The serotonergic network and SIDS

Written by:
Maren R.Tandsæther stud.med.
Mars 2012

UIO

Under the supervision of:
Dr.philos Siri Hauge Opdal

Abstract

Cot death or Sudden Infant Death Syndrome (SIDS) is fortunately a rare phenomenon. Still it makes up a measurable fraction of the annual deaths among children up to the age of three, since the mortality rate in this age group is low. The shock is also that much worse when a seemingly healthy child suddenly dies. For several decades one has tried to find factors that can trigger and predispose to this condition. Research groups in the US, Japan and Norway among others have conducted research on a cellular and molecular level in affected infants. The scope has not yet been limited to one field only, but most researches base their investigations on a principle proposed by R.J. Wedgewood in 1970, the triple-risk hypothesis. Here he proposes that an infant exposed to an external risk factor, with a genetic predisposition and at a vulnerable stage in development is at risk of SIDS. The external risk factors are fairly well mapped out. Mothers are advised not to smoke during pregnancy, and parents told to take care when co-sleeping and to avoid the prone sleeping position when putting the child to sleep. The vulnerable stage refers to the development of regulatory functions of the nervous system such as breathing, alertness and consciousness, and development of the immune system. The most controversial part of the hypothesis is the genetic predisposition, and this review will discuss some of the studies made on genetic polymorphisms (variants of genes found in the population), more specifically the 5-HT (serotonin) related polymorphisms. During the last two decades researchers have found anatomical discrepancies in the brainstem of infants affected by SIDS and described several polymorphisms related to the serotonergic network in the brainstem of SIDS victims.

Index

Introduction

I had the idea of this review after reading in Brodals "Sentralnervesystemet" about the reticular formation. I learned of the connection this part of the brain has to the regulation of our basic regulatory functions. After looking this up online I came across a genetic researcher at the Department of Forensic Pathology, dr.philos Siri Hauge Opdal, and discovered that she among others had published several papers on the subject of brainstem serotonin and sudden infant death syndrome. I contacted her and she agreed to be my supervisor for this review. I am most grateful for the recommendations, advice and time she has given me. The material publised in this field is vast, and I could not have done the selection without her help. The data on which this review is written are not systematically searched by protocol. My searches and choices on which papers and studies to include has however been based on her recommandations and experience on research done in this field in the last decades.

SIDS pathogenesis

Sudden infant death syndrome, SIDS, is defined as "the sudden unexpected death of an infant, with the onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including performance of a complete autopsy, and a review of the circumstances of death and the clinical history"(1).

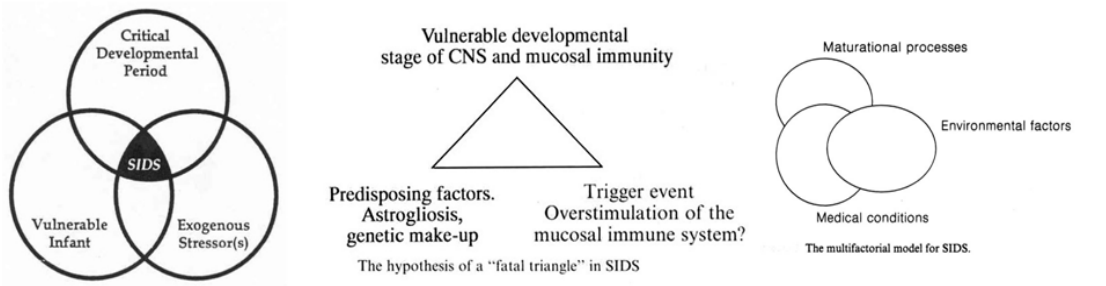
The SIDS-rate, given as number of cases per 1000 live births, differs between different countries. This may have several different explanations, as both the age span for using the SIDS diagnosis, the SIDS definition, and also the content and use of death-scene investigation and autopsy protocols differ between countries (2).

Table 1. SIDS rate in 13 different countries as of 2005

Argentina	**0,49**
Australia	**0,32**
Canada	**N/A (2004 rate was 0,24)**
England/Wales	**0,30**
Germany	**0,43**
Ireland	**0,38**
Japan	**0,16**
Netherlands	**0,10**
New Zealand	**0,80**
Norway	**0,30**
Scotland	**0,39**
Sweden	**0,23**
United States	**0,53**

The table is based on numbers from Hauck et al (2).

The etiology of SIDS is most likely multifactorial. In light of this the first triple-risk hypothesis was proposed by R.J. Wedgewood and presented at the Bedson Symposium in Cambridge, 1970 (3). Rognum and Saugstads (1993) fatal triangle, the triple-risk model by Filiano and Kinney (1994) and the multifactorial model of Kahn all emphasize approximately the same (4-7). (fig 1).



The tripple risk model for SIDS
Filiano JJ, Kinney HC, Biology of the Neonate 1994; 65: 194-197

Rognum TO, Saugstad OD. Acta Pædiatr Suppl 1993; 389: 82-85

Kahn A Groswasser J, Kelmanson I. In: Sudden Infant Death Syndrome. New Trends in the Nineties. Ed. TO Rognum. Scandinavian University Press 1995: pp 132-137

Fig 1. The triple risk hypothesis in SIDS.

According to the triple risk hypothesis, SIDS may occur when an infant at the same time is at a vulnerable developmental stage, has a predisposing factor and is exposed to a trigger event. The vulnerable developmental stage refers to an age where an infant will be at a crucial stage in the development of both the central nervous system and the immune system.The age distribution for SIDS peaks between 2 and 4 months of age, although this has partly changed into a more even age distribution through the first year of life after the SIDS risk-reducing campaigns starting in the 1990`s (8, 9).

Most likely the genetic predisposition for SIDS represent a polygenic inheritance pattern, and so far genes involved in regulation of the immune system, cardiac function, the serotonergic network, and brain function and development have emerged as the most important with regard to SIDS (10).

External risk factors or trigger events for SIDS include prone sleeping position, co-sleeping, soft covering, thermal stress, maternal smoking, and slight infection.

Prone sleeping position is by far the most associated. Campaigns to reduce SIDS by

converting from prone to supine sleeping position was launched in the 1990`s, in Norway the first in 1989. The effect was remarkable, reducing the number of SIDS cases by fifty percent in one year (11). Similar results have been seen elsewhere in Europe and in the US.

A Norwegian study comparing numbers from two six-year periods before and after the supine sleeping campaign found a strong reduction in total number of deaths (12). The biggest reduction was found in the 3rd and 4th months of life, and the number of deaths during the first two months of life now make up a much larger percentage of total deaths than previously (from 4,5% to 18%). More infants were found dead in a co-sleeping position than before the campaign, an increase from 7% to 35%. The risk increase was found among the youngest infants, during the two first months of life. Also in this study the risk was further increased by maternal smoking.

Unsafe co-sleeping is another trigger event for SIDS. Arnestad et al published a paper on changes in epidemiological patterns for SIDS cases in south-eastern Norway in 2001 (9). According to their findings, an increase was seen in the number of infants found dead while co-sleeping in the late 1990's. This may be due to an increased risk of SIDS with co-sleeping, or it might refer to a general increase in the number of infants co-sleeping with a parent. The study also disclosed a positive relationship between co-sleeping at time of death and maternal smoking during pregnancy.

Thermal stress (increased temperature in the child's sleeping environment) has been revealed as a possible risk factor, mostly during interviews with parents of SIDS victims. One of the first to argue this was Stanton (13).He investigated cases of cot death in the Oxford and Scarborough areas from 1981 to 1984. The most important finding was that almost all the investigated cases had extra risk factors for overheating at the time of death. The risk factors being a warm sleeping environment, overdressing/wrapping, victim hot/sweaty when discovered, or terminal infective illness (13).

There are also studies indicating that a raised temperature has a negative impact on respiratory reflexes during hypoxia. An analysis published by Guneroth and Spiers in 2001 based on publications on thermal stress in infants concludes that a strong association exist between thermal regulation and ventilatory control in relation to

prolonged apnoea (14).

Another well-known external risk factor for SIDS is maternal smoking during pregnancy. In a Swedish study published in 1990, Haglund and Cnattingius found an almost fifty percent increased risk of SIDS in infants whose mothers smoked between 1 and 9 cigarettes per day, and an almost tripled risk if the mothers smoked more than 10 cigarettes per day (15). Another paper published in 1996, by Blair et al., show similar results with the risk of SIDS increasing with the number of cigarettes smoked (16).

In relation to infection being an external risk factor, about half of SIDS victims show signs of slight infection prior to death. Several studies have looked into this. A case control study from 1989 of pulmonary immunoglobulin levels in post-mortem lung lavage samples from infants dying of SIDS showed raised levels of IgM, IgG, and to a lesser extent IgA, when compared to controls dying from non-pulmonary causes (17). Vege et al. published a paper in 1995 where they reported elevated levels of the cytokine IL-6 in the CSF (cerebrospinal fluid) in SIDS victims compared to controls (18). They proposed a mechanism in which the raised levels of cytokines trigger a pathological response in the brainstem of predisposed infants. This was followed up by a study published in 2009 with findings of abnormal IL-6R (IL-6 receptor) expression in the arcuate nucleus of SIDS infants, further suggesting an abnormal infectious response in the brainstem (19).

Kinney and co-workers have proposed that "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" (20).

Two approaches to the verification of the brainstem abnormality hypothesis are investigations of genetic polymorphisms in genes involved in the serotonergic network in cases of SIDS, searching for a possible genetic vulnerability, and the post mortem examination of brainstem tissue from SIDS victims, also here with the focus on serotonergic pathways in the brainstem. The rest of this review will focus on these two lines of research.

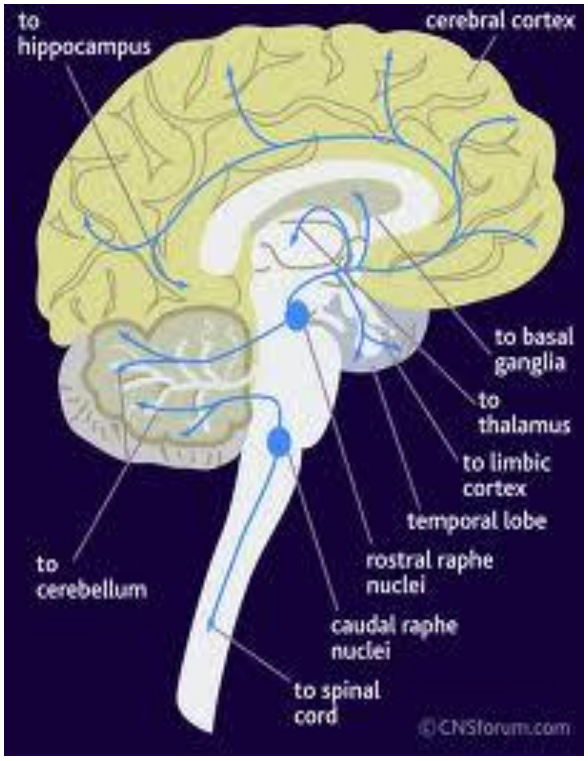


Fig 2. Projections of the serotonergic network.
http://www.cnsforum.com/imagebank/item/Neuro_path_SN/default.aspx

The brainstem and its structures

The brainstem controls many of our primary functions, including respiration, circulation and consciousness. The anatomical area defined as the brainstem can be divided into three structures, the medulla oblongata, the pons and the mesencephalon, with the medulla oblongata being the most inferior, situated on top of the medulla (spinal cord) and the mesencephalon the most superior, connecting with the diencephalon (midbrain).

The brainstem has a hollow central canal going through the three parts vertically, expanding into a cavity (the fourth ventricle) on passing the pons. This canal contains the cerebrospinal fluid (CSF) and runs from the base of the spine to the cerebral cortex (21).

Another structure found in all three parts of the brainstem is the reticular formation. This is a diffuse network connecting both upwards to the cerebrum and cerebellum and downwards to the spine. The reticular formation is centrally situated in the brainstem, filling out the parts not occupied by cranial nerve nuclei and motor and sensory pathways. Different functions can be found according to structural position. An example is the ventral respiratory group (VRG) situated in the ventrolateral, superior part of the medulla oblongata. The neurons in this area have been found to contain what we believe to be the rhythm generator for respiration, the pre-Botzinger complex. Though probably one of the most important, the VRG is only a part of the complex system controlling respiration.

As previously mentioned the brainstem also contains the nuclei of all 12 cranial nerves, with the exception of 1 and 2 which are located in the cerebrum. Axons (nerve threads) connecting the cerebrum and cerebellum can also be found in the pons. They form bundles or stalks called the crus cerebri and brachium pontis (22).

The brainstem is also thought to influence the state of wakefulness/consciousness. These functions can also be related to the reticular formation. In 1949 Moruzzi and Magoun found a cortical arousal response to electrode stimulation of the reticular substance in animal experiments (23). This was recorded through electroencephalography (EEG) readings showing a shift in brainwave activity. Moruzzi and Magoun proposed on the background of their findings, that the brainstem holds a mechanism for activation of the cortex in relation to arousal and attention.

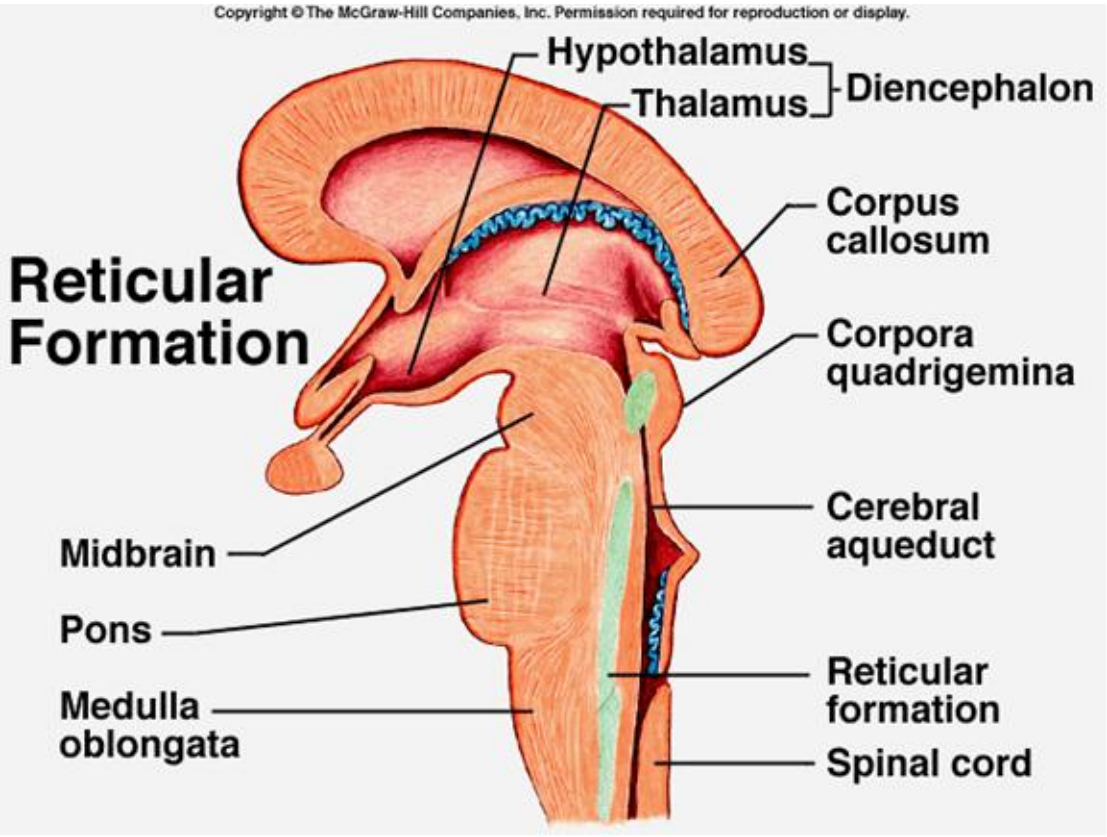


Fig.3 The reticular formation of the brainstem

http://legacy.owensboro.kctcs.edu/gcaplan/anat/Notes/API%20Notes%20L%20Central%20Nervous%20System-Brain.htm

Serotonin and its properties

Serotonin, or 5-hydroxytryptamine (5-HT), is a biological mediator produced within the body's cells from the amino-acid tryptophan. It was originally discovered as a vasoconstrictor, and the name sero-tonin was therefore given.

In sense of volume, the majority of bodily serotonin can be found in enterochromaffin cells imbedded in the gut lining. The release of serotonin from these cells increase peristaltic movement and triggers vomiting when an irritant is introduced into the stomach. In addition some serotonin can also be found in the myenteric plexus (a peripheral nerve plexus controlling the gut) where its function is to transmit excitatory signals (24).

Serotonin is an important neurotransmitter in the central nervous system (CNS). The discovery of this dates back to the beginning of the 1950`s when J.H. Gaddum showed the antagonist effect of LSD (a hallucinogenic drug produced from the fungus ergot) on serotonin in peripheral tissue. Knowing the hallucinogenic features of LSD, the thought that serotonin might also have a function within CNS was formed (25).This idea was also supported by an article presented by B.M.Twarog and I.Page in 1953, showing the existence of serotonin in the mammalian brain (26).

Because 5-HT is a hydrophilic substance it cannot pass the blood-brain barrier. As a consequence of this 5-HT must be produced internally in the brain in order to have access and effect within the CNS (25).

The first step in the synthesis of 5-HT is uptake of tryptophan by neuronal cells. From tryptophan the enzyme tryptophan hydroxylase produce 5-hydroxytryptophan. A non-specific amino-acid decarboxylase then synthesizes the final product 5-hydroxytryptamine (5-HT). The regulating steps in this process are tryptophan availability and tryptophan hydroxylase activity (25).

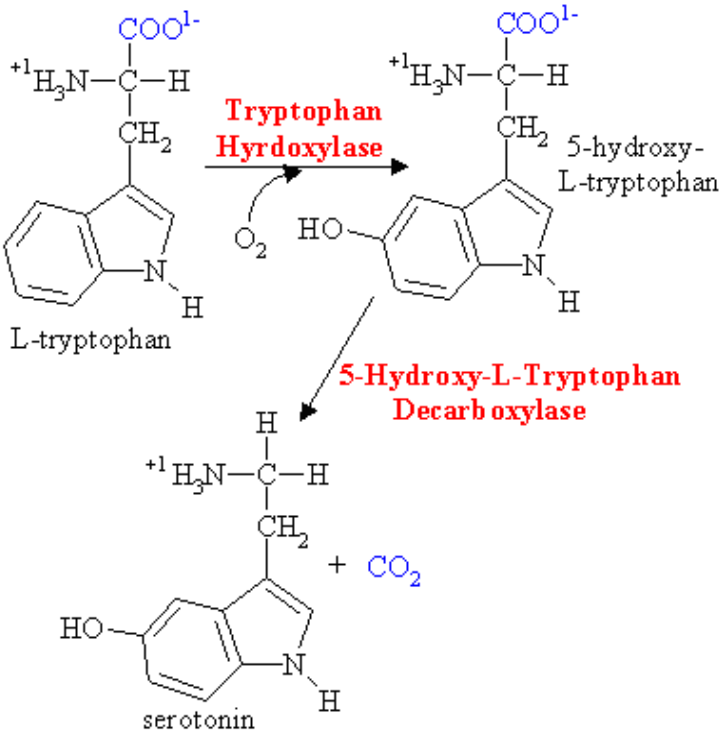


Fig 4. Overview over serotonin synthesis.
http://flipper.diff.org/app/pathways/info/1637

In the brain, serotonin is released into the synaptic cleft, where it either will bind to a receptor, or be subject to reuptake through the serotonin transporter (5-HTT) and further degradation by monoamine oxidase A (MAO-A). Alternatively serotonin can bind to auto receptors at the presynaptic surface and here regulate the release of serotonin by a feedback mechanism (fig 5).

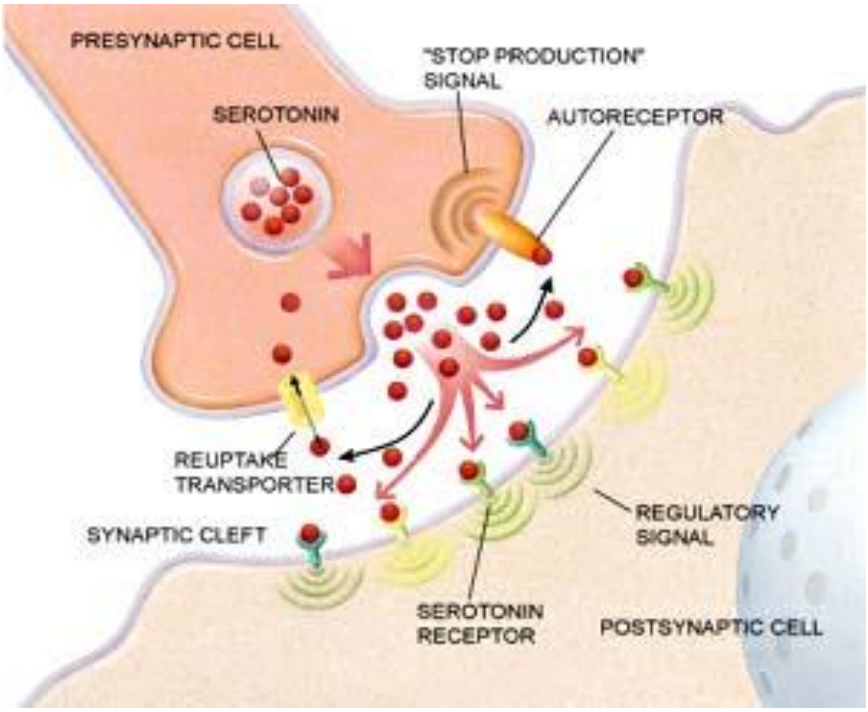


Fig 5. Overview over the serotonergic network.
http://www.bio.davidson.edu/Courses/genomics/2003/mccord/serotoninnerve.jpg

The neuroanatomy of 5-HT in the brain has been shown to consist of a medullary system, mainly in rostral and caudal parts of the brainstem (e.g. raphe, extra raphe and ventral surface).The cell bodies project upwards to the cerebral cortex, thalamus, hypothalamus, basal ganglia, hippocampus and amygdale, and downwards to other sites in the brainstem, cerebellum and spinal cord. Their positioning reflects connections to different functions. The rostral extension is connected to regulation of arousal, cognition, mood, motor activity and cerebral blood flow. The caudal part modulates cardiovascular function, respiration, chemo sensitivity, thermoregulation, upper airway reflexes, motor activity, pain and arousal (27).

Serotonin receptors

The serotonin receptor family consists of several subtypes, all acting through G-protein and second-messenger systems, with the exception of 5-HT_3, which is a ligand-gated ion channel. At least seventeen subtypes have been recognized so far. They are divided into the 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_{4-7} families, with the subtypes classified under each family (27). Most subtypes can be found within the CNS, with 5-HT_1 being the most CNS specific. The relevance of the different subtypes is shown through the fact that they display different responses to the same substance. As an example, the 5-HT_1 receptor subtype inhibits neuronal firing, while the 5-HT_2 receptors of CNS have an excitatory effect (24).

The neurotransmitter effect is conveyed by the release of serotonin from axonal varicoses (axonal swellings within the target area), diffusion, and further binding to receptors at postsynaptic terminals or extra synaptic receptors. This form of neurotransmission is called volume transmission and is different from the fast and precise transmission from pre to post synaptic terminal where the distance of diffusion is shorter. Volume transmission is typical to the modulating effects of metabotrope receptors which the above mentioned G-protein serotonin receptors are examples of. This effect is slow, diffuse and has a more modulating rather than deciding effect (28).

The serotonin transporter

The serotonergic network is under bottleneck control by the serotonin transporter (5-HTT), which regulates the level of available serotonin in the synaptic cleft between the cells. 5-HTT is a trans-membrane protein specific to serotonin, although related to the other monoamine transporters, DAT (dopamine transporter) and NAT (noradrenalin transporter) transporters (28). 5-HTT is coded for by a single gene located on chromosome 17. Several polymorphisms affecting the expression and function of 5-HTT have been described, the most important is a length variation in the promoter region of the gene. This is a deletion/insertion polymorphism which gives rise to two allelic variants, denoted short (S) and long (L), consisting of 14 and 16 repeats respectively. It has been shown that the L allele and the L/L genotype results in an enhanced expression

of the gene, which results in a more effective reuptake of serotonin, and thus a lower serotonin level in the synapses (29).

Foetal development of 5-HT in the medulla

Kinney's research group at Harvard Medical School have analyzed brainstem tissue collected from 30 different stages throughout foetal development into infancy (30).Their findings suggests an appearance of the first 5-HT neurons early in the first trimester, and that a mature pattern is appearing at 20 weeks of gestation, after which the only change seemed to be qualitative in the form of subtype differentiation and internal migration within the 5-HT system, with the total number of neurons being unchanged. This process however continued throughout the span of their data set, meaning that the serotonergic network is not fully developed at birth. Thus the vulnerable developmental period seem to span from the embryonic period throughout the first year of infancy.

These findings are important as confirmation of the existence and location of a medullary 5-HT pathway, and as a stepping stone towards mapping the significance of its effect on homeostatic functions. Pinpointing the time of development is also important as it allows more fine-tuned research into which factors affect the foetus at different times of gestation, and why defects in the 5-HT system occur. In their paper , Kinney et al. underline the importance of exogenous stressors affecting the foetus at an early time in gestation when the mother is not aware of the pregnancy and therefore do not take the precautions (e.g. exposure to nicotine, alcohol, SSRI's) as she later on would when becoming aware of the pregnancy.

Brain stem abnormalities in SIDS

The brainstem hypothesis can be traced back to 1987 when Hunt and Brouillette speculated that SIDS was related to a brainstem abnormality in the neuroregulation of cardiorespiratoy control (31). This was at the time a consolidated hypothesis and its position is even more strengthened today.

Hannah Kinney and her research group at Harvard Medical School has tried to approach the pathology behind SIDS through the brainstem hypothesis, more precisely put, that a defect in the brainstem cause a diminished response to hypercapnia and hypoxia and can therefore possibly lead to asphyxiation under periods of stress (prone sleeping position, soft bedcovering, infection, overheating). Their line of research has focused on the 5-HT medullary pathway, its location, development and possible abnormalities detected between SIDS victims and controls (27).

Kinney and her colleagues at Boston Childen Hospital have since 2000 published several papers on three independent datasets, comparing SIDS victims to controls in respect of brainstem abnormalities. One of the papers published on the first of these three data sets was published in the Journal of Neuropathology & Experimental Neurology and tested the hypothesis of 1) decreased serotonergic receptor binding in the arcuate nucleus, and 2) an increase or decrease of binding in the raphè obscurus in SIDS victims compared to controls (32). Both regions showed decreased binding compared with controls. In addition decreased binding was also found in the nucleus gigantocellularis, nucleus paragigantocellularis lateralis and intermediate reticular zone.

The abovementioned results spurred further research into medullary serotonergic abnormalities. In 2003 Kinney and colleagues published a new paper, this time from a dataset in a high risk group, the Northern Plains Indians (33). In this study they presented three hypotheses: 1) that there would be a decreased serotonergic receptor binding on average in SIDS victims compared to controls, 2) also, there would be a decreasing binding in SIDS victims with increasing age compared to controls with no such decrease, and 3) a serotonergic receptor binding decrease in SIDS victims correlate with maternal cigarette and/or alcohol use during pregnancy, linking these as teratogens in respect to abnormalities in the medullary serotonergic network. The material was collected from 23 SIDS victims and 6 controls.

The study revealed that serotonergic receptor binding was found to be significantly lower

in the arcuate nucleus of SIDS victims when compared to controls. Decrease in binding with increasing age was found in the nucleus gigantocellularis, nucleus paragigantocellularis lateralis, intermediate reticular zone and raphè dorsalis. This decline in binding was not found in the control group. A significantly lower mean binding was found in mothers who smoked compared to non-smokers. Only a marginal lowering in binding capacity was found when comparing mothers that drank alcohol to those who did not.

A third dataset consisting of 31 SIDS cases and 10 controls investigated 5-HT cellular defects related to the previously found receptor binding abnormalities (34).The authors measured 5-HT neuron count, density, 5-HT receptor binding density (5-HT$_{1A}$) and 5-HTT binding density, all samples were taken from the medulla. A significantly higher number and density of 5-HT neurons were found in the samples taken from the raphè, extraraphè and ventral surface of both rostral and mid-medulla levels of SIDS cases when compared to samples from controls. Binding density to 5-HT $_{1A}$ were significantly decreased in SIDS cases compared to controls (not in the principal olivary nucleus). In addition to this they found a significantly lower binding density in SIDS males when compared to SIDS females. This is an interesting observation as males make up the majority of SIDS victims.

Regarding the serotonin transporter, 5-HTT, there was not an absolute decrease, but a relative binding decrease in relation to number of neurons was observed in the raphè obscurus of SIDS cases when compared to controls.

The study also reported findings to support the external risk part of the triple risk hypothesis. 97% of the SIDS victims were reported to have a minimum of 1 abnormal risk factor, the risk factors tested being 1) male gender, 2) prematurity, 3) prone sleeping position, 4) face down position, 5) bed sharing, 6) sick with minor illness last week before death (34).

The cause for this increased number and density of 5-HT neurons have been speculated to be compensatory, meaning the increased number is caused by a deficiency of 5-HT and therefore during development the brain compensates through increasing the number and density of neurons. The composition of different subtypes of 5-HT neurons was also found to differ between controls and SIDS victims in this study. In the latter, more granular and less multipolar 5-HT cells were identified, the granular cells being a more rudimentary form than the multipolar. The significance of this is unclear, but seen in the light of their findings on reduced receptor binding capacity in SIDS victims, one can

speculate on a dysfunction of these cells causing a greater number and density of 5-HT neurons in an attempt to compensate for 5-HT deficiency.

On the second of February 2010, JAMA published a paper by the same research group (35). This latest paper were of significant findings of decreased medullary 5-HT and TPH2 (a precursor of 5-HT) in SIDS victims, compared to controls. 26 % lower 5-HT levels in the PGCL (nucleus paragigantocellularis lateralis) and raphè obscurus, and 22% lower TPH2 in the raphè obscurus. They did however not find excess levels of the degradation metabolite of 5-HT, 5-HIAA, indicating a low production rather than an excess degradation of 5-HT. In this study a control group and a group of hospitalized infants with chronic oxygenation disorders were used for comparison. In the hospitalized group, levels of 5-HT were higher than the SIDS group and the TPH2 levels lower. They also compared 5-HT_{1A} - receptor binding in the brainstem, and found reductions in the SIDS group compared to controls in the hypoglossal nucleus, solitary tract nucleus and dorsal motor nucleus of vagus. Furthermore a relative decrease in 5-HT receptor binding in PGCL, gigantocellularis and intermediate reticular zone with increasing age of the victim was seen in the SIDS cases and not in the control group.

Genetic investigations of the serotonergic network in SIDS

A review on implicated genes in SIDS was published in 2007 by Weese-Mayer et al. (36) reflecting the last two decades of research in this field. Five groups of genes were identified as candidates. 1) genes for ion channel proteins, 2) genes regulating the 5-HT transporter, 3) genes involved in embryologic development of the autonomic nervous system, 4) genes for nicotine metabolizing enzymes and 5) genes regulating inflammatory, thermal and hypoglycaemic processes.

In a summary on genes involved in SIDS from 2011, Opdal and Rognum highlight three areas of interest, namely cardiac function, the immune system and 5-HT network related genes (37). They also emphasize a polygenetic inheritance pattern as a predisposing factor as opposed to one single gene defect as seen in for instance long QT-syndrome or MCAD (medium-chain acyl-coenzyme A dehydrogenase deficiency).

As mentioned earlier, the serotonin transporter (SERT or 5-HTT) is responsible for serotonin reuptake from the synaptic cleft, and thus responsible for the regulation of available serotonin in the cleft. The findings of reduced serotonin levels and receptor binding in cases of SIDS (32-35) may indicate that also specific variants of the genes encoding the different components involved in the serotonergic network may be involved in SIDS.

The first study regarding this was a Japanese study investigating an insertion/deletion polymorphism in the promoter of the 5-HTT gene in 27 SIDS cases and 115 controls (38). This study revealed an association between the L-allele and SIDS, suggesting a lower level of available serotonin in SIDS victims compared to controls.

Similar results were also presented by Weese-Mayer et al. (39) in a cohort study of 84 SIDS cases in the US published in 2003. They showed a positive association between L/L genotype and L-allele and SIDS cases. They also found a negative association between the S/S genotype and SIDS, thus indicating a reduced risk of SIDS in infants carrying the S/S genotype.

Furthermore this group found an association to another polymorphism involved in regulating 5-HTT expression. Having a 12 versus a 10 repeat sequence in a VNTR in intron 2 of the gene was more common among SIDS victims when compared to controls

(40). The 12 repeat haplotype was more common among Afro-Americans, both SIDS victims and controls compared to the Caucasian group. The latter has however not been confirmed by later studies.

In 2008 Marzano et al. published results of a study of 20 SIDS cases and 150 controls looking into several polymorphisms including the promoter and intron 2 VNTRs of the 5-HTT gene (41). They found a significant difference in distribution of genotype L/L and allele L between the SIDS group and the control group. The intron 2 polymorphism was also increased in the SIDS group but the findings were not significant.

This was again confirmed in a study by Opdal and Rognum, also in 2008 (42). With larger groups of both SIDS and controls, 163 and 243 respectively, they also investigated polymorphisms in the promoter and intron 2 of the 5-HTT. They found a tendency for the L allele and the L/L genotype to be more frequent in SIDS cases than in controls ($p= 0.05$ and 0.07, respectively). In addition they noticed a different distribution of genotype in regards to the infants sleeping position at the time of death. More victims found in other sleeping positions than prone had the L/L genotype, and more S/S victims were found prone, suggesting perhaps that the L/L victims were less dependent on this known risk factor, and therefore more intrinsically predisposed than victims found in the prone position. Regarding the intron 2 polymorphism there were no differences between the groups (42).

In contrast to the abovementioned results two studies have later found no significant difference in allelic or genotype distribution between SIDS and control groups.
Haas et al. investigated 145 SIDS cases and 58 controls, but found contradictory results with an increased frequency of both L/L genotype and L allele in the control group as opposed to the SIDS group (43). Paterson et al. investigated 179 SIDS cases, 139 dead controls and 299 living controls and also found no significant increase in frequency of the L/L genotype or the L allele in their SIDS population (44). They also tested the link previously described between sleeping position and genotype/allele, but did not find any significant difference in distribution (44).

Weese-Mayer et al. has also used the same dataset (39, 40, 45) to investigate a 3`UTR polymorphism in the 5-HTT. No association was found between haplotype distribution of the 3`UTR polymorphism and SIDS (45).

In addition this dataset has been used to investigate the genes coding for some of the

5-HT receptors occurring in the brainstem. In relation to SIDS two such studies have investigated the HTR1A and HTR2A (genes coding for the 1A and 2A 5-HT receptors) (46, 47). In both cases they found no specific gene polymorphism more common among SIDS victims than among controls in the polymorphisms investigated.

Discussion

This review has tried to summarize two strings of research within the brain stem serotonin hypothesis in SIDS. The immunohistochemical studies have revealed a definite pathology in relation to distribution of 5-HT networks and activity in the brain stem of SIDS victims. The question is what direct consequence this has to the physiology of the infant. In looking at polymorphisms of genes regulating the amount of 5-HT we try to come closer by determining the functional consequences of these seen anatomical discrepancies. A decrease of 5-HT can be explained by increased activity of the 5-HTT. How reliable are the results, especially in light of contradictive findings?

SIDS is, when speaking in absolute numbers, a rare disorder, and access to research material is in a lot of cases difficult on the background of this. The ethical and moral dilemmas of getting access to the available material by getting permission from grieving parents also makes the accumulation of data difficult in some countries. While we in Norway have a law securing that all children under the age of 18 dying of an unknown cause go through an autopsy, some countries like the UK have laws demanding all material taken at autopsy be returned to the body, making it impossible to retain samples for research. All the studies in this review are case-control studies. A randomization process is therefore in these cases not possible. However, in the process of analyzing results very few have mentioned blinding the analysis process or mentioned this as a possible systematic bias to their results.

The immunohistochemical studies have been extensive and meticulous, however they are a time consuming way of conducting research. The downside in this is a limited number of samples being investigated. The first dataset from 2000 (32) has 52 SIDS cases and 27 controls. To their benefit they have blinded the analysis of measuring 5-HT binding, selecting 19 brainstems to be analyzed, however 19 in itself is a fairly low number to base calculation of means on.

The Northern Plains study from 2003 (33) brings reliability to the previous study and confirm the results but cannot be generalized outside the American Indian population. Also in the 2006 (34) and 2010 (35) publications the consistent findings of abnormalities in 5-HT neuron density, distribution and binding sites are confirmed. Combined they yield a strong likelihood of such an abnormality actually existing. Their findings on gender discrepancies, as well as the hypothesis that decreased 5-HT levels are due to

reduced production as opposed to reduced breakdown, will need further confirmation.

The Narita study from 2001 (38) has a relatively small sample but concentrates on one ethnic population only. Although small this means the results can be transferred to a genetically identical population The Weese-Mayer study (39) has a larger sample of both Caucasian and African-American cases and the findings are significant with a p-value of 0.022 in regards to genotype and 0.005 for allele. This reflects a strong significance, the transference value to the general population is however weakened by the mixing of two ethnic groups.

The Marzano study (41) is criticized for a low number of SIDS cases, only 20, and in addition has a genotype and allelic distribution in their control-group that differ from the control groups in other studies of same ethnic background (36, 39, 41-43). It is difficult to speculate how this has come about, but is does unfortunately make it more difficult to compare to similar studies made.

The Opdal and Rognum study (42) has by far the largest sample among the three studies showing significant differences between SIDS and control groups. The significance is strongest here for the allelic association with a p-value of 0.05, with the near significant genotype association at p= 0.07. This sample is based upon Caucasians only.

The Haas group (43) has a large SIDS group of 145 cases, but only 58 control cases and is also based on a Caucasian population only.

The study by Paterson et al. (44) has a large, multi-ethnic sample in both SIDS and control group and their findings are non-significant for difference in allelic and genotype distribution in the promoter of 5-HTT, also in the case of association to sleeping position. A non-significant finding in such a large sample is curious when looking at former studies made. A possible explanation could be that mixing of ethnicities cloaks the existing genetic differences. It must also be argued that as always a lack of a significant finding does not exclude that such an association exists.

Opdal and Rognum (42) suggest an indirect link connected to polymorphisms of genes affecting the 5-HTT polymorphism itself. Also mentioned and interesting is the discovery of a subdivision of the L and S alleles by an SNP (single nucleotide polymorphism)

(r225531) within the VNTR of the 5-HTT, giving an L_A and a L_G, and the same for the S allele, with the L_G allele yielding the same expression of the gene as the S allele, namely reduced levels of 5-HTT mRNA leading to lower activity while only the L_A allele gives increased activity (48). This could if correct explain why studies have showed contradictive findings in relation to allelic and genotype distribution but is at this time only speculative.

As previously mentioned in the introductory chapters, a multifactorial approach must be taken when discussing SIDS. The combination of a predisposition, a vulnerable developmental period and a trigger event still stands as the reasonable approach to this enigma. What this predisposition consist of is still an important field of research, but considering the histopatological findings and the genetic studies, the brain stem serotonergic network is most likely a part of what predisposes a child to SIDS.

References

1. Krous HF, Beckwith JB, Byard RW, et al. Sudden infant death syndrome and unclassified sudden infant deaths: a definitional and diagnostic approach. Pediatrics 2004;114:134-238

2. Hauck FR, Tanabe KO. International Trends in Sudden Infant Death Syndrome: Stabilization of Rates Requires Further Action**.** Pediatrics 2008;122;660-666

3. R.J Wedgewood. Sudden and unexpected deaths in infancy (cot deaths) Report of the Proceedings of the Sir Samuel Bedson Symposium, Addenbrooke`s Hospital, Cambridge, 1970

4. Rognum TO, Saugstad OD. Biochemical and immunological studies in SIDS victims. Clues to the understanding of the death mechanism. Acta Pædiatr Suppl 1993;389:82–85

5. Filiano JJ, Kinney HC. A perspective on neuropathologic findings in victims of the sudden infant death syndrome: the triple risk model. Biol Neonate 1994;65:194-197

6. Kahn A, Groswasser J, Franco P et al. Sudden infant deaths: arousal as a survival mechanism. Sleep Medicine 2002;Vol. 3, Suppl.2:s11-s14

7. Guneroth W.G, Spiers PS. The triple risk hypothesis in sudden infant death syndrome. Pediatrics 2002;110(5):e64

8. Rogum TO, Byard RW. Sudden infant Death Syndrome, Etiology and Epidemiology. Encyclopaedia of Forensic and Legal Medicine, 2005;117-129

9. Arnestad M, Andersen M, Vege A, Rognum T. Changes in the epidemiological pattern

of sudden infant death syndrome in southeast Norway, 1984-1998: implications for future prevention and research. Arch Dis Child 2001;85:108-115

10. Opdal SH, Rognum TO. Gene variants predisposing to SIDS: current knowledge Forensic Sci Med Pathol 2011;7:26–36

11. Markestad T, Skadberg B, Hordvik E, Moriold I, Inregn LM. Sleeping position and sudden infant death syndrome (SIDS): effect of an intervention programme to avoid prone sleeping. Acta Pædiatr 1995;84:375-378

12. Stray-Pedersen A, Arnestad M, Vege Å, Sveum L, Rognum TO. Bed sharing and sudden infant death. Tidsskr Nor Lægeforen 2005;125:2919-2921

13.Stanton AN. Overheating and Cot Death, The Lancet 1984;2:1199-1201

14. Guneroth WG, Spiers PS. Thermal Stress in Sudden Infant Death: Is there an ambiguity with the rebreathing hypothesis? Pediatrics 2001;107(4):693-698

15. Haglund B, Cnattingius S. Cigarette smoking as risk factor for sudden infant death syndrome: a population-based study. Am J Public Health 1990;80:29–32

16. Blair PS, Fleming PJ, Bensley D, et al. Smoking and the sudden infant death syndrome: results from 1993–5 case-control study for confidential inquiry into stillbirths and deaths in infancy. BMJ 1996;313:195–198

17. Forsyth KD, Weeks SC, Koh L, Skinner J, Bradley J. Lung immunoglobulins in the sudden infant death syndrome. BMJ 1989;298:23-6

18. Vege A, Rognum TO, Scott H, Aasen AO, Saugstad OD. SIDS cases have increased levels of interleukin-6 in cerebrospinal fluid. Acta Paediatr. 1995;84(2):193-196

19. Rognum IJ, Hayes RL, Vege A, Yang M, Rognum TO, Kinney HC. Interleukin-6 and the serotonergic system of the medulla oblongata in the sudden infant death syndrome. Acta Neuropathol 2009;118:519-530

20. Kinney HC, Richerson GB, Dymecki SM, Darnall RA, Nattie EE. The brainstem and serotonin in the sudden infant death syndrome. Annu Rev. Pathol. 2009;4:517-550

21. Putz R, Pabst R. Sobotta Atlas of Human Anatomy: Vol 1-Head, Neck, Upper limb 2006 München : Elsevier Urban & Fischer

22. Brodal P. Sentralnervesystemet. 3rd edition, chapters 3, 16, 17. 2001 Universitetsforlaget

23. Moruzzi G, Magoun HW. Brain stem reticular formation and activation of the EEG. Electroencephalogr Clin Neurophysiol 1,1949, 455-473

24. Rang HP, Dale MM, Ritter JM,. Moore PK. Pharmacology, 5th edition, chapter 12. 2003 Churchill Livingstone, Elsevier Limited

25. Siegel et al. Basic Neurochemistry. Molecular, cellular and medical aspects. 6th edition. Chapter 13;1999 Lippincott, Williams and Wilkins. Web edition. http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=bnchm&part=A946

26. Twarog BM, Page I. Serotonin Content of Some Mammalian Tissues and Urine and a Method for Its Determination Am J Physiol 1953;175: 157-161

27. Kinney HC. Abnormalities of the Brainstem Serotonergic System in the Sudden Infant Death Syndrome: A review. Pediatric and Developmental Pathology 2005;8:507-524.

28. Brodal P. Sentralnervesystemet. 3rd edition, chapter 2;2001 Universitetsforlaget

29. Heils A, Teufel A, Petri S, et al. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996;66:2621-24

30. Kinney HC, Belliveau RA, Trachtenberg FL, Rava LA, Paterson DS. The development of the medullary serotonergic system in early human life. Autonomic Neuroscience: Basic and Clinical 2007 132;81-102

31. Hunt CE, Brouillette RT. Sudden infant death syndrome: 1987 perspective. Journal of Pediatrics 1987 110:669-78

32. Panigrahy A, Filiano J, Sleeper LA, et al. Decreased serotonergic receptor binding in rhombic lip-derived regions of the medulla oblongata in the sudden infant death syndrome. 2000 Journal of Neuropathology and Experimental Neurology 2000 59;377-84

33. Kinney HC, Randall LL, Sleeper LA, et al. Serotonergic brainstem abnormalities in Northern Plains Indians with the sudden infant death syndrome. Journal of Neuropathology and Experimental Neurology 2003 62;1178-91

34. Paterson DS, Trachtenberg FL, Thompson EG. et al. Multiple serotonergic brainstem abnormalities in sudden infant death syndrome JAMA 2006 296;2124-32

35. Duncan JR, Paterson DS, Hoffman JM. et al. Brainstem Serotonergic Deficiency in Sudden Infant Death Syndrome. JAMA 2010 303;430-437

36. Weese-Mayer DE, Ackerman MJ, Marazita ML, Berry-Kravis EM. Sudden infant death syndrome: Review of implicated genetic factors. Am J Med Genet

2007;143:771-88

37. Opdal SH, Rognum TO Gene variants predisposing to SIDS: current knowledge 2011 Forensic science, medicine, and pathology. 2011 7(1):26-36.

38. Narita N, Narita M, Takashima S. et al. Serotonin Transporter Gene Variation Is a Risk Factor for Sudden Infant Death Syndrome in the Japanese Population. Pediatrics 2001 107;690-692

39. Weese-Mayer DE, Berry-Kravis EM, Maher BS et al. Sudden infant death syndrome: association with a promoter polymorphism of the serotonin transporter gene. Am J Med Genet. 2003 117A:268-274

40.Weese-Mayer DE, Berry-Kravis EM, Maher BS et al. Association of the serotonin transporter gene with sudden infant death syndrome: a haplotype analysis. Am J Med Genet. 2003 122A:238-245

41.Nonnis Marzano F, Maldini M, Filonzi L. et al. Genes regulating the serotonin metabolic pathway in the brain stem and their role in the etiopathogenesis of the sudden infant death syndrome. Genomics 2008 91:485–491

42.Opdal SH, Vege A, Rognum TO. Serotonin transporter gene variation in sudden infant death syndrome. Acta Paediatr 2008 97:861–865

43.Haas C, Braun J, Ba¨r W, Bartsch C. No association of serotonin transporter gene variation with sudden infant death syndrome (SIDS) in Caucasians. Leg Med (Tokyo) 2009 11:S210–S212

44.Paterson DS, Rivera KD, Broadbelt KG. et al. Lack of Association of the Serotonin Transporter Polymorphism With the Sudden Infant Death Syndrome in the San Diego

Dataset. Pediatric Research 2010 68 (5) (409-413)

45. Maher BS, Marazita ML, Rand C, Zhou L, Berry-Kravis EM, Weese-Mayer DE. 3`UTR Polymorphism of the Serotonin Transporter Gene and Sudden Infant Death Syndrome: Haplotype analysis. Am J Med Genet 2006 Part A;140A:1453-57

46. Morley ME, Rand CM, Berry-Kravis EM, Zhou L, Fan W, Weese-Mayer DE. Genetic variation in the HTR1A gene and sudden infant death syndrome. Am J Med Genet 2008 Part A

47. Rand CM, Berry-Kravis EM, Fan W, Weese-Mayer DE. HTR2A variation and sudden infant death syndrome: a case-control analysis. Acta Paediatrica 2009:98:58-61

48. Hu X, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD. Serotonin Transporter Promoter Gain-of-Function Genotypes Are Linked to Obsessive-Compulsive Disorder. The American Journal of Human Genetics 2006:78:815-26