EXPRESSION OF SUBSTANCE P IN BRAINSTEMS OF VICTIMS OF SUDDEN UNEXPLAINED PERINATAL DEATH

A THESIS

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"God has two dwellings; one in heaven and the other in a meek and thankful heart".

"All our dreams can come true if we have the courage to pursue them"(Walt Disney).

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Abstract

Sudden perinatal death including SIDS is a rare but lethal syndrome and there is no symptom of this disorder until the fatal outcome has occurred. Epidemiological, genetic, molecular and pathological studies conducted so far give us some possible explanations about it but are inadequate to explain it completely. Brainstem etiology is a mostly accepted hypothesis to induce sudden perinatal death. We investigated the immunohistochemical expression of substance P (SP) in the brainstems of 56 subjects aged from 17 gestational weeks to 10 postnatal months, died of unknown (sudden unexplained perinatal deaths and SIDS) and known causes (controls). The goals of this study were to obtain basic information about the expression of the SP during the first phases of human nervous system development; to evaluate whether there are altered manifestations of this neuromodulator in victims of sudden death; to verify the correlation with maternal cigarette smoking and to see the evolutionary aspects of SP gene (TAC1) through computational analysis.

Immunohistochemistry demonstrated SP-immunoreactivity in correspondence of the caudal trigeminal nucleus area, with progressive increase in density of positive fibers of the corresponding tract from fetal life to first postnatal months. So, we first delineated the structure of the human trigeminal nucleus, so far little investigated, and provided essential data on its morphologic and functional development. Nevertheless, a negativity or low SP-positivity of the tract fibres was detectable in a wide subset of SIDS and, conversely, high SP-expression in a wide subset of sudden fetal deaths. Therefore we postulate, on the basis of these results, the functional importance of the SP in the early phases of central nervous system development and in the regulation of autonomic functions.

Besides, the observation of a significant correlation between sudden unexplained death, altered SP staining and maternal smoking, prompted us to suppose a close relation between smoking absorption in utero and decrease of the functional activity of the trigeminal nucleus, leading to sudden death during pregnancy and in the first months of life. Computational analysis suggests that SP encoding gene (TAC1) is a singleton, appeared in vertebrates and is more prone to induce neuropathologies along with its interactors, if mutated or functionally altered, as it is located mostly in brain

CHAPTER I

1. An overview of sudden unexplained perinatal and infant death

Perinatal mortality includes fetal (still-births or fetal death), neonatal and post-neonatal infant mortality (sudden infant death syndrome or SIDS). According to WHO, "stillbirth is birth of a deceased baby weighing more than 500 grams and born after 22nd gestational weeks" (WHO 2005). Fetal death or stillbirth is divided into antenatal (antepartum) and intranatal (intrapartum or during birth) death (Lavezzi et al. 2004). Sudden Infant Death Syndrome (SIDS), also called as crib or cot death, has been defined as, "the sudden death of an infant younger than 1 year that remains unexplained after thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical history (Willinger et al. 1991)". It remains a diagnosis of exclusion (Molz 1997) and is the most common and leading cause of infant mortality. Its incidence in United States is 0.57 deaths per 1000 live births annually (Willinger et al. 1991).

Every year more than 133 million babies are born and 3 million are stillborns (one quarter of these are intrapartum deaths), 2.8 million die during first week of life and approximately one million die during the next three weeks. Incidence of these deaths is more in developing countries (WHO 2007). Still births account for 70% of perinatal deaths and one third of these deaths are unexplained. Incidence of stillbirth is 10 times higher as compared to SIDS (http://www.stillbirthalliance.org).

When we look at the characteristic features of SIDS, we find that a normally healthy infant with an age range of 1-6 months dies suddenly and unexpectedly due to unknown causes. There is no inheritance pattern of any disease and autopsy procedures also fail to identify a lethal cause. There is more risk for an infant who is male, premature and has symptoms of respiratory infections. Victims are mostly found dead while asleep, usually belongs to economically poor family and incidence is high in winter (Froggatt and James 1973). Anatomo-pathological studies reveal oedema in the lungs (Kerenyi and Fekete 1969), petechiae on infant's heart, lungs, and thymus (Ferris et al. 1973; Anderson, Bouton et al. 1974) brain-stem gliosis (Kinney etal. 1983), upper respiratory tract infection (Froggatt and James 1973), neuropathological (Lavezzi et al. 2010) and conduction system abnormalities (Ottaviani and Bergui 2009).

1.1. Historical Perspective

Although the term "Sudden Infant Death Syndrome" is established in 1969 but the documentary record of SIDS dates back to Biblical times and it is written in Holy Bible, where the 'Judgment of Solomon' is described, "and this woman's child died because she overlaid it" (1st Kings, chapter 3, verse 19). Roman Catholic Church announced punishment for killing the infants by overlaying, although it was considered a small sin at that time (Guntheroth 1989).

Record of SIDS is also found from Euripides (a Greek Tragedian of classical Athens 480 BC-406 BC), "what greater pain can mortals bear than this; to see their children die before their eyes?" Diodurus Siculus (a Greek historian who lived in 1st century BC) reported that Eygptian mothers were blamed for overlaying their babies. Demon god Larbatu was held responsible for SIDS in Babylonia (Russell-Jones 1985).

Medieval book by Welsh priest Giraldus Cambrensis (Irish musician born in 12th century) also gives records about SIDS. According to a German Placard cited by Sudhoff in 13th century (Dresden Catalogue 6375), mothers were not allowed to take infants under 3 years into their beds at night (Russell-Jones 1985). Throughout 14th century overlaying of baby was considered a forgivable sin. In sixteenth century it was considered a major sin. During the 17th century, SIDS was attributed to mechanical suffocation and certain measures were taken such as arcuccio (arch made of wood and iron that was placed over the infant's head during sleep). This practice of using arch continued till the end of 19th century.

During 18th and 19th century, mothers were usually blamed for overlaying their babies while drunk. SIDS as a medical issue gained more attention in 19th century. In 1892, Templeman, a Scottish pathologist, analysed 258 cases of sudden death in infants, in the town of Dundee and attributed these deaths to 'suffocation' by 'overlaying'. His main focus was on carelessness, drunkenness of the caretaker and overcrowding in small place due to low socioeconomic status (Templeman 1892). A British Physician, Anthony Leared MD (1862) and Brendon Curgenven (1871) attributed SIDS to mechanical suffocation (due to bedsheets, under the breast, overclothing), overlaying and carelessness of the caretaker (Russell-Jones 1985).

Since 17th century till the early and mid 20th century, overlying or suffocation was considered as the only cause of SIDS. Paltauf (1889) proposed theory of "Status lymphaticus" which states that hypertrophy of thymus gland is the cause of sudden death of an infant but it was rejected by Hammar (Hammar 1906). In 1918, Pritchard (Pritchard 1918) suggested that

hypertrophy of suprarenals instead of thymus is responsible for SIDS but this theory also didn't get much approval. Werne and Garrow in 1940 for the first time proposed that inflammatory responses may be the cause of death in infants and not only the suffocation (Werne 1942).

White suggested a combination of factors namely infection, congenital abnormality, suffocation and aspiration of regurgitated food as the causes of SIDS (White 1948). By the end of 1950s, the theory of infection of upper airway as cause of SIDS started to get more attention (Crowley and Emery 1956). Parish in 1960 proposed that sudden death in infancy may be due to allergic reaction to foreign protein in the form of cow's milk (Parish et al. 1960). Three hypothesis were given in late 1950s and 1960s, regarding SIDS: an overwhelming infection, suffocation from soft bedding and an anaphylactic type of reaction resulting from inhalation of cow's milk by a milk-sensitized child (Carpenter and Shaddick 1965; Sutton and Emery 1966; Parish et al. 1960).

Morgan proposed the following possible causes of SIDS: infection, pneumonia, allergic shock, laryngeal spasm, inhalation of vomitus, overlaying, thymus gland pressure, cardiac arrhythmias, inherited cardiac conduction anomalies and adrenal insufficiency (Morgan 1969). Froggatt suggested three factors responsible for SIDS: asphyxia from laryngospasm or nasal obstruction, cardiac conduction disturbance, and some hypersensitivity or aberrant immunological reaction (Froggatt et al. 1971). Money proposed vitamin E and selenium deficiency as a possible cause of SIDS. He pointed that human milk has more selenium and Vitamin E as compared to cow's milk and bottle fed infants suffer its deficiency and have more risk of getting hit by SIDS (Money 1971). Rhead rejected this hypothesis (Rhead et al. 1973). Frais suggested hypokalemia as a cause of SIDS (Frais 1972).

Myeloid leukaemia was reported by Dr. Alice Stewart as a numerically important cause of cot deaths (Stewart 1975) but it was also not widely accepted. Apnea theory was the dominant hypothesis during 1970s and 1980s (Mitchell 2009). According to Tonkin (Tonkin 1975) and Tapp (Tapp et al. 1975): in addition to apnea (Steinschneider 1972), nasal obstruction or laryngeal spasm (Shaw 1970), inflammation of the respiratory tract is also involved in sudden infant deaths due to suspension in respiration. Although surgical repair of tetralogy of fallot results in an improvement in the duration and quality of life for most patients, late sudden deaths are known to occur (Sondheimer et al. 1976).

Abnormalities of cardiac conduction system and cardiorespiratory control centers got attention at the end of 1960s and start of 1970s when James first pointed out the involvement of conducting tissue of the heart in SIDS (James 1968). Now it is known that dysfunctions, maldevelopment of cardiac structures, mutations, polymorphisms in genes encoding receptors and subunits for cardiac channels (Killen et al. 2010; Liu et al. 2010; Plant et al. 2006) are involved in SIDS.

In 1984, a disorder of fatty acid oxidation—medium chain acyl CoA dehydrogenase (MCAD) deficiency—was the first example of an inherited metabolic disorder to be recognised as the cause of death in an infant who had originally been classified as having died of SIDS (Howat et al. 1985). At the end of 1970s, there was an increase in attention to brainstem hypothesis of SIDS (Naeye 1976). Irrespective of the triggering factor, neural control of cardiac and/or respiratory system was ultimately found to be the underlying mechanism (Becker et al. 1993).

1.2 Pathophysiology and Triple Risk Model

The first grouping of SIDS risk factors was done by Wedgwood in 1972. He proposed a "triple risk hypothesis", consisting of general vulnerability, age-specific risks, and precipitating factors (Wedgwood 1972). The "fatal triangle" presented by Rognum and Saugstad (Rognum and Saugstad 1993) had the same groupings like previous triple risk hypothesis, but he added mucosal immunity under a vulnerable developmental stage of the baby. The Triple risk hypothesis of Filiano and Kinney (Filiano and Kinney 1994) is famous and widely accepted in which they have included prenatal injury of the brainstem as a risk factor. The National Institute of Child Health and Development SIDS Strategic Plan 2000, states that "SIDS is a developmental disorder. Its origins are during fetal development." All of these hypothesis are same and the only difference is the prenatal origin (Guntheroth and Spiers 2002). Filiano and Kinney's model suggests that an interaction of genetic, environmental and biological factors is required for SIDS to occur and these factors include (Fig. 1), "a vulnerable infant who possesses intrinsic abnormalities in cardiorespiratory control, a critical period of development of homeostatic control mechanisms, and exogenous stressors."

1.3 Risk factors involved in SIDS

Theories on the possible causes of SIDS are more than hundered. It is widely accepted that SIDS is a combination of multifactors that occur during the period of increased vulnerability

and may cause the fatal outcome in some infants (Otto-Buczkowska 2002). "Bergman (1970) also argued that SIDS did not depend on any, "single characteristic that ordains an infant for death," but on an interaction of risk factors with variable probabilities (Bergman 1970). Pathophysiology of SIDS remains unexplained (Hunt and Brouillette 1987). Following are some factors (Fig. 1) known to be involved in the etiology of SIDS.

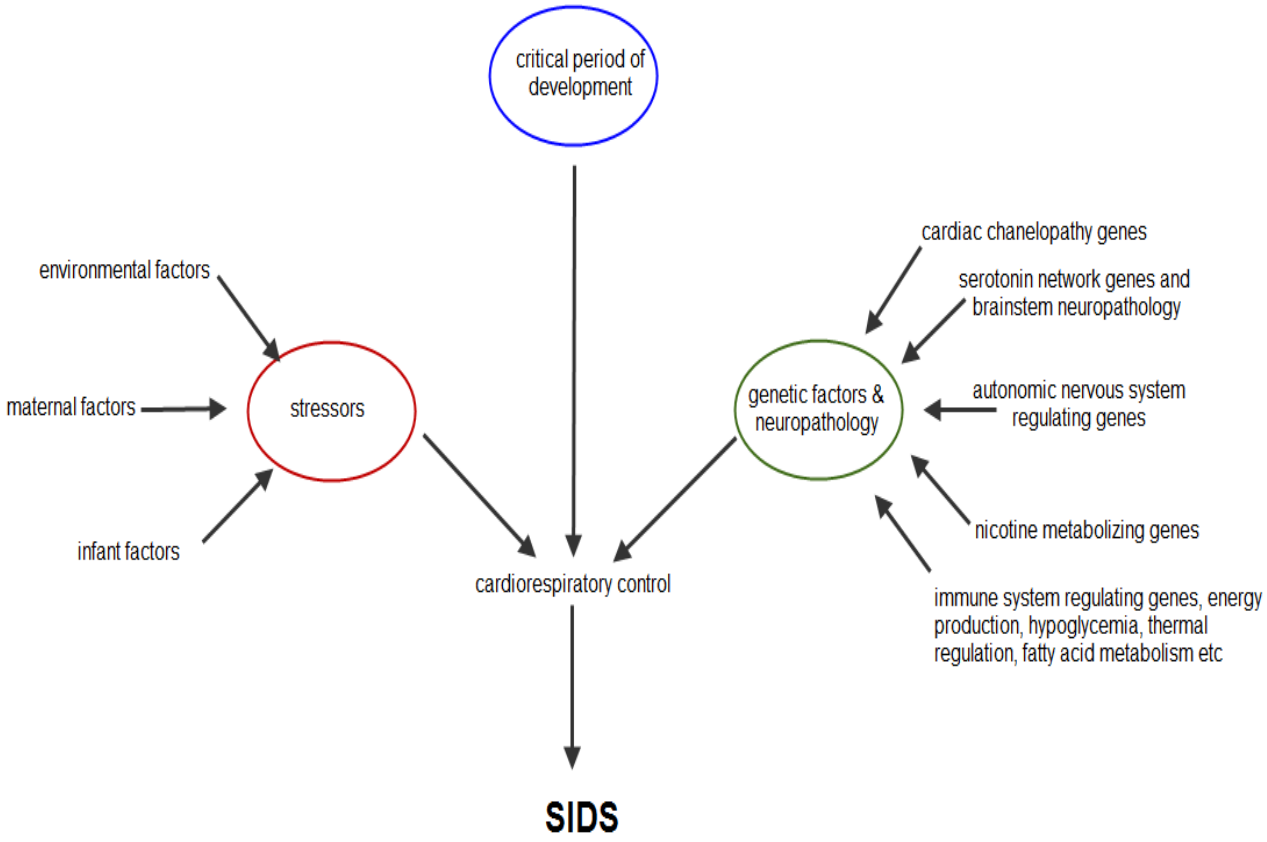


Figure 1: Triple risk model showing epidemiological and genetic factors affecting the cardiorespiratory control and leading to SIDS

1.3.1 Infant factors

1.3.1.1 Race/ethnicity

In US, there was a high incidence of SIDS among African-Americans than for white or oriental. Inappropriate prenatal care, co-sleeping or poor living conditions may be the underlying mechanisms of high prevalence of SIDS in African-Americans (Unger et al. 2003; Borhani et al. 1973; Kitsantas and Gaffney 2010). Different ethnic groups were observed to have different risk profiles for adverse pregnancy outcomes in Canada (Luo et al. 2004).

1.3.1.2 Prematurity

Low birth weight, prematurity and low APGAR (Appearance, Pulse, Grimace, Activity and Respiration) scores are mostly associated with infant mortality (Kapoor et al. 1994; Cheron et al. 1993; Borhani et al. 1973). Many structures of the heart and brain that play vital role in sleep/arousal or cardiorespiratory control are not fully developed in premature infants and may lead to the death of infant (Lavezzi et al. 2010).

1.3.1.3 Infant Botulism (Intoxication)

A neurotoxin botulinum produced by *Clostridium botulinum* blocks the neurotransmission in the cholinergic synapses (Fischer et al. 2004) and cause weakness of muscles, paralysis and has been implicated in SIDS also. Infant botulism and SIDS is associated in 20% of the cases (Bartram and Singer 2004). Honey and raw vegetables are a source of *Clostridium botulinum*, but as the infant has no vegetable intake, so the only source can be honey. Honey should not be given to infant less than 6 months to prevent botulism.

1.3.1.4 Male sex

Male infants dying of SIDS are in consistently 50% in excess per 1000 live births of each sex but the reason of this high male prevalence is not well understood. The only explanation available for the excess of male SIDS victims as compared to the females suggests the dominant X-linkage hypothesis for SIDS (Mage and Donner 2004).

1.3.1.5 Heart related abnormalities

Thomas James in 1968 firstly emphasized the role of cardiac conduction system as a contributing factor to SIDS. He investigated conduction systems of 56 infants, out of which 40 were SIDS cases. He found partial “resorptive degeneration” of the A-V node and left portion of His bundle in all of the SIDS cases (James 1968). Investigations of SIDS cases revealed heart related anomalies or dysfuncions such as myocarditis (Carturan et al. 2007), cardiac arrhythmias, cadiacmyopathy, hypoxia-related changes, disturbances of the rhythmogenic function and cardiac dysregulation as the most frequent finding. Disturbances of cardiac rhythmogenic function due to LQTS were among the most speculative (Bajanowski et al. 2003). Cardiac arrhythmias accounted for 5-10% (Thach 2005) and myocarditis was diagnosed in 25% of SIDS cases (Madea 2009).

Conduction system anomalies found in SIDS are resorptive degeneration, fetal dispersion, hypoplasia of cardiac conduction system, splitting of the atrioventricular node or of the His bundle and a Zahn node, accessory atrioventricular pathways (mostly Mahaim fibres), cartilaginous hypermetaplasia and junctional islands (Matturri et al. 2008; Ottaviani and Matturri 2008; Ottaviani and Bergui 2009; Lavezzi et al. 2005; Ottaviani 2010).

1.3.1.6 Neuropathology

Role of brainstem in pathophysiology of SIDS got attention in 1976 when Naeye's reported astrogliosis in 50% of SIDS victims (Naeye 1976). Some anatomical structures and areas of central nervous system (CNS) are involved in the regulation of autonomic function and respiration during a critical developmental stage and anomalies in any of these structures can induce SIDS, still birth or sudden intrauterine death (SIUD) (Duncan et al. 2010; Guntheroth and Spiers 2002). Due to these abnormalities, an infant becomes unable to respond to any stressor during sleep (e.g. hypoxia, hypercarbia) and dies suddenly during a vulnerable period of postnatal life (Kinney et al. 2009). In order to understand the mechanisms of sudden perinatal death, we should first know about neuropathology along with epidemiological and genetic factors.

According to Kinney, brain research in SIDS follows three directions: "analysis to determine a primary (neural) cause of death (developmental anomaly), analysis to uncover secondary changes that points to the primary cause, either within the brain or in other organ systems (oxygen deficiency events), and analysis to determine the age of onset of the chain of events prenatally and/or postnatally that results in sudden death in the vulnerable postnatal period" (Kinney 2009). Insufficient oxygen supply, immunological reactions and apoptotic neurodegeneration are the underlying mechanisms for neuropathological lesions in perinatal stage leading to sudden death. Examinations of brains of sudden perinatal death victims gives us an insight into the neuropathological events that can cause brain lesions in liveborn as well as stillborn infants and that cause brain disorders and SIDS in liveborn infants (Grafe and Kinney 2002; Sparks and Hunsaker 2002).

Morphology and/or functional alterations of one or more of the brainstem structures that regulate sleep-awake cycle through cardiorespiratory control centers, or failure to arouse or both may be involved in the pathology of SIDS (Sparks and Hunsaker 2002). Lavezzi and Matturri reported developmental alterations (hypoplasia, neuronal immaturity, and gliosis) of the arcuate

nucleus (Arc) (Matturri et al. 2002) (Fig. 2), Inferior olivary nucleus (ION) (Fig. 2), Dentate nucleus (DN) (Lavezzi et al. 2007), pre-Botzinger complex (Lavezzi and Matturri 2008) (Fig. 2), triangle of Guillain and Mollaret (G-Mt) a neuronal brainstem/cerebellum network (from the dentate nucleus to red nucleus and inferior olivary nucleus), the hypoglossal nucleus (HGN) (Fig. 2), intermediolateral nucleus (ILN) (Lavezzi et al. 2010), vagal dorsal motor nucleus (Lavezzi et al. 2003), locus coeruleus (LC) (Lavezzi et al. 2005) and the parabrachial Kolliker-Fuse complex in the pons (Lavezzi et al. 2004) of stillborns, unexplained perinatal and SIDS victims. Neuropathological changes provides a link between antenatal factors such as smoking and SIDS (Lavezzi et al. 2010). Recently, Lavezzi et al 2010, reported a possible role of damaged ependyma, the lining providing a protective barrier and filtration system separating brain parenchyma from cerebrospinal fluid, in SIDS because ependyma is the first to get affected before other autonomic nervous system centers, by the noxious agents entering to the fetus from mother (Lavezzi et al. 2010).

1.3.1.7 Cardiorespiratory control

All the factors whether maternal, infant, environmental or genetic, interfere with the cardiorespiratory control (Fig. 1) leading to final common pathway (death). Mechanisms underlying SIDS appear to originate in fetal period of development resulting in neural damage and affect breathing or blood pressure during sleep later on (Harper et al. 2000). Critical periods in the human development is from 2-4 months of gestation and at this stage, fetus is more vulnerable and at high risk to environmental, genetic and biochemical factors. These traumatic factors may alter the normal development of the respiratory control centers related to spinal constriction and compression following birth trauma and may contribute to SIDS (Banks et al. 1987).

Anomalies and immaturity of neuroregulator neuronal systems such as monoaminergic and peptidergic systems in medulla oblongata (Kopp et al. 1994), catecholaminergic systems (Kopp et al. 1992) in vegetal central areas of brainstem, muscarinic (Kinney et al. 1995) and serotonergic systems (Kinney et al. 2001) in brainstem have been observed and provide a possible mechanism of death in SIDS. Serotonin provides a key role in mediating protective responses to homeostatic stressors via medullary circuits (Kinney et al. 2009). Changes in dendritic synapses, delayed myelinisation (O'Brien 1993), some neurotransmitters, some

peptides, their enzymes and receptors may adversely affect cardiorespiratory swallowing control systems chemoreception (Harper 2000).

Vulnerable infants are born with respiratory centre immaturity which in combination with other problems such as illness, head colds, exposure to cold, air or smoke, may result in cessation of breathing (Nattie and Kinney 2002). More emphasis should be given to the understanding of mechanisms of breathing control in neonate and the maturation of cardiorespiratory control during infancy (Ozawa and Takashima 2002). Defects in brainstem neural circuits involved in cardiorespiratory control may be one of the leading cause of SIDS (Kinney and Filiano 1988).

1.3.1.8 Infection

Templeman in 1892 analysed 258 SIDS cases and found 74% of the cases with upper respiratory tract infections (Templeman 1892). Adelson and Kinney (Adelson and Kinney 1956) also noticed 55% of SIDS cases they investigated with upper airway infections within one week before death. The authors concluded that "no single bacterial organism or group of organisms was cultured from any site with a degree of consistency sufficient to indicate that it had probable etiological significance".Viral infection is not the only cause of SIDS but an association of upper respiratory tract infection and SIDS was also noted by enhanced detection rate of viruses in SIDS cases as compared to the controls (Fleming 1992). But no single bacterial or viral agent has been causally linked to SIDS (Williams et al. 1984).

1.3.1.9 Deformational plagiocephaly

Approximately half of the infants, who sleep in supine position, get affected by deformational plagiocephaly (flattening of the occiput). This problem has increased since the back to sleep campaign started. In order to prevent the negative effects of this problem e.g skull deformities, infants should be given "tummy time," in which they should spend maximum time on their stomach instead of backs while being awake (Persing et al. 2003).

1.3.2 Maternal factors

SIDS incidence is normally high in infants whose mothers are less than twenty years (Borhani et al. 1973) or over 35 years of age (http://www.stillbirthalliance.org), obese, have visited the prenatal care unit after the forth gestational months (Gunther 1975), use illegal drugs such as methadone (Burns et al. 2010) and alcohol (Phillips et al. 2010) during pregnancy as compared to the control group.

1.3.2.1 Pregnancy related factors

Maternal health during pregnancy is an important factor and mothers with shortened gestational age (Cheron et al. 1993), primiparity, high parity, multiple pregnancies (Gunther 1975) or anemia are more likely to have hypoxia and brainstem injury and could have endangered pregnancy outcomes (Froggatt et al. 1971; Guntheroth and Spiers 2002).

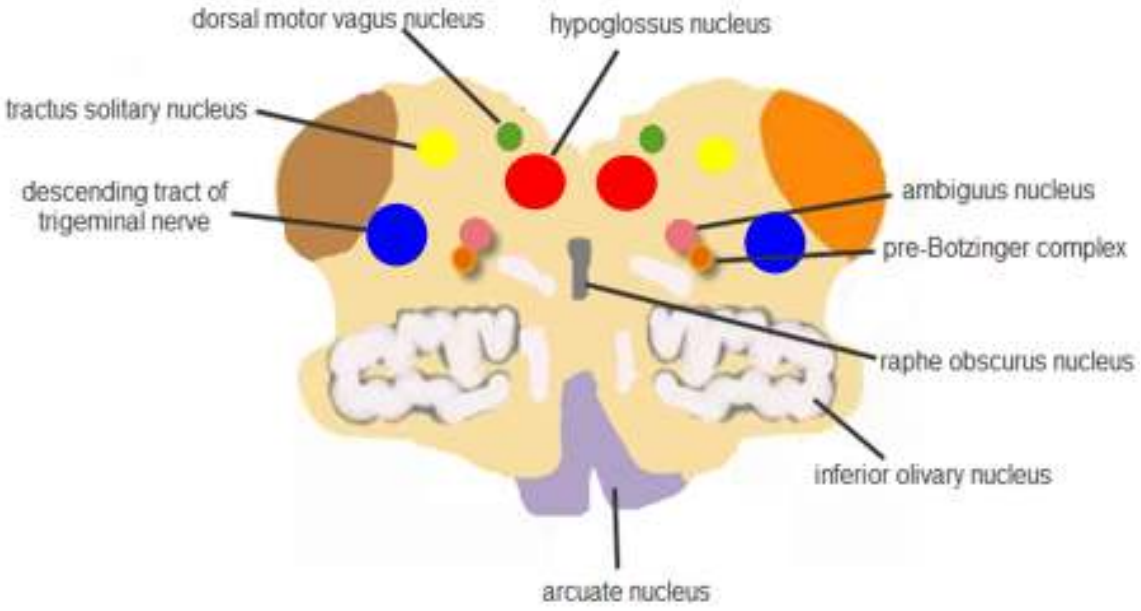


Figure 2: Schematic representation of a transverse section of the medulla oblongata (at the obex level) showing the localization of the principal nuclei

In many cases of SIDS, mothers had previous history of sexually transmitted diseases, urinary tract infections (Stewart et al. 1995), abnormal and complicated pregnancy, suffered from influenza, had received anaesthezia at the time of delivery and had Vasa praevia (a rare obstretic complication that causes antepartum haemmorhage) during pregnancies (Pallewela 1974). Mode of delivery of infant is also a crucial factor and many of the SIDS infants were found to have non vertex or breech presentation (Kapoor et al. 1994). An increase in the rate of SIDS was observed for labor more than 16 hours (Buck et al. 1991).

1.3.2.2 Maternal smoking

Maternal smoking also contributes to the pathology of SIDS (Krous 1984) and victims of SIDS show a higher incidence of respiratory control abnormalities including central apneas, delayed arousal responses and diminished ventillatory chemoreflexes. Nicotine is most probably a causal link between maternal smoking and sudden death (Eugenin et al. 2008). Several

experiments have revealed the fact that exposure to nicotine prenatally alters the central chemosensitivity and changes the muscarinic receptor to nicotinic. It also alters the systems of serotonin, noradrenaline, GABA (Gamma amino butyric acid), glycine and glutamine. Maternal tobacco smoking alters the respiratory control of perinatal infant by two main mechanisms: reorganization of Neurotransmitter (NT) systems and remodeling of neural circuits. These changes make breathing more vulnerable to fail in early postnatal life, which could be related to pathogenesis of SIDS (Campos et al. 2009). Intra-uterine growth retardation (IUGR) that is a major risk factor for SIDS is caused by maternal smoking. Carbon monoxide and nicotine are among the largely investigated components of cigarette smoke. They can directly affect the development of fetal brain or indirectly by inducing oxgen insufficiency (Gressens et al. 2003). In utero fetal exposure to tobacco smoke adversely affects growth and accounts for about one-third of perinatal deaths (Mitchell and Milerad 2006).

1.3.3 Environmental or Postnatal factors

1.3.3.1 Prone sleeping position

SIDS is typically associated with sleep (Kinney et al. 2009). Firstly, the association between supine sleeping position and SIDS was observed in 1965 but the recommendations to support it were issued in 1990s (Mitchell et al. 1991). Prone sleeping position has been known as a cause of SIDS since then. After the introduction of "Back to Sleep Campaign," there has been a dramatic reduction in SIDS (Blatt et al. 1999). Sleeping position is most modifiable risk factor (Adams et al. 2009). Several reports from well developed countries like Norway and Britain show that by putting the babies at their back for sleep can reduce the risk by half (Haaland and Thoresen 1992; Willinger et al. 1994). An infant in supine sleeping position is at reduced risk for reflex apena during upper airway infection leading to SIDS as compared to the infant sleeping prone (Crooks et al. 2004).

1.3.3.2 Day, Time, Month and Place

Prenatal deaths are mostly observed during the weekends as compared to the working days. More deaths were reported on saturday night (Peterson 1966), sunday night (Froggatt et al. 1971) and monday morning (Glasgow et al. 2006). Mothers are usually drunk during weekends and overlay babies. Most of the infant mortalities occur during midnight when the child is asleep. He is mostly found dead in the morning (Wedgwood 1972). A study showed that 50% of the infants died from midnight to 8am, 36% between 8am and 4pm, and 14% between 4pm and

midnight (Froggatt et al. 1971; Hodges 1972). Templeman found that 62% of the victims died during October to March (Templeman 1892). More prevalence was found between December to March in a Canadian study (Kraus et al. 1967) and many other studies also reported that SIDS is common in colder months of the year (Fedrick 1973). In one study, over 70% of infants were found dead at home, 6.8 % on way to hospital and 22-3% at hospital (Fedrick 1973). This also indicates delay in taking the infant to the hospital and lack of health care facilities may be the possible causes, as most deaths were at home.

1.3.3.3 Geographical link

Incidence of SIDS varies with geographical location. Sudden unexplained perinatal deaths were more prevalent in the higher latitudes (Mitchell 2009). Sweden has the lowest rate of SIDS which is reported as being as low as 0.6 SIDS deaths for 1000 live births. In comparison Ontario, Canada had a reported 3 SIDS deaths per 1000 live births. The actual mechanism is not known but improved living standard may be a possible cause of low incidence of perinatal deaths in Canada and Scandanavia.

1.3.3.4 Socio-economic status/ Poverty

A significant association between adverse social circumstances and SIDS has been observed (Spencer and Logan 2004). A population based chohort study was conducted in US during two periods, 1989-1991 and 1996-1998 using US linked birth/Infant death data sets. Social class of mother was measured by mother's education level. Results indicated that SIDS rate was associated with lower social class (Pickett et al. 2005). Templeman in 1892 pointed out that death incidence was more in areas with poor housing conditions and he concluded that these deaths are due to suffocation and overlying because many family members slept in small place to keep themselves warm (Templeman 1892). Unemployment, low social class, car ownership, rented accommodation and overcrowding are five census-based indicators of Deprivation Index. Many infants born to unmarried (illegitimate) ,divorced or widow mothers are more prone to die of SIDS due to poor health care facilities and low income (Gunther 1975).

1.3.3.5 Softbedding and overheating/overclothing

Werne and Garrow proposed that overlying may be responsible for SIDS and for a long time suffocation by covering the baby in bedsheets was considered as the cause (Werne and Garrow 1947). Overheating is a possible factor to have an effect on the sudden deaths (Gotestam 1991; Peduto et al. 1994). Correlation between the use of soft bedding and SIDS has been

noticed in victims in different Nordic and other countries too (Gotestam 1991). In two independent studies, it was found that infants who died of SIDS had more clothes on; heads were covered with bed clothes and blankets covering them. Possible mechanism is that overheating may interfere with the respiratory control (Haaland and Thoresen 1992). Prevalence of headcovering in final sleep was observed in 24.6% as compared to 3.2% among controls. Covering of head is a main modifiable risk factor related to SIDS (Blair et al. 2008).

1.3.3.6 Lack of breast feeding

Infants who are not breastfed are at more risk for SIDS than breastfed infants. It is an important modifiable risk factor to reduce SIDS (Stuebe 2009). Recommended time period for breastfeeding by American Academy of Pediatrics (AAP) (Gartner et al. 2005) and the American Academy of Family Physicians (AAFP) (Vennemann et al. 2009) is first six months exclusively and two years by the World Health Organization (WHO) (Stuebe 2009).

1.3.3.7 Bedsharing/cosleeping

Co-sleeping is a controversial matter among health professionals and parents. Increased rate of breastfeeding (Haycock and Greenough 2007) and parent child bond (Horsley et al. 2007) are among the positive affects of bedsharing while it can also cause sudden death of infant due to overlaying. The AAP (American Association of Pediatrics) discouraged co-sleeping of parents and infant as well as infant with an older child in 2005 by stating that it is the cause of SIDS in United States in one half of the SIDS cases (2005).

1.3.3.8 Use of pacifiers

A great reduction in the rate of SIDS was observed after using pacifiers, dummies or soothers during the final sleep (Sexton and Natale 2009). Mechanism underlying this observation is not known. Use of soothers may also lower the other risk factors related to SIDS such as prone sleeping position. It was evidenced in various studies that infants who were sleeping prone without pacifiers had greater risk of sudden death (Li et al. 2006). Some researchers oppose the use of pacifiers by stating that it may increase the risk of SIDS by decreasing the duration of breastfeeding but this issue remains controversial (Mitchell et al. 2006). According to the most accepted concept, the use of pacifiers should be suggested in infants from one to 6 months of age but not after 6 months to prevent otitis media (Marter and Agruss 2007; Sexton and Natale 2009).

1.3.4 Genetic studies in SIDS

Candidate genes of SIDS are divided into 5 categories by Weese-Mayer (Fig. 1), as follows: genes for ion channel proteins based on electrocardiographic evidence of prolonged QT intervals in SIDS victims, gene for serotonin transporter based on decreased serotonergic receptor binding in brainstems of SIDS victims, genes pertinent to the early embryology of the autonomic nervous system (ANS) (and with a link to the 5-HT system) based on reports of ANS dysregulation in SIDS victims, genes for nicotine metabolizing enzymes, genes regulating inflammation, energy production, hypoglycemia, and thermal regulation based on reports of postnatal infection, low birth weight, and/or overheating in SIDS victims. More genetic polymorphisms in serotonin transporter (5-HTT) are found to have a correlation with defected neural pathways components of serotonin binding (Weese-Mayer et al. 2007).

Genetic polymorphisms of voltage-gated sodium channel cardiac cells and type V alpha (SCN5A), designated S1103Y in Black American infants is associated with an increased risk of SIDS. Both wild and mutant SCN5A channels are functional under normal invitro conditions but exposure to low intracellular pH produced altered gain of function delayed reopenings of S1103Y channels, a behavior usually related to cardiac arrhythmias. Lower pH can cause conditions like respiratory acidosis which is a known factor to increase SIDS rate (Makielski 2006). Genetic modifications of cardiac channels, give new observations after thorough molecular autopsy of potassium channel genes linked to long QT syndrome in SIDS (Tester and Ackerman 2009). Potentially lethal cardiac channelopathies are responsible for about 10% of SIDS cases and most of these victims have genetically modified cardiac sodium channel due to beta subunit mutations. Na (V) beta subunits have a role in cardiac arrhythmias and the 4 genes (SCN-1B to 4B) encoding them are candidate genes for SIDS pathogenesis responsible for 1% of SIDS cases (Tan et al. 2010). Dominant X-Linked inheritance need further study in future to investigate the candidate genes for high SIDS prevalence in male infants (Mage and Donner 2004).

CHAPTER II

2 Tachykinins

Tachykinin (TK) family of neuropeptides is one of the largest peptide families that are described in the animals. Till now, more than forty tachykinin (TK) peptides have been identified in the animal kingdom including invertebrates, protochordates and vertebrates (Severini et al. 2002). Substance P like immunoreactivity (SP-LI) was localized in nervous systems of Hydra (Pierobon et al. 1989), locust (Benedeczky et al. 1982), cockroach (Verhaert and De Loof 1985), lobster (Mancillas et al. 1981), blowfly (Lundquist et al. 1994) and several other invertebrates but all of these were not authentic tachykinins while TKs identified in octopods *E. moschata* and *Eledone aldovrandi* and mosquito *Aedes aegypti* (Champagne and Ribeiro 1994) containing eledoisin, sialokinins I and II, are authentic because they have signature terminal pentapeptide sequence F(X)GLMNH2 (Erspamer and Anastasi 1962) and have more resemblance to the mammalian TKs and also bind to the same receptors (Severini et al. 2002).

A significantly less SP-LI is found in *Amphioxus lanceolatus* (Lembeck et al. 1985) and ascidian *Ciona intestinalis* (Protochordate) but they are not like mammalian TK peptides (Severini et al. 2002). Among submammalian vertebrates; reptiles, amphibians, fish, and agnatha contain authentic NKA and NKB peptides. First submammalian TK, physalaemin, was identified in 1964 by Erspamer from the skin of frog (Erspamer et al. 1964) and two more TKs, scyliorhinins I and II were isolated from dogfish intestine 22 years later (Conlon et al. 1986). By the end of 20th century, 24 TKs were identified from these vertebrates, 12 from the brain and 12 from digestice tract (Severini et al. 2002).

Substance P (SP), Neurokinin A (NKA) and Neurokinin B (NKB) are the best known members of this family (Page 2004) in mammals. This family of neuropeptides is enlarged by the later addition of newly discovered Endokinins (EK), Hemokinins (HK) (Page 2004), Virokinins (VK) (Zimmer et al. 2003; Nelson and Bost 2004) and Chromosome 14 tachykinin like peptide-1 (C14TKL-1) (Nelson and Bost 2004). Neuropeptide K (NPK) and Neuropeptide gamma (NP*g*) (Kage et al. 1988) are N-terminally extended forms of NKA. Three gene related peptides, EK-2 (Page 2004) in rabbit, EKC and EKD in human are identified (Page et al. 2003). Virokinin (VK) is a converted derivative of the bovine respiratory syncytial virus (BRSV) fusion protein (Zimmer et al. 2003).

TKs are evolutionary conserved excitatory neuropeptides or peptide hormones characterised by a common, specific C-terminal signature motif: FXGLMNH2. X (Fig.24, Table 1) is an aromatic or aliphatic amino acid. This hydrophobic and amidated sequence is important for the specific responses and generation of signal pathways. It is also necessary for TK receptor binding.

Tachykinins	**Amino acid sequence**
SP	RPKPQQFFGLM-NH2
NKA	HKTDSFVGLM-NH2
NPγ	DAGHGQISHKRHKTDSFVGLM-NH2
NPK	DADSSIEKQVALLKALYGHGQISHKRHKTDSFVGLM-NH2
NKB	DMHDFFVGLM-NH2
HK- 1 (Mouse)	RSRTRQFYGLM-NH2
HK-1 (human)	TGKASQFFGLM-NH2
EKA/EKB (human)	TGKASQFFGLM-NH2
C14TKL-1(human)	RHRTPMFYGLM-NH2
VK	GIPELIHYTRNSTKKFYGLM-NH2
TK gene-related peptides	
EKC (human)	KKAYQLEHTFQGLL-NH2
EKD (human)	VGAYQLEHTFQGLL-NH2

Table 1: Amino acid sequences of Tachykinins

Tachykinins (TKs) are widely distributed throughout the mammalian body in both the central nervous system (CNS) and peripheral nervous system (PNS) (Howard et al. 2006). TKs are ubiquitous and are identified in many species of vertebrates and invertebrates (Holmgren and Jensen 2001).Capsaicin-sensitive sensory nerves have been considered as the principal source of TKs at the peripheral level (Patacchini et al. 1998). Previously, they were considered to be synthezied and released from CNS neurons, capsaicin sensitive primary afferent neurons and capsaisin-insensitive intrinsic neurons of the gastrointestinal tract (Maggi 1995; Holzer and Holzer-Petsche 1997) but now it is known after several studies, that TKs are also found in non-neuronal and non-innervated tissues (Patak et al. 2003) such as bone marrow stem cells

(BMSCs), mature blood cells (Zhang et al. 2006), epithelial cells, immune cells (Weinstock et al. 1988), endothelial muscle cells, macrophages, neutrophils, tumor cells, vascular endothelial cells (Linnik and Moskowitz 1989), inflammatory cells (Maggi 1997), somatotroph and thyrotroph cell populations of the anterior pituitary gland (Brown et al. 1991), in blood circulation (Pernow 1983) and even in placenta which is a tissue completely devoid of nerves (Page et al. 2000).

SP is mostly found in limbic regions and brainstem nuclei such as the dorsal raphe nucleus (DRN) (Froger et al. 2001) and it is synthesized in B cells of the dorsal root ganglion (DRG) cells (Liu et al. 2007), neuronal and glial cells of human nervous system (Lazarczyk et al. 2007). The presence of SP in rat cerebellum is 3 times more than NKA and NKB (Otsuka and Yoshioka 1993). NKB is confined mostly to the CNS (Goubillon et al. 2000). Hemokinin-1 (HK-1) and endokinins are expressed in non-neuronal cells (Patak et al. 2003). Chromosone 14 tachykinin like peptide 1 (C14TKL-1) mRNA has been detected in many human central and peripheral tissues (Groneberg et al. 2006).

Tachykinins have been associated with many physiological processes in the cardiovascular, nervous, respiratory and immune systems (Pernow 1983). In addition to mediating certain physiological functions, SP and its receptors are also involved in some pathophysiological conditions (Liu et al. 2007). TKs are synthesised in neuronal and glial cells of the human central and peripheral nervous system (Lazarczyk et al. 2007). TKs are linked to neurological conditions, such as depression (Sergeyev et al. 2005), anxiety (Ebner and Singewald 2006), Parkinsonian syndrome, Parkinson's disease (Barker 1989) and Alzheimer's disease. The TKs have also been implicated in traumatic brain injury (TBI) (Zacest et al. 2010), ischemic stroke and epileptic seizures (Ko et al. 1991). At the level of CNS, SP has been found to be involved in conditions such as nausea, vomiting and pain (Harrison and Geppetti 2001).

2.1 Tachykinin Genes

Three genes namely TAC1,TAC3 and TAC4 (Table 3), belonging to the family of preprotachykinins (PPTs), encode TKs (Nowicki et al. 2007). Previously these genes were called PPT-A, PPT-B and PPT-C respectively (Nawa et al. 1983). PPTs are larger precursor molecules that produce many peptides after differential RNA splicing. TAC1 and TAC3 PPTs are two homologous genes (Nawa et al. 1983; Kotani et al. 1986). TAC4 was discovered in 2000 by Zhang (Zhang et al. 2000). Mammalian substance P is derived from the preprotachykinin-A (PPT-A) or TAC1 gene (Fig. 3), which originates from a common ancestral gene by duplication

(Carter and Krause 1990). The tachykinin peptides substance P (SP), neurokinin A (NKA), neurokinin A (3-10), neuropeptide K (NPK), and neuropeptide gamma (NPγ) are produced from a single preprotachykinin gene PPT-A or TAC1 as a result of differential RNA splicing and differential posttranslational processing (Helke et al. 1990).

PPT-A/TAC1 comprises of seven exons, which can be alternately spliced and modified to form four transcripts: α, β, γ, and δ (Table 2; Fig. 3). SP is encoded by exon 3, present in each transcript, and NKA is encoded by exon 6, present only in transcripts β and γ (Nowicki et al. 2007). NPK is produced by β*TAC*1 and γNP by γPPT-A/γ*TAC*1 (Kawaguchi et al. 1986; Lai et al. 1998) by differential processing at the N-terminal dibasic cleavage site of NKA (Krause et al. 1989). mRNA of each uses a different exon corresponding to the protein coding region. αPPT-A contains all the exons except 6 while exon 4 is missing in γPPT-A mRNA. βPPT-A mRNA comprise of all the 7 exons (Fig. 3) (Harrison 2001). α PPT-A mRNA expression is more abundant in the brain, while βPPT-A and γPPT-A mRNAs are predominately expressed in peripheral tissues (Kotani et al. 1986). Expression of PPT-A mRNA in nodose (Hamid et al. 1991), trigeminal (Kiyama et al. 1988) and dorsal root ganglia (DRG) (Sternini 1991) has been demonstrated.

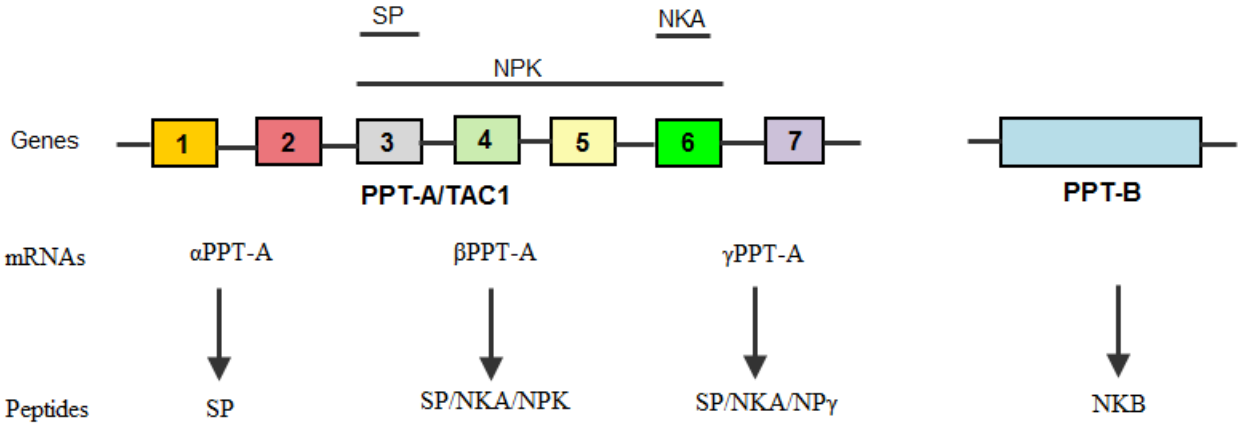


Figure 3: Structure of TAC1 gene (Harrison and Geppetti 2001)

TAC3 or PPT-B gene also originates from the same common ancestral gene as PPT-A. However, the PPT-B gene encodes for NKB only (Fig. 3) (Nakanishi 1987), while TAC2 is the gene encoding NKB in mouse (Nelson and Bost 2004). 3 different splice variants are formed after proteplytic cleavage of PPT-B or TAC3 mRNA in humans, namely α, β and γTAC3 (Page 2004). A TAC3 also comprise of 7 exons and only exon 5 encodes for NKB. TAC3 is expressed

in brain and peripheral tissues (Nowicki et al. 2007). TAC4 or PPT-C is relatively a new member of PPT family. It was identified in 2000 by Zhang (Zhang et al. 2000). It encodes for newly discovered TK members, Hemokinin-1 (HK-1), Endokinin A (EKA), Endokinin B (EKB), Endokinin C (EKC), and Endokinin D (EKD) (Pinto et al. 2004; Page 2004). TAC4 expression is mainly observed in peripheral tissues such as adrenal gland and placenta (Page et al. 2003).

Species	Gene	Chromosomal location	mRNA transcript	Protein product
Human	TAC1	7q21-q22	αTAC1 βTAC1 γTAC1 δTAC1	SP SP, NKA, NPγ SP,NKA,NPK SP
Human	TAC3	12q13-q21	αTAC3 βTAC3 γ TAC3	NKB NKB -
Human	TAC4	17q21.33	TAC4 αTAC4 v1 αTAC4 v2 βTAC4 γ TAC4 δ TAC4	hHK-1,hHK-1 (4-11) EKA,EKC EKB,EKC EKB,EKD EKB EKB
Mouse	TAC4	11D cM	TAC4	HK-1
Rat	TAC4	10q31 cM	TAC4	HK-1
Rabbit	TAC4	-	αTAC4 βTAC4	EK-1,EK-2 EK-1

Table 2: Tachykinin genes

Preprotachykinin contains a signal peptide of 16-30 residues at N-terminal, one or several copies of a neuropeptide and one or more spacer parts and is formed after the translation of mRNA from PPT-A, PPT-B or PPT-C in nucleus. It is then transported to the endoplasmic reticulum where it is converted to propeptide after the removal of signal peptide. From here this propeptide is transported to Golgi complex where the spacer parts are clipped off to yield final active peptide sequence (Page et al. 2003). These active peptides are then packed into secretory

granules, leave the golgi apparatus and then transported through the axon to the nerve terminals (Holmgren and Jensen 2001).

2.2 Tachykinin receptors

All the physiological responses of TKs are mediated by three types of receptors namely Neurokinin receptor 1 (NK-1R), Neurokinin receptor 2 (NK-2R) and Neurokinin receptor 3 (NK-3R) which binds preferentially to SP, NKA and NKB, respectively (Table 3). HK-1, EKA and EKB also prefer NK-1R (Zhang et al. 2000). TK receptors belong to family 1 (Rhodopsin like) hydrophobic 7-transmembrane (TM1-TM7) ,G protein coupled receptors (GPCRs) (Lavagno et al. 2001).They have 3 extracellular and 3 intracellular loops, one extracellular amino terminus and one intracellular carboxy terminus (Maggi 1995) (Fig. 4).These receptors are involved in many biological processes (Krause et al. 1992).

Tachykinin neuropeptide, SP brings all of its cellular activities after binding to the G-protein coupled receptor NK-1R which is located on cell surface (Helke et al. 1990). Like all TK receptors, SP also binds to heterotrimeric G-protein complex with a preference for Gs and Gq. Binding of receptor to Gs stimulates adenylyl cyclase and cyclic AMP production while binding

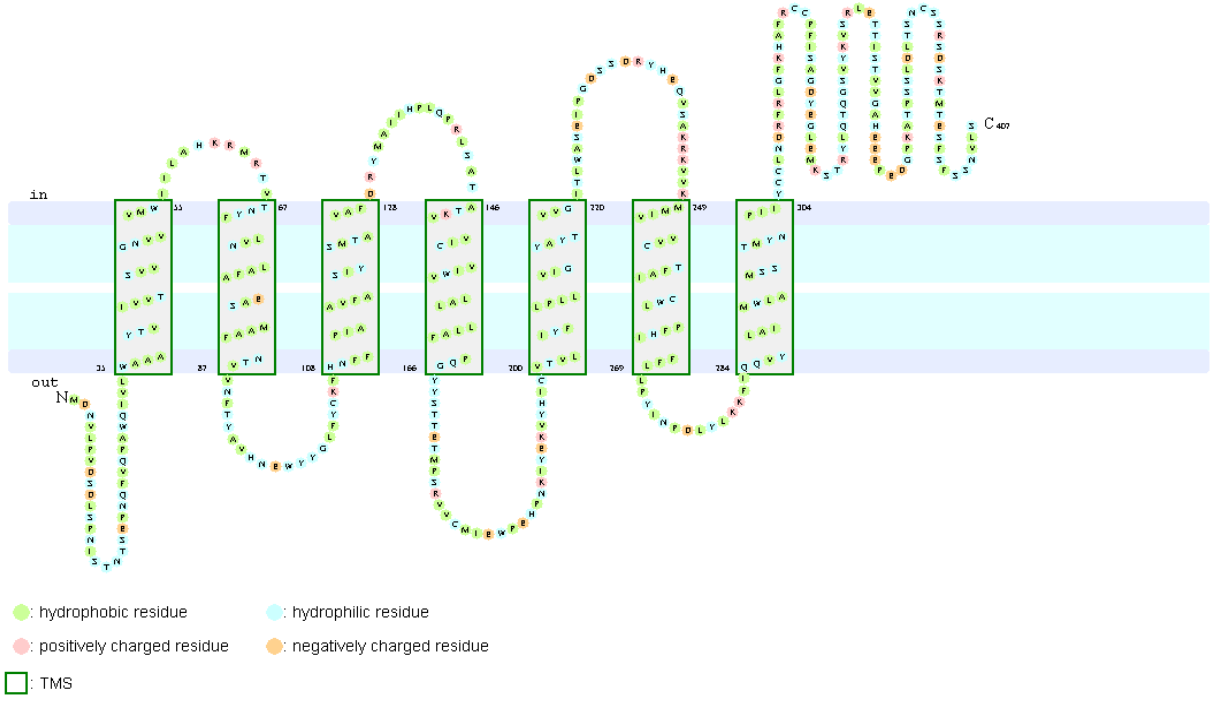


Figure 4: Transmembrane topology prediction of NK-1R by using ConPred II

to Gq initiates the phosphatidyl-inositol casade (Nakanishi 1991). Upon binding of SP to NK-1R, a signal transduction cascade is initiated by internalization of SP-NK-1R complex (Quartara and Maggi 1997) resulting in activation of Phospholipase C (PLC). PLC is a second messenger and produces inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) (Ramkissoon et al. 2006). IP3 stimulates endoplasmic reticulum to release intracellular calcium and initiates many signal transduction cascades. DAG activates protein kinase C (PKC) (Regoli et al. 1994; Radhakrishnan et al. 1995).

There are two distinct conformational isoforms of NK-1R: a full-length NK-1R (NK-1RF) isoform and a truncated NK-1R (NK-1RT) isoform, which lacks the terminal cytoplasmic 96-aa residues (Chernova et al. 2009). Both of these isoforms have same binding affinity for SP but different affinities for NKA. The NK-1R has a relatively long 5′ untranslated region compared to the other tachykinin receptors, which is preceded by a single TATAAA sequence (Hershey and Krause 1990). Three different genes termed TACR1, TACR2, and TACR3 (Table 3; Fig. 5) encode for the NK-1R, NK-2R, and NK-3R, respectively (Pennefather et al. 2004). All these genes are evolutionay conserved and have same structure with five exons interrupted by introns in identical positions (Hershey et al. 1991; Howard et al. 2006) (Fig. 5). TM1-3 are encoded by exon 1, TM4 by exon 2, TM5 by exon 3 and TM6,7 by exon 4. This feature of G-protein coupled receptors to retain the introns is very uncommon in this superfamily (Klassert et al. 2010).

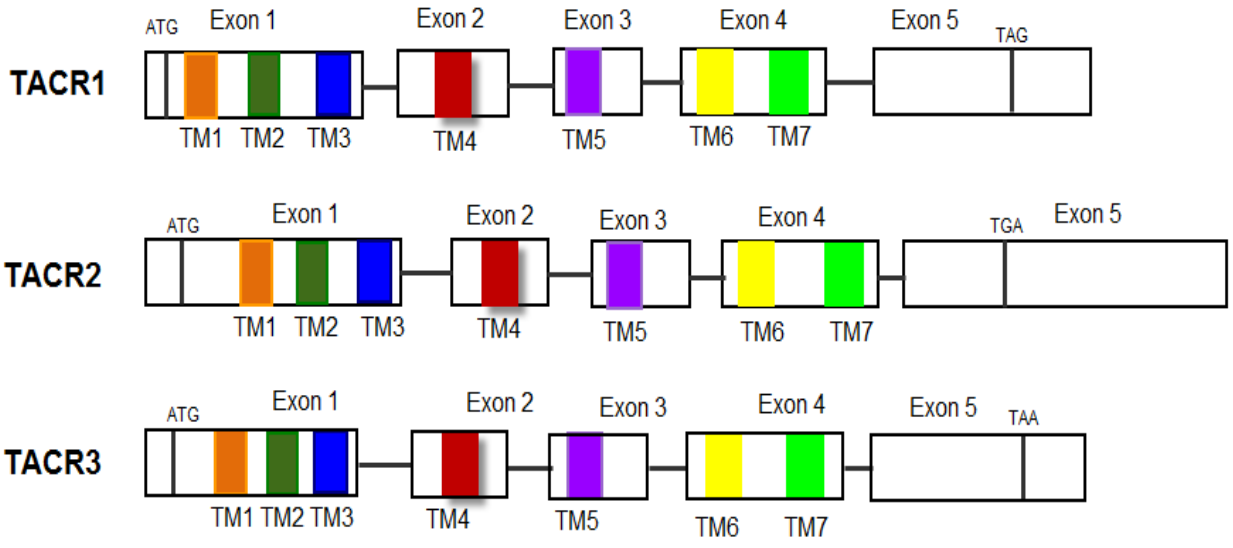


Figure 5: Tachykinin receptor genes, Modified (Howard et al. 2006)

TK receptors are uniquely distributed throughout the nervous system in neural as well as non-neural system (Patacchini and Maggi 1995). NK-1R is expressed in both central (CNS) and peripheral nervous system (PNS) (Tsuchida et al. 1990) while NK-2R is present mostly in PNS although some NK-2R immunoreactivity is also found in CNS (Naline et al. 1989; Klassert et al. 2008). NK-3R is mostly localized to CNS and less in peripheral tissues (Massi et al. 2000). A single substrate type can express more than one TK receptor, so that if blockade of one receptor occurs, the other receptor can compensate its functions (Lecci et al. 2006) because all these TK receptors have high structural specificity and homology.

TK receptors	Affinity for TKs	Location	Amino acids	Genes	Gene ID	Chromosome location	mRNA ID	Protein ID
NK-1R	SP>NKA>NKB	CNS/PNS	407	TACR1	6869	2p13.1-p12	NM_001058	NP_001049
NK-2R	NKA>NKB>SP	PNS	398	TACR2	6865	10q11-q21	NM_001057	NP_001048
NK-3R	NKB>NKA>SP	CNS	465	TACR3	6870	4q25	NM_001059	NP_001050

Table 3: Tachykinin receptors

2.3 Substance P

Substance P (SP) is the prototype and first discovered tachykinin. It is a neurotransmitter of the afferent sensory nervous system (Felderbauer et al. 2007). von Euler and Gaddum first identified SP as an atropine resistant factor in brain and intestine and reported that alcohol extracts of brain and intestine from the horse elicited a variety of strong pharmacological effects in animal and tissue models, most notably the contraction of smooth muscle and hypotension (lowered the BP by vasodilation) in rabbits (von Euler and Gaddum 1931). In 1949 Erspamer extracted a similarly acting substance from the salivary glands of the Mediterranean octopuses *Eledone moschata* and *Eledone aldovrandi* (Erspamer 1949). The structure of this non-mammalian substance 'Eledoisin' was resolved in 1962 (Anastasi and Erspamer 1962). A substance 'Physalaemin' with similar properties was characterized in the following year from the skin of frog *Physalaemus biligonigerus* (Anastasi et al. 1964). Later it was confirmed that all these three peptide hormones are highly related members of the largest peptide family, Tachykinins (Severini et al. 2002).

'Sialogen', a peptide that stimulate salivation was discovered in 1967 from bovine hypothalamus (Leeman and Hammerschlag 1967). Lembeck and Starke (Lembeck and Starke 1968) suggested that this peptide may be the same as SP. In 1970, Chang isolated and purified this substance and called it a sialogogic peptide because it can increase secretion of saliva (Chang and Leeman 1970). SP is a small peptide hormone consisting of 11 amino acids (Chang 1971; Pernow 1983) (Table 1). It is the most abundant TK peptide, neurotransmitter, neuromodulator or cotransmitter in CNS of mammals (Severini et al. 2002). It has been implicated in various physiological and pathophysiological processes (Ebner and Singewald 2006) and found in many central and peripheral neural pathways. SP-LI and NK1 was also observed in mammalian pineal gland for the first time in 2009 (Mukda et al. 2009).

2.3.1 Role of Substance P in Brain

SP-IR in mammalian brain was first detected in 70s (Hokfelt et al. 1975). SP-IR has been demonstrated in the rhinencephalon, telencephalon, basal ganglia, hippocampus, amygdala, septal areas, diencephalon, hypothalamus, mesencephalon, metencephalon, pons, myelencephalon and spinal cord (Shults et al. 1984), as well as periaqueductal gray matter (Hokfelt et al. 1982), dorsal raphe nucleus (Kachidian et al. 1991), locus coeruleus (Pickel et al. 1979), parabrachial nuclei, inferior olivary nucleus (Dean et al. 1993) and in the nucleus of the tractus solitarius. In lower brainstem of human fetus, an extensive network of SP-IR fibers were found in a number of areas (Nomura et al. 1982) (Fig. 6).

SP is abundant in the cerebral arteries of a number of species including man, supplying the dura mater, arachnoid and pia mater of all brain regions (Edvinsson et al. 1983) and may be involved in control of cerebral blood flow in man (Mejia et al. 1988). SP-IR bodies and terminal networks are distributed in most areas of the brain (Hokfelt et al. 1982). In different brain regions, SP frequently coexists in the same neuron with other neurokinins and with 'classical' neurotransmitters such as enkhaphalin (Pickel et al. 1979), glutamate, serotonin (Nicholas et al. 1992), dopamine, GABA (Hokfelt et al. 1982), acetylcholine, noradrenaline, or neuropeptides such as hydroxytryptamine and thyrotropin releasing hormone (Dean et al. 1993).

Some of the highest concentrations of SP in forebrain are found in areas where dopaminergic neurons arise or terminate (Iversen et al. 1980). It is suggested that SP mediates activation of dopaminergic pathway by exciting neurons of reticulata or reticular formation (Collingridge and Davies 1982). Increased levels of SP within substantia nigra induces release of

dopamine which in turn cause SP release by positive feedback mechanism (Thornton et al. 2010). Most if not all dendrites bearing membranous NK-1Rs appeared to be GABAergic (Lacoste et al. 2009). TKs activate or depress hippocampal activity by inhibiting GABAergic interneurons located in the CA1 region and CA1 pyramidal neurons via NK1 receptors. SP acts at the somatodendritic membrane of interneurons, rather than at their axon terminals (Ogier et al. 2008).

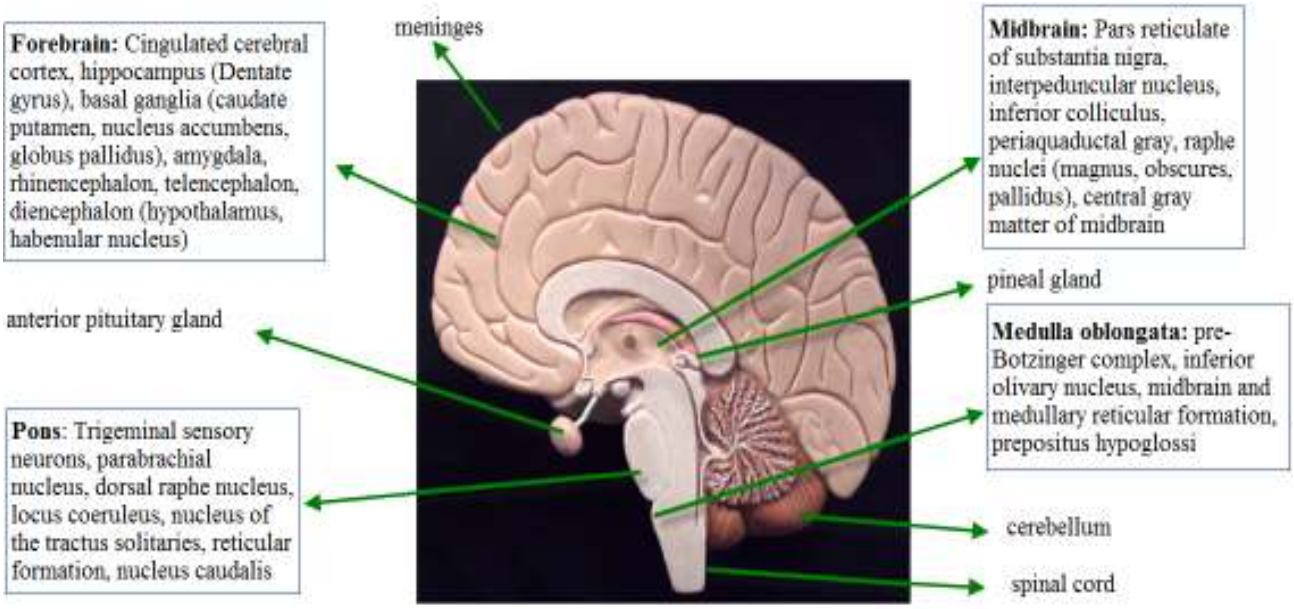


Figure 6: Distribution of SP in human brain

SP is a nociceptive afferent neurotransmitter and most of the brain areas in which SP-LI is detected, have implications in modulation of stress and anxiety reactions. SP and NK-1R have been involved in emotion, anxiety, stress, mood, migraine, emesis, pain, and seizures (Vink and van den Heuvel 2010). TKs exert their effects by binding to NK receptors (Almeida et al. 2004). SP level is increased in specific limbic structures such as amygdalae and septum and the magnitude of this effect is directly related to the severity of the stressor (Ebner and Singewald 2006). Interference with TK transmission can modulate emotional behavior and TK transmission is observed to be upregulated in suffering from stress-related disorders (Ebner et al. 2009). This undecapeptide modulates neuronal transmission of serotonin in dorsal raphe nucleus (DRN) via NK-1Rs and controls mood through habenular afferents (Lacoste et al. 2009). Fibers with SP and NKA are derived from the trigeminal ganglion (Uddman and Edvinsson 1989). SP-LI is present in the superficial and ventromedially located layers of nucleus caudalis. There is an abnormal

release of SP from all the three divisions of trigeminal nerve endings in response to nociceptive stimulation (Sicuteri et al. 1986). SP may have role in trigeminovascular nociceptive mechanisms of migraine pathophysiology (Fusayasu et al. 2007) by inducing vasodilation and plasma extravasation.

SP-IR in eye is originated from trigeminal ganglion (Sasaoka et al. 1984). SP-IR nerves in human eye are similar to those found in other mammals. Immunoreactivity of this neuromodulator peptide is present in different parts of eye such as sphincter muscle (Tornqvist et al. 1982), cornea, retina, ciliary body (Beuerman and Stern 2005) and limbal muscles but is more in iridic sphincter (Tervo 1981). Neurogenic inflammation is produced in eye through the release of SP, in exposure to pathogens, chemical irritants and mechanical disruption. SP breaks the blood-tissue barrier, cause edema and release polymorphonuclear leukocytes into the tears, thus bringing the first line of defence to the ocular surface (Beuerman and Stern 2005).

It was demonstrated in 2009 for the first time that SP plays an active role in memory and learning processes after injecting it into the ventral pallidum and is related to the globus pallidus (GP) and amygdaloid (AMY) (Kertes et al. 2009). GP may also be involved in reward-related processes. SP and its NK1 receptors play important roles in pallidal positive reinforcing mechanisms (Kertes et al. 2010). Activation of NK-1R in GP may be involved in positive role of SP in Parkinson's disease (Chen et al. 2009). Elevated level of SP is fatal for neuronal survival and cause motor dysfunction, that is characteristic of Parkinson's disease (Thornton et al. 2010).

Co-release of SP with lactate dehydrogenase, a marker of cell death, causes neurotoxin 6-hydroxydopamine (6-OHDA) induced cell death invitro (Thornton et al. 2010) and thus there is an implication of neuronal death by SP at cellular level. Neuropeptides and in particular SP is released after acute injury to the CNS (Vink and van den Heuvel 2010) and traumatic brain injury (Zacest et al. 2010) as part of neurogenic inflammatory response and plays crucial role to the development of morphological injury and functional deficits following traumatic brain injury by modulating blood-brain barrier permeability (Donkin and Vink 2010). It induces antidromic vasodilation, synaptic transmission in autonomic ganglia and cerebral edema during this process. Elevated levels of SP under trauma or stress conditions can help central, peripheral or enteric nervous system to adapt to those traumatic changes and help improve survival, adaptability and promote recovery after injury (Hokfelt and Kuteeva 2006). Lack of production of SP may cause impaired neurotransmission in patients with ischemic stroke due to damage of basal ganglia and

decreased swallowing response, leading to aspiration pneumonia, a major cause of death in stroke patients (Nishiyama et al. 2010).

2.3.2 Role of Substance P in regulation of Respiratory rhythm

Neuromodulator Substance P produces salivation, neuronal excitation, vasodilatation, increased vascular permeability and contraction of smooth muscles in the respiratory tract (Koch et al. 1999). In humans, NK-1R is involved in causing bronchoconstriction (Naline et al. 1996). TKs induce neurotransmission at ganglionic and postganglionic level via NK-1R and NK-2R respectively (Advenier et al. 1997). NK-1Rs mediate increases in secretion of mucous glands in human trachea (Rogers et al. 1989). Bronchoalveolar lavage fluid (Nieber et al. 1992) and sputum samples (Tomaki et al. 1995) taken from asthmatics after antigen challenge show an increase in SP-IR. Expression of NK-1R mRNA appears to be greater in asthmatic lung tissue as compared to non-asthmatic tissue and chronic inflammation in asthma may result in increased NK-1R gene expression (Adcock et al. 1993).

Elevated levels of SP and PPT-A mRNA were observed in the nodose ganglia of ovalbumin-sensitized guinea-pigs (Fischer et al. 1996) with increased neurogenic inflammation and bronchoconstriction produced by NK-1R (Harrison and Geppetti 2001). It suggests that SP, NK-1R and neurogenic inflammation is crucial in the development of airway hyper-responsiveness (AHR) as a consequence of allergen challenge (Bertrand and Geppetti 1996) and NK-1R antagonists attenuated the AHR and plasma extravasation in animal models invivo (Harrison and Geppetti 2001). The underlying mechanism in causing AHR may be the airway inflammation and interaction of SP and CGRP (calcitonin gene related peptide) (Wu et al. 2007). Mice deficient in NK-1Rs show reduced IgG mediated lung injury and neutrophil infiltration as compared to the control group (Bozic et al. 1996).

Substance P is present in bronchopulmonary C fibers (PCFs) and defend the lungs against injury from inhaled agents by a CNS reflex consisting of apnea, cough, bronchoconstriction, hypotension, bradycardia (Mutoh et al. 2000), secretion from seromucous glands, release of mediators (including prostaglandins and NO) from the airway epithelium (Geppetti et al. 1993) and bronchorelaxation (Figini et al. 1996). SP synthesis in vagal airway C fibers may be enhanced in pathological conditions such as allergic asthma and chronic bronchitis, and may be responsible for some of the associated respiratory symptoms stated above (Mutoh et al. 2000). SP and NKA produce bronchonconstriction and lung resistance (LR) in cynomolgus monkeys

(Mauser et al. 2001) and sheep (Rice et al. 2001) via NK-1 receptors and this effect is more pronounced when they are given by the intravenous route.

All three tachykinin receptors NK-1R, NK-2R and NK-3R are present in the nucleus of the solitary tract (nTS) (Fig. 2) and are involved in the central control of respiration (Mazzone and Geraghty 2000). NK-1Rs are present within the respiratory medullary network and in the phrenic nucleus, which controls the diaphragm and mediate the respiratory responses to SP. Prototype TK, SP induces an increase in respiratory frequency or an increase in inspiratory motor output in wild-type mice, in the invitro brainstem-spinal cord preparation but not in the NK-1R knockout (NK-1R-/-) mice (Ptak et al. 2000).

The study of invtro brainstem preparations revealed that neurokinin receptors (NK-Rs) (Table 3) have a vital role in the regulation of respiratory control and lung burst activity during the development of bullfrog from tadpole to adult stage (Chen and Hedrick 2008). Role of SP has been implicated in the development of plasticity of respiratory system and the regulation of respiratory rhythm. A functional SP-ergic system is necessary for the generation of sufficient ventillatory responses to hypoxia in newborn mice and during early maturation (Berner et al. 2007). Under increasing hypoxia, SP manifests as natural anti-hypoxant and is not only involved in nociception mechanisms but also in brain adaptation to oxygen deficiency (Vlasova and Torshin 2001).

SP-ergic system was found to be more active in regulating the respiratory responses during the early postnatal period in neonatal rat brainstem-spinal cord preparation (Shvarev and Lagercrantz 2006) and medullary slice preparations of newborn mice (Yasuda et al. 2001). But surprisingly, SP was not found to control ventillatory rhythm generation in fetal rats and it was hypothesized that may be SP doesnot modulate the generation of respiratory responses before birth and affects the phrenic motoneurons only after birth (Ptak et al. 1999). Many changes in the development of brainstem nuclei occur during the first two weeks of postnatal life in rat and there is a decrease in the expression of SP and NK-1R expression but this situation is changed at postnatal day 12. Level of excitatory neurotransmitters (e.g glutamate) fall and inhibitory neurotransmitters (e.g GABA) and SP rise sharply. Cytochrome oxidase activity also drops in respiratory neurons and subunit switches occur in many receptor types. All these dramatic changes make the respiratory system vulnerable to any external stressor and the stressor can cause failure of respiratory system too. If we relate these studies in animal models to

physiological processes of human then we can assume that the same changes also happen in human and may be a possible risk factor for SIDS (Wong-Riley and Liu 2005).

The preBötzinger complex (preBötC) (Fig. 2) is present in medulla oblongata and it has glutamatergic NK1R-expressing neurons (Morgado-Valle and Feldman 2004). It has a unique and pivotal role in the generation of respiratory rhythm patterns in neonates of mammalian and non-mammalian vertebrates and is sensitive to Substance P (SP) (Lavezzi and Matturri 2008; Kinkead 2009). SP directly acts on preBötC to modulate the respiratory activity. Any depletion in the level of SP within preBötC can attenuate the ventillatory rhythm generation in neonates (Morgado-Valle and Feldman 2004).

2.3.3 Role of Substance P in different systems

SP induces the release of cardiac mast cells (MC) and Angiotension II by cardiac inflammatory cells under the conditions of inflammation such as cardiac hypertrophy, fibrosis and hypertension (Levick et al. 2010). TKs such as hemokinins (HK) and SP causes the release of nitric oxide (NO) from coronary endothelium that relaxes the coronary vessels, decrease heart rate, and induces vasodilation and hypotensive responses via NK-1Rs in large arterial vessels. A decrease in TKs can reduce the endomyocardial NO content, which could cause reduce endothelium dependent vasodilation and an increased incidence of diastolic heart failure during aging, diabetes or posttransplantation (Paulus 2001). Alteration of vagal control of the heart by SP can perturb the cardiovascular regulation by increasing the sympathetic flow (Dzurik et al. 2007).

Substance P (SP) play a role in the immediate and late type of hypersensitivity as well as in neurogenic inflammation (Grzybowska-Chlebowczyk et al. 2003). Neurogenic inflammation provides the first line of defence to protect the tissue and contribute to the healing of wounds by activating the un-myelinated primary afferent sensory neurons and release of neuropeptides, among which SP is very important. Inflammatiory responses consist of hyperemia (Herbert and Holzer 2002; Birklein and Schmelz 2008) and oedema (Walsh and D 2006) accompanied by pain such as migraine (Fusayasu et al. 2007), asthma, fibromyalgia (Lieb et al. 2002), craniofacial pain, arthritis, complex regional pain syndrome (CRPS) type 2, pulmonary oedema, hypertension, pre-eclampsia, and stroke (Walsh and D 2006). Neurogenic inflammation induces plasma extravasation from postcapillary venules and vasodilation of arterioles (Birklein and Schmelz 2008). Two mechanisms interact with each other to cause neurogenic inflammation:

activation of the sympathetic nervous system and antidromic release of neuropeptides (e.g. tachykinin peptides) from sensory afferent end organs (Procacci etal., 1999). Whether the noxious stimulus is capsaicin, tobacco smoke, allergen, ozone, or stress, it causes substance P (SP), and other neuropeptides to release from the sensory nerve fibers.

Several inflammatory skin diseases including non-atopic nummular eczema (NE), psoriasis (Saraceno et al. 2006; Remrod et al. 2007; El-Nour et al. 2009), ear swelling (Inagaki et al. 2010), scratching behavior (Inagaki et al. 2010), allergic dermatitis, pruritus, psoriasis, atopic dermatitis (AD) (Ohshima et al. 2010) and human malignant melanomas (Munoz et al. 2010) occur due to neurogenic-mediated inflammation. SP nerves are found to be increased in epidermis of AD and NE lesions that consequently may stimulate keratinocytes to release cytokines which affect various cell types by increasing vascular permeability and causing neurogenic inflammation. Increased vascular permeability in the skin as a result of neurogenic inflammation involves mast cells in addition to SP. Within the epidermis, SP immunoreactive nerve fibres are found in the papillary layer near the epidermal basal membrane (Schulze et al. 1997). Although SP is found in innervated and non-innervated tissues but SP may possibly be more important in non-neuronal tissues in pathological conditions (Erin and Ulusoy 2009). Micromolar concentrations of SP are needed to evoke plasma extravasation in the skin (Petersen et al. 1994). Release of SP by cutaneous sensory nerves preferentially binds to NK-1Rs (Holzer 1998). Abnormal increase or decrease in NK-1R expression could elevate plasma extravasation, immune cells and increased sensitivity of cells to SP released by primary afferent neurons of small diameter (Yonehara et al. 1987) providing innervations to the epidermis (Harrison and Geppetti 2001).

Neuromodulator peptide SP, present in C fibers of skin, play a vital role in tissue repair (Delgado et al. 2005). SP modulates epithelial cell migration and restitution in vitro by the release of TGF-beta from fibroblasts (Felderbauer et al. 2007) and stimulation of CD29+ stromal like cells (Hong et al. 2009). In this way, SP contributes to the protective mechanism of tissue repair and maintains a mucosal homeostatis (Felderbauer et al. 2007). It induces cytokine release, dilates the blood vessels, increases their permeability and stimulates mast cells to release histamine and all these actions are carried by the activation of endothelial cells (Dunnick et al. 1996). Any dysfunction in the functioning of SP-ergic system may cause delayed wound healing

in humans and expression of SP-IR is elevated in wounds and injuries (Onuoha and Alpar 2001; Dunnick et al. 1996).

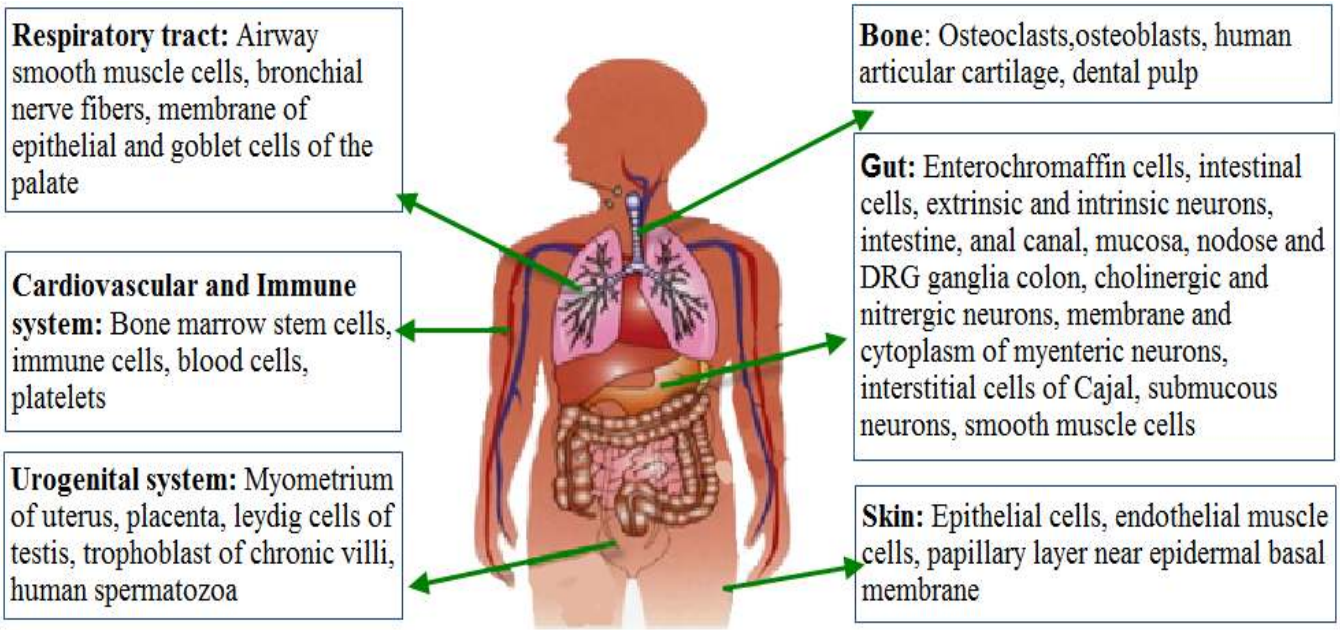


Figure 7: Distribution of SP in different systems of body

Tachykinins are called as brain-gut hormones (Tomita 2009) and SP is found in the gut in abundance (Schmidt et al. 2003). They exerts several pathophysiological effects on gastrointestinal motor disorders such as diarrhea, gastrointestinal inflammation, visceral sensitivity and pain (Holzer 1998), acute intestinal inflammation (Koon et al. 2005), Crohn's disease (CD) (Wang et al. 2006), ulcerative colitis (UC) (Jonsson et al. 2005), acute inflammation of colon (Landau et al. 2007), Irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). These disorders are attributed to imbalance of tachykininergic neurons, expression, their receptors (Holzer 1998) and neurogenic inflammation by the recruitment of immune cells (Sipos et al. 2008). In human histological sections of laryngopharynx and gastrointestine of sudden erethistic death and sudden death of heart attach as control, SP-IR was found more in the experimental group. Thus alterations in SP expression can serve as a diagnostic tool of sudden erethistic death (Sun et al. 2006).

In the digestive tract (Fig. 7), tachykinins (substance P, neurokinin A) are neurotransmitters which regulate motor activity and vascular functions (Holzer 1998). Gastrointestinal tract contains intrinsic and extrinsic neurons, both of which have SP-IR

innervations (Costa et al. 1986). SP serve as neuroimmunomodulator and controls intestinal motility (Lecci et al. 2006), secretion, sensory perception, immune function in intestine (Straub et al. 2006), maintains mucosal homeostasis, tissue repair (Felderbauer et al. 2007), mucosal permeability (Koon et al. 2008) and has protective role in colitis (Felderbauer et al. 2007). SP is involved in secretion of electrolytes and fluid into the lumen of colon by activating cholinergic and noncholinergic neurons and by releasing histamine (Kuwahara and Cooke 1990; Lecci et al. 2006). SP is believed to predominately mediate its effects via NK2 receptors, inducing excitation and contraction (Holzer and Maggi 1994), although NK1 may also play a minor role in this response (Maggi et al. 1994).

There are several reports for the involvement of TKs in reproduction (Patak et al. 2000). All the TKs and their receptors are found to be expressed in uterus of superovulated and unfertilized mice and may play a role in both male (Clement et al. 2009) and female reproductive system (Pintado et al. 2003) (Fig.7). There is a significant up-regulation of NK-1R protein at full term fetus and newborn infant with a peak at day 1 and it downregulates at 8^{th} day, which indicates that NK-1R may be involved in the mechanisms modulating the processes during labour and after birth. SP-IR has an opposite correlation with NK-1R protein expression in pregnant and uterus after birth (Schmidt et al. 2003). Another study has different finding and suggests that SP expression is elevated at term fetus and soon after birth and has a role in cervical ripening and labour mediated by estrogen-ER system, most probably ER-alpha, that is systhezised in DRG (Mowa et al. 2003). A role of SP in stress induced abortions, by causing neurogenic inflammation, has also been implicated. Under the conditions of stress, SP augments the synthesis of decidual Tumor Necrosis Factor (TNF)-alpha, which is a possible trigger of miscarriage (Fest et al. 2006; Joachim et al. 2001).

SP has a role in both normal and pathological hematopoiesis (Nowicki et al. 2007). Hematopoietic effects by TKs can be expressed in neural and non-neural tissues. SP is a hematopoietic modulator. SP activates bone marrow stromal cells to produce hematopoietic cytokines (Adamus 2009). Bone marrow nerve fibers containing SP are a neural SP source while stromal SP provides a non-neural source (Rameshwar and Gascon 1997). Hematopoietic effects of SP are mediated by NK-1R, bone marrow and growth factors (Rameshwar and Gascon 1995). Any imbalance in hematopoietic-neurotransmission immune axis can result in hematological malignancies (Adamus 2009). It is also found to be involved in the regulation of platelet function

and thrombus formation by mobilizing intracellular calcium and degranulation. Platelets play an important role in homeostasis, with inappropriate platelet activation being a major contributor to debilitating and often fatal thrombosis by causing myocardial infarction and stroke. SP-IR observed in platelets, is secreted during activation and stimulates platelet activation by positive feedback mechanism (Jones et al. 2008). SP-mediated platelet aggregation is mediated by NK-1R (Graham et al. 2004).

Metabolism of skeletal system is controlled by the nervous system. About 10 neurotransmitters have been identified in bone and SP is one of them (Liu et al. 2007). Tachykinin peptide SP and its receptor NK-1R has been found in bone cells (Goto et al. 2007) where they are known to stimulate the process of repair, formation and resorption of bone (Li et al. 2010) mediated by NK-1Rs at late stage bone formation (Goto et al. 2007). SP receptors are localized in osteoclasts (Liu et al. 2007). SP is also involved in nociceptive responses in bone e.g. pain produced by cervical facet joint distractions (Lee and Winkelstein 2009) and osteoporosis (OP) (Liu et al. 2008).

2.4 Trigeminal System

Trigeminal system is highly established and well studied system in mammals and birds (Davies 1988). SP-IR has been observed in trigeminal (Lee et al. 1985) and dorsal root ganglia (DRG) (Gibbins et al. 1987). Main feature of this system is the presence of two distinct groups of primary afferent neurons: Trigeminal Ganglion (TG) and Mesencephalic Trigeminal nucleus (MTN). Cell bodies of these primary afferent neurons are present in TG (Lazarov 2000) and few lie in MTN. MTN is involved mainly in proprioception (mainly orofacial musculature) (Nagy et al. 1986). TG dorsomedial part is involved in nociception, thermoreception and proprioception while TG ventrolateral part is involved in mechanoreception (Lazarov 2002). Signals from the trigeminal system are transmitted by second order neurons in brainstem to different regions of CNS pain centers (Eftekhari et al. 2010). The central processes of the TG terminate on several groups of second order neurons, whose impulse are conveyed to the somatosensory cortex via thalamus (Pfaller and Arvidsson 1988) (Fig. 8).

2.4.1 Trigeminal Ganglion

Trigeminal ganglion (TG) is accumulation of pseudounipolar neurons (Krastev 2008) and consists of neurons and their fibers. TG is the center of interest for studying the expression of SP due to the activation of the trigeminal nerve system found in this study in the brainstem of

perinatal death victims as compared to the normal ones. TG is a cranial analog of DRG in PNS (Lazarov 2002). Activation of TG nerves play a central role in most forms of orofacial pain (Takemura et al. 2006). A variety of transmitters and their receptors have been described in different subsets of TG neurons (Lazarov 2002). TG neurons supply innervations mostly to the mechanoreceptors, receptors for thermoregulation and pain regulation in face, oral and nasal cavity (Dubner 1978; Davies 1988). Glial cells also known as satellile cells (Pannese 1981) completely enclose the neuronal somata of TG neurons and thus they have no synaptic contacts (Lieberman 1976).

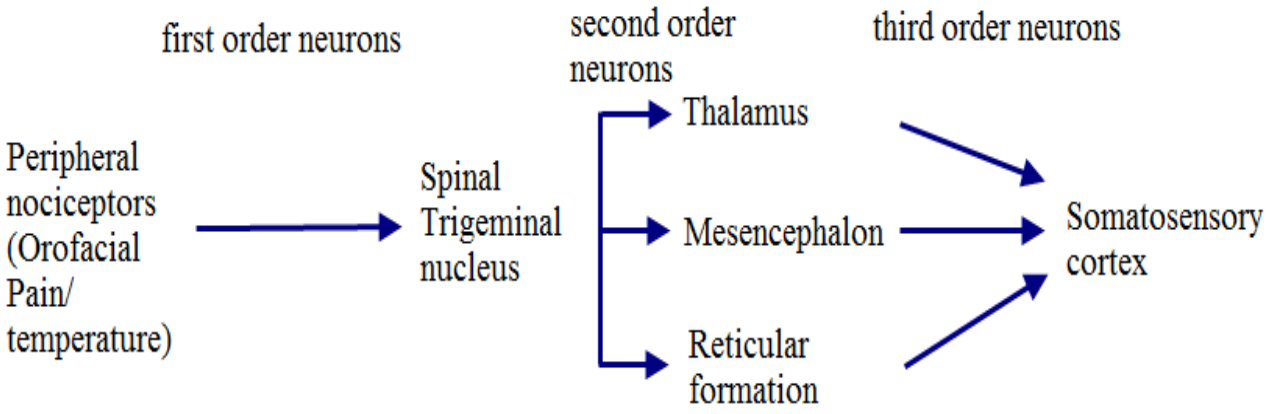
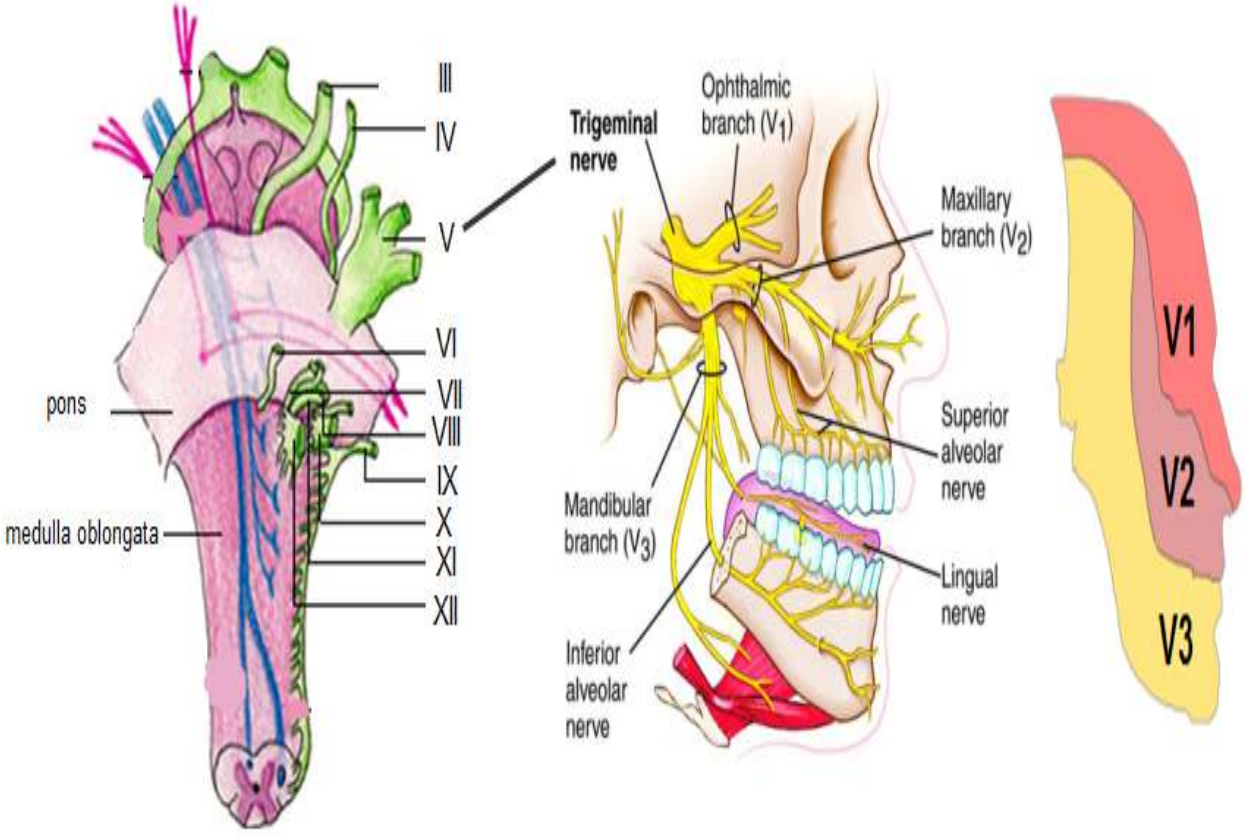


Figure 8: Processing of nociceptive stimulus within brain

Based on their appearance, ganglion cells are classified as follows: large light (A) and small dark (B) cells (Gaik and Farbman 1973). Thick myelinated fibers originate from large light A cells and thin myelinated and unmyelinated C fibers derive from small dark B cells (Scharf and Rowe 1958). Two primary afferent neurons subpopulations are noticed in TG: Small and medium sized neurons with small somata, including a number of Glutamate (Glu), Substance P (SP), Somatostatin (SOM), Neurokinin A (NKA), CGRP, Cholesystokinin (CCK), Vasoactive intestinal peptide (VIP) and Galanin-IR and larger sized neurons that are relatively less and includes neuropeptide Y (NPY) and Peptide 19 (PEP 19)-IR trigeminal neurons. The majority of large ganglion cells are surrounded by SP, CGRP, SOM, CCK, VIP, NOS and SER-IR periosmotic networks (Lazarov 2002). Presence of SP in small diameter primary afferent fibers and in nociceptive centers of brain gives us an idea of its nociceptive role (Hunt and Rossi 1985; Levine et al. 1993).

2.4.1.1 Trigeminal Nerve

Trigeminal ganglion provides somatosensory innervations of face and oral cavity through trigeminal nerve. Trigeminal nerve is 5th and the largest of cranial nerves and its name is derived from the fact that it has 3 major branches each providing inervation to distinct regions of head, face and oral cavity (Voogd and Glickstein 1998). V1 and V2 are purely sensory while V3 (Fig. 9) has both sensory and motor functions. V1 innervates forehead, upper eyelid, cornea, conjunctiva, mucosa of frontal ethmoid and sphenoid sinuses and dorsum of nose (Fig. 9). V2 innervates upper lip, lateral portions of nose, parts of oral cavity, mucosa of nasal cavity, maxillary sinus, upper jaw and roof of mouth and upper dental arch while V3 innervates lower lip, chin, cheek, lower teeth, gingival, mucosa of lower jaw, floor of mouth and anterior two thirds of tongue (Usunoff et al. 1997; Takemura et al. 2006) (Fig. 9).



A **B** **C**

Figure 9: Trigeminal nerve and its branches; **A:** Brainstem exhibiting 12 cranial nerves, trigeminal nerve is 5th, **B,C:** Trigeminal nerve providing innervations to forehead, eyes (V1); to upper lip, oral cavity, upper jaw (V2) and to lower jaw, chin, lower lip and tongue (V3)

2.4.1.2 Structural Organization of Trigeminal Nerve Nucleus

Trigeminal nerve nucleus is composed of three nuclei, mesencephalic, chief sensory and spinal trigeminal nucleus (Fig. 10) and two types of glial cells; satellite glial cells and schwann cells (Hanani 2005; Hanani 2010). Spinal trigeminal nucleus is located caudally and nociceptive sensory information from orofacial region is first processed here. This nucleus is further divided rostro-caudally into subnuclei oralis, interpolaris and caudalis (Vc) (Olszewski 1950) (Fig. 10). Nociceptive information from face and oral cavity is first processed in Vc (Sessle 2000) through the release of SP (Aita et al. 2005). Trigeminal primary afferent neurons conduct neurotransmission by peptide transmitters, calcium binding proteins and other neuroactive molecules (Lazarov 2000). Environmental factors and stressors alters the neuronal content of these transmitters (Copray et al. 1990). Among all the TKs, SP undoubtedly acts as a transmitter substance in primary sensory neurons (Konishi and Otsuka 1985). 10-20% of TG cells show strong IR to both SP and NKA (Tornwall et al. 1994). Earlier IHC studies indicate that SP is present in primary sensory neurons of TG in almost all mammals (Hokfelt et al. 1976), e.g. guinea pigs, rats, monkeys (Terenghi et al. 1985), rabbits (Tervo 1981), cats (Lazarov and Chouchkov 1996) and humans. Furthermore, in human TG, the percentage of SP containing cells varies with age, declining from about 24% in newborns to about 17% in adults (Del Fiacco et al. 1990). Neuropeptides in the trigeminal sensory neurons have significant functions in disease mechanisms associated with head pain in humans (Goadsby et al. 1988; Otsuka and Yanagisawa 1990).

The trigeminal subnucleus caudalis (Vc) is critical site for the processing of pain stimulus from orofacial region (Aita et al. 2005). Trigeminal pain processing has been extensively studied in trigeminal subnucleus caudalis (Vc) (Fig. 10) and now studies are focusing on other aspects of trigeminovascular system beyond Vc or MDH (Bereiter et al. 2000),(Dubner and Ren 2004),(Ren and Dubner 1999). Activation of neurons in ventral portion of Vi/Vc transition zone along with caudal part of Vc has been observed after orofacial injury (Strassman and Vos 1993),(Hathaway et al. 1995). Various studies suggest that glia and inflammatory cytokines are involved in the development of constant nociception (Ren and Dubner 2008; McMahon et al. 2005). Transition zone of Vi/Vc contributes to the deep orofacial pain processing (Chattipakorn, et al. 2002).

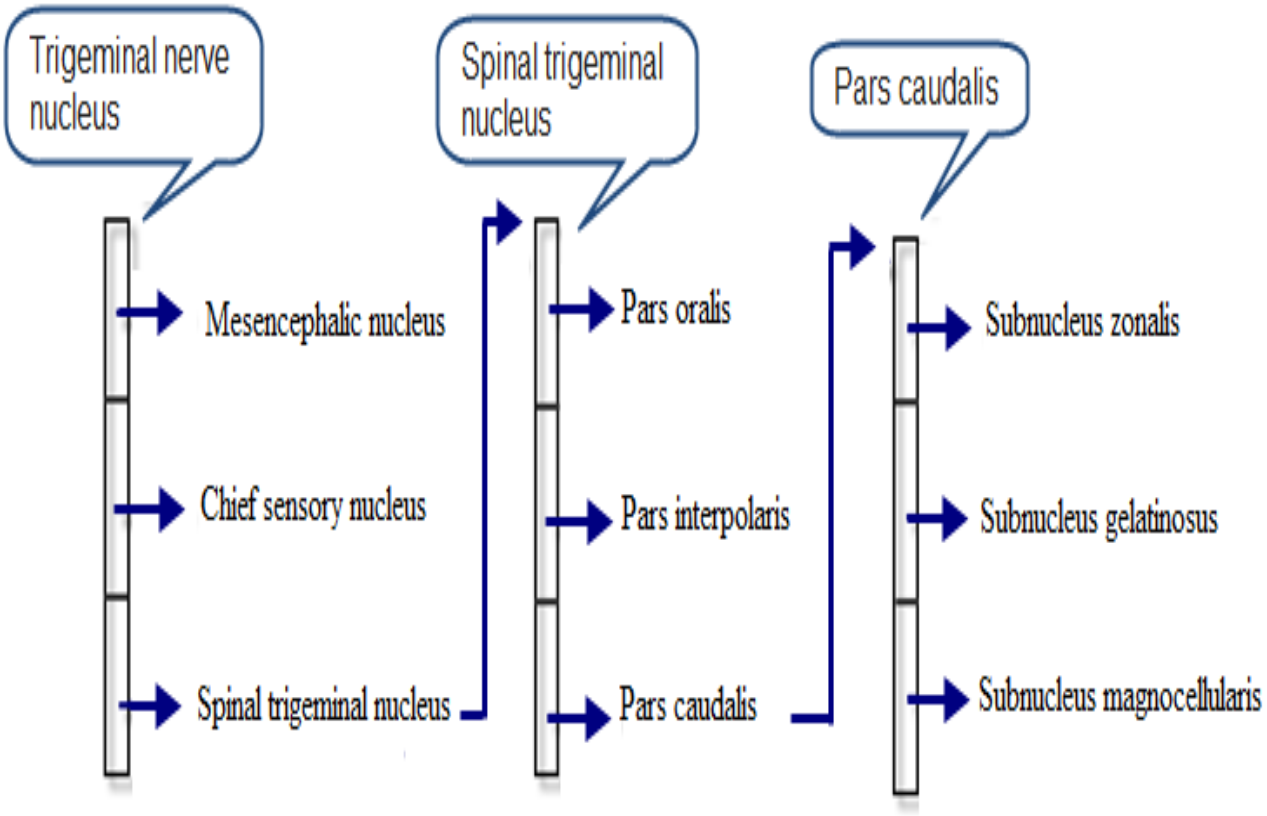


Figure 10: Graphical representation of Trigeminal nerve nucleus (TrN)

2.4.2 Mesencephalic Trigeminal Nucleus

Subpopulation of large sized neurons in Mesencephalic Trigeminal Nucleus (MTN) exhibit Glutamate immunoreactivity. Most of the interneurons in MTN are GABAergic and other neurotransmitters are not found here (Lazarov 2002). A single identified nucleus in CNS that has cell bodies of primary afferent neurons is MTN (Johnston 1909; Freeman 1925). Another peculiarity of MTN is that they are " proprioceptive neurons" of the trigeminal system (Dubner 1978; Davies 1988). MTN neurons mostly innervate muscle spindles in the masticatory (Capra et al. 1985; Alvarado-Mallart et al. 1975) and extraocular muscles (Alvarado-Mallart et al. 1975) and other types of receptors in the periodontal ligaments (Byers and Dong 1989; Linden et al. 1994) and dental pulp (Yoshino et al. 1989). MTN neurons are functionally homogeneous (Linden 1978) and without exception are involved in sensory aspects of orofacial proprioceptive information processing while trigeminal Primary afferent neurons are chemically heterogeneous (Lazarov 2000).

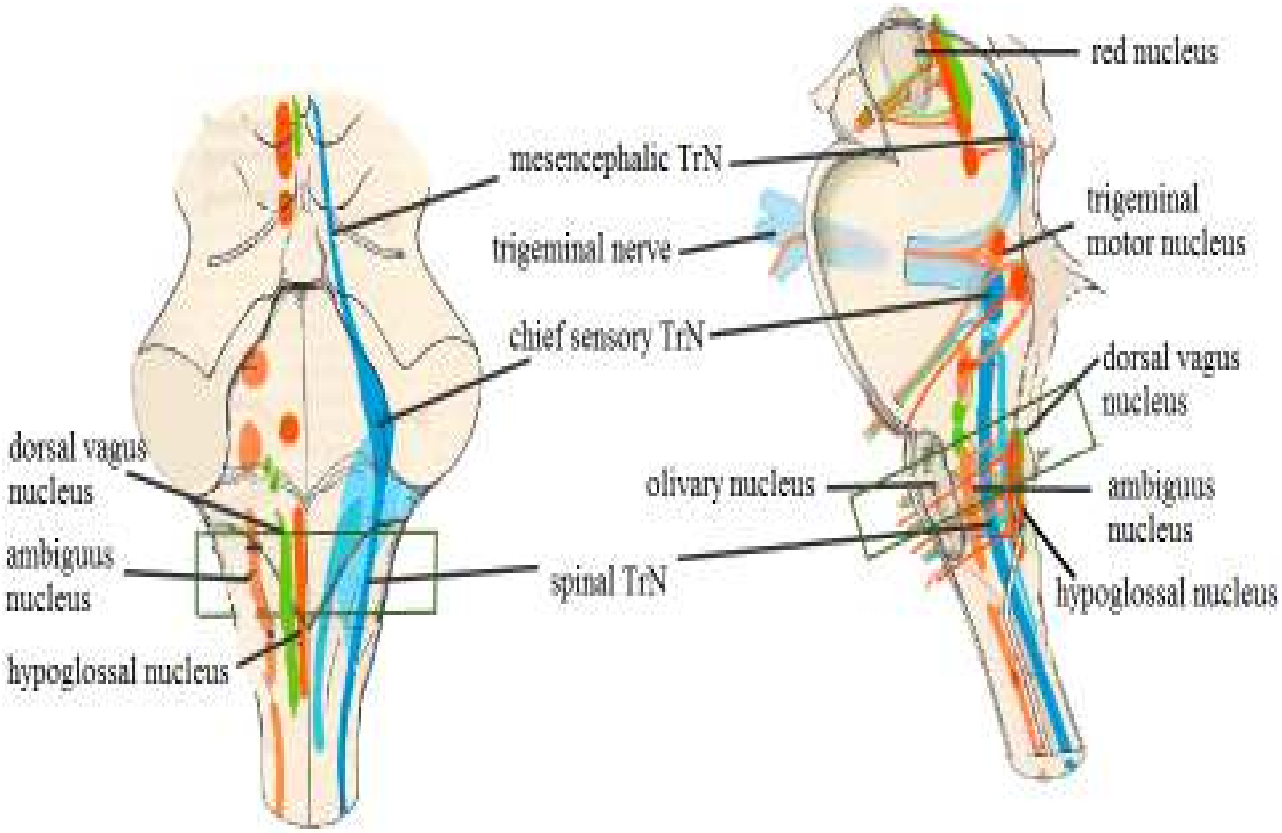


Figure 11: Brainstem showing trigeminal nucleus and also show the level of sampling A) Ventral view B) Side view: showing trigeminal nerve, mesencephalic, chief sensory and spinal trigeminal nucleus (Kahle and Frotscher 2005).

CHAPTER III

AIM OF STUDY

The goals of this study were:

1) to obtain basic information about the expression of the SP during the first phases of human nervous system development;

2) to evaluate whether there are altered manifestations of this neuromodulator in victims of sudden death;

3) to verify the correlation with maternal cigarette smoking.

4) to understand the network properties, evolution and duplicability of the gene encoding substance P (Tachykinin 1) by computational analysis.

3 Material and Methods

A total of 56 brains were collected from 24 fresh human fetuses (17-40 gestational weeks) and 32 infants aged 1-10 months. In 36 cases, after the in-depth anatomo-pathological examination the death remained totally unexplained. A diagnosis of SIUD (sudden intrauterine unexplained death) was established for 16 fetuses, who died suddenly before complete expulsion or retraction from the mother, and of SIDS (sudden infant death syndrome) for 20 infants who died within the first year of life. In the remaining 20 cases, 9 stillbirths, and 11 infant deaths, a precise cause of death was formulated at autopsy. These cases were used as controls. The related infant death diagnoses in this group were: congenital heart disease (n=5), severe bronchopneumonia (n=2), myocarditis (n=1), pulmonary dysplasia (n=2), and mucopolysaccharidosis type I (n=1). Specific diagnoses among the fetal deaths included: chorioamnionitis (n=6) and congenital heart disease (n=3).

This was a selected set of cases, all sent to "L.Rossi" Research Center in application of the 2006 guidelines stipulated by Italian law n.31 "**Regulations for Diagnostic Post Mortem Investigation in Victims of SIDS and Unexpected Fetal Death**". This law decrees that all infants with suspected SIDS who died suddenly in Italian regions within the first year of age, as well as all fetuses who died without any apparent cause, must undergo, after parental consent, an in-depth anatomo-pathological examination, particularly of the autonomic nervous system (Matturri et al. 2005; Matturri et al. 2008). Permission from ethic committee was not required for this study as our Research Center is the referral national center for the sudden unexplained fetal and infant death, according to above-mentioned Italian Law n. 31.

For every case, a complete clinical history was collected. Additionally, mothers were asked to complete a questionnaire on their smoking habit, detailing the number of cigarettes smoked before, during and after pregnancy. Fifteen of the 36 SIDS/SIUD mothers (42%) were active smokers before and during the pregnancy, smoking more than 3 cigarettes/ day. The remaining 21 mothers (58%) admitted no history of cigarette smoking. Four of the 20 mothers in the control group (20%) reported a smoking habit, while the remaining 16 mothers (80%) were non smokers.

3.1 Protocol for the examination of Brainstem

Anatomo-pathologic study of the brainstem (medulla oblongata, pons and midbrain) includes sampling of three specimens (Fig. 12), after fixation for 3-4 days in 10% buffered formalin. Structures participating in control of the vital functions are located (cardiorespiratory, arousal, upper digestive tract, etc) here. The first specimen, ponto-mesencephalic, includes the upper third of the pons and the adjacent portion of mesencephalon. The second extends from the upper third of the medulla oblongata to the portion adjacent to the pons. The third specimen takes as reference point the obex and extends 2-3 mm above it and below it (Fig. 12 and 13). In all cases, it is important to avoid pulling of leptomeninges, as it will cause damage to the ventral surface of medulla oblongata. This simple procedure of brainstem examination in both fetus and infant, provides a significant reduction of sections to just 3 samples of medulla oblongata and allow to examine the various nuclei in cranial, middle and caudal part of pontine-mesencephalic sample.

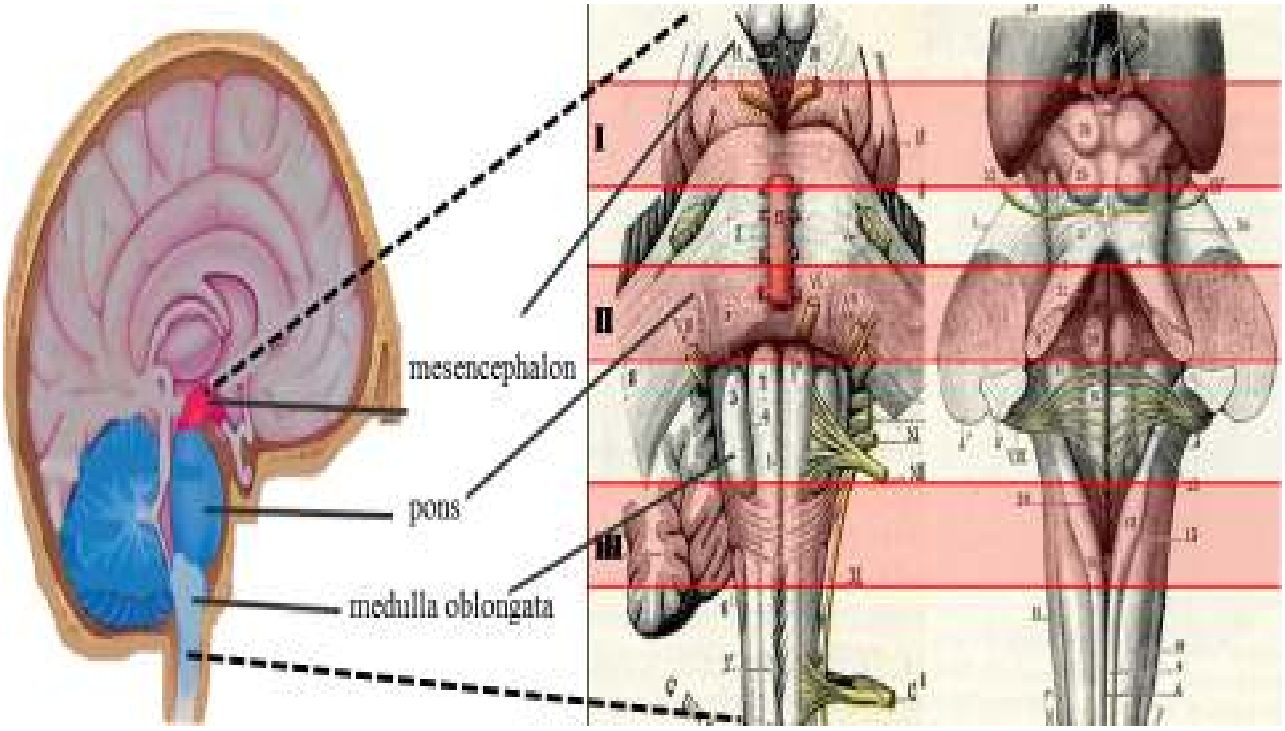


Figure 12: Sampling of the brainstem from brain ventral (left) and dorsal (right) surface. Structure of brainstem [Modified from L. Testut, "Anatomia Umana", Unione Tipografico-Editrice Torinese, Torino, 1923]

3.2 Preparation of solutions

Solutions were prepared with distilated water. Citrate buffer, Phophate buffered saline (PBS), Xylene, Acetone, Alcohol, Hydrogen peroxide were used in this study. All solutions used are named in the text.

3.2.1 Preparation of Phosphate buffered saline (PBS)

Dissolved one tablet of PBS (Sigma, Batch no. 124K8202) in 200 ml of distilled water and stored it at 5^{o}C

3.2.2 Preparation of Citrate buffer

Dissolved one tablet (Sigma, Lot no. 123K8209) in 100ml of deionized water to obtain 0.05 M phosphate citrate buffer (PH 5.0).

3.2.3 3% Hydrogen peroxide solution

For preparing 3% Hydrogen peroxide solution, I used 6ml of 30% Hydrogen peroxide (PROLABO) in 200ml of PBS.

3.2.4 Blocking Serum (Normal Serum)

Added three (3) drops (150 µl) of stock (yellow label from ABC kit) to 10 ml of buffer (PBS) in mixing bottle (yellow label). The preferred serum for blocking is prepared from the same species in which the biotinylated secondary antibody is made. Apart from blocking serum, ABC kit also has biotinylated secondary antibody and ABC reagent.

3.3 Primary Antibody

Rabbit Polyclonal Anti-Substance P 4-11, conjugated to thyroglobulin, ARP (American Resarch Products), Inc., 1:80

3.4 Secondary Antibody

Biotinylated secondary antibody from Avidin Biotin Complex kit (ABC kit Vectastain, Vector laboratories Inc. Burlin game, CA, USA). Added three (3) drops (150 µl) of normal blocking serum stock from ABC kit (yellow label) to 10 ml buffer (PBS) in mixing bottle and then added one (1) drop (50 µl) of biotinylated antibody stock (blue label from ABC kit).

3.5 Dyes, Substrates, Pretreatment, Embedding Media, Counterstains and Mountant

3.5.1 Embedding Media

BioPlast Paraffin wax was used for embedding (Bio-Optica) having a melting point of 57-58^{o}C.

3.5.2 STA-ON

Tissue section adhesive was put in hot water bath while shifting the sections from water bath to slides.

3.5.3 ABC Reagent

Added exactly two drops of REAGENT A (gray label from ABC Vectastain kit) to 5 ml of buffer (PBS) in the ABC Reagent large mixing bottle. Then added exactly two drops of REAGENT B (gray label) to the same mixing bottle, mix immediately, and allowed to stand the reagent for 30 minutes before use.

3.5.4 Horseradish Peroxidase Substrates

Diaminobenzidine (DAB) (Vector laboratories) substrate kit was used which yields a brown chromogenic precipitate. This solution is made immediately before use. To prepare the substrate solution, I took 5 ml of distilled water, added two drops of buffer stock solution and mixed it well. Then added 4 drops of DAB stock solution and mix it well again. Added 2 drops of hydrogen peroxidase solution in this solution and mixed it thoroughly.

3.5.5 Dyes and Counterstains

Methyl green was used for counterstaining of nuclei. It was prepared by dissolving 1g of methyl green powder in 1ml of glacial acetic acid and then it was mixed in 99ml of distilled water to make the volume 100ml. Solution was then mixed on stirrer without heating. Solution was filtered through a 0.45 or 0.2 micron filter prior to use.

3.5.6 Mountant

DPX mountant (BDH chemicals Ltd. Poole England).

3.6 Equipment

Unless stated otherwise, general lab equipment was used for the immunohistochemical techniques, including–stop watch, microscope, balances, heating and stirring hot plate, incubation ovens, fridge and refrigerators, micropipettes, pH meter, whatman filter paper, chemical hood, vortex mixer and waterbaths. Glassware included graduated cylinder, flask for filteration, volumetric bottles for solutions, slides, coverslips, coplin jars and beakers of different sizes. Plastic ware includes Wet chambers, slide racks with removeable handles, test tube racks, pipette stands, heat and chemical resistant coplin jars. Sections were obtained using the microtome LEITZ model 1400. A NIKON EClLIPSE E800M was used to document whole mount images.

3.7 Processing of Brainstem Manually

After fixation in 10% phosphate-buffered formalin, the processing of the brain stem and spinal cord was done manually, using different grades of alcohol for dehydration and xylene located under the hood and then samples were embedded in paraffin. This grading scale of alcohol was used for brainstem and many cases can be processed at the same time because the samples are in small plastic cages (megacassettes) or small bags and appropriately marked by the case number, and the sample inside. When samples are more, they should be put in larger containers and processed always under hood. Reagents were changed or atleast filled after each processing, when reagents level was decreased.

3.8 Embedding of Brainstem Tissue Sample

The brainstem and cerebellum, the main structures analyzed in our studies, were processed and embedded in paraffin. After embedding, it was allowed to cool down in fridge for overnight.

3.9 Protocol for Histology of Brainstem

Transverse serial sections of the midbrain, pons, medulla oblongata, and cerebellum samples were made at intervals of 20-30 μm. For each block, 15 levels were cut and 12 serial sections (1 HE+3 normal blank+8 treated with silane) of 5 μm each were obtained from each level by using STA-ON (in water bath). Slides were placed in oven at 37ºC overnight. In the next morning, took one slide for histological examination using hematoxylin-eosin, one was submitted to immunohistochemical study of SP and the remaining sections were saved and stained as deemed necessary for further investigations. Klüver-Barrera is done if required.

The routine histological evaluation of the brainstem was focused on the locus coeruleus and the parabrachial/Kölliker-Fuse complex in the rostral pons/caudal mesencephalon, on the retrotrapezoid nucleus, the superior olivary complex and the facial/parafacial complex in the caudal pons; on the hypoglossal, the dorsal motor vagal, the tractus solitarius, the ambiguus, the pre-Bötzinger, the inferior olivary, the raphe and the arcuate nuclei in the medulla oblongata. In the cerebellum, the cortex layers (external granular layer, molecular layer, Purkinje cell layer and internal granular layer) and the medullary deep nuclei (the dentate nucleus, the fastigial nucleus, the globose nucleus and the emboliform nucleus) were examined.

3.10 Hematoxylin-Eosin Staining

For HE staining, slides were placed in xylene I and II for 10 minutes each and then in different grades of alcohol in decreasing manner from absolute to 60% (100%,95%,80% and 60%) to hydrate them. Washed the slides with tap water for 5 minutes and then stained them with Mayer hematoxylin for 15 minutes. Slides were put under running water for 10 minutes. Colour of hematoxylin changed from violet to blue. Placed the slides in 0.5% Eosin for 13 seconds and dipped in water once. Dehydrated the slides by giving one dip in grades of alcohol in increasing manner (60%,80%,95% and 100%). Slided were put in xylene III and then xylene IV for 5 minutes each and then mounted.

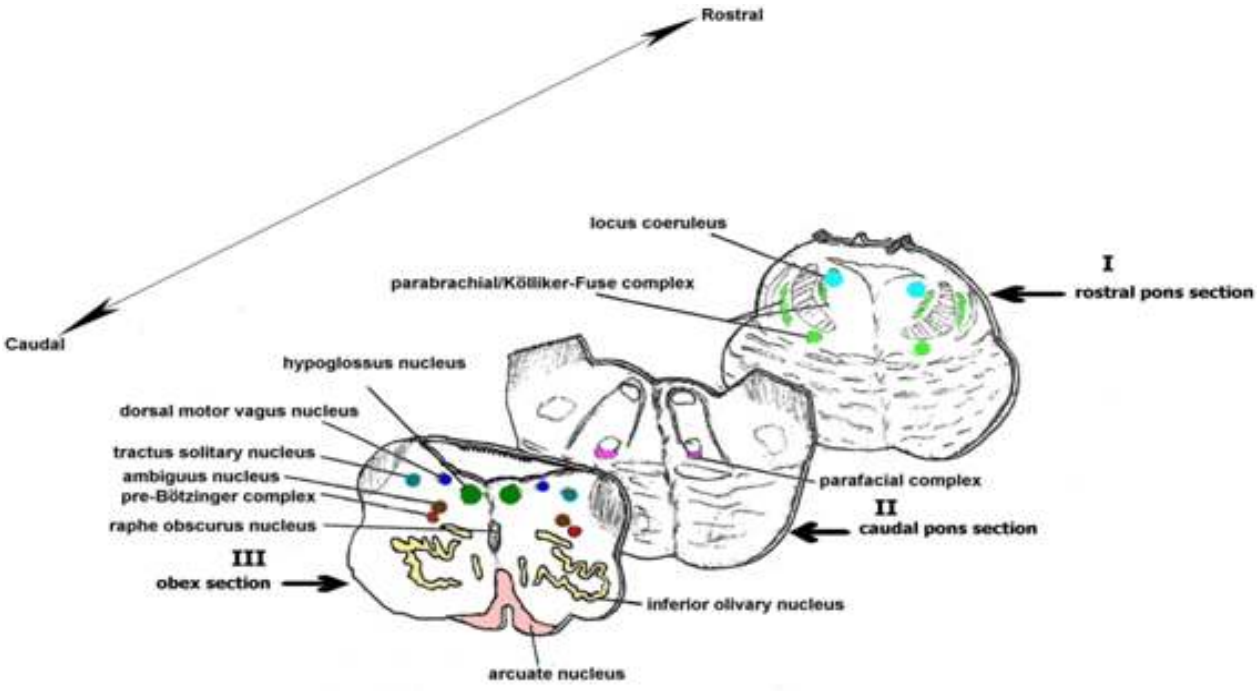


Figure 13: Diagram of the histological sections corresponding to the three fragments obtained from the sampling procedures of brainstem for the anatomo-pathologic examination, indicating the main nuclei and structures to be examined.

3.11 Preparation of Slides for immunohistochemistry

To inhibit binding of antibodies to slides and to help in adhesion of sections to slides stongly (during immunohistochemical reactions and heating in oven), slides were pre-treated with 3-Aminopropyl triethoxy silane (Sigma, Code No. 3648). Slides were put 3 times in 70% alcohol with HCl 1% for 10 minutes and then a solution of acetone I and chloroform (1:1) was used to wash slides for 10 seconds. Slides were then placed in a solution of 3-Aminopropyl

triethoxy silane and acetone (1:25). Slides were given another wash in solution of acetone + chloroform (1:1) for 30 seconds and in ditilated water for 15 seconds. Then slides were placed in oven at 37^{o}C overnight.

3.12 Substance P Immunohistochemistry (IHC)

Sections were deparrafinized with xylene 1, xylene II and different grades of alchohol (100%, 90%, 70%), washed in distilled water and then phosphate buffered saline (PBS). The slides were pretreated in a microwave oven using citrate buffer (PH=6) (3X for 1 minute at 440W, 4 minute at 250W). Slides were then allowed to cool for 20 minutes. Blocking of endogenous peroxidase was done by using 3% H_2O_2 for 30 minutes after washing with distilled water and phosphate buffered saline. Sections were incubated overnight with primary rabbit polyclonal anti-substance P (4-11) antibody at a dilution of 1:80 after washing with phophate buffered saline. Immunohistochemical staining was performed by using peroxidase-antiperoxidase method and the avidin biotin complex technique (ABC kit Vectastain, Vector laboratories Inc. Burlin game, CA, USA) for 30 minutes. Diaminobenzidine (DAB, Vector Laboratories Inc.) was used as chromogen substrate and sections were incubated in DAB for 15 minutes and counterstained with methyl green for 15 minutes at 60ºC. Sections were given quick wash in acetone I and then again in acetone II for 10 minutes. Negative controls of the same tissue were done using phosphate-buffered saline instead of primary antibody. Sections were washed in xylene I and II for 5 minutes each and mounted.

The examination of slides was performed in blinded fashion, without initial knowledge of the cause of death, age or other clinicopathologic information. Only after the histologic and immunohistochemical assessment of the brainstem and cerebellum had been completed, the findings were matched with the corresponding records.

3.13 Statistical analysis

The association between immunohistochemical data, the main target of this study, and victim groups was evaluated by Cox regression analysis. The statistical value of the correlation was established at the $p<0.05$ level using one way analysis of variance followed by t-test or Student's t-test.

3.14 Data mining for TAC1

I have also looked at the system level properties such as genomic duplication, network properties and evolution of TAC1 gene. Firstly, I searched orthologs of TAC1 by using eggNOG

Version 2.0 (http://eggnog.embl.de). I found TAC1 gene in 26 species of vertebrates and then multiple sequence alignment was performed for the subsequent computational analysis.

3.14.1 Genomic duplicability of TAC1 gene

To find duplicates or paralogs of TAC1 in human genome, I have used UCSC (University of California Santa Cruz) genome browser (www.genome.ucsc.edu). It uses BLAT (BLAST like Alignment tool) for the sequence alignment of proteins and DNA. I considered a gene as duplicate if it has additional hit on the genome and as singletion if it has only one hit on the genome.

3.14.2 Phylogenetic analysis.of TAC1 gene

MAFFT, online version 6.0 (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/), was employed for multiple protein alignments using the E-INS-i strategy with the default parameters. To estimate the phylogenetic relationships of the sequences I performed distance-based analyses using the Neighbor Joining (NJ) (a bottom up clustering method used for the construction of phylogenetic trees) programs.

3.14.3 Network properties of TAC1 gene

Predictions for TAC1 interactors were performed using STRING 8.3 database (string-db.org). It is a database of known and predicted protein-protein interactions. The interactions include direct (physical) and indirect (functional) associations. They are derived from the sources of gemomic context, high throughput experiments, conserved coexpression and PUBMED.

3.14.4 Generation of Amino acid Sequence Logos

Sequence logos were generated using a web-based program, Weblogo, version 3.0 (http://weblogo.threeplusone.com/) developed by Crooks (Crooks et al. 2004) and Schneider and Stevens (Schneider and Stephens 1990). A logo was generated with amino acid sequences from preprotachykinin 1 gene of 26 vertebrate species (Fig. 23) including homosapiens. It is a web based application designed to generate sequence logos. Sequecne logos are a graphical representation of an amino acid or nucleic acid multiple sequence alignment. Each logo consists of stacks of symbols, one stack for each position in the sequence. The overall height of the stack indicates the sequence conservation at that position while the height of the symbols within the stack indicates the relative frequency of each amino or nucleic acid at that position.

CHAPTER IV

4 RESULTS

Firstly, we proceeded to define the localization of the SP-IR (Substance P immunoreactivity), the target of this study, in the brainstem of fetal and infant control cases. SP-positivity appeared mainly in fiber-structures, mostly comprised of dot-like varicosities and with densities varying from low to very high. These SP-IR fibers, generally surround non-IR neurons. Only a low number of SP-immunoreactive perikarya was sometimes found. Strong immunopositivity was constantly observable, in the area of the trigeminal nucleus (TrN) in the dorsolateral part of the medulla and in rostral spinal cord both before and after birth.

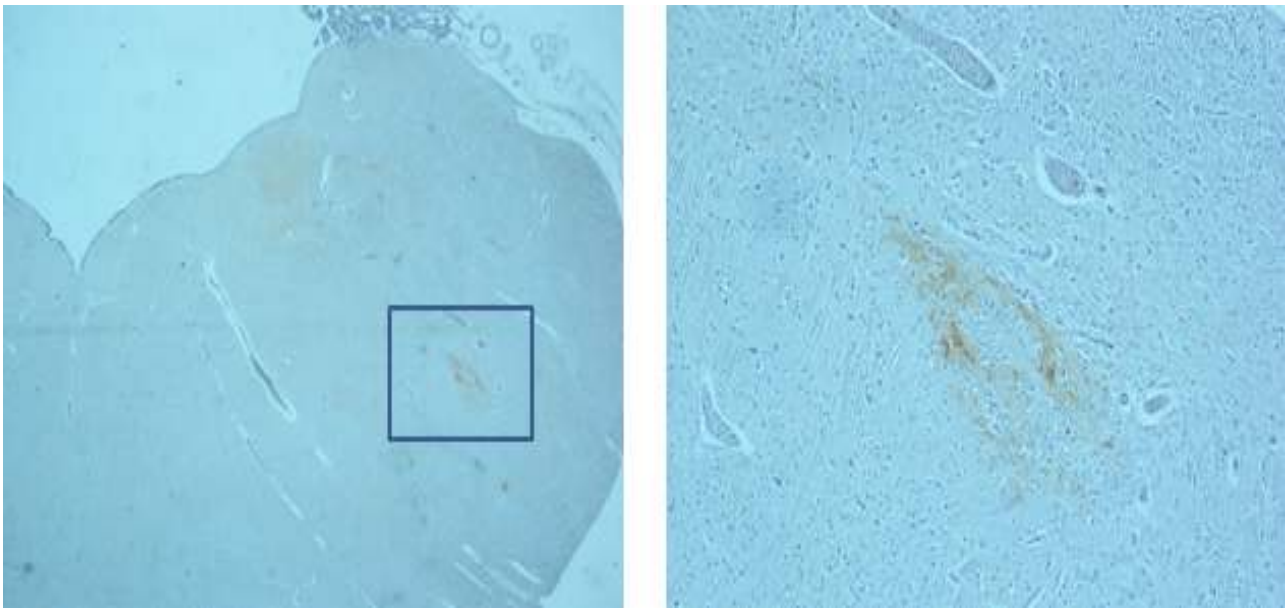


Figure 14: Photomicrograph of a transverse histological hemisection of medulla oblongata (dorsolateral portion) of a fetus of control group (died at 18th gestational weeks), showing in the boxed area (magnified in B) the trigeminal nucleus with poor number of positive fibres of the tract. Substance P immunostain. Magnification: **A)** 10x; **B)** 20x

Given the constant presence of SP-immunostaining in the TrN (Trigeminal nucleus), we tried, with the help of the immunohistochemistry and following the indications exclusively provided by experimental studies in this field (Luccarini et al. 1998; Phelan and Falls 1989; Athanassiadis et al. 2005), to elicit basic data on the morphological and functional developmental steps of this nucleus, so difficult to identify in histological sections of human brainstem, due to its indefinite boundaries. Classical studies in rodents consider this nucleus as a

neuronal complex including, throughout its extension, the “principal sensory nucleus” in the pons and the “spinal trigeminal nucleus” located in the medulla and rostral segments of the spinal cord. The spinal TrN is divided into three subnuclei, the oral, the interpolar and the caudal (Fig. 10), adjacent to the spinal trigeminal tract that appears to be layered on transversal sections. The spinal caudal subnucleus, in turn, includes a magnocellular core, composed of medium-large sized cells with abundant Nissl substance.

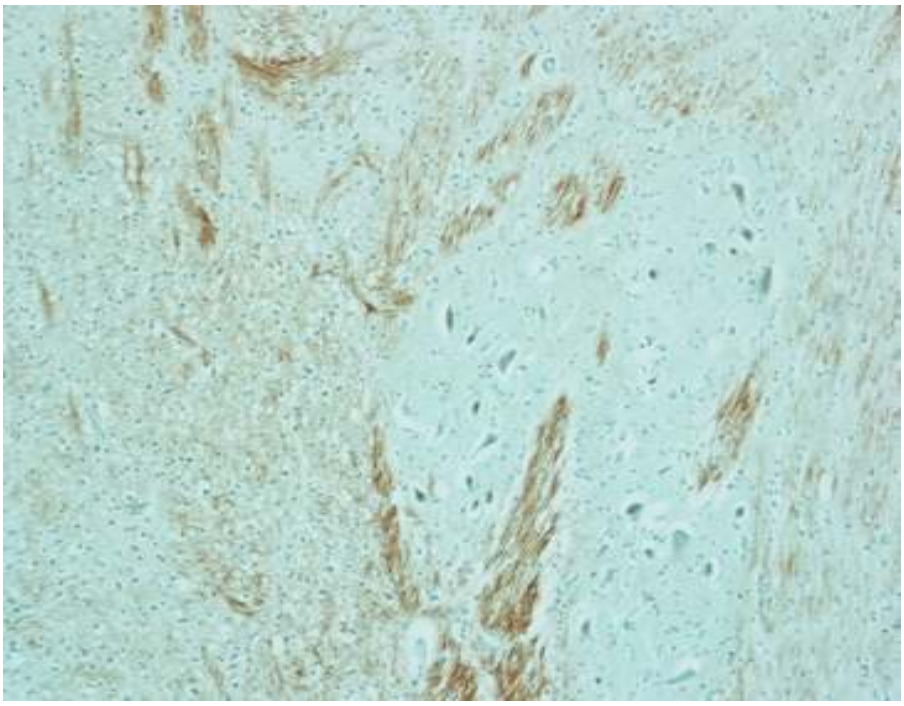


Figure 15: SP-IR of fibers in trigeminal nucleus, with interstitial island of immunonegative neurons, in an infant died at 4 months of life of bronchopneumonia. Substance P immunostain. Magnification: 40x

4.1 Developmental cytoarchitecture of the human spinal TrN in control fetal and infant deaths

At the earliest observation (17^{th}-18^{th} gestational week) the TrN in the coronal sections of medulla oblongata and rostral spinal cord (corresponding to the “spinal TrN” in rodents) shows low number of positive fibres in correspondence of the tract, surrounding undifferrentiated small rounded neurons, without defined outlines (Figure 14 A and B). This cytoarchitectonic feature persists in the following gestational weeks, with the only difference that the tract is increasingly

thickened and stratified. The marginal immune-negative cells frequently appear medium-sized and pear-shaped or polygonal with sketchy axons and dendrites.

The most conspicuous positive SP-fibre plexus of the spinal TrN is found at birth and in the first months of life, with interstitial island of immunonegative neurons embedded in bundles of the tract (Figure 15). Precisely, SP-immunoreactive fibres, that appear to be layered in transversal sections, form distinct pericellular arrays around the somata and dendrites of neurons. These interstitial cells show various edges: triangular, rounded, polygonal or star-shaped. They send thin dendrites interconnecting the cells and achieving the plexus.

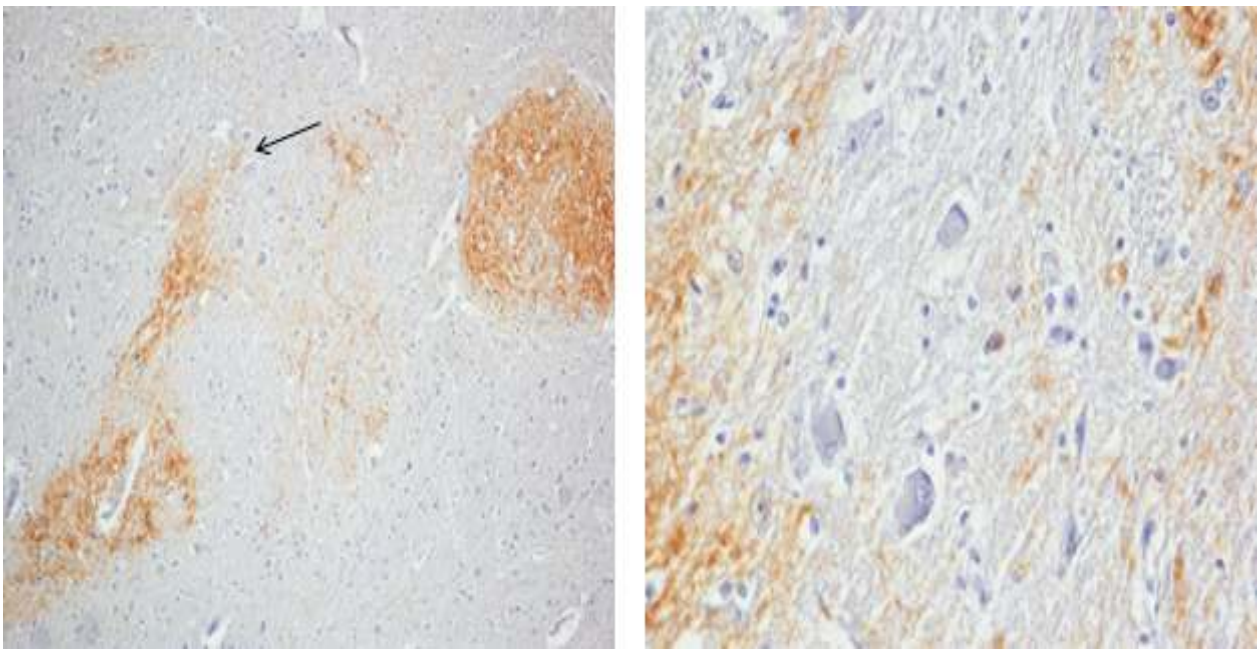


Figure 16: Magnocellular core (see arrow) of spinal trigeminal nucleus in infant of contol group aged 2 months at **A:** lower (20X) and **B:** higher (40X) magnification of large neurons. Substance P immunostain.

A magnocellular group consisting of large lightly stained neurons with peripheral Nissl substance, either grouped or disseminated, are also found at these levels, corresponding to the magnocellular core of the caudal subnucleus in rodents (Figure 16 A and B). We can identify the oral limit of the human spinal caudal subnucleus and the shift to the interpolar TrN in correspondence of the disappearance of these neurons. Overall, we observed during development a slight, progressive increase of the spinal TrN outlines, and in particular of the tract and

neuronal cell body areas, whereas the neuronal density remained steady. In infant deaths, in addition to the TrN, the raphé complex and the reticular formation displayed a variable density of SP-immunoreactivity (Figure 17).

4.2 Neuropathology of the spinal TrN in unexplained perinatal death and SIDS

We observed different distribution of SP-binding sites limited to the spinal TrN in 21 victims of unexplained death (33%) compared with the age-matched controls. Precisely, dense plexus of SP immunoreactive fibres in the TrN area were found in 10 SIUD victims (Figure 18) and, conversely, depletion or fewer reactive fibres in 11 SIDS subjects (Figure 19). In addition, in 5 of these 11 cases we diagnosed hypoplasia of the TrN, given the presence of rare interstitial neurons and total absence of large cells in the magnocellular area of the caudal trigeminal subnucleus (Figure 20).

Figure 17: SP-immunopositivity of the raphe obscurus nucleus and of the reticular formation in control infant aged 3 months. Substance P immunostain. Magnification: 10x

4.3 Relation with smoking exposure

A significant correlation was observed between altered expression of SP in TrN and the mother's smoking habit. In fact, 17 of the 21 victims of sudden death with TrN neuropathology had a smoker mother ($p < 0.05$).

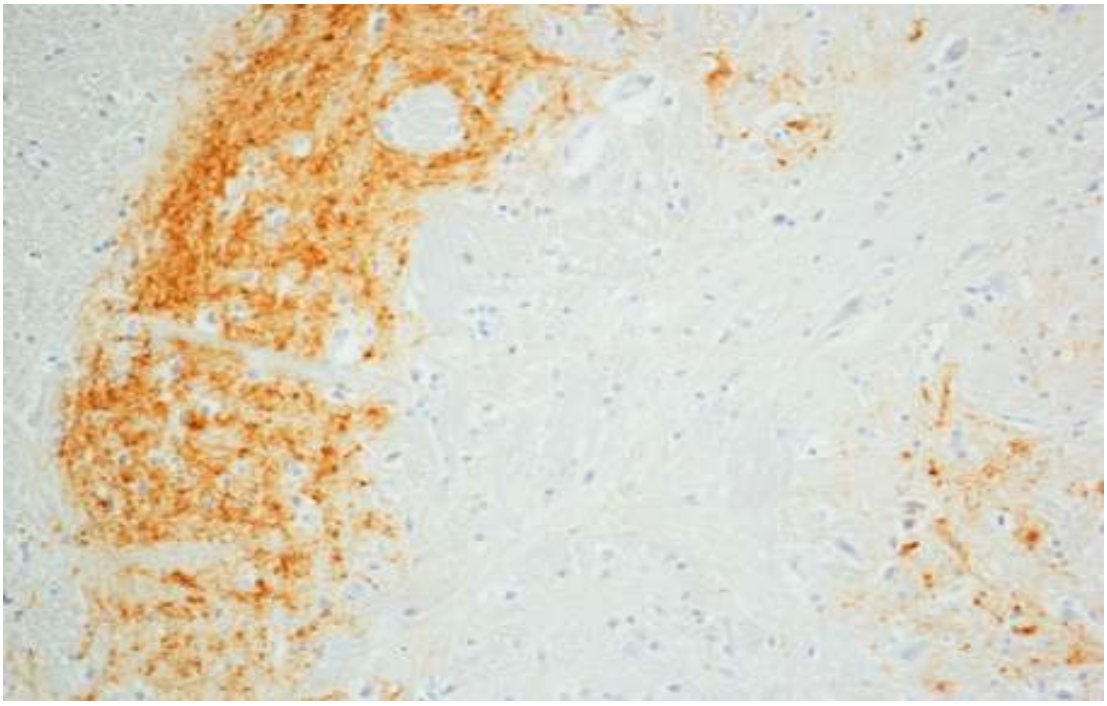


Figure 18: Dense plexus of SP-IR fibers in the trigeminal nucleus area in a SIUD victim aged 32 gestational weeks.Substance P immunostain. Magnification: 20x

4.4 Brainstem pathological results overall

Table 4 summarizes all the neuropathological findings in the brainstem. Besides to alterations of the spinal TrN, a subset of SIDS cases had hypoplasia of the medullary arcuate nucleus or of the pre-Botzinger complex, or different raphe nuclei. A subset of SIUD cases had hypoplasia of the pre-Botzinger complex or the parafacial nucleus or different raphe nuclei. A small subset of controls, both fetal and infant deaths, had hypoplasia of the arcuate nucleus and/or the raphé obscurus nucleus. In all, a significantly greater proportion of neurological alterations were observed in SIDS-SIUD cases compared with controls ($p<0.01$). The more frequent association was between alterations of SP signalling in TrN and pre-Bötzinger complex hypoplasia. In fact, 17 of the 21 victims of sudden death with alterations of TrN SP-IR (including the 5 cases with TrN hypoplasia) showed hypoplasia/agenesis of the pre-Bötzinger complex ($p<0.01$).

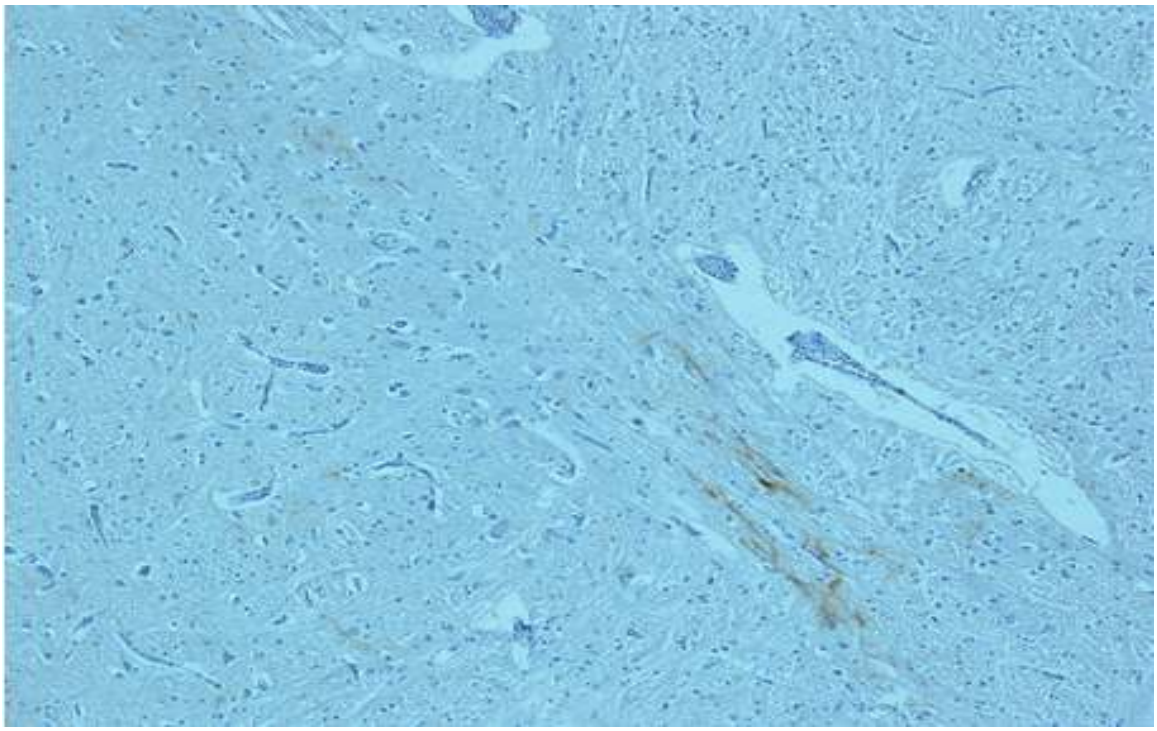


Figure 19: Poor immunopositivity of fibers in the trigeminal nucleus area in SIDS victim 3 months old. Substance P immunostain: Magnification 20X

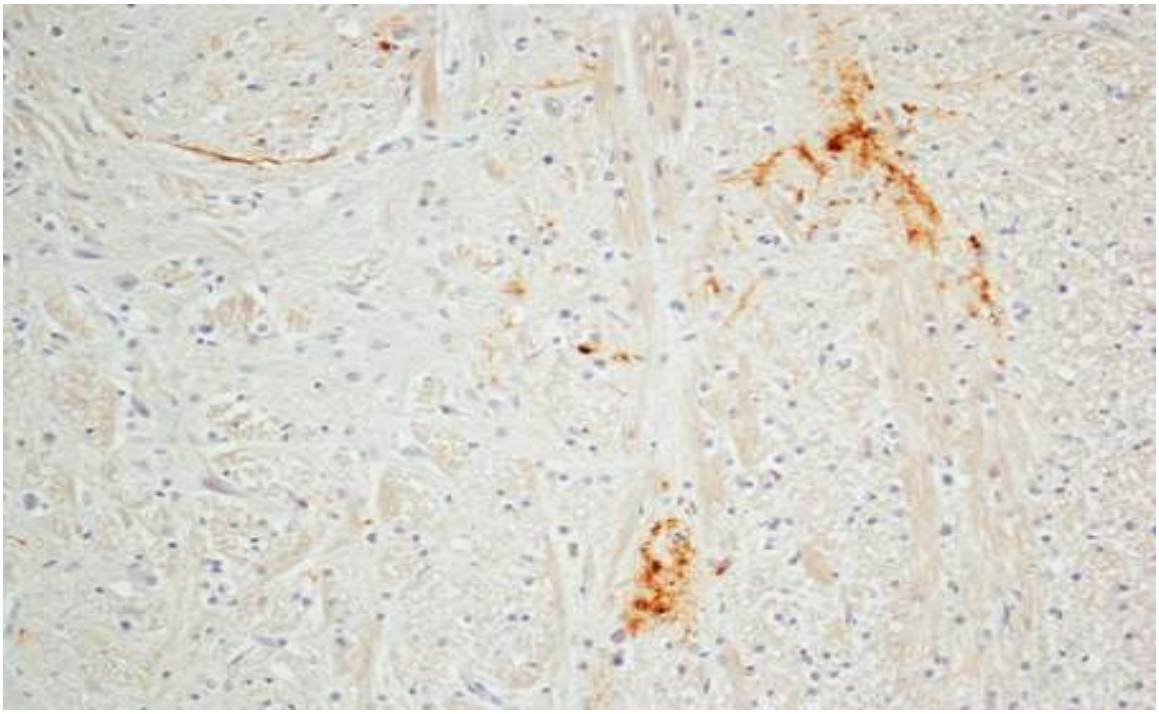


Figure 20: Hypoplasia of the trigeminal nucleus with depletion of immunopositive fibers and rare interstitial neurons. Substance P immunostain: Magnification 20X

Study group	Arcuate nucleus hypoplasia number of cases (%)	Pre-Bötzinger complex hypoplasia number of cases (%)	Parafacial nucleus hypoplasia number of cases (%)	Raphé nuclei hypoplasia number of cases (%)	SP alterations in trigeminal nucleus number of cases (%)
SIDS n=20	9 (45%)	9 (45%)	-	7 (35%)	11[b] (55%)
Infant Controls n=11	2 (18%)	-	-	3 (%)	-
SIUD n=16	7 (44%)	8 (50%)	6 (37%)	4 (25%)	10[a] (65%)
Fetal Controls n=9	2 (22%)	-	-	1 (11%)	-

[a] : increased SP-expression [b] : decreased expression

Table 4: Overall neuropathological brainstem findings in 20 SIDS, 16 SIUD and 20 controls

4.5 COMPUTATIONAL ANALYSIS

I have also studied the system level properties, such as gene duplicability, gene appearance in evolution, protein-protein interaction network and amino acid sequence conservation in prepro-tachykinin 1 precursor gene (TAC1) from 26 vertebrate species and structure of its receptor NK-1R (Fig. 4). I found by using BLAT (Fig. 21) that TAC1 has no additional hit on human genome, suggesting that it is a singleton gene and a single copy in the genome makes it functionally essential (Fig. 21). A small perturbation to this gene could lead to fatal outcome. By looking at the evolutionary tree (Fig. 23) of TAC1 suggests that it has appeared at the level of vertebrates and is a new gene. It also suggests that it has a role specific to the organisms with advanced nervous systems and it is brain specific, required for the sophisticated functions. We already know that TKs are present in both lower and higher vertebrates but they are much more advanced and authentic in mammals. They are highly diversified and established in mammals. In mammals they are involved in regulation of several important functions such as developmental processes, differentiation of cell, brain development, axon guidance, neurotransmission, immune modulation, mood control, behavioural responses, gastrointestinal contractions, lung and airway functions, response to environmental stress, learning and memory.

Human BLAT Results

BLAT Search Results

ACTIONS	QUERY	SCORE	START	END	QSIZE	IDENTITY	CHRO	STRAND	START	END	SPAN
browser details	Homo	358	1	129	129	99.8%	7	++	97361925	97369229	7305

Figure 21: BLAT results of human TAC1 gene

TAC1 protein-protein interaction network (Fig. 22) shows that it interacts with 10 proteins, that includes TACR1, TACR2, TACR3, TAC3, TAC4, CALCB (calcitonin gene related peptide precursor 2), CALCA (calcitonin gene related peptide precursor 1), NGFB (Beta nerve growth

factor precusor), VIP (Vasoactive intestinal protein) and NR3C1(glucocorticoid receptor). Among 10 protein interactors 5 belongs to TK family and other interactors such as CGRP (CALCA and CALCB), NFGB, NR3C1 and VIP also have similar roles such as TAC1 encoded product SP like vasodilation, inflammation, muscle contractility. CGRP and NGFB are abundant in CNS and have important roles in development of CNS and PNS like SP. WebLogo diagram of TAC1 gene (Fig. 24) depicts the conserved amino acid sequence F-X-GLM, particular of tachykinin family in these 26 vertebrate species.

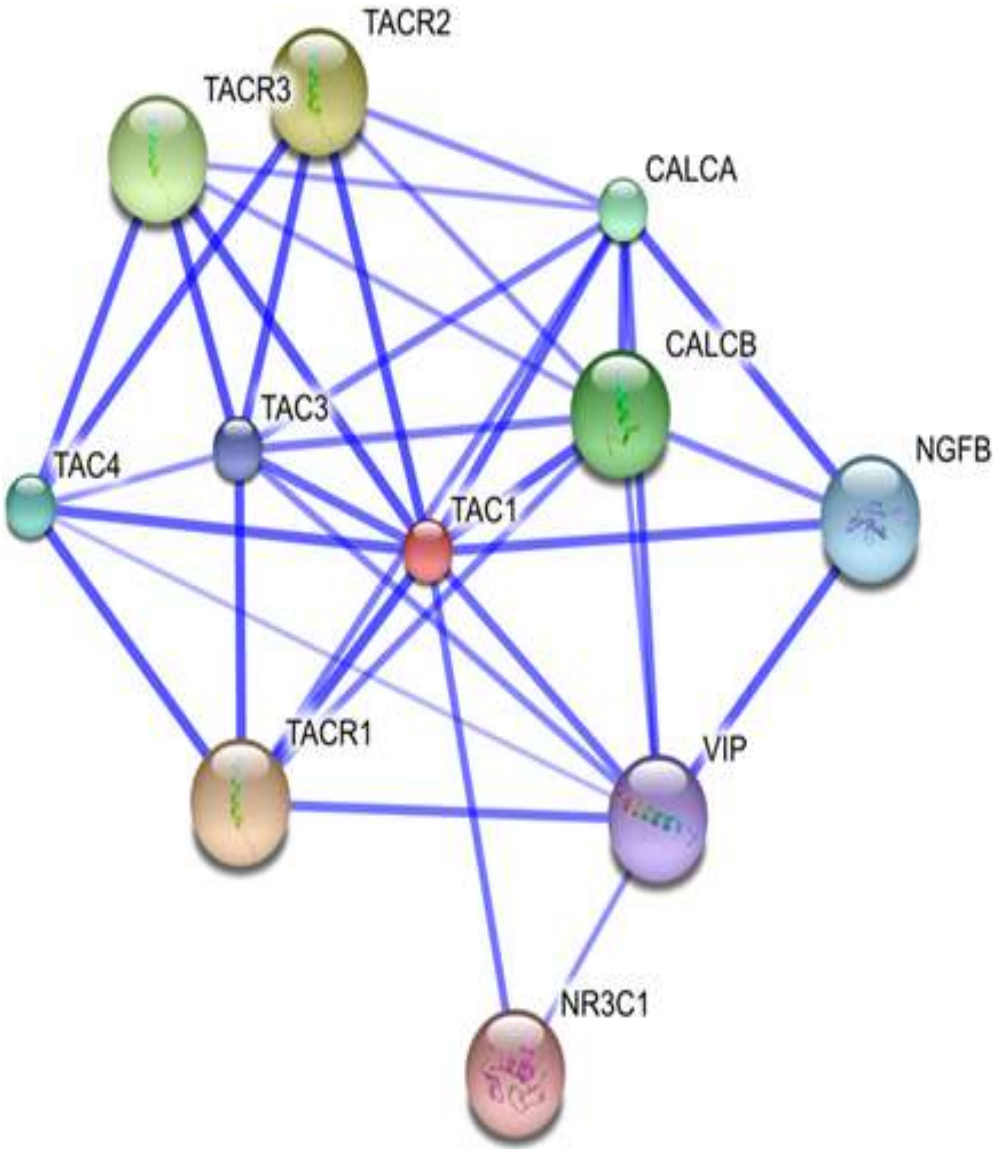


Figure 22: Protein-protein interaction of TAC1 gene by using STRING 8.3

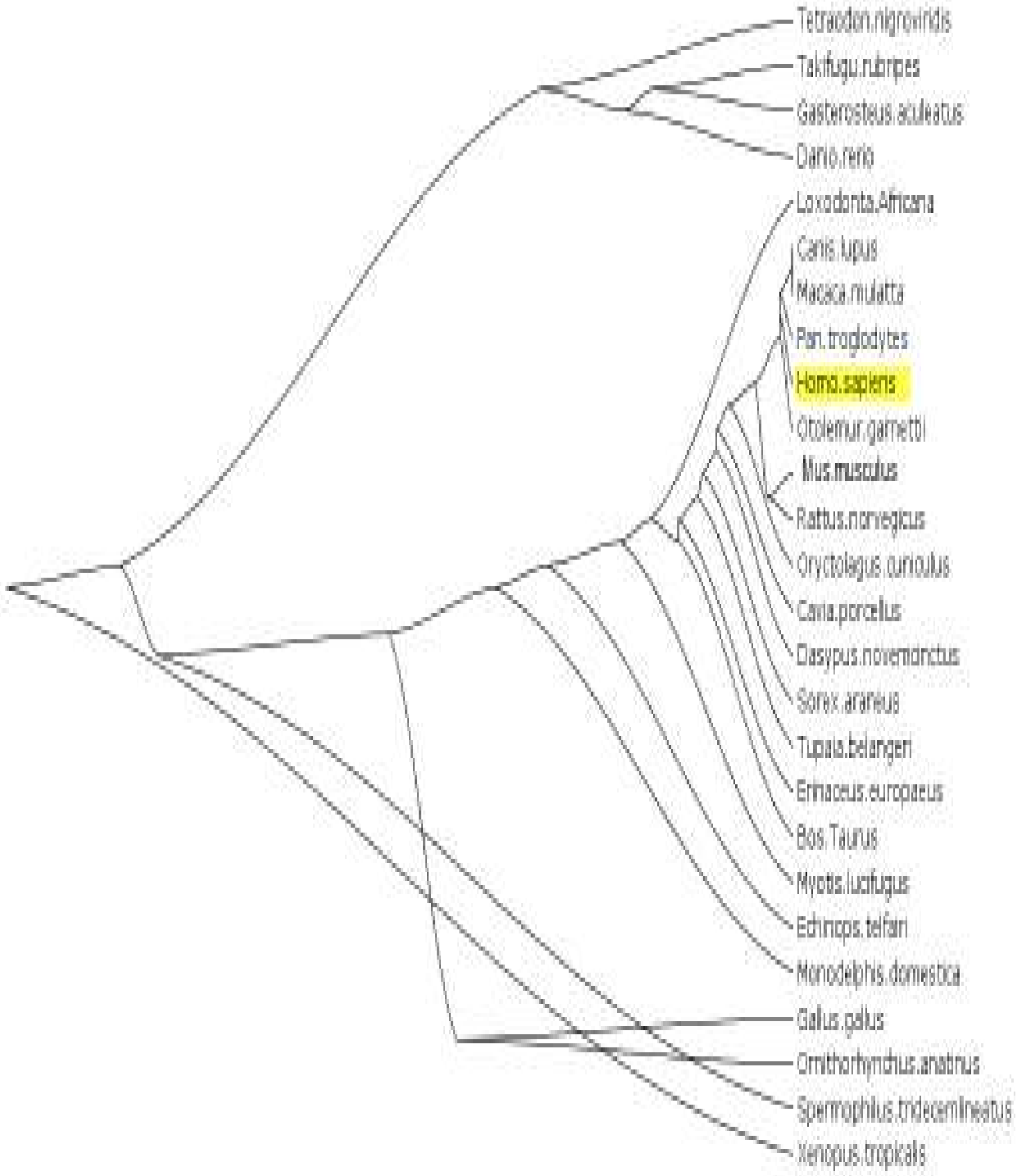


Figure 23: Phylogenetic tree of TAC1 gene by using MAFFT online version 6.0

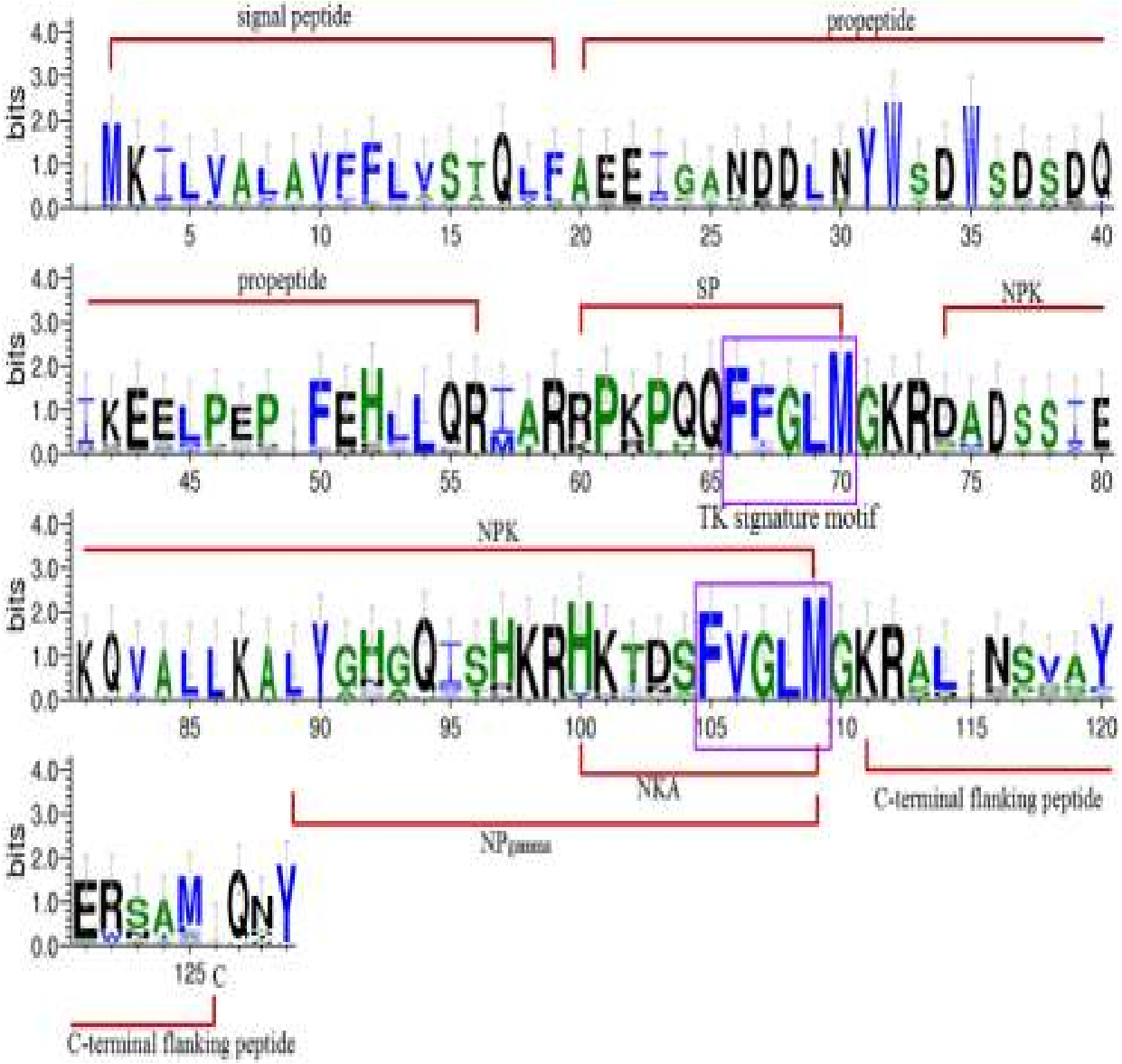


Figure 24: Amino acid sequence conservation in the vertebrate prepro- TAC1 gene from 26 species. Conservation is displayed as a sequence logo. Sequence logos were generated as described (Kraemer et al. 2008) by using WebLogo version 3.0 (http://weblogo.threeplusone.com). In this representation, the relative frequency with which an amino acid appears at a given position is reflected by the height of its one letter amino acid code in the logo, with a total height at a given position proportional to the level of sequence conservation (see material and method). Tachykinin 1 (TAC1) encodes for SP, NP gamma, NPK, NKA and this preprotachykinin 1 sequence also shows signal peptide, C terminal flanking sequence and propeptide. Preprotachykinin is cleaved into mature peptides after differential splicing. Sequence in purple box shows highly conserved signature motif of tachykinin family.

5 DISCUSSION

The respiratory control system is influenced by classical neurotransmitters and by neuromodulators. The neuromodulators are neuroactive substances that can be secreted at a distance from their receptors and can have a non-synaptic transmission via the intercellular spaces. Neuromodulators that are considered seriously as natural participants in central respiratory control include dopamine, adenosine, endorphins, serotonin, and substance P (SP) (Moss and Inman 1989; Moss et al. 1986; Hilaire and Duron 1999; Bonham 1995). SP in particular is a member of the tackykinin family of neuropeptides that, besides to play a role in the modulation of hemodynamic function and neuronal pathways related to pain sensation (Nicoll et al. 1980), is involved in the breathing acceleration under conditions of hypoxemia (Lagercrantz et al. 1991; Hedner et al. 1981). It can be highlighted, such as many neurotransmitters, by a specific immunohistochemical method.

Abnormal distribution of different neurotransmitters involved in breathing control (namely somatostatin, serotonin, catecholamines) (Lavezzi et al. 2004, 2005, 2009) has been observed, by our lab previously, by immunohistochemistry in the brainstem of sudden perinatal death and SIDS victims. These alterations were thought to cause drawback of vital functions and/or induction of fatal breathing in prenatal life and worsening of ventilatory control in newborns, easily leading to irreversible apnea.

In the present study we aimed to enlarge our knowledge by evaluating the expression of SP in these pathologies. Therefore we investigated the SP-immunoreactivity (SP-IR) in subjects aged from 17 gestational weeks to 10 postnatal months, who had died of both known and unknown causes. Our aim was, besides to obtain basic information about the distribution of SP binding sites in the first phases of the human nervous system development, to evaluate the possible presence of SP alterations in cases of sudden perinatal and infant death, in addition to the morpho-functional troubles of the autonomic nervous system already reported (Lavezzi et al. 2004; 2005; 2006; 2007; 2009; Matturri et al. 2002; Lavezzi and Matturri 2008).

A topographical mapping of SP immunoreactive structures in the human brain have been reported in the past by several authors but with many disagreements in the demonstration of the SP-binding sites (Chigr et al. 1991; Jordan et al. 1995; Del Fiacco et al. 1984). This study provides a detailed report of the distribution of SP-immunoreactivity (SP-IR) within the human fetal and infant brainstem. We detected SP positivity from early stages of ontogenesis mainly in

the dorsolateral part of the medulla oblongata and of the rostral spinal cord exclusively in the area corresponding to the spinal trigeminal nucleus (TrN). These observations testify the high specificity of the localization of the SP in the human brain.

Only a few authors have focused attention on the TrN in man (Rusu 2004; Dallel et al. 2003). The structure of this nucleus, including a cellular component and a group of fibres (tract), is in fact difficult to identify, given its undefined boundaries. However, the TrN recognition is facilitated by the expression of its major neurotransmitter, the SP, in the tract. Thus, by using immunohistochemistry, we firstly defined the localization and traced the cytoarchitectonic features of this nucleus, providing essential data on its morphologic and functional developmental dynamics in subjects who had died of known aetiology from 17 gestational weeks to 10^{th} month of life. The comparative study of the fetus and newborn coronal sections of medulla revealed that, while in fetuses the TrN was barely recognizable, due to limited presence of fibres expressing SP, a clear structural organization of this nucleus with interstitial island of neurons, embedded in a dense plexus of positive fibres in the tract,was evident in newborns.

The different SP densities, varying from low in the intrauterine life to very high in the first postnatal months, underlines the attainment of a precise functional program in the coordination of the nervous system developmental steps. The SP released by neurons of the TrN might thus be involved in critical periods for brain plasticity and modulation of different autonomic vital functions. Experimental studies in rodents have showed that the TrN is involved in a variety of rhythmic behaviors, such as suckling, mastication, swallowing, and breathing (Goldberg and Chandler 1990; Lund et al. 1998). Breathing in particular is a fundamental mammalian activity, and must be functional at birth (Feldman et al. 2003).

Humans usually breathe only through the nasal airway route, without using the oral airway route. Oral breathing along with jaw movements, aimed at maintaining upper airway resistance, is required under loaded respiratory conditions, such as severe hypoxia (Bartlett 1986). Accordingly, the trigeminal respiratory activity is fundamentally involved in the network controlling oral breathing and plays a critical role in the regulation of upper airway patency. Several authors have reported that trigeminal motor activity is dramatically altered during respiratory disorders, such as sleep apnea (Chandler et al. 1980; Chamberlin and Saper 1998).

In the present study we detected, in spite of what occurs in control cases, dense plexuses of SP immunoreactive fibres in the spinal TrN area in SIUD victims and, conversely, depletion

of SP-IR fibres in TrN in SIDS, frequently in the context of a hypoplasia of the whole nucleus. Previously, while studying the brain expression of the somatostatin, another neuropeptide involved in the control of respiration, we similarly have observed differential pattern of positivity of this neurotransmitter in the hypoglossal nucleus before and after birth in more than 50% of cases of sudden perinatal and infant death compared to cases with a known etiology of death (Lavezzi et al. 2004).

The hypoglossus nucleus, even if not considered as a classic breathing center, similarly to the TrN, contains motoneurons with respiratory-related rhythmical discharges. In particular it controls the extrinsic muscles of the tongue, mainly the genioglossus that is important in maintaining a patent airway, especially during inspiration (Withington-Wray et al. 1988; Roda et al. 2002). The TrN abnormalities, in about half the cases of this study, were associated to the pre-Bötzinger complex hypoplasia. This close connection is supported by experimental studies. Koizumi and coworkers (Koizumi et al. 2009) while working in rat brainstem-sectioning experiments, suggested that the origin of trigeminal respiratory activity is the pre-Bötzinger complex in the medulla. Their observations confirmed that trigeminal respiratory activity disappeared when the region corresponding to the pre-Bötzinger complex was completely removed. The essential role of the pre-Bötzinger complex for the generation of the respiratory rhythm, as well as for the modulation of eupneic breathing, has been suggested by the previous work of our lab (Lavezzi and Matturri 2008). In particular we reported structural and/or functional developmental defects (hypoplasia with a decreased neuronal number and/or dendritic hypodevelopment of the reticular formation, abnormal neuronal morphology, immunonegativity of neurotransmitters, and agenesis) of this nucleus in a high percentage of sudden fetal and infant deaths. Our next purpose will be to confirm, by using SP-immunohistochemistry in the recorded brainstem inclusions of these victims, the concomitant presence of TrN alterations.

Discordant works have been performed with regard to the SP-IR in the brainstem of SIDS victims, all performed on a limited number of cases (Obonai et al. 1996; Ozawa and Takashima 2002; Yamanouchi et al. 1993; Sawaguchi et al. 2003; Takashima et al. 1994). Only a few authors have observed immunopositivity in TrN. In particular, Obonai (Obonai et al. 1996) and Ozawa (Ozawa and Takashima 2002) reported increased expression of SP in trigeminal fibres, compared with age-matched controls. Conversely, in the study of Sawaguchi (Sawaguchi et al. 1993), no significant correlation was found between the density of SP in the TrN and SIDS.

Our studies and previous works reported in the literature provide insight into the attribution to chronic hypoxia as the cause of the neuronal abnormalities and of the pathogenetic mechanism of the perinatal sudden death (Matturri et al. 2002; Lavezzi et al. 2003; Kinney and Thach 2009; Hunt 1992). Severe hypoxia can rouse oral breathing by means of the TrN activation. A possible determining mechanism for hypoxic events arises from maternal cigarette smoking in pregnancy (Lavezzi et al. 2005; Wisborg et al. 2001). Finally, our previous observations of a significantly increased incidence of morphological and functional alterations in the brainstems of stillborns and SIDS victims with smoker mothers compared with victims with nonsmoker mothers (Lavezzi et al. 2005a,b; 2004;2007), prompted us to verify whether maternal cigarette smoking could also be related to SP abnormalities. Also in this study we observed a significant correlation between tobacco smoke exposure in utero and alterations of the SP expression in spinal TrN.

In case of maternal smoking in pregnancy, carbon monoxide, a gaseous combustion product of nicotine, may readily cross the placenta by passive diffusion, where it binds to hemoglobin. Consequently, the carboxyhemoglobin, that is present in fetal compartment with concentrations generally 15% higher than maternal levels (Lambers and Clark 1996), inhibits the release of oxygen into fetal tissues causing hypoxia with consequent delayed maturation of all the organs, especially of the brain. Besides, the nicotine is one of the few lipid-soluble substances able to go beyond the blood-brain barrier by concentration gradient (Cutler and Spertell 1982; Dawson 1996), and act directly on expression of genes that control the developing brain. Therefore, among the numerous compounds present in cigarette smoke, carbon monoxide and nicotine could affect the fetal brain through indirect and/or direct action (Gressens et al. 2003).

Our observations of TrN alterations in some victims of non-smoking mothers could be attributable to the fact that many women in pregnancy are exposed to passive smoking. Furthermore, it should be considered that retrospective assessment of maternal smoking habit, mainly if performed after the fatal event, is sometimes unavoidable (Heath et al. 2003; Walsh et al. 1996). This may be caused by the fact that smoking mothers are reluctant to honestly report their tobacco use, possibly because of feelings of guilt. Besides, other risk factors, such as air pollution, may contribute to defects in the nervous system. Since most of the victim's mothers and of infants of this study lived in large industrialized cities, we can hypothesize that also

atmospheric pollution may have contributed to a wrong development of the TrN. Nevertheless, we think that fetal nicotine exposure is one of the possible preventable factors with a strong potential influence on the developmental alterations of the brainstem vital centers and consequently on the incidence of stillbirths and infant deaths.

Evolutinary analysis suggests that TAC1 is a new gene and is present in advanced animals such as vertebrates specially the mammals who have well established nervous system. It also shows that these genes are regulating important nervous system functions in these animals and not in the organisms with poorly developed nervous system. CGRP, Glutamate and VIP, which are found to be the colocalized and coreleased with TAC1 encoded product SP in trigeminal ganglion confirmed by previous studies (Lazarov 2002), is also evident from protein-protein interaction analysis in this study. As TAC1 is a singleton, it shows that it is a fragile point in the genome which means that if there is some mutation in this gene then there is no other gene with similar function that can compensate for its function. It can lead to deleterious effects and much pathology such as neuropathological and mental disorders. If the genes and nuclei regulating the nervous system development and cardiorespiratory control are affected, it may cause embryonic lethality and SIDS too because TAC1 and the interacting genes are mainly expressed in central nervous system.

CONCLUSION AND OUTLOOK

In conclusion, all pregnant women should be advised that smoking places their unborn children in serious danger to undergo a variety of morphological, genetic and functional abnormalities of the brainstem that can be cause of stillbirth and neonatal death. Moreover, the low success rate of smoking cessation among pregnant women, suggests that efforts to reduce the tobacco use in pregnancy should focus on preventing cigarette smoking among teenage girls. Infact, most women begin smoking early in the teenage years, and find it difficult to quit, even during pregnancy. Computational analysis reveals that SP encoding gene TAC1 is very important functionally and a mutation or alteration in this gene can lead to a fatal outcome. It also suggests that TAC1 is controlling many important pathways in human nervous system regulation. This study also suggests that trigeminal nucleus hypoplasia and SP expression alteration in SIDS and SIUD victims may be one of the possible mechanisms for sudden death pathology.

7 REFERENCES

(2005). "The changing concept of sudden infant death syndrome: diagnostic coding shifts, controversies regarding the sleeping environment, and new variables to consider in reducing risk." Pediatrics 116(5): 1245-1255.

Adams, S. M., M. W. Good, et al. (2009). "Sudden infant death syndrome." Am Fam Physician 79(10): 870-874.

Adamus, M. (2009). "[Substance P as a regulatory peptide of hematopoiesis and blood cell functions]." Postepy Hig Med Dosw (Online) 63: 106-113.

Adcock, I. M., M. Peters, et al. (1993). "Increased tachykinin receptor gene expression in asthmatic lung and its modulation by steroids." J Mol Endocrinol 11(1): 1-7.

Adelson, L. and E. R. Kinney (1956). "Sudden and unexpected death in infancy and childhood." Pediatrics 17(5): 663-699.

Advenier, C., V. Lagente, et al. (1997). "The role of tachykinin receptor antagonists in the prevention of bronchial hyperresponsiveness, airway inflammation and cough." Eur Respir J 10(8): 1892-1906.

Aita, M., T. Maeda, et al. (2005). "Postnatal development of substance P-immunoreaction in the trigeminal caudalis of neonatally capsaicin-treated mice." Arch Histol Cytol 68(4): 311-320.

Aita, M., K. Seo, et al. (2005). "Postnatal changes in the spatial distributions of substance P and neurokinin-1 receptor in the trigeminal subnucleus caudalis of mice." Brain Res Dev Brain Res 155(1): 33-41.

Almeida, T. A., J. Rojo, et al. (2004). "Tachykinins and tachykinin receptors: structure and activity relationships." Curr Med Chem 11(15): 2045-2081.

Alvarado-Mallart, M. R., C. Batini, et al. (1975). "Trigeminal representations of the masticatory and extraocular proprioceptors as revealed by horseradish peroxidase retrograde transport." Exp Brain Res 23(2): 167-179.

Anastasi, A. and V. Erspamer (1962). "Occurrence and some properties of eledoisin in extracts of posterior salivary glands of Eledone." Br J Pharmacol Chemother 19: 326-336.

Anastasi, A., V. Erspamer, et al. (1964). "ISOLATION AND AMINO ACID SEQUENCE OF PHYSALAEMIN, THE MAIN ACTIVE POLYPEPTIDE OF THE SKIN OF PHYSALAEMUS FUSCUMACULATUS." Arch Biochem Biophys 108: 341-348.

Anderson, R. H., J. Bouton, et al. (1974). "Sudden death in infancy: a study of cardiac specialized tissue." Br Med J 2(5911): 135-139.

Athanassiadis, T., K. G. Westberg, et al. (2005). "Physiological characterization, localization and synaptic inputs of bursting and nonbursting neurons in the trigeminal principal sensory nucleus of the rat." Eur J Neurosci 22(12): 3099-3110.

Bajanowski, T., C. Ortmann, et al. (2003). "Pathological changes of the heart in sudden infant death." Int J Legal Med 117(4): 193-203.

Banks, B. D., R. W. Beck, et al. (1987). "Sudden infant death syndrome: a literature review with chiropractic implications." J Manipulative Physiol Ther 10(5): 246-252.

Barker, R. (1989). "Adrenal grafting for Parkinson's disease: a role for substance P." Int J Neurosci 46(1-2): 47-51.

Bartlett, D. J. (1986). "Upper airway motor systems". *In: Handbook of physiology. Section 3: The respiratory system. Vol. II: Control of breathing*. Fishman AP, editor. Bethesda, MD, USA: American Physiological Society, 223–245.

Bartram, U. and D. Singer (2004). "[Infant botulism and sudden infant death syndrome]." Klin Padiatr 216(1): 26-30.

Becker, L. E., W. Zhang, et al. (1993). "Delayed maturation of the vagus nerve in sudden infant death syndrome." Acta Neuropathol 86(6): 617-622.

Benedeczky, I., J. Z. Kiss, et al. (1982). "Light and electron microscopic localization of substance P-like immunoreactivity in the cerebral ganglion of locust with a monoclonal antibody." Histochemistry 75(1): 123-131.

Bereiter, D. A., H. Hirata, et al. (2000). "Trigeminal subnucleus caudalis: beyond homologies with the spinal dorsal horn." Pain 88(3): 221-224.

Bergman, A. B., Beckwith, J. B., and Ray, C. G. (Ed.) 1(1970). "Sudden; Infant Death Syndrome." Proceedings of the second international conference on. causes of sudden Death in infants. Seattle: University of Washington Press.

Berner, J., Y. Shvarev, et al. (2007). "Altered respiratory pattern and hypoxic response in transgenic newborn mice lacking the tachykinin-1 gene." J Appl Physiol 103(2): 552-559.

Bertrand, C. and P. Geppetti (1996). "Tachykinin and kinin receptor antagonists: therapeutic perspectives in allergic airway disease." Trends Pharmacol Sci 17(7): 255-259.

Beuerman, R. W. and M. E. Stern (2005). "Neurogenic inflammation: a first line of defense for the ocular surface." Ocul Surf 3(4 Suppl): S203-206.

Birklein, F. and M. Schmelz (2008). "Neuropeptides, neurogenic inflammation and complex regional pain syndrome (CRPS)." Neurosci Lett 437(3): 199-202.

Blair, P. S., E. A. Mitchell, et al. (2008). "Head covering - a major modifiable risk factor for sudden infant death syndrome: a systematic review." Arch Dis Child 93(9): 778-783.

Blatt, S. D., V. Meguid, et al. (1999). "Sudden infant death syndrome, child sexual abuse, and child development." Curr Opin Pediatr 11(2): 175-186.

Bonham, A. C. (1995). "Neurotransmitters in the CNS control of breathing." Respir Physiol 101(3): 219-230.

Borhani, N. O., P. A. Rooney, et al. (1973). "Post-neonatal sudden unexplained death in a California community." Calif Med 118(5): 12-16.

Bozic, C. R., B. Lu, et al. (1996). "Neurogenic amplification of immune complex inflammation." Science 273(5282): 1722-1725.

Brown, E. R., K. A. Roth, et al. (1991). "Substance P is distributed between somatotrophs and thyrotrophs in a sexually dimorphic manner in rat." Ann N Y Acad Sci 632: 366-369.

Buck, G. M., A. M. Michalek, et al. (1991). "Labor and delivery events and risk of sudden infant death syndrome (SIDS)." Am J Epidemiol 133(9): 900-906.

Burns, L., E. Conroy, et al. (2010). "Infant mortality among women on a methadone program during pregnancy." Drug Alcohol Rev 29(5): 551-556.

Byers, M. R. and W. K. Dong (1989). "Comparison of trigeminal receptor location and structure in the periodontal ligament of different types of teeth from the rat, cat, and monkey." J Comp Neurol 279(1): 117-127.

Campos, M., E. Bravo, et al. (2009). "Respiratory dysfunctions induced by prenatal nicotine exposure." Clin Exp Pharmacol Physiol 36(12): 1205-1217.

Capra, N. F., K. V. Anderson, et al. (1985). "Localization and morphometric analysis of masticatory muscle afferent neurons in the nucleus of the mesencephalic root of the trigeminal nerve in the cat." Acta Anat (Basel) 122(2): 115-125.

Carpenter, R. G. and C. W. Shaddick (1965). "ROLE OF INFECTION, SUFFOCATION, AND BOTTLE-FEEDING IN COT DEATH; AN ANALYSIS OF SOME FACTORS IN THE HISTORIES OF 110 CASES AND THEIR CONTROLS." Br J Prev Soc Med 19: 1-7.

Carter, M. S. and J. E. Krause (1990). "Structure, expression, and some regulatory mechanisms of the rat preprotachykinin gene encoding substance P, neurokinin A, neuropeptide K, and neuropeptide gamma." J Neurosci 10(7): 2203-2214.

Carturan, E., C. Basso, et al. (2007). "[Molecular investigation of sudden death]." G Ital Cardiol (Rome) 8(12): 752-759.

Chamberlin, N. L. and C. B. Saper (1998). "A brainstem network mediating apneic reflexes in the rat." J Neurosci 18(15): 6048-6056.

Champagne, D. E. and J. M. Ribeiro (1994). "Sialokinin I and II: vasodilatory tachykinins from the yellow fever mosquito Aedes aegypti." Proc Natl Acad Sci U S A 91(1): 138-142.

Chandler, S. H., M. H. Chase, et al. (1980). "Intracellular analysis of synaptic mechanisms controlling trigeminal motoneuron activity during sleep and wakefulness." J Neurophysiol 44(2): 359-371.

Chang, M. M. and S. E. Leeman (1970). "Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P." J Biol Chem 245(18): 4784-4790.

Chang, M.M., S. E. Leeman and H. D. Niall (1971).” Amino acid sequence of substance P.” Nat New Biol 232(29):86-7.

Chattipakorn, S. C., A. Sigurdsson, et al. (2002). "Trigeminal c-Fos expression and behavioral responses to pulpal inflammation in ferrets." Pain 99(1-2): 61-69.

Chen, A. K. and M. S. Hedrick (2008). "Role of glutamate and substance P in the amphibian respiratory network during development." Respir Physiol Neurobiol 162(1): 24-31.

Chen, L., Q. L. Cui, et al. (2009). "Neurokinin-1 receptor activation in globus pallidus." Front Neurosci 3: 58.

Chernova, I., J. P. Lai, et al. (2009). "Substance P (SP) enhances CCL5-induced chemotaxis and intracellular signaling in human monocytes, which express the truncated neurokinin-1 receptor (NK1R)." J Leukoc Biol 85(1): 154-164.

Cheron, G., C. Rambaud, et al. (1993). "[Sudden crib death. Experience in a referral center 1986-1991]." Arch Fr Pediatr 50(4): 293-299.

Chigr, F., M. Najimi, et al. (1991). "Anatomical distribution of substance P-immunoreactive neurons in human brainstem during the first postnatal year." Brain Res Bull 26(4): 515-523.

Clement, P., M. Peeters, et al. (2009). "Role of the neurokinin-1 receptors in ejaculation in anesthetized rats." J Sex Med 6(1): 126-134.

Collingridge, G. L. and J. Davies (1982). "Actions of substance P and opiates in the rat substantia nigra." Neuropharmacology 21(7): 715-719.

Conlon, J. M., C. F. Deacon, et al. (1986). "Scyliorhinin I and II: two novel tachykinins from dogfish gut." FEBS Lett 200(1): 111-116.

Copray, J. C., R. S. Liem, et al. (1990). "Dopaminergic afferents to the mesencephalic trigeminal nucleus of the rat: a light and electron microscope immunocytochemistry study." Brain Res 514(2): 343-348.

Costa, M., J. B. Furness, et al. (1986). "Chemical coding of enteric neurons." Prog Brain Res 68: 217-239.

Crooks, G. E., G. Hon, et al. (2004). "WebLogo: a sequence logo generator." Genome Res 14(6): 1188-1190.

Crowley, E. M. and J. L. Emery (1956). "Clinical histories of infants reported to coroner as cases of sudden unexpected death." Br Med J 2(5008): 1518-1521.

Cutler, R. W. and R. B. Spertell (1982). "Cerebrospinal fluid: a selective review." Ann Neurol 11(1): 1-10.

Dallel, R., L. Villanueva, et al. (2003). "Neurobiologie de la douleur trigeminale." Med/Sci 19:67-74.

Davies, A. M. (1988). "The trigeminal system: an advantageous experimental model for studying neuronal development." Development 103 Suppl: 175-183.

Dawson, H. and M. B. Seegal (1996)." Physiology of CSF and blood-brain barriers". CRP Press, London.

Dean, C., L. Marson, et al. (1993). "Distribution and co-localization of 5-hydroxytryptamine, thyrotropin-releasing hormone and substance P in the cat medulla." Neuroscience 57(3): 811-822.

Del Fiacco, M., M. L. Dessi, et al. (1984). "Topographical localization of substance P in the human post-mortem brainstem. An immunohistochemical study in the newborn and adult tissue." Neuroscience 12(2): 591-611.

Del Fiacco, M., M. Quartu, et al. (1990). "Substance P-like immunoreactivity in the human trigeminal ganglion." Neurosci Lett 110(1-2): 16-21.

Delgado, A. V., A. T. McManus, et al. (2005). "Exogenous administration of Substance P enhances wound healing in a novel skin-injury model." Exp Biol Med (Maywood) 230(4): 271-280.

Krastev, D. (2008).“Trigeminal ganglium-electronmicroscopy of large light pseudounipolar neurons. J IMAB *Annual proceeding (Scientific Papers) book1*.

Donkin, J. J. and R. Vink (2010). "Mechanisms of cerebral edema in traumatic brain injury: therapeutic developments." Curr Opin Neurol 23(3): 293-299.

Dubner, R. (1978). "Neurophysiology of pain." Dent Clin North Am 22(1): 11-30.

Dubner, R. and K. Ren (2004). "Brainstem mechanisms of persistent pain following injury." J Orofac Pain 18(4): 299-305.

Duncan, J. R., D. S. Paterson, et al. (2010). "Brainstem serotonergic deficiency in sudden infant death syndrome." JAMA 303(5): 430-437.

Dunnick, C. A., N. S. Gibran, et al. (1996). "Substance P has a role in neurogenic mediation of human burn wound healing." J Burn Care Rehabil 17(5): 390-396.

Dzurik, M. V., A. Diedrich, et al. (2007). "Endogenous substance P modulates human cardiovascular regulation at rest and during orthostatic load." J Appl Physiol 102(6): 2092-2097.

Ebner, K., S. B. Sartori, et al. (2009). "Tachykinin receptors as therapeutic targets in stress-related disorders." Curr Pharm Des 15(14): 1647-1674.

Ebner, K. and N. Singewald (2006). "The role of substance P in stress and anxiety responses." Amino Acids 31(3): 251-272.

Edvinsson, L., S. Rosendal-Helgesen, et al. (1983). "Substance P: localization, concentration and release in cerebral arteries, choroid plexus and dura mater." Cell Tissue Res 234(1): 1-7.

Eftekhari, S., C. A. Salvatore, et al. (2010). "Differential distribution of calcitonin gene-related peptide and its receptor components in the human trigeminal ganglion." Neuroscience 169(2): 683-696.

El-Nour, H., A. Santos, et al. (2009). "Neuronal changes in psoriasis exacerbation." J Eur Acad Dermatol Venereol 23(11): 1240-1245.

Erin, N. and O. Ulusoy (2009). "Differentiation of neuronal from non-neuronal Substance P." Regul Pept 152(1-3): 108-113.

Erspamer, V. (1949). "[Not Available.]." Farmaco 4(2): 179-181.

Erspamer, V. and A. Anastasi (1962). "Structure and pharmacological actions of eledoisin, the active endecapeptide of the posterior salivary glands of Eledone." Experientia 18: 58-59.

Erspamer, V., G. Bertaccini, et al. (1964). "BIOGENIC AMINES AND ACTIVE POLYPEPTIDES IN THE SKIN OF TEN JAPANESE AMPHIBIAN SPECIES." Jpn J Pharmacol 14: 467-473.

Eugenin, J., M. Otarola, et al. (2008). "Prenatal to early postnatal nicotine exposure impairs central chemoreception and modifies breathing pattern in mouse neonates: a probable link to sudden infant death syndrome." J Neurosci 28(51): 13907-13917.

Fedrick, J. (1973). "Sudden unexpected death in infants in the Oxford record linkage area. An analysis with respect to time and place." Br J Prev Soc Med 27(4): 217-224.

Felderbauer, P., K. Bulut, et al. (2007). "Substance P induces intestinal wound healing via fibroblasts--evidence for a TGF-beta-dependent effect." Int J Colorectal Dis 22(12): 1475-1480.

Feldman, J. L., G. S. Mitchell, et al. (2003). "Breathing: rhythmicity, plasticity, chemosensitivity." Annu Rev Neurosci 26: 239-266.

Ferris, J. A., W. A. Aherne, et al. (1973). "Sudden and unexpected deaths in infants: histology and virology." Br Med J 2(5864): 439-442.

Fest, S., A. C. Zenclussen, et al. (2006). "Stress and substance P but not the substance P-metabolite SP5-11 trigger murine abortion by augmenting TNF-alpha levels at the feto-maternal interface." Scand J Immunol 63(1): 42-49.

Figini, M., C. Emanueli, et al. (1996). "Evidence that tachykinins relax the guinea-pig trachea via nitric oxide release and by stimulation of a septide-insensitive NK1 receptor." Br J Pharmacol 117(6): 1270-1276.

Filiano, J. J. and H. C. Kinney (1994). "A perspective on neuropathologic findings in victims of the sudden infant death syndrome: the triple-risk model." Biol Neonate 65(3-4): 194-197.

Fischer, A., G. P. McGregor, et al. (1996). "Induction of tachykinin gene and peptide expression in guinea pig nodose primary afferent neurons by allergic airway inflammation." J Clin Invest 98(10): 2284-2291.

Fischer, D., A. Freislederer, et al. (2004). "[Sudden death of twins: botulism because of contamination by pap vegetables]." Klin Padiatr 216(1): 31-35.

Fleming, K. A. (1992). "Viral respiratory infection and SIDS." J Clin Pathol 45(11 Suppl): 29-32.

Frais, J. A. (1972). "Cot deaths and hypokalemia." Br Med J 3(5827): 646.

Freeman, W. (1925). "The relationship of the radix mesencephalica trigemini to the extraocular muscles." Arch Neurol Psychiat 14, 111–113.

Froger, N., A. M. Gardier, et al. (2001). "5-hydroxytryptamine (5-HT)1A autoreceptor adaptive changes in substance P (neurokinin 1) receptor knock-out mice mimic antidepressant-induced desensitization." J Neurosci 21(20): 8188-8197.

Froggatt, P. and T. N. James (1973). "Sudden unexpected death in infants. Evidence on a lethal cardiac arrhythmia." Ulster Med J 42(2): 136-152.

Froggatt, P., M. A. Lynas, et al. (1971). "Epidemiology of sudden unexpected death in infants ('cot death') in Northern Ireland." Br J Prev Soc Med 25(3): 119-134.

Froggatt, P., M. A. Lynas, et al. (1971). "Sudden unexpected death in infants ("cot death"). Report of a collaborative study in northern Ireland." Ulster Med J 40(2): 116-135.

Fusayasu, E., H. Kowa, et al. (2007). "Increased plasma substance P and CGRP levels, and high ACE activity in migraineurs during headache-free periods." Pain 128(3): 209-214.

Gaik, G. C. and A. I. Farbman (1973). "The chicken trigeminal ganglion. I. An anatomical analysis of the neuron types in the adult." J Morphol 141(1): 43-55.

Gartner, L. M., J. Morton, et al. (2005). "Breastfeeding and the use of human milk." Pediatrics 115(2): 496-506.

Geppetti, P., C. Bertrand, et al. (1993). "Characterization of tachykinin receptors in ferret trachea by peptide agonists and nonpeptide antagonists." Am J Physiol 265(2 Pt 1): L164-169.

Gibbins, I. L., J. B. Furness, et al. (1987). "Pathway-specific patterns of the co-existence of substance P, calcitonin gene-related peptide, cholecystokinin and dynorphin in neurons of the dorsal root ganglia of the guinea-pig." Cell Tissue Res 248(2): 417-437.

Glasgow, J. F., A. J. Thompson, et al. (2006). "Sudden unexpected death in infancy: place and time of death." Ulster Med J 75(1): 65-71.

Goadsby, P. J., L. Edvinsson, et al. (1988). "Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system." Ann Neurol 23(2): 193-196.

Goldberg, L. J. and S. H. Chandler (1990). "Central mechanisms of rhythmic trigeminal activity". *In: Neurophysiology of jaws and teeth*. Taylor A, editor. London, Macmillan, 268-321

Gotestam, K. G. (1991). "[Sudden infant death in Norway--does bedding play any role?]." Tidsskr Nor Laegeforen 111(29): 3544-3545.

Goto, T., K. Nakao, et al. (2007). "Substance P stimulates late-stage rat osteoblastic bone formation through neurokinin-1 receptors." Neuropeptides 41(1): 25-31.

Goubillon, M. L., R. A. Forsdike, et al. (2000). "Identification of neurokinin B-expressing neurons as an highly estrogen-receptive, sexually dimorphic cell group in the ovine arcuate nucleus." Endocrinology 141(11): 4218-4225.

Grafe, M. R. and H. C. Kinney (2002). "Neuropathology associated with stillbirth." Semin Perinatol 26(1): 83-88.

Graham, G. J., J. M. Stevens, et al. (2004). "Tachykinins regulate the function of platelets." Blood 104(4): 1058-1065.

Gressens, P., V. Laudenbach, et al. (2003). "[Mechanisms of action of tobacco smoke on the developing brain]." J Gynecol Obstet Biol Reprod (Paris) 32(1 Suppl): 1S30-32.

Groneberg, D. A., S. Harrison, et al. (2006). "Tachykinins in the respiratory tract." Curr Drug Targets 7(8): 1005-1010.

Grzybowska-Chlebowczyk, U., H. Wos, et al. (2003). "[Basal concentration of Substance P (SP) and Vasoactive Intestinal Peptide (VIP) in the blood serum of children with allergic dermatitis]." Med Wieku Rozwoj 7(1): 43-48.

Gunther, M. (1975). "The neonate's immunity gap, breast feeding and cot death." Lancet 1(7904): 441-442.
Guntheroth, W. G. (1989). "Theories of cardiovascular causes in sudden infant death syndrome." J Am Coll Cardiol 14(2): 443-447.
Guntheroth, W. G. and P. S. Spiers (2002). "The triple risk hypotheses in sudden infant death syndrome." Pediatrics 110(5): e64.
Haaland, K. and M. Thoresen (1992). "[Crib death, sleeping position and temperature]." Tidsskr Nor Laegeforen 112(11): 1466-1470.
Hamid, Q., M. G. Belvisi, et al. (1991). "Localization of beta pre-protachykinin mRNA in nodose ganglion." Neuropeptides 20(3): 145-150.
Hammar, J. A. (1906). " Ueber Gewicht, Involution, und Persistenz der Thymus im Postfdtalleben des Menschen ." ArchivffirAnatomische Entwicklungsgeschichte, 91-182 (quoted in Rolleston, op. cit., note 20 above, p. 439).
Hanani, M. (2005). "Satellite glial cells in sensory ganglia: from form to function." Brain Res Brain Res Rev 48(3): 457-476.
Hanani, M. (2010). "Satellite glial cells in sympathetic and parasympathetic ganglia: In search of function." Brain Res Rev.
Harper, R. M. (2000). "Sudden infant death syndrome: a failure of compensatory cerebellar mechanisms?" Pediatr Res 48(2): 140-142.
Harper, R. M., H. C. Kinney, et al. (2000). "Sleep influences on homeostatic functions: implications for sudden infant death syndrome." Respir Physiol 119(2-3): 123-132.
Harrison, S. and P. Geppetti (2001). "Substance p." Int J Biochem Cell Biol 33(6): 555-576.
Hathaway, C. B., J. W. Hu, et al. (1995). "Distribution of Fos-like immunoreactivity in the caudal brainstem of the rat following noxious chemical stimulation of the temporomandibular joint." J Comp Neurol 356(3): 444-456.
Haycock, G. and A. Greenough (2007). "Sudden infant death, bed-sharing and dummies: authors' reply." Arch Dis Child 92(6): 560.
Heath, A. C., V. S. Knopik, et al. (2003). "Accuracy of mothers' retrospective reports of smoking during pregnancy: comparison with twin sister informant ratings." Twin Res 6(4): 297-301.
Hedner, T., J. Hedner, et al. (1981). "Evidence suggesting a role for substance P in central respiratory regulation in the rat." Acta Physiol Scand 112(4): 487-489.
Helke, C. J., J. E. Krause, et al. (1990). "Diversity in mammalian tachykinin peptidergic neurons: multiple peptides, receptors, and regulatory mechanisms." FASEB J 4(6): 1606-1615.
Herbert, M. K. and P. Holzer (2002). "[Neurogenic inflammation. II. pathophysiology and clinical implications]." Anasthesiol Intensivmed Notfallmed Schmerzther 37(7): 386-394.
Hershey, A. D., P. E. Dykema, et al. (1991). "Organization, structure, and expression of the gene encoding the rat substance P receptor." J Biol Chem 266(7): 4366-4374.
Hershey, A. D. and J. E. Krause (1990). "Molecular characterization of a functional cDNA encoding the rat substance P receptor." Science 247(4945): 958-962.
Hilaire, G. and B. Duron (1999). "Maturation of the mammalian respiratory system." Physiol Rev 79(2): 325-360.
Hodges, F. B. (1972). "Sudden infant death syndrome." Calif Med 116(1): 85-86.
Hokfelt, T., J. O. Kellerth, et al. (1975). "Substance p: localization in the central nervous system and in some primary sensory neurons." Science 190(4217): 889-890.
Hokfelt, T. and E. Kuteeva (2006). "Substance P: a neuropeptide." Am J Psychiatry 163(4): 578.

Hokfelt, T., B. Meyerson, et al. (1976). "Immunohistochemical evidence for substance P-containing nerve endings in the human cortex." Brain Res 104(1): 181-186.

Hokfelt, T., S. Vincent, et al. (1982). "Distribution of substance P in brain and periphery and its possible role as a co-transmitter." Ciba Found Symp(91): 84-106.

Holmgren, S. and J. Jensen (2001). "Evolution of vertebrate neuropeptides." Brain Res Bull 55(6): 723-735.

Holzer, P. (1998). "Neurogenic vasodilatation and plasma leakage in the skin." Gen Pharmacol 30(1): 5-11.

Holzer, P. (1998). "Tachykinins as targets of gastroenterological pharmacotherapy." Drug News Perspect 11(7): 394-401.

Holzer, P. and U. Holzer-Petsche (1997). "Tachykinins in the gut. Part II. Roles in neural excitation, secretion and inflammation." Pharmacol Ther 73(3): 219-263.

Holzer, P. and C. A. Maggi (1994). "Synergistic role of muscarinic acetylcholine and tachykinin NK-2 receptors in intestinal peristalsis." Naunyn Schmiedebergs Arch Pharmacol 349(2): 194-201.

Hong, H. S., J. Lee, et al. (2009). "A new role of substance P as an injury-inducible messenger for mobilization of CD29(+) stromal-like cells." Nat Med 15(4): 425-435.

Horsley, T., T. Clifford, et al. (2007). "Benefits and harms associated with the practice of bed sharing: a systematic review." Arch Pediatr Adolesc Med 161(3): 237-245.

Howard, M., K. Haddley, et al. (2006).

Howat, A. J., M. J. Bennett, et al. (1985). "Defects of metabolism of fatty acids in the sudden infant death syndrome." Br Med J (Clin Res Ed) 290(6484): 1771-1773.

Hunt, C. E. (1992). "The cardiorespiratory control hypothesis for sudden infant death syndrome." Clin Perinatol 19(4): 757-771.

Hunt, C. E. and R. T. Brouillette (1987). "Sudden infant death syndrome: 1987 perspective." J Pediatr 110(5): 669-678.

Hunt, S. P. and J. Rossi (1985). "Peptide- and non-peptide-containing unmyelinated primary afferents: the parallel processing of nociceptive information." Philos Trans R Soc Lond B Biol Sci 308(1136): 283-289.

Inagaki, N., N. Shiraishi, et al. (2010). "Depletion of substance P, a mechanism for inhibition of mouse scratching behavior by tacrolimus." Eur J Pharmacol 626(2-3): 283-289.

Iversen, L. L., C. M. Lee, et al. (1980). "Regulation of neuropeptide release." Proc R Soc Lond B Biol Sci 210(1178): 91-111.

James, T. N. (1968). "Sudden death in babies: new observations in the heart." Am J Cardiol 22(4): 479-506.

Joachim, R. A., M. Hildebrandt, et al. (2001). "Murine stress-triggered abortion is mediated by increase of CD8+ TNF-alpha+ decidual cells via substance P." Am J Reprod Immunol 45(5): 303-309.

Johnston, J.B. (1909). "The radix mesencephalica trigemini." J Comp Neurol 19, 593–64

Jones, S., K. L. Tucker, et al. (2008). "Peripheral tachykinins and the neurokinin receptor NK1 are required for platelet thrombus formation." Blood 111(2): 605-612.

Jonsson, M., O. Norrgard, et al. (2005). "Substance P and the neurokinin-1 receptor in relation to eosinophilia in ulcerative colitis." Peptides 26(5): 799-814.

Jordan, D., I. Kermadi, et al. (1995). "Regional distribution of substance P binding sites in the brainstem of the human newborn." Brain Res 695(2): 117-124.

Kachidian, P., P. Poulat, et al. (1991). "Immunohistochemical evidence for the coexistence of substance P, thyrotropin-releasing hormone, GABA, methionine-enkephalin, and leucin-enkephalin in the serotonergic neurons of the caudal raphe nuclei: a dual labeling in the rat." J Neurosci Res 30(3): 521-530.

Kage, R., G. P. McGregor, et al. (1988). "Neuropeptide-gamma: a peptide isolated from rabbit intestine that is derived from gamma-preprotachykinin." J Neurochem 50(5): 1412-1417.

Kahle, W. and M. Frotscher (2005). "Taschenatlas Anatomie: 3 Nervensystem und Sinnesorgane." Thierne Publishers 106-107.

Kapoor, S. K., K. Anand, et al. (1994). "Risk factors for stillbirths in a secondary level hospital at Ballabgarh, Haryana: a case control study." Indian J Pediatr 61(2): 161-166.

Kawaguchi, Y., M. Hoshimaru, et al. (1986). "Sequence analysis of cloned cDNA for rat substance P precursor: existence of a third substance P precursor." Biochem Biophys Res Commun 139(3): 1040-1046.

Kerenyi, N. and J. Fekete (1969). "Sudden unexpected death in infancy." Canad J Pub Hlth 60: 357-361.

Kertes, E., K. Laszlo, et al. (2009). "Effects of substance P microinjections into the globus pallidus and central nucleus of amygdala on passive avoidance learning in rats." Behav Brain Res 198(2): 397-403.

Kertes, E., K. Laszlo, et al. (2010). "Positive reinforcing effects of substance P in the rat globus pallidus revealed by conditioned place preference." Behav Brain Res.

Killen, S. A., J. Kunic, et al. (2010). "SCN5A allelic expression imbalance in African-Americans heterozygous for the common variant p.Ser1103Tyr." BMC Med Genet 11: 74.

Kinkead, R. (2009). "Phylogenetic trends in respiratory rhythmogenesis: insights from ectothermic vertebrates." Respir Physiol Neurobiol 168(1-2): 39-48.

Kinney, H. C. (2009). "Neuropathology provides new insight in the pathogenesis of the sudden infant death syndrome." Acta Neuropathol 117(3): 247-255.

Kinney, H. C., P. C. Burger, etal. (1983). "Reactive gliosis in the medulla oblongata of victims of the sudden infant death syndrome." Pediat 72(2): 181-187.

Kinney, H. C. and J. J. Filiano (1988). "Brainstem research in sudden infant death syndrome." Pediatrician 15(4): 240-250.

Kinney, H. C., J. J. Filiano, et al. (1995). "Decreased muscarinic receptor binding in the arcuate nucleus in sudden infant death syndrome." Science 269(5229): 1446-1450.

Kinney, H. C., J. J. Filiano, et al. (2001). "Medullary serotonergic network deficiency in the sudden infant death syndrome: review of a 15-year study of a single dataset." J Neuropathol Exp Neurol 60(3): 228-247.

Kinney, H. C., G. B. Richerson, et al. (2009). "The brainstem and serotonin in the sudden infant death syndrome." Annu Rev Pathol 4: 517-550.

Kinney, H. C. and B. T. Thach (2009). "The sudden infant death syndrome." N Engl J Med 361(8): 795-805.

Kitsantas, P. and K. F. Gaffney (2010). "Racial/ethnic disparities in infant mortality." J Perinat Med 38(1): 87-94.

Kiyama, H., Y. Morita, et al. (1988). "Demonstration of rat preprotachykinin A mRNA in the rat trigeminal ganglion by in situ hybridization histochemistry." J Chem Neuroanat 1(3): 125-132.

Klassert, T. E., S. A. Patel, et al. (2010). "Tachykinins and Neurokinin Receptors in Bone Marrow Functions: Neural-Hematopoietic Link." J Receptor Ligand Channel Res 2010(3): 51-61.

Klassert, T. E., F. Pinto, et al. (2008). "Differential expression of neurokinin B and hemokinin-1 in human immune cells." J Neuroimmunol 196(1-2): 27-34.

Ko, F. J., C. H. Chiang, et al. (1991). "Somatostatin, substance P, prolactin and vasoactive intestinal peptide levels in serum and cerebrospinal fluid of children with seizure disorders." Gaoxiong Yi Xue Ke Xue Za Zhi 7(8): 391-397.

Koch, B. L., A. A. Edvinsson, et al. (1999). "Inhalation of substance P and thiorphan: acute toxicity and effects on respiration in conscious guinea pigs." J Appl Toxicol 19(1): 19-23.

Koizumi, H., K. Nomura, et al. (2009). "Regulation of trigeminal respiratory motor activity in the brainstem." J Dent Res 88(11): 1048-1053.

Konishi, S. and M. Otsuka (1985). "Blockade of slow excitatory post-synaptic potential by substance P antagonists in guinea-pig sympathetic ganglia." J Physiol 361: 115-130.

Koon, H. W., D. Zhao, et al. (2008). "Substance P-mediated expression of the pro-angiogenic factor CCN1 modulates the course of colitis." Am J Pathol 173(2): 400-410.

Koon, H. W., D. Zhao, et al. (2005). "Substance P-stimulated interleukin-8 expression in human colonic epithelial cells involves protein kinase Cdelta activation." J Pharmacol Exp Ther 314(3): 1393-1400.

Kopp, N., L. Denoroy, et al. (1994). "Studies of neuroregulators in the brain stem of SIDS." Biol Neonate 65(3-4): 189-193.

Kopp, N., L. Denoroy, et al. (1992). "[Abnormalities of the nervous system in sudden infant death syndrome]." Pediatrie 47(6): 429-439.

Kotani, H., M. Hoshimaru, et al. (1986). "Structure and gene organization of bovine neuromedin K precursor." Proc Natl Acad Sci U S A 83(18): 7074-7078.

Kraemer, A. M., L. R. Saraiva, et al. (2008). "Structural and functional diversification in the teleost S100 family of calcium-binding proteins." BMC Evol Biol **8**: 48.

Kraus, A. S., R. Steele, et al. (1967). "Sudden, unexpected death in infancy in Ontario. II. Findings regarding season, clustering of deaths on specific days and weather." Can J Public Health 58(8): 364-371.

Krause, J. E., M. R. MacDonald, et al. (1989). "The polyprotein nature of substance P precursors." Bioessays 10(2-3): 62-69.

Krause, J. E., Y. Takeda, et al. (1992). "Structure, functions, and mechanisms of substance P receptor action." J Invest Dermatol 98(6 Suppl): 2S-7S.

Krous, H. F. (1984). "Sudden infant death syndrome: pathology and pathophysiology." Pathol Annu 19 Pt 1: 1-14.

Kuwahara, A. and H. J. Cooke (1990). "Tachykinin-induced anion secretion in guinea pig distal colon: role of neural and inflammatory mediators." J Pharmacol Exp Ther 252(1): 1-7.

Lacoste, B., M. Riad, et al. (2009). "Trafficking of neurokinin-1 receptors in serotonin neurons is controlled by substance P within the rat dorsal raphe nucleus." Eur J Neurosci 29(12): 2303-2314.

Lagercrantz, H., M. Srinivasan, et al. (1991). "Functional role of substance P for respiratory control during development." Ann N Y Acad Sci 632: 48-52.

Lai, J. P., S. D. Douglas, et al. (1998). "Identification of a delta isoform of preprotachykinin mRNA in human mononuclear phagocytes and lymphocytes." J Neuroimmunol 91(1-2): 121-128.

Lambers, D. S. and K. E. Clark (1996). "The maternal and fetal physiologic effects of nicotine." Semin Perinatol 20(2): 115-126.

Landau, A. M., K. Yashpal, et al. (2007). "Sensory neuron and substance P involvement in symptoms of a zymosan-induced rat model of acute bowel inflammation." Neuroscience 145(2): 699-707.

Lavagno, L., G. Bordin, et al. (2001). "Tachykinin activation of human monocytes from patients with rheumatoid arthritis: in vitro and ex-vivo effects of cyclosporin A." Neuropeptides 35(2): 92-99.

Lavezzi, A. M., V. Casale, et al. (2009). "Sudden infant death syndrome and sudden intrauterine unexplained death: correlation between hypoplasia of raphe nuclei and serotonin transporter gene promoter polymorphism." Pediatr Res 66(1): 22-27.

Lavezzi, A. M., M. Corna, et al. (2009). "Neuropathology of the Guillain-Mollaret Triangle (Dentato-Rubro-Olivary Network) in Sudden Unexplained Perinatal Death and SIDS." Open Neurol J 3: 48-53.

Lavezzi, A. M., M. Corna, et al. (2010). "Study of the human hypoglossal nucleus: normal development and morpho-functional alterations in sudden unexplained late fetal and infant death." Brain Dev 32(4): 275-284.

Lavezzi, A. M., M. F. Corna, et al. (2010). "Ependymal alterations in sudden intrauterine unexplained death and sudden infant death syndrome: possible primary consequence of prenatal exposure to cigarette smoking." Neural Dev 5: 17.

Lavezzi, A. M., M. F. Corna, et al. (2010). "Neuropathology of the intermediolateral nucleus of the spinal cord in sudden unexplained perinatal and infant death." Int J Dev Neurosci 28(2): 133-138.

Lavezzi, A. M. and L. Matturri (2008). "Functional neuroanatomy of the human pre-Botzinger complex with particular reference to sudden unexplained perinatal and infant death." Neuropathology 28(1): 10-16.

Lavezzi, A. M., G. Ottaviani, et al. (2004). "Preliminary study on the cytoarchitecture of the human parabrachial/Kolliker-fuse complex, with reference to sudden infant death syndrome and sudden intrauterine unexplained death." Pediatr Dev Pathol 7(2): 171-179.

Lavezzi, A. M., G. Ottaviani, et al. (2003). "Identification of neurons responding to hypoxia in sudden infant death syndrome." Pathol Int 53(11): 769-774.

Lavezzi, A. M., G. Ottaviani, et al. (2004). "Involvement of somatostatin in breathing control before and after birth, and in perinatal and infant sudden unexplained death." Folia Neuropathol 42(2): 59-65.

Lavezzi, A. M., G. Ottaviani, et al. (2005a). "Adverse effects of prenatal tobacco smoke exposure on biological parameters of the developing brainstem." Neurobiol Dis 20(2): 601-607.

Lavezzi, A. M., G. Ottaviani, et al. (2006). "Alterations of biological features of the cerebellum in sudden perinatal and infant death." Curr Mol Med 6(4): 429-435.

Lavezzi, A. M., G. Ottaviani, et al. (2007). "Biopathology of the dentate-olivary complex in sudden unexplained perinatal death and sudden infant death syndrome related to maternal cigarette smoking." Neurol Res 29(6): 525-532.

Lavezzi, A. M., G. Ottaviani, et al. (2005b). "Analysis of the human locus coeruleus in perinatal and infant sudden unexplained deaths. Possible role of the cigarette smoking in the development of this nucleus." Brain Res Dev Brain Res 154(1): 71-80.

Lavezzi, A. M., G. Ottaviani, et al. (2004). "Hypoplasia of the parabrachial/kolliker-fuse complex in perinatal death." Biol Neonate 86(2): 92-97.

Lazarczyk, M., E. Matyja, et al. (2007). "Substance P and its receptors -- a potential target for novel medicines in malignant brain tumour therapies (mini-review)." Folia Neuropathol 45(3): 99-107.

Lazarov, N. E. (2000). "The mesencephalic trigeminal nucleus in the cat." Adv Anat Embryol Cell Biol 153: iii-xiv, 1-103.

Lazarov, N. E. (2002). "Comparative analysis of the chemical neuroanatomy of the mammalian trigeminal ganglion and mesencephalic trigeminal nucleus." Prog Neurobiol 66(1): 19-59.

Lazarov, N. E. and C. N. Chouchkov (1996). "Peptidergic innervation of the mesencephalic trigeminal nucleus in the cat." Anat Rec 245(3): 581-592.

Lecci, A., A. Capriati, et al. (2006). "Tachykinins and tachykinin receptors in the gut, with special reference to NK2 receptors in human." Auton Neurosci 126-127: 232-249.

Lee, K. E. and B. A. Winkelstein (2009). "Joint distraction magnitude is associated with different behavioral outcomes and substance P levels for cervical facet joint loading in the rat." J Pain 10(4): 436-445.

Lee, Y., Y. Kawai, et al. (1985). "Coexistence of calcitonin gene-related peptide and substance P-like peptide in single cells of the trigeminal ganglion of the rat: immunohistochemical analysis." Brain Res 330(1): 194-196.

Leeman, S. E. and R. Hammerschlag (1967). "Stimulation of salivary secretion by a factor extracted from hypothalamic tissue." Endocrinology 81(4): 803-810.

Lembeck, F., G. Bernatzky, et al. (1985). "Characterization of substance P-like immunoreactivity in submammalian species by high performance liquid chromatography." Peptides 6 Suppl 3: 231-236.

Lembeck, F. and K. Starke (1968). "[Substance P and salivary secretion]." Naunyn Schmiedebergs Arch Exp Pathol Pharmakol 259(5): 375-385.

Levick, S. P., D. B. Murray, et al. (2010). "Sympathetic nervous system modulation of inflammation and remodeling in the hypertensive heart." Hypertension 55(2): 270-276.

Levine, J. D., H. L. Fields, et al. (1993). "Peptides and the primary afferent nociceptor." J Neurosci 13(6): 2273-2286.

Li, D. K., M. Willinger, et al. (2006). "Use of a dummy (pacifier) during sleep and risk of sudden infant death syndrome (SIDS): population based case-control study." BMJ 332(7532): 18-22.

Li, J., M. Ahmed, et al. (2010). "Occurrence of substance P in bone repair under different load comparison of straight and angulated fracture in rat tibia." J Orthop Res.

Lieb, K., Y. Treffurth, et al. (2002). "Substance P and affective disorders: new treatment opportunities by neurokinin 1 receptor antagonists?" Neuropsychobiology 45 Suppl 1: 2-6.

Lieberman, A. R. (1976). "Sensory ganglia." In *The Peripheral Nerve,* ed. D. N. Landon 188-278. London: Chapman and Hall.

Linden, R. W. (1978). "Properties of intraoral mechanoreceptors represented in the mesencephalic nucleus of the fifth nerve in the cat." J Physiol 279: 395-408.

Linden, R. W., B. J. Millar, et al. (1994). "A comparative physiological and morphological study of periodontal ligament mechanoreceptors represented in the trigeminal ganglion and the mesencephalic nucleus of the cat." Anat Embryol (Berl) 190(2): 127-135.

Linnik, M. D. and M. A. Moskowitz (1989). "Identification of immunoreactive substance P in human and other mammalian endothelial cells." Peptides 10(5): 957-962.

Liu, D., L. S. Jiang, et al. (2007). "Substance P and its receptors in bone metabolism." Neuropeptides 41(5): 271-283.

Liu, D., H. Li, et al. (2008). "Changes of substance P-immunoreactive nerve fiber innervation density in the sublesional bones in young growing rats at an early stage after spinal cord injury." Osteoporos Int 19(4): 559-569.

Liu, J. F., A. J. Moss, et al. (2010). "Mutation-specific risk in two genetic forms of type 3 long QT syndrome." Am J Cardiol 105(2): 210-213.

Luccarini, P., R. Cadet, et al. (1998). "Effects of lesions in the trigeminal oralis and caudalis subnuclei on different orofacial nociceptive responses in the rat." Brain Res 803(1-2): 79-85.

Lund, J. P., A. Kolta, et al. (1998). "Brainstem mechanisms underlying feeding behaviors." Curr Opin Neurobiol 8(6): 718-724.

Lundquist, C. T., F. L. Clottens, et al. (1994). "Locustatachykinin immunoreactivity in the blowfly central nervous system and intestine." J Comp Neurol 341(2): 225-240.

Luo, Z. C., R. Wilkins, et al. (2004). "Risks of adverse pregnancy outcomes among Inuit and North American Indian women in Quebec, 1985-97." Paediatr Perinat Epidemiol 18(1): 40-50.

Madea, B. (2009). "Sudden death, especially in infancy--improvement of diagnoses by biochemistry, immunohistochemistry and molecular pathology." Leg Med (Tokyo) 11 Suppl 1: S36-42.

Mage, D. T. and E. M. Donner (2004). "The fifty percent male excess of infant respiratory mortality." Acta Paediatr 93(9): 1210-1215.

Maggi, C. A. (1995). "The mammalian tachykinin receptors." Gen Pharmacol 26(5): 911-944.

Maggi, C. A. (1997). "Tachykinins as peripheral modulators of primary afferent nerves and visceral sensitivity." Pharmacol Res 36(2): 153-169.

Maggi, C. A., R. Patacchini, et al. (1994). "Tachykinin NK1 and NK2 receptor antagonists and atropine-resistant ascending excitatory reflex to the circular muscle of the guinea-pig ileum." Br J Pharmacol 112(1): 161-168.

Makielski, J. C. (2006). "SIDS: genetic and environmental influences may cause arrhythmia in this silent killer." J Clin Invest 116(2): 297-299.

Mancillas, J. R., J. F. McGinty, et al. (1981). "Immunocytochemical localization of enkephalin and substance P in retina and eyestalk neurones of lobster." Nature 293(5833): 576-578.

Marter, A. and J. C. Agruss (2007). "Pacifiers: an update on use and misuse." J Spec Pediatr Nurs 12(4): 278-285.

Massi, M., I. Panocka, et al. (2000). "The psychopharmacology of tachykinin NK-3 receptors in laboratory animals." Peptides 21(11): 1597-1609.

Matturri, L., I. Minoli, et al. (2002). "Hypoplasia of medullary arcuate nucleus in unexpected late fetal death (stillborn infants): a pathologic study." Pediatrics 109(3): E43.

Matturri, L., G. Ottaviani, et al. (2005). "Techniques and criteria in pathologic and forensic-medical diagnostics in sudden unexpected infant and perinatal death." Am J Clin Pathol 124(2): 259-268.

Matturri, L., G. Ottaviani, et al. (2008). "Guidelines for neuropathologic diagnostics of perinatal unexpected loss and sudden infant death syndrome (SIDS): a technical protocol." Virchows Arch 452(1): 19-25.

Mauser, P. J., S. Skeans, et al. (2001). "Effect of tachykinins on airway function in cynomolgus monkeys." Pulm Pharmacol Ther 14(2): 121-127.

Mazzone, S. B. and D. P. Geraghty (2000). "Respiratory actions of tachykinins in the nucleus of the solitary tract: characterization of receptors using selective agonists and antagonists." Br J Pharmacol 129(6): 1121-1131.

McMahon, S. B., W. B. Cafferty, et al. (2005). "Immune and glial cell factors as pain mediators and modulators." Exp Neurol 192(2): 444-462.

Mejia, J. A., J. Pernow, et al. (1988). "Effects of neuropeptide Y, calcitonin gene-related peptide, substance P, and capsaicin on cerebral arteries in man and animals." J Neurosurg 69(6): 913-918.

Mitchell, E. A. (2009). "SIDS: past, present and future." Acta Paediatr 98(11): 1712-1719.

Mitchell, E. A. (2009). "What is the mechanism of SIDS? Clues from epidemiology." Dev Psychobiol 51(3): 215-222.

Mitchell, E. A., P. S. Blair, et al. (2006). "Should pacifiers be recommended to prevent sudden infant death syndrome?" Pediatrics 117(5): 1755-1758.

Mitchell, E. A. and J. Milerad (2006). "Smoking and the sudden infant death syndrome." Rev Environ Health 21(2): 81-103.

Mitchell, E. A., R. Scragg, et al. (1991). "Results from the first year of the New Zealand cot death study." N Z Med J 104(906): 71-76.

Molz, G. (1997). "[Sudden infant death]." Ther Umsch 54(5): 286-292.

Money, D. F. (1971). "Cot deaths and deficiency of vitamin E and selenium." Br Med J 4(5786): 559.

Morgado-Valle, C. and J. L. Feldman (2004). "Depletion of substance P and glutamate by capsaicin blocks respiratory rhythm in neonatal rat in vitro." J Physiol 555(Pt 3): 783-792.

Morgan, F. A. (1969). "Crib deaths." Can Med Assoc J 100(20): 968-969.

Moss, I. R., M. Denavit-Saubie, et al. (1986). "Neuromodulators and transmitters in respiratory control." Fed Proc 45(7): 2133-2147.

Moss, I. R. and J. G. Inman (1989). "Neurochemicals and respiratory control during development." J Appl Physiol 67(1): 1-13.

Mowa, C. N., S. Usip, et al. (2003). "Substance P in the uterine cervix, dorsal root ganglia and spinal cord during pregnancy and the effect of estrogen on SP synthesis." Peptides 24(5): 761-771.

Mukda, S., M. Moller, et al. (2009). "The modulatory effect of substance P on rat pineal norepinephrine release and melatonin secretion." Neurosci Lett 461(3): 258-261.

Munoz, M., M. Rosso, et al. (2010). "The NK-1 receptor is expressed in human melanoma and is involved in the antitumor action of the NK-1 receptor antagonist aprepitant on melanoma cell lines." Lab Invest 90(8): 1259-1269.

Mutoh, T., A. C. Bonham, et al. (2000). "Substance P in the nucleus of the solitary tract augments bronchopulmonary C fiber reflex output." Am J Physiol Regul Integr Comp Physiol 279(4): R1215-1223.

Naeye, R. L. (1976). "Brain-stem and adrenal abnormalities in the sudden-infant-death syndrome." Am J Clin Pathol 66(3): 526-530.

Nagy, J. I., M. Buss, et al. (1986). "On the innervation of trigeminal mesencephalic primary afferent neurons by adenosine deaminase-containing projections from the hypothalamus in the rat." Neuroscience 17(1): 141-156.

Nakanishi, S. (1987). "Substance P precursor and kininogen: their structures, gene organizations, and regulation." Physiol Rev 67(4): 1117-1142.
Nakanishi, S. (1991). "Mammalian tachykinin receptors." Annu Rev Neurosci 14: 123-136.
Naline, E., P. Devillier, et al. (1989). "Characterization of neurokinin effects and receptor selectivity in human isolated bronchi." Am Rev Respir Dis 140(3): 679-686.
Naline, E., M. Molimard, et al. (1996). "Evidence for functional tachykinin NK1 receptors on human isolated small bronchi." Am J Physiol 271(5 Pt 1): L763-767.
Nattie, E. and H. Kinney (2002). "Nicotine, serotonin, and sudden infant death syndrome." Am J Respir Crit Care Med 166(12 Pt 1): 1530-1531.
Nawa, H., T. Hirose, et al. (1983). "Nucleotide sequences of cloned cDNAs for two types of bovine brain substance P precursor." Nature 306(5938): 32-36.
Nelson, D. A. and K. L. Bost (2004). "Non-neuronal mammalian tachykinin expression." Front Biosci 9: 2166-2176.
Nicholas, A. P., V. A. Pieribone, et al. (1992). "Serotonin-, substance P- and glutamate/aspartate-like immunoreactivities in medullo-spinal pathways of rat and primate." Neuroscience 48(3): 545-559.
Nicoll, R. A., C. Schenker, et al. (1980). "Substance P as a transmitter candidate." Annu Rev Neurosci 3: 227-268.
Nieber, K., C. R. Baumgarten, et al. (1992). "Substance P and beta-endorphin-like immunoreactivity in lavage fluids of subjects with and without allergic asthma." J Allergy Clin Immunol 90(4 Pt 1): 646-652.
Nishiyama, Y., A. Abe, et al. (2010). "Nicergoline increases serum substance P levels in patients with an ischaemic stroke." Cerebrovasc Dis 29(2): 194-198.
Nomura, H., S. Shiosaka, et al. (1982). "Distribution of substance P-like immunoreactivity in the lower brainstem of the human fetus: an immunohistochemical study." Brain Res 252(2): 315-325.
Nowicki, M., D. Ostalska-Nowicka, et al. (2007). "The significance of substance P in physiological and malignant haematopoiesis." J Clin Pathol 60(7): 749-755.
O'Brien, M. J. (1993). "SIDS--a developmental neurological perspective." Early Hum Dev 34(1-2): 125-132.
Obonai, T., S. Takashima, et al. (1996). "Relationship of substance P and gliosis in medulla oblongata in neonatal sudden infant death syndrome." Pediatr Neurol 15(3): 189-192.
Ogier, R., L. J. Wrobel, et al. (2008). "Action of tachykinins in the hippocampus: facilitation of inhibitory drive to GABAergic interneurons." Neuroscience 156(3): 527-536.
Ohshima, M., M. Miyake, et al. (2010). "Development of mechanisms associated with neurogenic-mediated skin inflammation during the growth of rats." Pediatr Res 67(4): 363-368.
Olszewski, J. (1950). "On the anatomical and functional organization of the spinal trigeminal nucleus." J Comp Neurol 92(3): 401-413.
Onuoha, G. N. and E. K. Alpar (2001). "Levels of vasodilators (SP, CGRP) and vasoconstrictor (NPY) peptides in early human burns." Eur J Clin Invest 31(3): 253-257.
Otsuka, M. and M. Yanagisawa (1990). "Pain and neurotransmitters." Cell Mol Neurobiol 10(3): 293-302.
Otsuka, M. and K. Yoshioka (1993). "Neurotransmitter functions of mammalian tachykinins." Physiol Rev 73(2): 229-308.

Ottaviani, G. (2010). "Sudden infant and perinatal unexplained death: are we moving forward yet?" Cardiovasc Pathol.

Ottaviani, G. and G. C. Bergui (2009). "Sudden unexpected death in infancy (SUDI): a new anatomo-clinical approach." Europace 11(3): 395.

Ottaviani, G. and L. Matturri (2008). "Histopathology of the cardiac conduction system in sudden intrauterine unexplained death." Cardiovasc Pathol 17(3): 146-155.

Otto-Buczkowska, E. (2002). "[Sudden infant death syndrome]." Pol Merkur Lekarski 13(78): 524-525.

Ozawa, Y. and S. Takashima (2002). "Developmental neurotransmitter pathology in the brainstem of sudden infant death syndrome: a review and sleep position." Forensic Sci Int 130 Suppl: S53-59.

Page, N. M. (2004). "Hemokinins and endokinins." Cell Mol Life Sci 61(13): 1652-1663.

Page, N. M., N. J. Bell, et al. (2003). "Characterization of the endokinins: human tachykinins with cardiovascular activity." Proc Natl Acad Sci U S A 100(10): 6245-6250.

Page, N. M., R. J. Woods, et al. (2000). "Excessive placental secretion of neurokinin B during the third trimester causes pre-eclampsia." Nature 405(6788): 797-800.

Pallewela, C. S. (1974). "Antepartum diagnosis of vasa praevia--report of a case causing sudden foetal death." Postgrad Med J 50(589): 723-725.

Paltauf, A. (1889). " Über die Beziehung der Thymus zum plötzlichen Tod." Wien Klin Wochenschr 2:877-881.

Pannese, E. (1981). "The satellite cells of the sensory ganglia." Adv Anat Embryol Cell Biol 65: 1-111.

Parish, W. E., A. M. Barrett, et al. (1960). "Hypersensitivity to milk and sudden death in infancy." Lancet 2(7160): 1106-1110.

Patacchini, R. and C. A. Maggi (1995). "Tachykinin receptors and receptor subtypes." Arch Int Pharmacodyn Ther 329(1): 161-184.

Patacchini, R., P. Santicioli, et al. (1998). "Excitatory motor and electrical effects produced by tachykinins in the human and guinea-pig isolated ureter and guinea-pig renal pelvis." Br J Pharmacol 125(5): 987-996.

Patak, E., M. L. Candenas, et al. (2003). "Tachykinins and tachykinin receptors in human uterus." Br J Pharmacol 139(3): 523-532.

Patak, E. N., J. N. Pennefather, et al. (2000). "Effects of tachykinins on uterine smooth muscle." Clin Exp Pharmacol Physiol 27(11): 922-927.

Paulus, W. J. (2001). "The role of nitric oxide in the failing heart." Heart Fail Rev 6(2): 105-118.

Peduto, V. A., M. Musu, et al. (1994). "[Sudden infant death syndrome (SIDS): risk conditions and intervention strategies]." Minerva Anestesiol 60(7-8): 393-402.

Pennefather, J. N., A. Lecci, et al. (2004). "Tachykinins and tachykinin receptors: a growing family." Life Sci 74(12): 1445-1463.

Pernow, B. (1983). "Substance P." Pharmacol Rev 35(2): 85-141.

Persing, J., H. James, et al. (2003). "Prevention and management of positional skull deformities in infants. American Academy of Pediatrics Committee on Practice and Ambulatory Medicine, Section on Plastic Surgery and Section on Neurological Surgery." Pediatrics 112(1 Pt 1): 199-202.

Petersen, L. J., L. K. Poulsen, et al. (1994). "The use of cutaneous microdialysis to measure substance P-induced histamine release in intact human skin in vivo." J Allergy Clin Immunol 94(4): 773-783.

Peterson, D. R. (1966). "Sudden, unexpected death in infants. An epidemiologic study." Am J Epidemiol 84(3): 478-482.

Pfaller, K. and J. Arvidsson (1988). "Central distribution of trigeminal and upper cervical primary afferents in the rat studied by anterograde transport of horseradish peroxidase conjugated to wheat germ agglutinin." J Comp Neurol 268(1): 91-108.

Phelan, K. D. and W. M. Falls (1989). "The interstitial system of the spinal trigeminal tract in the rat: anatomical evidence for morphological and functional heterogeneity." Somatosens Mot Res 6(4): 367-399.

Phillips, D. P., K. M. Brewer, et al. (2010). "Alcohol as a risk factor for sudden infant death syndrome (SIDS)." Addiction.

Pickel, V. M., T. H. Joh, et al. (1979). "Electron microscopic localization of substance P and enkephalin in axon terminals related to dendrites of catecholaminergic neurons." Brain Res 160(3): 387-400.

Pickett, K. E., Y. Luo, et al. (2005). "Widening social inequalities in risk for sudden infant death syndrome." Am J Public Health 95(11): 1976-1981.

Pierobon, P., M. Kemali, et al. (1989). "Substance P and hydra: an immunohistochemical and physiological study." Comp Biochem Physiol C 92(2): 217-221.

Pintado, C. O., F. M. Pinto, et al. (2003). "A role for tachykinins in female mouse and rat reproductive function." Biol Reprod 69(3): 940-946.

Pinto, F. M., T. A. Almeida, et al. (2004). "mRNA expression of tachykinins and tachykinin receptors in different human tissues." Eur J Pharmacol 494(2-3): 233-239.

Plant, L. D., P. N. Bowers, et al. (2006). "A common cardiac sodium channel variant associated with sudden infant death in African Americans, SCN5A S1103Y." J Clin Invest 116(2): 430-435.

Pritchard, E. (1918). "Enlarged Suprarenals and Sudden Death in an Infant." Proc R Soc Med 11(Sect Study Dis Child): 35-36.

Procacci, P., M. Marcesca (1999). " Referred pain from somatic and visceral strutures." Curr Rev Pain 3:96–99.

Ptak, K., E. Di Pasquale, et al. (1999). "Substance P and central respiratory activity: a comparative in vitro study on foetal and newborn rat." Brain Res Dev Brain Res 114(2): 217-227.

Ptak, K., S. P. Hunt, et al. (2000). "Substance P and central respiratory activity: a comparative in vitro study in NK1 receptor knockout and wild-type mice." Pflugers Arch 440(3): 446-451.

Quartara, L. and C. A. Maggi (1997). "The tachykinin NK1 receptor. Part I: ligands and mechanisms of cellular activation." Neuropeptides 31(6): 537-563.

Radhakrishnan, V., K. Yashpal, et al. (1995). "Implication of a nitric oxide synthase mechanism in the action of substance P: L-NAME blocks thermal hyperalgesia induced by endogenous and exogenous substance P in the rat." Eur J Neurosci 7(9): 1920-1925.

Rameshwar, P. and P. Gascon (1995). "Substance P (SP) mediates production of stem cell factor and interleukin-1 in bone marrow stroma: potential autoregulatory role for these cytokines in SP receptor expression and induction." Blood 86(2): 482-490.

Rameshwar, P. and P. Gascon (1997). "Hematopoietic modulation by the tachykinins." Acta Haematol 98(2): 59-64.

Ramkissoon, S. H., H. J. Patel, et al. (2006). "G protein-coupled receptors in haematopoietic disruption." Expert Opin Biol Ther 6(2): 109-120.

Regoli, D., Q. T. Nguyen, et al. (1994). "Neurokinin receptor subtypes characterized by biological assays." Life Sci 54(26): 2035-2047.

Remrod, C., S. Lonne-Rahm, et al. (2007). "Study of substance P and its receptor neurokinin-1 in psoriasis and their relation to chronic stress and pruritus." Arch Dermatol Res 299(2): 85-91.

Ren, K. and R. Dubner (1999). "Central nervous system plasticity and persistent pain." J Orofac Pain 13(3): 155-163; discussion 164-171.

Ren, K. and R. Dubner (2008). "Neuron-glia crosstalk gets serious: role in pain hypersensitivity." Curr Opin Anaesthesiol 21(5): 570-579.

Rhead, W. J., G. N. Schrauzer, et al. (1973). "Letter: Sudden death in infancy and vitamin E deficiency." Br Med J 4(5891): 548-549.

Rice, A. J., P. N. Reynolds, et al. (2001). "Tachykinin-induced bronchoconstriction in sheep is NK-1 receptor mediated and exhibits tachyphylaxis." Respirology 6(2): 113-123.

Roda, F., C. Gestreau, et al. (2002). "Discharge patterns of hypoglossal motoneurons during fictive breathing, coughing, and swallowing." J Neurophysiol 87(4): 1703-1711.

Rogers, D. F., B. Aursudkij, et al. (1989). "Effects of tachykinins on mucus secretion in human bronchi in vitro." Eur J Pharmacol 174(2-3): 283-286.

Rognum, T. O. and O. D. Saugstad (1993). "Biochemical and immunological studies in SIDS victims. Clues to understanding the death mechanism." Acta Paediatr Suppl 82 Suppl 389: 82-85.

Russell-Jones, D. L. (1985). "Sudden infant death in history and literature." Archives of Disease in Childhood 60(3): 278-281.

Rusu, M. C. (2004). "The spinal trigeminal nucleus--considerations on the structure of the nucleus caudalis." Folia Morphol (Warsz) 63(3): 325-328.

Saraceno, R., C. E. Kleyn, et al. (2006). "The role of neuropeptides in psoriasis." Br J Dermatol 155(5): 876-882.

Sasaoka, A., I. Ishimoto, et al. (1984). "Overall distribution of substance P nerves in the rat cornea and their three-dimensional profiles." Invest Ophthalmol Vis Sci 25(3): 351-356.

Sawaguchi, T., Y. Ozawa, et al. (2003). "Substance P in the midbrains of SIDS victims and its correlation with sleep apnea." Early Hum Dev 75 Suppl: S51-59.

Scharf, J. H. and C. P. Rowe (1958). "[Distribution of carbohydrates and some enzymes in the semilunar ganglion of cattle.]." Acta Histochem 5(5-8): 129-145.

Schmidt, C., E. Lobos, et al. (2003). "Pregnancy-induced changes in substance P and neurokinin 1 receptor (NK1-R) expression in the rat uterus." Reproduction 126(4): 451-458.

Schmidt, P. T., M. Lordal, et al. (2003). "Tachykinins potently stimulate human small bowel blood flow: a laser Doppler flowmetry study in humans." Gut 52(1): 53-56.

Schneider, T. D. and R. M. Stephens (1990). "Sequence logos: a new way to display consensus sequences." Nucleic Acids Res 18(20): 6097-6100.

Schulze, E., M. Witt, et al. (1997). "Immunohistochemical detection of human skin nerve fibers." Acta Histochem 99(3): 301-309.

Sergeyev, V., S. Fetissov, et al. (2005). "Neuropeptide expression in rats exposed to chronic mild stresses." Psychopharmacology (Berl) 178(2-3): 115-124.

Sessle, B. J. (2000). "Acute and chronic craniofacial pain: brainstem mechanisms of nociceptive transmission and neuroplasticity, and their clinical correlates." Crit Rev Oral Biol Med 11(1): 57-91.

Severini, C., G. Improta, et al. (2002). "The tachykinin peptide family." Pharmacol Rev 54(2): 285-322.

Sexton, S. and R. Natale (2009). "Risks and benefits of pacifiers." Am Fam Physician 79(8): 681-685.

Shaw, E. B. (1970). "Sudden unexpected death in infancy syndrome." Am J Dis Child 119(5): 416-418.

Shults, C. W., R. Quirion, et al. (1984). "A comparison of the anatomical distribution of substance P and substance P receptors in the rat central nervous system." Peptides 5(6): 1097-1128.

Shvarev, Y. N. and H. Lagercrantz (2006). "Early postnatal changes in respiratory activity in rat in vitro and modulatory effects of substance P." Eur J Neurosci 24(8): 2253-2263.

Sicuteri, F., D. Renzi, et al. (1986). "Substance P and enkephalins: a creditable tandem in the pathophysiology of cluster headache and migraine." Adv Exp Med Biol 198 Pt B: 145-152.

Sipos, G., P. Sipos, et al. (2008). "Correlation and immunolocalization of substance P nerve fibers and activated immune cells in human chronic gastritis." Anat Rec (Hoboken) 291(9): 1140-1148.

Sondheimer, H. M., T. Izukawa, et al. (1976). "Conduction disturbances after total correction of tetralogy of Fallot." Am Heart J 92(3): 278-282.

Sparks, D. L. and J. C. Hunsaker, 3rd (2002). "Neuropathology of sudden infant death (syndrome): literature review and evidence of a probable apoptotic degenerative cause." Childs Nerv Syst 18(11): 568-592.

Spencer, N. and S. Logan (2004). "Sudden unexpected death in infancy and socioeconomic status: a systematic review." J Epidemiol Community Health 58(5): 366-373.

Steinschneider, A. (1972). "Prolonged apnea and the sudden infant death syndrome: clinical and laboratory observations." Pediatrics 50(4): 646-654.

Sternini, C. (1991). "Tachykinin and calcitonin gene-related peptide immunoreactivities and mRNAs in the mammalian enteric nervous system and sensory ganglia." Adv Exp Med Biol 298: 39-51.

Stewart, A. (1975). "Infant leukaemias and cot deaths." Br Med J 2(5971): 605-607.

Stewart, A. J., S. M. Williams, et al. (1995). "Antenatal and intrapartum factors associated with sudden infant death syndrome in the New Zealand Cot Death Study." J Paediatr Child Health 31(5): 473-478.

Strassman, A. M. and B. P. Vos (1993). "Somatotopic and laminar organization of fos-like immunoreactivity in the medullary and upper cervical dorsal horn induced by noxious facial stimulation in the rat." J Comp Neurol 331(4): 495-516.

Straub, R. H., R. Wiest, et al. (2006). "The role of the sympathetic nervous system in intestinal inflammation." Gut 55(11): 1640-1649.

Stuebe, A. (2009). "The risks of not breastfeeding for mothers and infants." Rev Obstet Gynecol 2(4): 222-231.

Sun, H. W., C. R. Gao, et al. (2006). "[Expression of substance P in human laryngopharynx and gastrointestine in sudden erethistic death]." Fa Yi Xue Za Zhi 22(3): 180-182.

Sutton, R. N. and J. L. Emery (1966). "Sudden death in infancy: a microbiological and epidemiological study." Arch Dis Child 41(220): 674-677.

Takashima, S., T. Mito, et al. (1994). "Developmental brain-stem pathology in sudden infant death syndrome." Acta Paediatr Jpn 36(3): 317-320.

Takemura, M., S. Sugiyo, et al. (2006). "Mechanisms of orofacial pain control in the central nervous system." Arch Histol Cytol 69(2): 79-100.

Tan, B. H., K. N. Pundi, et al. (2010). "Sudden infant death syndrome-associated mutations in the sodium channel beta subunits." Heart Rhythm 7(6): 771-778.

Tapp, E., D. M. Jones, et al. (1975). "Interpretation of respiratory tract histology in cot deaths." J Clin Pathol 28(11): 899-904.

Terenghi, G., J. M. Polak, et al. (1985). "Distribution and origin of calcitonin gene-related peptide (CGRP) immunoreactivity in the sensory innervation of the mammalian eye." J Comp Neurol 233(4): 506-516.

Templeman, C. (1892). "Two hundred fifty-eight cases of suffocation in infants." Edinburgh Med J 18: 125-130.

Tervo, K. (1981). "Effect of prolonged and neonatal capsaicin treatments on the substance P immunoreactive nerves in the rabbit eye and spinal cord." Acta Ophthalmol (Copenh) 59(5): 737-746.

Tester, D. J. and M. J. Ackerman (2009). "Cardiomyopathic and channelopathic causes of sudden unexplained death in infants and children." Annu Rev Med 60: 69-84.

Thach, B. T. (2005). "The role of respiratory control disorders in SIDS." Respir Physiol Neurobiol 149(1-3): 343-353.

Thornton, E., T. T. Tran, et al. (2010). "A substance P mediated pathway contributes to 6-hydroxydopamine induced cell death." Neurosci Lett 481(1): 64-67.

Tomaki, M., M. Ichinose, et al. (1995). "Elevated substance P content in induced sputum from patients with asthma and patients with chronic bronchitis." Am J Respir Crit Care Med 151(3 Pt 1): 613-617.

Tomita, R. (2009). "Regulation of vasoactive intestinal peptide and substance P in the human pyloric sphincter." Hepatogastroenterology 56(94-95): 1403-1406.

Tonkin, S. (1975). "Post-neonatal infant deaths in the Auckland Hospital Board area 1972." N Z Med J 81(534): 187-190.

Tornqvist, K., A. Mandahl, et al. (1982). "Substance P-immunoreactive nerve fibres in the anterior segment of the rabbit eye. Distribution and possible physiological significance." Cell Tissue Res 222(3): 467-477.

Tornwall, J., H. Uusitalo, et al. (1994). "The distribution and origin of nerve fibers immunoreactive for substance P and neurokinin A in the anterior buccal gland of the rat." Cell Tissue Res 277(2): 309-313.

Tsuchida, K., R. Shigemoto, et al. (1990). "Tissue distribution and quantitation of the mRNAs for three rat tachykinin receptors." Eur J Biochem 193(3): 751-757.

Uddman, R. and L. Edvinsson (1989). "Neuropeptides in the cerebral circulation." Cerebrovasc Brain Metab Rev 1(3): 230-252.

Unger, B., J. S. Kemp, et al. (2003). "Racial disparity and modifiable risk factors among infants dying suddenly and unexpectedly." Pediatrics 111(2): E127-131.

US, V. E. and J. H. Gaddum (1931). "An unidentified depressor substance in certain tissue extracts." J Physiol 72(1): 74-87.

Usunoff, K. G., E. Marani, et al. (1997). "The trigeminal system in man." Adv Anat Embryol Cell Biol 136: I-X, 1-126.

Vennemann, M. M., T. Bajanowski, et al. (2009). "Does breastfeeding reduce the risk of sudden infant death syndrome?" Pediatrics 123(3): e406-410.

Verhaert, P. and A. De Loof (1985). "Substance P-like immunoreactivity in the central nervous system of the blattarian insect Periplaneta americana L. revealed by a monoclonal antibody." Histochemistry 83(6): 501-507.

Vink, R. and C. van den Heuvel (2010). "Substance P antagonists as a therapeutic approach to improving outcome following traumatic brain injury." Neurotherapeutics 7(1): 74-80.

Vlasova, I. G. and V. I. Torshin (2001). "[Antihypoxic properties of opiates and substance P]." Patol Fiziol Eksp Ter(2): 13-15.

Voogd, J. and M. Glickstein (1998). "The anatomy of the cerebellum." Trends Neurosci 21(9): 370-375.

Walsh, D. A. and F. M. D (2006). "Tachykinins and the cardiovascular system." Curr Drug Targets 7(8): 1031-1042.

Walsh, R. A., S. Redman, et al. (1996). "The accuracy of self-report of smoking status in pregnant women." Addict Behav 21(5): 675-679.

Wang, W. F., Y. S. Yang, et al. (2006). "Alternation of substance P-containing neural pathways in a rat model of irritable bowel syndrome with rectal distension." Chin J Dig Dis 7(4): 211-218.

Wedgwood, R. J. and Bendtrr, E. P. (Ed.) (1965). "Sudden Death in Inifants." Proceedings of the Conference on Causes of Sudden Dea.th in Infants, September 1963, Seattle, Washington. Public Health Service Publications No. 1412, Department of Health, Education and Welfare, Washington D.C.

Wedgwood, J. R. (1972). "In Sudden and Unexpected Deaths in Infancy." Ed. Camps, F. E. & Carpenter, R. G. Bristol: John Wright & Sons.

Weese-Mayer, D. E., M. J. Ackerman, et al. (2007). "Sudden Infant Death Syndrome: review of implicated genetic factors." Am J Med Genet A 143A(8): 771-788.

Weinstock, J. V., A. Blum, et al. (1988). "Eosinophils from granulomas in murine schistosomiasis mansoni produce substance P." J Immunol 141(3): 961-966.

Werne, J. (1942). "Postmortem evidence of acute infection in unexpected death in infancy." Am J Pathol Bacteriol 18:759.

Werne, J. and I. Garrow (1947). "Sudden deaths of infants allegedly due to mechanical suffocation." Am J Public Health 37(6): 675-687.

White, L. L. (1948). "Sudden death in infancy." Proc R Soc Med 41(12): 866-868.

WHO, UNICEF, UNFPA and the World Bank (2007). "Maternal mortality in 2005", (http://www.who.int/reproductive-health/publications/maternal_mortality_2005/index.html, accessed 14 August 2008).

Williams, A. L., E. C. Uren, et al. (1984). "Respiratory viruses and sudden infant death." Br Med J (Clin Res Ed) 288(6429): 1491-1493.

Willinger, M., H. J. Hoffman, et al. (1994). "Infant sleep position and risk for sudden infant death syndrome: report of meeting held January 13 and 14, 1994, National Institutes of Health, Bethesda, MD." Pediatrics 93(5): 814-819.

Willinger, M., L. S. James, et al. (1991). "Defining the sudden infant death syndrome (SIDS): deliberations of an expert panel convened by the National Institute of Child Health and Human Development." Pediatr Pathol 11(5): 677-684.

Wisborg, K., U. Kesmodel, et al. (2001). "Exposure to tobacco smoke in utero and the risk of stillbirth and death in the first year of life." Am J Epidemiol 154(4): 322-327.

Withington-Wray, D. J., S. W. Mifflin, et al. (1988). "Intracellular analysis of respiratory-modulated hypoglossal motoneurons in the cat." Neuroscience 25(3): 1041-1051.

Wong-Riley, M. T. and Q. Liu (2005). "Neurochemical development of brain stem nuclei involved in the control of respiration." Respir Physiol Neurobiol 149(1-3): 83-98.

Wu, H., C. Guan, et al. (2007). "Upregulation of substance P receptor expression by calcitonin gene-related peptide, a possible cooperative action of two neuropeptides involved in airway inflammation." Pulm Pharmacol Ther 20(5): 513-524.

Yamanouchi, H., S. Takashima, et al. (1993). "Correlation of astrogliosis and substance P immunoreactivity in the brainstem of victims of sudden infant death syndrome." Neuropediatrics 24(4): 200-203.

Yasuda, K., D. M. Robinson, et al. (2001). "Modulation of hypoglossal motoneuron excitability by NK1 receptor activation in neonatal mice in vitro." J Physiol 534(Pt. 2): 447-464.

Yonehara, N., T. Shibutani, et al. (1987). "Contribution of substance P to heat-induced edema in rat paw." J Pharmacol Exp Ther 242(3): 1071-1076.

Yoshino, K., S. Andoh, et al. (1989). "Innervation of the tooth pulp by the mesencephalic trigeminal nucleus in the cat: a retrograde horseradish peroxidase study." Brain Res 503(1): 152-155.

Zacest, A. C., R. Vink, et al. (2010). "Substance P immunoreactivity increases following human traumatic brain injury." Acta Neurochir Suppl 106: 211-216.

Zhang, Y., A. Berger, et al. (2006). "Tachykinins in the immune system." Curr Drug Targets 7(8): 1011-1020.

Zhang, Y., L. Lu, et al. (2000). "Hemokinin is a hematopoietic-specific tachykinin that regulates B lymphopoiesis." Nat Immunol 1(5): 392-397.

Zimmer, G., M. Rohn, et al. (2003). "Virokinin, a bioactive peptide of the tachykinin family, is released from the fusion protein of bovine respiratory syncytial virus." J Biol Chem 278(47): 46854-46861.

Apendix

8 Abbreviations

AHR, airway hyperresponsiveness

AMY, amygdaloid

ARC, Arcuate nucleus

CD, Crohn's disease

CNS, central nervous system

CRPS, complex regional pain syndrome

C14TKL-1, chromosome 14 tachykinin-like peptide 1

DN, Dentate nucleus

DRG, Dorsal root ganglion

DRN, Dorsal raphe nucleus

EK, Endokinin

GABA, gamma-amino butyric acid

G-Mt, Guillain-Mollaret triangle

GP, globus pallidus

GPCR, G-protein coupled receptor

HK, Hemokinin

IBD, inflammatory bowel disease

IBS, Irritable bowel syndrome

IHC, Immunohistochemistry

ILN, Intermediolateral nucleus

ION, Inferior olivary nucleus

IP3, inositol 1,4,5-triphosphate

IUGR, Intra-uterine growth retardation

KB, Kluver-Barrera

MC, Mast cell

MTN, Mesencephalic Trigeminal Nucleus

NK, Neurokinin

NK-R, Neurokinin Receptor

NPK, Neuropeptide K

NP*g,* Neuropeptide gamma

nTS, nucleus tractus solitarious

PKC, protein kinase C

PPT, Preprotachykinin

RN, Red nucleus

SIDS, Sudden infant death syndrome

SP, Substance P

SP-IR, Substance P immunoreactivity

SP-LI, Substance P like immunoreactivity

SUID, Sudden unexplained infant death

SIUD, sudden intrauterine death

TAC, Tachykinin gene

TACR, Tachykinin rFigure 25eceptor gene

TG, Trigeminal ganglion

TK, Tachykinin

TH, Tyrosine hydroxylase

TNF, tumor necrosis factor

TrN, trigeminal nucleus

VK, Virokinin

UC, ulcerative colitis

9 CURRICULUM VITAE

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OBJECTIVE

To work in a challenging and dynamic environment towards a successful career in research field related to genetics and neurosciences by making the best out of my abilities and interpersonal skills. I want to see myself as an active contributor to a team of ambitious people and thereby enhance my knowledge and personality.

RESEARCH INTERESTS

- Correlation between Substance P and SIDS (Immunohistochemical, genetic, computational and comparative aspects).
- Correlation between alterations in Intermediolateral nucleus and sudden death
- Role of Parvalbumin in GABAergic interneurons in human brainstem and its relation to SIDS.
- Founder effect analysis of disease haplotypes in DFNB23/USH1F linked Pakistani families.
- Morphological and histological changes in chick embryo by induced doses of organocholorines.

KEY FOCUS AREAS

- Genetics (mutational analysis, inherited disorders e.g deafness, pandred syndrome, usher syndrome, founder effect analysis, linkage analysis, haplotype analysis).
- Molecular cell biology.
- Neuropathology.
- Neurodegenerative diseases.
- Developmental biology and toxicology.
- Compartitive genomics.
- Evolutionary Analysis.
- Data mining and Bioinformatics analysis.

SOFTWARE EXPERTISE

Bioinformatics skills	Data mining, Phylogenetic & Evolutionary analysis, Motif prediction, Comparative genomic, Homology modelling
Bioinformatics softwares & Databases:	BLAST, Ncbi, UCSC genome browser, EggNog, Genecards, Transmembrane prediction tools, Struture prediction tools, Sequence Alignment, PDB, Uniprot, Swissprot, Gene Scan, Reactome, STRING, , Pedigree formation by Cyrillic
Operating Systems:	Windows 9x /2000/XP/Server 2003/2007
Technical Writing:	MS Office, ENDNOTE
Graphics:	Powerpoint

Laboratory Skills

- Histology
- PCR (Single, Multiplex)
- Immunohistochemistry
- Insitu-hybridization
- Karyotyping
- DNA, RNA extraction
- Staining
- Gel electrophoresis
- Genotyping
- Haplotyping
- Linkage analysis
- Human brain sampling
- Organotypic brain slice culturing of mouse

EDUCATION

PhD in Neuropathology, University of Milan, Italy.
2007 – todate

Master in Philosophy (Mphil) in Molecular Biology
2006 – 2007

National Center of Excellence in Molecular Biology, Lahore, Pakistan

MSc in Zoology
2002 – 2004

University of Punjab, Lahore Pakistan

BSc (Zoology, Botany, Chemistry)
2000 – 2002
Queen Marry College, Lahore, Pakistan.

RESEARCH PUBLICATION

- **Developmental alterations of the trigeminal nucleus disclosed by Substance P immunohistochemistry in perinatal and infant sudden unexplained deaths.**
  Lavezzi AM, **Mehboob R**, Matturri L

  Neuropathology, (submitted)

- **Neuropathology of the intermediolateral nucleus of the spinal cord in sudden unexplained perinatal and infant death.**
  Lavezzi AM, Corna MF, **Mehboob R**, Matturri L

  International journal of Developmental Neuroscience, Volume 28, issue 2, pp 133-138, April 2010

- **Effects of Tenekil Plus, an organochlorine, on development of chick.**
  Asmatullah, **Mehboob R**, Andleeb S
  Punjab University Journal of Zoology, Vol 20 (1), pp 7-19, 2005

HONORS AND AWARDS

- Merit scholarship holder during MSc (Punjab University, Lahore, Pakistan)
- Second position (Silver Medalist) holder in Msc.

PERSONAL PROFILE

- Date of Birth: 14-Nov-1981
- Sex: Female
- Nationality: Pakistani
- Language: English, Urdu, Italian (basics)

WORKSHOPS AND SEMINARS

Sostegno Alla Vita Fragile (Pre-E Post Natale), a conference about sudden unexpected pre and post natal death of infants (16th Nov., 2009)

"Lino Rossi" Research Center for The Study and Prevention of Unexpected Perinatal Death and SIDS – Department of Surgical, Reconstructive and Diagnostic Sciences, University of Milan, Via della Commenda, 19, Milan 20122, Italy

7th International Stem Cell School in Regenerative Medicine, "Stem cells, biomaterials and nanotechnologies in regenerative medicine"(2-4 Nov., 2009)

Institute of Experimental Medicine, Academy of Sciences of Czech Republic Prague, Czech Republic

4th DIMI workshop, Molecular Imaging in Drug Discovery and Preclinical Development (22-23 June, 2009)

Center of Excellence on Neurodegenerative Diseases,

University of Milan, Italy.

6th International Stem Cell School in Regenerative Medicine (14-16 June, 2009)

University of Southern Denmark, Odense, Denmark

Training Course in Organotypic brain Slice Culturing (17-19th June, 2009)

Odense University Hospital, Odense, Denmark

5th International Stem Cell School in Regenerative Medicine (20th-22nd October, 2008)

Center for Mental Health Disease, Albrech-Kossel_Institute for Neuroregeneration

University of Rostock, Berlin, Germany

2nd ENFIN Advanced course on systems Modeling (8-10 July, 2008)

Ecole Normale Superieure

Organized by European Network of Excellence ENFIN on Systems Biology

EMBL - European Bioinformatics Institute

Wellcome Trust Genome Campus

Cachan, France

REFERENCES

Will be furnished upon request.