



Expression of Substance P and the Tachykinin NK1 Receptor in the Medullary Serotonergic
Network of the Human Infant During Development; Implications for Sudden Infant Death
Syndrome (SIDS)

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Thesis Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work in the future will be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Preface

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Thesis Dedication

In memory of

~ **River Jak Adam Waddell** ~

02.07.2011 - 07.11.2011

And each and every infant lost to unexpected infant death, accidental death
and infants tragically lost to circumstances beyond their control.

This Ph.D. thesis is dedicated sincerely to Alexandra and Karl Waddell, the
extended River's Gift family and to all of the families and communities
who have tragically lost infants and children suddenly and unexpectedly in
Australia and around the world.

~There is no footprint too small that cannot leave an imprint in this world~

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10. **Bright F**, Byard R, Vink R, Paterson D. Poster presentation. Developmental abnormalities in substance p neurokinin 1 receptor binding in critical medullary nuclei contributing to respiratory failure and reduced head movement in SIDS. University of Adelaide, National Wine Centre, Florey Post Graduate Research Conference 2016

Glossary Of Terminology

ARC	Arcuate nucleus
BH-SP	Bolton Hunter Substance P
CNS	Central nervous system
COCH	Cochlear nuclei
DAO	Dorsal accessory olivary nuclei
DMX	Dorsal motor nucleus of vagus nerve
DRN	Dorsal Raphe nucleus
GA	Gestational age
GC	Gigantocellularis lateralis nuclei
HG	Hypoglossal nuclei
IO	Inferior olivary nuclei
IF	Immunofluorescence
IHC	Immunohistochemistry
IRZ	Intermediate reticular zone
MAO	Medial accessory olivary nuclei
NK	Neurokinin
NK1R	Tachykinin NK-1 receptor
NTS	Nucleus tractus solitarii
PBC	Prebotzinger complex
PCA	Post-conceptual age
PGCL	Paragigantocellularis lateralis nuclei
PIO	Principal inferior olivary nuclei
PMI	Post mortem interval
PNA	Postnatal age
RS	Rett's syndrome
SIDS	Sudden infant death syndrome
5-HT	Serotonin
SP	Substance P

SUB	Subtrigeminal nucleus
SUDC	Sudden unexpected death in childhood
SUID	Sudden unexpected death in infancy

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Introduction

Sudden infant death syndrome (SIDS) is a devastating and unexpected event in which a seemingly healthy infant dies in the first year of life during a sleep period, with no warning or prior indication of any adverse pathology to cause alarm (Kinney and Thach, 2009a). It is one of the most significant causes of post neonatal mortality in developed countries, profoundly affecting families and their communities. SIDS is complex, heterogenous and a diagnosis based solely on exclusion where the exact cause of death remains largely unexplained following complete post mortem examination and investigation of the circumstances of death (Krous 2004). By attempting to identify those children who may be at risk, medical professionals and scientific researchers endeavour to uncover and understand the pathogenesis of SIDS not only to prevent its occurrence, but also to provide some form of closure for families who are left to make sense of not only the death of their child but the heartache and stigma that comes with the “nonentity” of SIDS (Thach, 2008, Wender, 2012).

Multiple definitions, theories, animal and human studies have been established in an attempt to decipher the pathogenesis of SIDS. Unfortunately there are no available biomarkers for SIDS; no single universally accepted definition or theory and the direct cause remains relatively unknown. However, multiple neuropathologic studies have provided evidence that a certain subset of SIDS infants are not entirely 'normal' prior to death (Filiano and Kinney, 1994, Takashima and Becker, 1985, Sridhar et al., 2003, Paterson et al., 2006b). Instead these infants possess some form of underlying vulnerability exposing them to an increased risk for sudden death (Kinney, 2009a, Kinney and Thach, 2009a, Paterson et al., 2006b). It is thought that SIDS or a certain subset of SIDS is caused by some form of underlying neural or systematic abnormality in medullary homeostatic control that impairs critical responses to life-threatening challenges such as hypoxia during a sleep period (Kinney and Thach, 2009a). This failure is thought to result from abnormalities in a multi-neurotransmitter network of neural pathways in the medulla oblongata that control respiration, chemosensitivity, autonomic function and arousal. Indeed abnormalities in various brainstem neurochemicals including catecholaminergic, nicotinic, muscarinic, cholinergic, glutamatergic and neuropeptide systems have been reported (Kinney, 2009b, Kinney et al., 2009b). Abnormalities in the medullary serotonergic (5-HT) system have been the most significantly and consistently observed in the brainstem of SIDS infants, however it remains unclear whether these abnormalities are the primary event in SIDS or an epiphenomenon, with the underlying pathogenesis of these specific abnormalities still undetermined.

The neuropeptide substance P (SP) functions within key medullary nuclei to regulate cardiorespiratory and autonomic function in conjunction with 5-HT and other neurochemicals.

Actions of SP are primarily mediated by tachykinin NK1 receptors (NK1R) in the CNS and SP is recognized as a primary excitatory neurotransmitter and central mediator of cardiovascular reflexes such as baroreceptor sensitivity and chemoreceptor reflex modulation in response to hypoxia. Abnormalities in SP neurotransmission may play, therefore, a role in homeostatic dysfunction in SIDS. Previous studies analyzing SP and NK1R in the brainstem in SIDS have, however, been inconsistent and inconclusive. Furthermore a potential functional relationship between the 5-HT and SP neurotransmitter systems may be of critical importance to the pathogenesis of SIDS, where deficiencies in 5-HT which is already well established in the literature, may stimulate a compensatory response by SP. Previous animal studies and post-mortem human infant tissue research have investigated both 5-HT and SP individually in relation to homeostatic control and failure underlying the pathogenesis of SIDS, however the role of SP in association with the medullary 5-HT network in SIDS has not been fully examined.

In a collaborative effort combining two independent cohorts of human infant brainstem tissue and associated digital autopsy databases from Australia and the USA, the overall objective of this research was to investigate the expression of SP and its NK1R in the medullary 5-HT network during neurodevelopment, with specific investigation of the potential role of both neurotransmitter systems in contributing to a multi-transmitter medullary homeostatic network dysfunction in a subset of SIDS cases. The thesis is comprised of three core studies, each of which are closely interrelated. Collectively these studies resulted in significant outcomes that contribute immensely to the continued investigation of the underlying pathogenesis of SIDS and have provided a foundation for promising future research directions.

Thesis Research Aims and Methods overview

Chapter 2: Normative distribution investigation

Normative distribution of substance P and its tachykinin NK-1 receptor in the medullary serotonergic network of the human infant during development

Specific Aim:

Characterization of the normative distribution of SP and the NK1R in the medullary 5-HT network of the human infant medulla during development in control cases (non-SIDS).

Hypothesis:

The NK1R and SP are extensively co-distributed but do not co-localize with 5-HT neurons in the nuclei of the medullary 5-HT network.

Methods:

1. Descriptive analysis using formalin-fixed paraffin-embedded human infant medullae specimens (non-SIDS controls) (N=10) obtained from Forensic Science South Australia (FSSA). Single labelled immunohistochemistry (IHC) was performed for TPH2 (5-HT neurons), SP and NK1R and double label immunofluorescence (IF) performed for SP relative to 5-HT and NK1R relative to 5-HT respectively. Descriptive immunohistochemical distribution of SP and NK1R was assessed within the medullary 5-HT system.

2. Quantitative analysis using fresh frozen human infant medullae specimens (non-SIDS controls) (N=15) accrued from autopsy services at the Department of Pathology, Boston Children's Hospital and the office of chief medical examiner San Diego, CA. SP specific binding density was performed using 0.15nM [¹²⁵I]- Bolton Hunter labelled SP autoradiography and quantitative densitometry analysis of total and non-specific binding density performed using MCID core computer based software, to determine the normative distribution of SP binding to NK1R in medullary 5-HT network nuclei.

Chapter 3. SIDS vs. Controls study: Medullary SP/NK1R study

Developmental abnormalities in SP NK-1 receptor binding in brainstem nuclei in sudden infant death syndrome related to sex and prematurity

Specific Aim:

Determination of NK1R binding density in the medullary 5-HT network in the human infant medulla in SIDS vs. non-SIDS control cases.

Hypothesis:

NK1R binding density, and thus putatively SP neurotransmission, is significantly altered in key autonomic and respiratory control nuclei in the medulla oblongata of SIDS cases compared to controls.

Method:

Quantitative analysis using fresh frozen infant brainstem specimens (n=76) accrued from the Office of the Chief Medical Examiner in San Diego, from three separate tissue datasets over the period 2004-2015. Tissue section autoradiography with 0.15nM [¹²⁵I] Bolton Hunter labelled SP and quantitative densitometry with MCID core software, was used to determine NK1R binding density (fmol/mg) in 14 medullary nuclei in 55 SIDS and 21 non-SIDS control infant brainstems. Binding results were adjusted for age, sex, prematurity and post-mortem interval.

Chapter 4. SIDS vs. Controls study: Medullary 5-HT study

Serotonin neuron abnormalities in the medullary serotonergic network in a South Australia SIDS cohort

Specific Aim:

To determine if the number and density of 5-HT neurons in the medullary 5-HT network is altered in an independent cohort of SIDS cases from South Australia replicating the same methodology as previously published by Paterson et al., 2006.

Hypothesis:

A significant medullary 5-HT abnormality exists in South Australian SIDS cases characterized by a significantly higher number and density of 5-HT neurons and altered neuron morphology in SIDS cases compared to controls.

Method:

Formalin-fixed paraffin-embedded medullae from infants who died from SIDS (41) and control cases (39) who died from definitive causes of death other than SIDS, were obtained from FSSA. Sections of medulla (4µm) were cut and immunostained for tryptophan hydroxylase (TPH2) using PH8 antibody to label 5-HT neurons. Quantitative 5-HT neuron cell count analysis was then performed using the NeuroLucida (MBF bioscience) computer based software. Number, density and different morphological types of 5-HT neuron were compared in SIDS cases to controls. Results were adjusted for age, sex, prematurity and post-mortem interval.