divided into two neuropeptides (orexin A and orexin B) (Hunt et al., 2015). Orexin's functions include sustaining an alert or sleeping status, balancing levels of arousal, and contributing to appropriate respiration during sleep (Hunt et al., 2015). Since crib death almost exclusively occurs during sleep, either at night or a nap during the day, some researchers have examined the potential dysfunction of these neuropeptide systems.

A recent study investigated the hypothalamus and the pons (part of the brainstem), in order to shed light on a possible relationship. Although the hypothalamus is not part of the brainstem, it is the area of the brain that houses the neurons that produce orexin, and these neurons have axonal projections all over the CNS (Hunt et al., 2015). Interestingly, it was found that there was no statistical difference between SIDS and non-SIDS victims in the morphology of orexin-generating neurons or the total number of neurons (both orexin and non-orexin) (Hunt et al., 2015). However, the study did detect a statistically significant decrease in the percentage of overall orexin immunoreactivity in fiber staining of both the hypothalamus (21%: all three components, orexin A/B $p < 0.05$) and the pons (40 – 50%: all six components, orexin A $p < 0.01$ and orexin B $p < 0.05$) in

the SIDS cases (Hunt et al., 2015). Since the morphology of neurons was not visibly affected, the investigators concluded that the immunoreactivity differential in SIDS and non-SIDS samples must have come from either the density of orexin-producing cells, or the limited capacity to express the neuropeptides (Hunt et al., 2015). An important discovery to note is that unlike previously mentioned defects in the CNS of SIDS patients, this study found that there was no true association between decreased orexin immunoreactivity and the major known risk factors (either maternal nicotine use or sleeping in the prone position) (Hunt et al., 2015). Perhaps these neuropeptide deficits contribute to an unknown mechanism of sudden infant death that is outside the realm of the triple-risk theory.

Another neuropeptide under investigation is brain-derived neurotrophic factor (BDNF), which comes from a specific family of growth factors called neurotrophins (Tang et al., 2008). BDNF has a vital position in regulating nervous system development and has been implicated as a contributor to respiration (Tang et al., 2008). This growth factor can be found in either a pro-BDNF or mature-BDNF arrangement with p75^{NTR} and TrkB being their respective receptors (Tang et al., 2008). These two forms of BDNF, in combination with their receptors, mediate either survival with subsequent differentiation (mature-BDNF) or apoptosis (pro-BDNF) as a means of selectively promoting proper circuitry in the brain (Tang et al., 2008). One study researched the possible relationship between known risk factors and irregularities of the BDNF system in piglet brainstems as a model for potential dysfunction in human infants.

The investigators concentrated on five or six nuclei of the caudal medulla in the brainstem by immunostaining for pro-BDNF, rhBDNF (mature), and TrkB in accordance with nicotine exposure, intermittent hypercapnic hypoxia (IHH), and the combination of the two (Tang et al., 2008). Mature-BDNF is represented by rhBDNF because the researchers used antibodies that were raised against rhBDNF but sequence comparison revealed a 100% homology between the two (Peiris et al., 2004). It was found that only TrkB (mature-BDNF receptor) had a statistically significant decrease ($p < 0.05$) after nicotine exposure alone or after the combination of nicotine and IHH, in either gender (Tang et al., 2008). It was also discovered that results for immunopositivity of pro-BDNF and mature-BDNF had more noticeable changes (both statistically significant and not) for the male samples than the female samples (Tang et al., 2008). This study was slightly less conclusive in that there were numerous changes among the five or six nuclei being studied, however, the results did point to a trend that suggests prior nicotine exposure in a maturing brainstem could greatly influence its ability to express BDNF and TrkB while encountering hypoxic injury (Tang et al., 2008). Complete results are outlined in the table below (**Table 2**).

This analysis could lend insight as to how perinatal nicotine exposure could provoke the necessary physiological changes in the brainstem for an exogenous stressor, such as hypoxia, to overpower the homeostatic centers. Given the results of these studies, and the roles of orexin and BDNF in sleep and respiration regulation, more neuropeptide signaling molecules may be researched in the future. Aside from these larger proteins

though, numerous smaller neurotransmitters have been looked at as well, in the hopes of understanding SIDS.

Table 2: Pro-BDNF, Mature-BDNF, and TrkB Immunostaining

This table displays the comprehensive results of the study on the BDNF system in the brainstem of piglets, as a model for human infants. Direct attention to the (*) which indicates a statistically significant difference ($p < 0.05$) between an exposure group and the control group for a particular gender. Significant findings are more concentrated in the nicotine and IHH combination exposure, possibly highlighting the true etiology of SIDS (adapted from Tang et al., 2008).

Pro-BDNF in six nuclei of the caudal medulla comparing gender in each exposure group								
Nucleus	Controls		Nicotine		IHH		Nic+IHH	
	Males	Females	Males	Females	Males	Females	Males	Females
XII	64.4±10.9	61.0±3.8	74.2±5.4	65.8±10.7	70.0±13.9	74.4±8.4	77.2±3.3	77.8±7.7
DMNV	68.8±4.8	46.5±1.9	57.8±10.7	51.4±8.3	66.3±16.2	58.8±8.5	59.7±5.7	86.0±3.5
NTS	64.0±4.8	53.5±2.1	40.0±3.0	52.8±6.9	49.3±11.8	49.8±9.4	44.2±5.0	59.8±12.4
Grac	61.6±5.6	51.5±6.7	63.0±10.0	53.4±5.8	46.0±8.8	62.2±8.3	57.2±2.9	74.0±5.7
Cun	71.2±6.9	57.3±1.9	52.7±9.6	60.2±9.5	45.5±9.6	66.5±14.3	50.0±5.6	70.8±7.0
ION	59.6±5.3	37.9±1.6	59.0±9.2	33.8±5.1	34.3±7.0	40.0±9.6	58.7±5.3	58.4±11.4
Mature-BDNF in five nuclei of the caudal medulla comparing gender in each exposure group								
Nucleus	Controls		Nicotine		IHH		Nic+IHH	
	Males	Females	Males	Females	Males	Females	Males	Females
XII	75.5±7.3	75.3±6.6	79.0±5.1	67.5±4.2	69.3±11.7	73.3±7.1	68.0±6.3	75.3±6.0
DMNV	62.2±6.1	65.5±6.6	76.8±6.8	70.3±5.8	70.8±3.8	73.5±4.3	68.3±3.9	81.8±3.7
NTS	42.0±7.8	38.7±3.8	29.0±9.3	42.7±4.5	32.3±6.8	39.3±15.3	37.4±4.6	42.0±23.0
Grac	48.0±6.8	44.8±9.1	45.2±3.5	53.3±5.4	21.8±4.0	43.7±21.7	51.9±7.3	72.0±10.4
ION	66.3±4.2	74.5±3.8	70.3±2.6	78.8±3.2	71.3±3.8	68.0±5.1	63.9±3.1	70.0±4.9
TrkB in six nuclei of the caudal medulla comparing gender in each exposure group								
Nucleus	Controls		Nicotine		IHH		Nic+IHH	
	Males	Females	Males	Females	Males	Females	Males	Females
XII	83.7±5.2	71.0±8.0	59.8±2.8	69.3±4.8	82.5±6.1	76.5±7.6	77.9±6.5	81.0±4.8
DMNV	80.8±1.4	76.3±8.0	63.4±6.2	63.5±4.3	81.5±4.0	80.8±2.0	68.1±4.9	82.8±2.9
NTS	60.0±4.0	45.3±4.1	44.8±7.7	57.9±3.6	42.0±7.8	63.8±7.6	49.1±3.8	67.5±11.7
Grac	58.0±5.3	56.5±7.5	46.2±7.0	61.4±4.6	53.0±3.1	53.8±4.6	43.9±2.8	67.3±9.4
Cun	64.1±7.1	41.5±11.1	50.8±6.2	48.9±6.6	34.3±4.5	43.3±17.9	25.4±5.6	53.3±14.0
ION	77.0±4.3	78.8±1.5	79.8±0.7	77.8±4.2	84.0±1.7	81.8±3.6	77.3±2.9	82.0±2.3

C.) Amino Acid Neurotransmitters

The body utilizes numerous types of molecules for the transduction of signals within the nervous system. As discussed above, larger proteins are often used in this type of communication, however, smaller amino acid neurotransmitters predominate in the

CNS (Singh et al., 2016). These NTs can be either excitatory or inhibitory depending on the post-synaptic effect they elicit (Singh et al., 2016). Due to the magnitude of this particular class of NTs, research has been conducted here as well to shed light on possible connections to crib death.

The carboxylate anion form of glutamate functions as the leading type of excitatory NT in the human CNS (Kinney et al., 2002). This NT has a few families of receptors that it can interact with, but the concentration of N-methyl-D-aspartate (NMDA) receptors in the brainstem have drawn the most attention (Fanous et al., 2006). A study involving piglet brainstems as a model for human infants focused on the immunopositivity of both NMDA receptor 1 (NR1) proteins and mRNA in seven nuclei as it pertains to nicotine use and sleep position (Fanous et al., 2006). The investigators discovered that with nicotine contact, only two of seven brainstem nuclei showed significant mRNA increases; meanwhile, mRNA increases were noted in five of seven nuclei following IHH exposure and three of seven after the combination of the two (Fanous et al., 2006). In contrast to this, NR1 protein immunopositivity was only increased in one brainstem nucleus (inferior olivary nucleus) following exposure to IHH alone ($p < 0.05$) and the combined exposure of nicotine and IHH ($p < 0.01$) (Fanous et al., 2006). According to this data, the mRNA of NR1 is effected to a greater extent than the full expression of NR1 to its protein structure (Fanous et al., 2006). This lends one to believe that these results on the glutamatergic-NR1 system are inconclusive, however, special attention must be paid to the one particular nucleus that showed significant mRNA and protein changes.

As previously discussed, the inferior olivary nucleus of the brainstem is the nucleus that communicates with the cerebellum. This is also the nucleus that displayed the highest amount of NR1 mRNA and protein expression following the experimental exposures (mostly IHH alone) (Fanous et al., 2006). It is believed that this circuitry plays a key role in the upper airway movement and blood pressure control during hypotensive events, and therefore the malformation of this brainstem nucleus due to IHH or nicotine use could eventually lead to SIDS (Fanous et al., 2006). The significant increase of full NR1 expression in the inferior olivary nucleus would seem counter intuitive to dysfunction, however, as a result of being an excitatory NT, investigators believe this could cause cell death from over-activation (“excitotoxicity”) (Fanous et al., 2006).

Staying within the realm of amino acid NTs, inhibitory chemicals play an important role on the other side of this spectrum. These NTs modulate neuronal communication by decreasing post-synaptic excitability (Singh et al., 2016). Two of these NTs under examination are glycine and GABA, with GABA being the inhibitory equivalent of glutamate, in terms of magnitude, in the human CNS (Neff et al., 2004). Investigators out of George Washington University studied the association between prenatal nicotine exposure and the activity of the cardioinhibitory vagal neurons (CVNs) in the preganglionic brainstem (Neff et al., 2004). Glycine and GABA are the two main inhibitory NT systems in the CVNs, and they both experience a specific pattern of conductivity during hypoxic events (Neff et al., 2004).

The pattern is a biphasic response that entails the momentary increase of inhibitory post-synaptic currents (IPSCs), followed by a modest decrease as it closely

tracks one's heart rate and respiratory frequency (Neff et al., 2004). This response is tailored as an adaptive measure that allows one's body to lower its metabolic needs during times of low oxygen (Neff et al., 2004). However, if there is a dysfunction with the commanding NT systems, the physiologic downtick of heart rate and respiration could become amplified, resulting in a life-threatening situation (Neff et al., 2004). Using rodent brainstems as a model for human infants, it was found that prenatal nicotine exposure did indeed produce irregularities, but only in one of the two systems. Although unexpected, glycinergic IPSCs recorded no statistically significant ($p > 0.05$) alterations between *in utero* nicotine exposure and the control group (Neff et al., 2004). However, when examining the GABA IPSCs, a steep decrease was noted in the downtick of the exposed group that was statistically significant ($p < 0.01$) (Neff et al., 2004). This magnified downtick in conductivity represents the potential dysfunction necessary for an infant's inability to cope with hypoxic stress (Neff et al., 2004). Although glycine did not produce substantial results in this study, the data pointing to GABA contributions were consistent with the findings of subsequent research.

In another study, researchers hypothesized that evidence of asphyxia in sudden and unexplained infant death would show differences in neurotransmitter function when compared to sudden and unexplained infant death that lacked evidence of hypoxia (Randall et al., 2013). The investigators believed that experiencing asphyxia would elicit a change in the neuroanatomy of these infants. Unfortunately, when comparing the two groups, they were unable to identify any noteworthy differences (Randall et al., 2013). While the study did not yield the outcomes expected by the research team, it did highlight

a substantial difference in the activity of specific NT systems in SIDS patients when compared to infants who passed away from known causes.

GABA receptor binding was one of these systems that generated interesting results (Randall et al., 2013). Using autoradiography, three brainstem nuclei (raphe obscurus, nucleus of the solitary tract, and hypoglossal nucleus) displayed statistically significant ($p < 0.05$) differences of GABA receptor activity between SIDS and non-SIDS samples (Randall et al., 2013). When compared to the ten brainstem nuclei overall that were studied, this may not appear to be a major discrepancy; however, much like the previous study, particular attention must be paid to the specific nuclei that did change. The raphe obscurus has common circuitry with the cerebellum and is implicated in respiration (Randall et al., 2013). The nucleus of the solitary tract is involved with cardio-respiratory functioning, and the hypoglossal nucleus deals with the intricate musculature of the tongue (Randall et al., 2013). These are all functionalities that have been linked, in some way or another, to the possible etiology of SIDS.

On that note, other studies have also scrutinized GABA receptor binding in the brainstem of SIDS victims. One such study examined GABA interactions on serotonergic (5-HT) neurons in the brainstem, because they are directly involved in the maintenance of homeostasis (Broadbelt et al., 2011). These 5-HT neurons receive inhibitory input from GABA neurons in the medulla, where they share a common circuitry that regulates blood pressure, respiratory chemo-sensitivity, and upper airway musculature (Broadbelt et al., 2011). The investigators focused on the GABA_A receptor family because they are found in high concentration on 5-HT neurons in the medulla and are known to be markers of

operational GABA activity (Broadbelt et al., 2011). The results of this study indicated an abnormality in the medullary GABA_A receptor binding density (Broadbelt et al., 2011). In seven of the ten medullary nuclei that were studied, autoradiography revealed a statistically significant ($p < 0.05$) decrease of 25% - 52% in GABA_A receptor activity for the SIDS victims (**Table 3** for full results) (Broadbelt et al., 2011). Additionally, western blotting exposed a statistically significant ($p < 0.05$) decrease of roughly 46% in a constituent (GABA_{A α 3}) of the 5-HT system in the brainstem of SIDS cases when compared to the controls (Broadbelt et al., 2011). The results show, assuming no systemic bias in the study design, that this association between crib death and decreased GABA functioning is too extensive to be due by random chance.

Table 3: GABA_A Receptor Activity in SIDS

This table outlines the results from the study on GABA_A receptor density when comparing SIDS victims to non-SIDS controls. It shows statistically significant results for seven of the ten brainstem nuclei. Important note: 3-way p-values represent significant difference between SIDS cases and combined receptor binding of acute and chronic controls (adapted from Broadbelt et al., 2011).

	Age-Adjusted SIDS Cases (n = 28)	Acute Controls (n = 5)	Chronic Control (n = 3)	3-Way p*
Medullary 5-HT source nuclei				
Raphé obscurus	25.4 ± 2.8	41.9 ± 6.4	54.9 ± 9.9	0.006
Paragigantocellularis lateralis	26.9 ± 3.4	48.5 ± 7.7	73.8 ± 12.0	<0.001
Gigantocellularis	32.7 ± 3.1	60.1 ± 7.0	56.0 ± 11.0	0.002
Intermediate reticular zone	32.7 ± 3.1	60.6 ± 6.9	63.6 ± 10.9	<0.001
Arcuate nucleus	27.3 ± 3.0	22.8 ± 7.0	35.7 ± 11.0	0.610
Medullary 5-HT projection nuclei				
Medial accessory olive	38.1 ± 2.5	53.6 ± 7.5	48.0 ± 7.6	0.100
Nucleus of the solitary tract	41.9 ± 2.7	77.2 ± 7.9	81.9 ± 8.1	<0.001
Hypoglossal nucleus	44.2 ± 3.7	78.3 ± 11.0	85.5 ± 11.2	<0.001
Dorsal accessory olive	34.6 ± 2.7	36.8 ± 6.2	64.6 ± 9.6	0.020
Principal inferior olive	28.9 ± 2.4	38.9 ± 5.7	39.0 ± 8.9	0.200

These discussed studies, as well as numerous others, all attempt to shed light on the potential contributions of amino acid NTs to the pathology of SIDS. By virtue of glutamate and GABA being the predominate form of excitatory and inhibitory signals respectively, it is understandable why investigators have examined these NT systems. However, the last report mentioned above did not only suggest irregularities in the activity and concentration of GABA receptors, it also touched on one of the most well reviewed signaling molecules concerning homeostasis, serotonin.

D.) Monoamine Neurotransmitters

The brainstem houses one of the largest concentrations of serotonin in the body, besides the gastro-intestinal system (Jafurulla et al., 2016). Serotonin is a monoamine NT, and due to its pivotal role in communication among numerous brainstem neurons, it has a well-established relationship with maintaining homeostasis (Medel-Matus et al., 2017). This affiliation has been the focal point of various research papers, and the 5-HT connection has been explored in a number of ways. One of these means is through the interaction of damaged glial cells around major 5-HT nuclei (Sawaguchi et al., 2002).

Gliosis is the systematic way in which glial cells, or cells other than neurons found in the CNS, change or respond to injury (Sawaguchi et al., 2002). This process can lead to the formation of glial scars, which have been known to elicit inhibitory effects on proper neuronal function or development (Sawaguchi et al., 2002). A study was conducted to examine the relationship between sleep apnea, prone sleeping positions, and gliosis of major 5-HT brainstem nuclei (Sawaguchi et al., 2002). It was hypothesized that sleep apnea or a prone sleeping position would introduce a hypoxic event that could

injure the CNS of an infant, precipitating as gliosis in specific locations (Sawaguchi et al., 2002). Gliosis was measured as the concentration of astrocytes that reacted positively for glial fibrillary acidic protein (GFAP) (Sawaguchi et al., 2002). The results of the study were not conclusive of a definitive link between potential IHH and gliosis of all key 5-HT neurons, however, there were two important findings. It was discovered that there was a statistically significant association ($p < 0.001$) between the occurrence of obstructive apnea and the prone sleeping position, as well as a statistically significant connection ($p < 0.001$) between those hypoxic events and gliosis in the raphe nuclei of SIDS cases when compared to controls (Sawaguchi et al., 2002).

The raphe nucleus is a small gathering of neuronal cell bodies in the brainstem whose principle responsibility is to moderate alertness with 5-HT release (Sawaguchi et al., 2002). Although this study only showed a link between CNS injury and gliosis of one brainstem nuclei, a different study illuminated a relationship between gliosis and another 5-HT center. This research was investigating the association between gliosis in infants who passed away from crib death and nicotine usage of their mother (Storm et al., 1999). It was found that there was a 41% probability ($p < 0.01$) that SIDS cases displayed increased gliosis in the nucleus olivaris inferior of the brainstem when the mother smoked during pregnancy (Storm et al., 1999). The nucleus olivaris inferior is known to be involved in cardio-respiration, as well as sharing common circuitry with the cerebellum, so increased gliosis at a critical age could result in improper 5-HT neuron development (Storm et al., 1999).

Glial cells are not directly involved in neuronal communication, rather they assist in maintaining a healthy CNS environment (Storm et al., 1999). When gliosis occurs, due to CNS injury, these cells can no longer provide the appropriate setting for optimal neuronal health or communication (Storm et al., 1999). In this case, these two studies identified a statistically significant association between both of crib death's two major known risk factors and gliosis of the astrocytes surrounding two brainstem 5-HT nuclei. It is possible that these regional concentrations of gliosis lend insight to possible mechanisms of 5-HT dysfunction. Regardless, this is only one way in which researchers examined 5-HT's role in SIDS pathology.

In pursuit of crib death's etiology, investigators have also turned to the genetic contributions of the 5-HT system. Instead of examining the genetic influences of the 5-HT NT or its varying receptors, researchers have studied the serotonin transporter (5-HTT) gene (Mecchia et al., 2013). This gene codes for the protein transporter located on the presynaptic terminal of 5-HT neurons (Narita et al., 2001). The 5-HTT is responsible for the maintenance of proper 5-HT levels in the synapse by selectively reclaiming NTs for breakdown or repackaging in the presynaptic terminal (Narita et al., 2001). This gene is variably expressed depending on its promoter sequence, which is polymorphic in nature by either containing short (S), long (L), or extra-long (XL) repeats (14, 16, and 18-20 repetitive elements respectively), and it is here that some scientists have focused their attention (Narita et al., 2001).

The classification of the promoter sequence is directly associated to the extent at which the 5-HTT gene is expressed (Narita et al., 2001). The longer the promoter repeat

sequence (L or XL) the higher the level of expression, and vice versa (Narita et al., 2001). In a case report of an unfortunate sudden infant death, the researchers specifically attempted to identify the victim's 5-HTT genotype (Mecchia et al., 2013). It was found that the infant was heterozygous for the promoter sequence, having both the L and S alleles (Mecchia et al., 2013). This discovery raises an interesting question about how variable 5-HTT gene expression could possibly contribute to SIDS. In a study examining genetic risk factors among a Japanese population, the results suggested a more solid relationship between the 5-HTT gene promoter sequence and SIDS. The investigation uncovered a statistically significant difference in both genotype distribution ($p < 0.01$) and allele frequency ($p < 0.01$) when comparing SIDS victims to age-matched controls (**Table 4**) (Narita et al., 2001).

Table 4: 5-HT Transporter Genotype and Allele Differences

Full results from the study of genetic differences amongst sudden infant death victims and age-matched controls. There were statistically significant differences in both genotype distribution and allele frequencies. This study displayed a higher occurrence of L and XL promoter sequences in crib death cases (adapted from Narita et al., 2001).

	Controls	SIDS	Chi-Squared	P-Value	Fisher's P-Value
Genotype	N=115	N=27	12.49	0.006	0.009
XL/S	1 (0.9%)	3 (11.1%)			
L/L	2 (1.7%)	2 (7.4%)			
L/S	27 (23.5%)	8 (29.6%)			
S/S	85 (73.9%)	14 (51.9%)			
Allele	N=230	N=54	11.36	0.003	0.006
XL	1 (0.4%)	3 (5.6%)			
L	31 (13.5%)	12 (22.2%)			
S/S	198 (86.1%)	39 (72.2%)			

The results of this research showed that the frequency of L and XL alleles were meaningfully increased in infants who succumbed to crib death (Narita et al., 2001). This outcome is continuous with the findings of the previous case report and has shed light on an association between a genetic risk factor and the development of SIDS. However, the important fact to consider is how these genotypes potentially contribute to this syndrome. As was previously mentioned, the larger the repeat sequence the greater the level of 5-HTT gene expression (Narita et al., 2001). Therefore, SIDS victims tend to have a higher density of 5-HTT proteins on the pre-synaptic terminal (Narita et al., 2001). This higher density equates to a larger amount of 5-HT NTs being removed from the synapse, and decreasing the NT-receptor interactions (Mecchia et al., 2013). Due to 5-HT's role in homeostasis and cardio-respiration, the increased gene expression and decreased 5-HT interactions in the brainstem could precipitate as sudden and unexplained death in susceptible infants.

In addition to selective gliosis and genetic vulnerabilities, investigators have also researched particular 5-HT receptor families while attempting to unearth the underlying pathology of SIDS. Two of these classifications (1A and 2A) were looked at specifically in a study that examined receptor density and major risk factors while utilizing piglet brainstems as a model for human infants (Say et al., 2007). This investigation concentrated on the receptor immunoreactivity of four brainstem nuclei (hypoglossal nucleus, inferior olivary nucleus, nucleus of the solitary tract, and dorsal motor nucleus of the vagus) across three different exposure categories (nicotine, IHH, and nicotine/IHH combination) (Say et al., 2007). The researchers discovered substantial physiological

effects in the nuclei among all three exposure classes. In the IHH group, 5-HT receptor 2A immunoreactivity was not meaningfully altered, however 5-HT receptor 1A immunoreactivity was significantly decreased ($p < 0.05$) in all four medullary nuclei (Say et al., 2007). In the nicotine group, 5-HT receptor 2A immunoreactivity was decreased in two of the four nuclei ($p < 0.05$) and three of the four nuclei displayed reduced immunoreactivity of 5-HT receptor 1A ($p < 0.01$) (Say et al., 2007). Finally, in the nicotine and IHH combination group, there were again no meaningful changes in the 5-HT receptor 2A density, but 5-HT receptor 1A immunoreactivity was statistically decreased in three of the four medullary nuclei ($p < 0.01$) (Say et al., 2007). These results depict a profound reduction of 5-HT receptor density in numerous vital homeostatic centers of SIDS victims when compared to controls.

In a similar study, 5-HT receptor 1A immunoreactivity was again examined in accordance with major known risk factors (sleep position and nicotine exposure) (Machaalani et al., 2008). Guinea pig polyclonal antibodies for 5HT receptor 1A were used to establish histological reactivity, and immunostaining was recorded in seven nuclei of the caudal medulla and eight nuclei of the rostral medulla (**Figure 6**) (Machaalani et al., 2008). The findings of this investigation point towards extensive reductions in medullary 5-HT receptor density (Machaalani et al., 2008). When compared to the control group, 5-HT receptor 1A positivity in the inferior olivary nucleus of the caudal medulla was significantly decreased ($p < 0.05$) among SIDS victims (Machaalani et al., 2008). In the rostral medulla, five of the eight examined nuclei displayed statistically significant reductions ($p < 0.05$) in 5-HT receptor density (Machaalani et al.,

2008). Considering the exposure categories, nicotine produced a meaningful decrease in immunostaining in one of the rostral nuclei ($p < 0.05$), and SIDS infants found in a prone sleeping position had a significantly lower 5-HT receptor density in two caudal nuclei ($p < 0.05$) (Machaalani et al., 2008). These results are in agreement with the previous report of reduced receptor immunoreactivity.

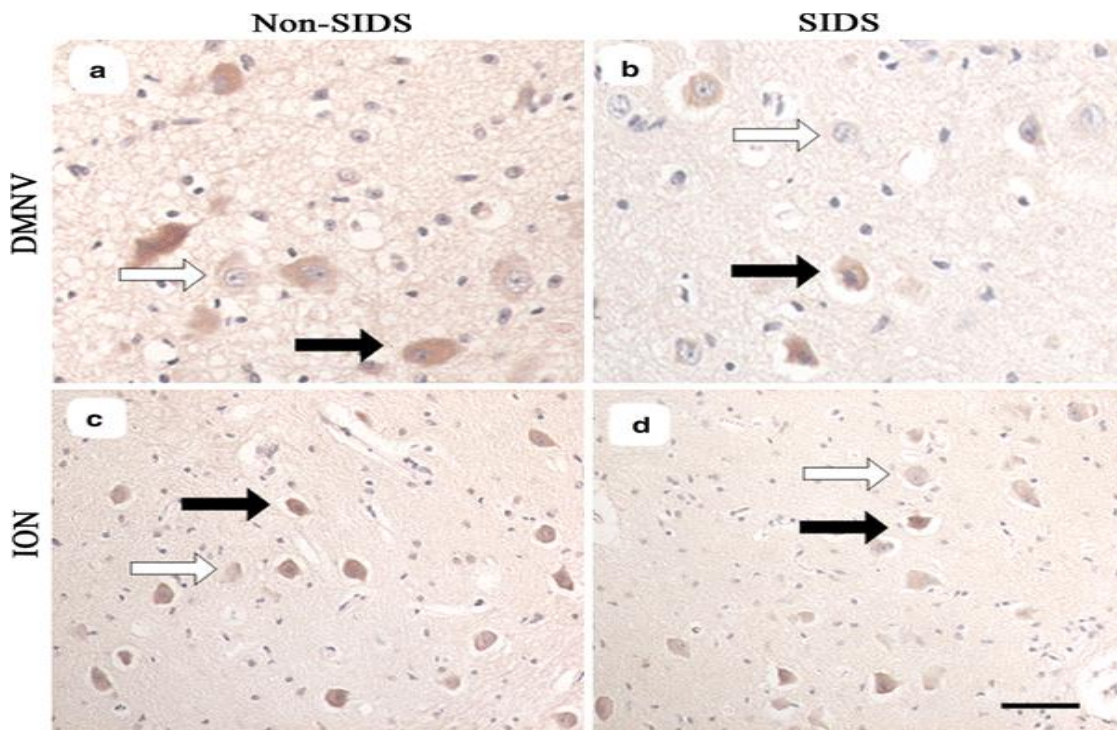


Figure 6: 5-HT Receptor 1A Immunoreactivity

This figure shows an example of two medullary nuclei that were stained with 5-HT receptor 1A antibodies in the histological slides of SIDS and non-SIDS brainstems. The black arrows indicate dark brown centers with high levels of positive reactivity. The White arrows indicate light brown centers with low levels of positive reactivity. Notice the higher concentration of immunoreactivity in the non-SIDS cases (adapted from Machaalani et al., 2008).

The 5-HT system has been extensively studied in a variety of ways. Whether the investigators focused on the gliosis of neighboring astrocytes, the polymorphism of the 5-HTT promoter sequence, or the density of specific 5-HT receptors, the results appear to suggest a connection between serotonin and crib death. These studies are only but a few

of the numerous investigations undertaken by pediatric and neurologic researchers in hopes of finding a true pathology to this syndrome. With the evidence of the reviewed published literature in mind, the next section will discuss the validity of the triple-risk theory for SIDS.

DISCUSSION AND FUTURE DIRECTIONS

The cause of Sudden Infant Death Syndrome has evaded medical experts for years. It has largely been a “diagnosis of exclusion,” meaning that physicians do not categorize an infant’s death as SIDS unless an autopsy, medical history, and place of death analysis offers no other indication (Kinney et al., 2009). Most medical professionals would probably be content with this particular type of diagnosis if crib death were a rare event. However, this syndrome is the leading cause of death amongst infants aged from one month to one year in the United States, and tolerance of an unknown pathology is unacceptable (Kinney et al., 2009). Besides narrow theories that suggest specific causes of crib death, there is one hypothesis that incorporates the diversity of observed irregularities in the victims as well as the known major risk factors, the triple-risk model.

The triple-risk hypothesis states that sudden and unexplained death precipitates from a combination of three events that must all occur in order for it to be categorized as SIDS (Paterson et al., 2006). These factors include an inherent vulnerability in the infant’s homeostatic centers, an exogenous stressor, and that it must be during a critical period of life (Paterson et al., 2006). The third factor of timing is easily understood because SIDS is normally only established for the sudden death of an infant from birth to one year. Also, for the published literature discussed above, all research was conducted on either infant animals or humans up to one year post-birth as a means of qualifying this criterion. This time frame is considered to be a key period of life because the infant’s body is adapting to life outside the uterus and experiencing a host of new external

stressors (Kinney et al., 2009). This then leaves the other two factors of the triple-risk model to be examined more carefully.

Maintaining a consistent environment within the body is predicated on the healthy and accurate communication that occurs within one's central nervous system. The published literature section touched on a number of structures in the CNS that are pertinent to this life-sustaining process, and the research suggested that dysfunction in many of these contributors were highly associated with SIDS. As previously stated, the cerebellum, and more specifically Purkinje cells, has an established network of communication with the brainstem as it aids in integration and propagation of homeostatic demands. The PCs of several SIDS victims were found to have both visible malformations, inappropriate migration patterns, and irregular division (Lavezzi et al., 2013). The abnormalities of brainstems in crib death cases were even more diverse and plentiful. The research showed dysfunction in numerous brainstem structures, such as the fourth ventricle/human choroid plexus, neuropeptides, amino acid NTs, and monoamine NTs (specifically serotonin). The evidence was not always conclusive of a complete relationship, but the sheer amount of alterations in cell morphology and receptor density in different brainstem nuclei are deserving of additional scrutiny.

It is this diversity of abnormal CNS structures in SIDS cases that constitute the factor of inherent homeostatic vulnerability in the triple-risk theory. An important aspect to note is that there is no solid consistency between all the alternations, besides that they all occur in some member of the team that deals with homeostasis. This shows that SIDS might be a syndrome based more on its outcome, death, than any one particular

dysfunction. All of these different irregularities fall within the realm of an intrinsic weakness, and lends credibility to the triple-risk model in that maybe being too specific with a cause is narrow-sighted, and that SIDS is potentially a syndrome of confluence. The reviewed literature also points out the connection to the risk factor of maternal tobacco smoking. It has been long known that SIDS is associated with maternal nicotine use, but what was discovered in the research was that CNS alteration was also strongly associated with perinatal nicotine exposure. If smoking cigarettes while pregnant, with associated nicotine exposure, can interrupt fetal CNS development, then investigators can finally start to piece together an accurate mechanism that accounts for the varied disruption of brainstem nuclei.

In summary, the published literature have satisfied at least two of the three factors in the triple-risk hypothesis. However, there is still one other event that must occur in order for an infant to suddenly pass away, according to the theory, and that is the induction of an external stressor. This may be the variable in the model that SIDS researchers are already the most aware of. As mentioned previously, there was a massive “Back to Sleep” campaign that urged mothers to have their infants sleep on their back to avoid rebreathing low oxygen air or asphyxiating situations while in the prone position (Corwin et al., 2003). These conditions, including tight swaddling with its associated over-heating, are the exogenous stressors that must be present to challenge the dysfunctional homeostatic center of a vulnerable infant.

Multiple studies had an interest in the association between periods of intermittent hypercapnic-hypoxia and the development of SIDS. The results showed that, with the

support of statistical analysis, there was indeed a meaningful connection between the two events, which was a common theme amongst similar reports. There was also a slight relationship between these stressful events and the furthering of physiologic alterations to the CNS, but this was much less conclusive. It appears that the IHH exposure in infants is more a stressful situation occurred during sleep, than a driving force for the development of inherent vulnerabilities. Regardless, the research presented here, as well as the previous understanding, all point to sleep position and tight swaddling as being an extremely hazardous risk factor that easily qualifies as the third and final factor in the triple-risk hypothesis.

Before a conclusion is drawn on the validity of the triple-risk hypothesis, it is important to consider possible limitations of the reviewed literature. One important note is that the sample sizes of most investigations were relatively small because it is difficult to acquire consent from a grieving parent to obtain brainstem samples from their recently deceased infant. Another important thing to consider is that neurologic research on crib death victims can only happen once death has occurred and a sample is secured from their CNS. The true cause of SIDS could be a fleeting event that is difficult to track as time goes on, and in the time from death to histologic investigation, other confounding variables or biases could intervene. Finally, one more thing to note is that much of the pertinent past history for each infant (maternal nicotine use, for example) is received from the parents, and this is a major source of bias as parents may be ashamed to admit nicotine use while pregnant or in an emotional state that systematically interferes with

their recall. Besides these common limitations, and other specific ones for each particular investigation, there is enough evidence to draw a definite conclusion.

In light of the reviewed literature, and the firm rationale behind each key aspect of the triple-risk theory, one should find this hypothesis to be the most accurate understanding of the sudden infant death syndrome to this day. What makes this hypothesis truly valid is that it tackles one of the more elusive aspects of the disease, which is the diversity of victim characteristics. By having all three events of the triple-risk model as a requirement for death to occur, it successfully explains why some infants whose mothers smoked tobacco do not always perish during the night, and also why some infants who sleep in the prone position do not always pass away either. This model then gives a framework to connect different cases of SIDS, regardless of how each of the three key aspects are satisfied. The inherent vulnerability can occur from maternal nicotine use, use of other illicit substances, or random genetic mutation, and the external stressor can occur from sleep position, tight swaddling, or any other over heating/asphyxiating event. Since SIDS rarely occurs after about one year post-birth, the critical age period of the hypothesis also holds true.

The triple-risk model has been able to uncover a coherent pathology that has gone unknown for a long time. If this hypothesis is accepted on a global scale, then SIDS can cease to be a diagnosis of exclusion, and more focused research can be conducted in hopes of fighting this unfortunate syndrome. The “Back to Sleep” campaign has already proven the success that can come from widespread public health interventions, and with the understanding of this hypothesis, new campaigns directed at other risky activities

could potentially decrease the incidence of SIDS altogether. Investigators finally have a solid direction to move in with their SIDS research, and hopefully with new screening techniques and/or public health interventions in the near future, the fear of waking up to your infant having inexplicitly passed away over the night will be a distant memory.

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