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List of Abbreviations

AAV	Adeno-associated viral vector
ANOVA	Analysis of Variance
AP	Anterior-posterior
CGRP	Calcitonin-gene related peptide
CTb	Cholera Toxin-beta subunit
DV	Dorsal-ventral
EGFP	Enhanced Green Fluorescent Protein
fMRI	Functional Magnetic Resonance Imaging
HSV-1 H129	Herpes Simplex Virus 1 strain H129
ML	Medial-lateral
nTS	Nucleus of the Solitary Tract
OCT	Optimal Cutting Temperature compound
Pa5	Paratrigeminal Nucleus
PAG	Periaqueductal nucleus
PBN	Parabrachial Nucleus
PBS	Phosphate Buffer Solution
PFA	Paraformaldehyde
VPL	Ventral posterolateral thalamic nuclei
VPM	Ventral posteromedial thalamic nuclei

1. Introduction

Respiratory diseases constituted a third of deaths in Australia in 2011 (Australian Bureau of Statistics, 2013) and asthma alone affects around 235 million people across the globe (World Health Organization, 2014). Therefore, there is an increasing need for research to improve our understanding of how respiratory diseases develop and the underlying physiology of their associated symptoms. To date most studies have focused on the immunology of respiratory disease. However, it is well accepted that many respiratory symptoms manifest due to altered neural control of the airways and lungs, and this has been studied considerably less, especially the neural pathways that generate airway sensations leading to behavioural modulations in respiration, such as cough (Mazzone et al., 2013). Understanding these processes may lead to the identification of new targets for alternate therapeutics. This project will assess brainstem projections of airway sensory neural pathways responsible for reflex and behavioural modulation of respiration.

2. Background

2.1 Neurophysiology of respiratory sensation

The respiratory system has substantial innervations from the nervous system. Pulmonary sensory and motor neurons work together to detect and respond to damaging stimuli through respiratory reflexes, such as cough (McGovern et al., 2012a). The sensory neurons originate in the vagal sensory ganglia and are broadly classified as either mechanosensors (myelinated A fibres) or chemosensors (unmyelinated C fibres) (Ho et al., 2001; McGovern et al., 2012a; Nassenstein et al., 2010). Mechanosensors generally only respond to mechanical stimulation of the airways such as lung inflation, deflation or punctate (touch-like) stimuli and typically have large somal diameters and fast conduction velocities because they are myelinated (Ho et al., 2001). Alternately, chemosensors generally only respond to chemical irritants such as capsacin (the active ingredient in chillies) and a

variety of mediators including pro-inflammatory mediators such as bradykinin and prostaglandins (Ho et al., 2001). These neurons also differ to mechanosensors in that they have small somal diameters, they express one or more neuropeptides and they have slow conduction velocities because they are un-myelinated (Ho et al., 2001). Therefore, these neurons can be differentiated using immunohistochemistry with antibodies for neuropeptides and neurofilament (a marker for myelin) (Riccio et al., 1996).

2.2 The origin, characterization and role of the vagal ganglia

There are two vagal ganglia; the inferior (or nodose) and the superior (or jugular) ganglia (see Figure 1) (Ho et al., 2001; Riccio et al., 1996). These two structures develop from different embryological origins. The nodose originates from the epibranchial placode while the jugular originates from the neural crest (Ho et al., 2001). This difference in embryological origin translates into functionally and phenotypically distinct neurons (Riccio et al., 1996). For example, jugular and nodose neurons are part of the somatic and visceral nervous systems, respectively (Ichikawa and Sugimoto, 2003). Studies using retrograde neuronal tracers to label and characterize airway neurons in the vagal ganglia have discovered that the jugular ganglia contain somatic chemosensors while the nodose ganglia contain visceral chemosensors and mechanosensors (Lieu et a., 2011; Riccio et al., 1996). The origin of the airway sensory neuron subtypes therefore allows insight into their possible function. The central projections of the vagal ganglia terminate in two brainstem nuclei; the nTS and the Pa5 (McGovern et al., 2012a). Although the airway neurons in the vagal ganglia have been well described, studies have not determined whether neurons from different vagal ganglia project equally to the same brainstem nuclei or if they have specific brainstem projections.

2.3 Secondary Airway Sensory Neurons in the Brainstem: Nucleus of the Solitary Tract (nTS) and the Paratrigeminal nucleus (Pa5)

The intermediate and caudal regions of the nTS contain second order airway sensory neurons (see Figure 1) (Hermes et al., 2006; Subramanian et al., 2007). This nucleus has been studied extensively for its roles in the cardiac and respiratory systems (Balan et al., 2004; McCulloch and Panneton, 1997). With respect to respiration, the nTS consists of inspiratory and expiratory neurons, which are controlled by a balance between excitatory and inhibitory neurotransmitters (Subramanian et al., 2007). For example, Sekizawa et al. (2003) have shown that substance P is released from vagal sensory nerve terminals within the intermediate and caudal nTS causing a net excitatory impact on respiratory function. In addition, research has shown that vagal stimulation creates an inhibitory feedback loop with inspiratory cells in the nTS (Subramanian et al., 2007). However, little is known about the types of neurons that project to this nucleus and specifically which subcortical and cortical regions it in turn projects to.

Similarly, the Pa5 has been shown to receive vagal sensory neuron projections, including those arising from the airways (Armstrong and Hopkins, 1998; McGovern et al., 2012a). This nucleus is located in the medulla oblongata at the level of obex, extending slightly rostral to this landmark, which is the most cause porint of the fourth ventricle in the brain (see Figure 1) (Armstrong and Hopkins, 1998; Ma et al., 2005). Similarly to the nTS, the Pa5 plays a role in many biological functions. The most established of these is its role in the cardiac system. Balan et al. (2004), tested unit activity in response to increased arterial blood pressure to determine the role of the Pa5 in cardiac function. The results showed that 72% of the neurons in the Pa5 are baroreceptor sensitive, similar to the nTS, and hence implicated the Pa5 in cardiac function (Balan et al., 2004).

On the other hand, the alternate roles of the Pa5 are not as well determined. These include the role of the Pa5 in pain and respiration (Armstrong and Hopkins, 1998; Cacus et al., 2001). Recently the

Pa5 has been specifically studied in regards to pain inasmuch as lesioning of pain related nerve fibers in rat tooth pulp leads to degenerating terminals in the Pa5 (Lapa and Watanabe, 2005). This result suggests that pain fibers project to the Pa5 for processing. In addition, multiple studies have used a combination of retrograde and anterograde neuronal tracers to determine the afferent and efferent connections of the Pa5 reporting projections to and from the nTS, parabrachial nucleus (PBN) and the ventral posteromedial (VPM) thalamic nucleus, which are regions well known for their involvement in cardiovascular and nociceptive processing (Cacus et al., 2001; Menetrey et al., 1987).. However, there is a distinct lack of information about the specifc role of the Pa5 in regulating respiration or airway defensive reflexes.

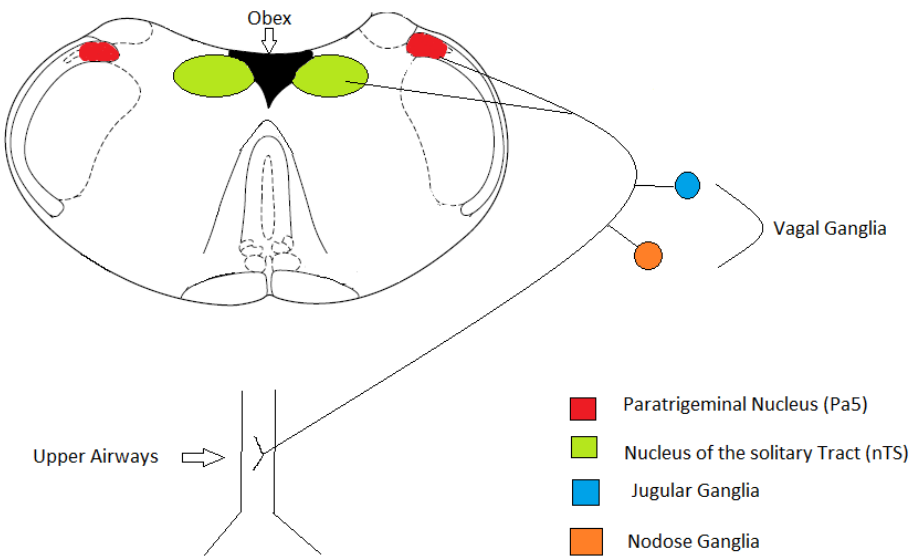

Figure 1: Airway sensory afferents originating in the vagal ganglia projecting to the airways and to two brainstem nuclei; the Nucleus of the Solitary tract (nTS) and the Paratrigeminal Nucleus (Pa5) in the brainstem. Adapted from Mazzone et al. (2013) and Paxinos and Watson (1986).

2.4 Higher brain projections from the Nucleus of the Solitary Tract (nTS) and the Paratrigeminal Nucleus (Pa5)

Ascending airway pathways originating from the nTS and Pa5 can be traced into multiple subcortical and cortical brain regions. A recent transneuronal tracing study by McGovern et al. (2012a) has clearly identified the cortical projections from the respiratory brainstem nuclei in rats by tracing airway sensory neurons with a herpes simplex virus strain H129 (HSV-1 H129). In addition, a study prior to this also captured the cortical projections of airway sensory pathways from the brainstem in humans using Functional Magenetic Resonane Imaging (fMRI) (Mazzone et al., 2011). The results from both of these studies have highlighted regions of the brain that are consistently implicated in respiratory function (see Table 1 for specific areas) (Mazzone et al., 2011; McGovern et al., 2012a). However, these projection pathways do not differentiate subcircuits arising from the nTS and the Pa5, but rather show the ascending circuitry in its entirety. Delineating the airway specific networks supplied by projections originating from the nTS versus Pa5 may provide novel insights into the functional roles of these two brainstem respiratory nuclei.

The nTS consists of multiple subnuclei each with distinct roles (Ter Horst et al., 1989). Anterograde tracing studies have shown that the rostral nTS mainly projects to brainstem nuclei such as the PBN and the locus coeruleus (Ter Horst et al., 1989). On the other hand, the caudal portion projects to hypothalamic and forebrain regions (Ter Horst et al., 1989). The hypothalamic nuclei include the paraventricular, dorsomedial and lateral nuclei (Geerling and Loewy, 2006). In addition, the posterior nTS projects to the medial and central amygdala nuclei, Nucleus Accumbens and the thalamic paraventricular nucleus (Geerling and Loewy, 2006; Ter Horst et al., 1989). Finally, the periaqueductal gray (PAG) is a central projection region of the nTS (Geerling and Loewy, 2006;Ter Horst et al., 1989). Alternately, anterograde tracing studies have shown that the Pa5 generally projects to a range of brainstem nuclei including the PBN, reticular nucleus, the ambiguous nucleus and the VPM (Cacus et al., 2001; de Sousa Buck et al., 2001). Although, these are the general higher

order projections of the nTS and Pa5, it remains unknown which of these regions receives input from specific airway sensory pathways via the nTS or the Pa5. For this reason, the focus of this project is to determine the airway specific projection pathways from the nTS versus the pa5.

Table 1: Specific cortical and subcortical regions involved in the processing of airway sensory afferents (McGovern et al., 2012a).

Overarching Brain Region	Specific Regions
Midbrain	PAG Interstitial Nucleus of the medial longitudinal fasciculus Anterior Pretectal Nucleus Inferior Coliculi Supraoptic Nuclei
Forebrain - Thalamus	Ventral Posterpmedial (VPM) nucleus Ventral Posterolater (VPL) nucleus Mediodorsal nucleus Reticular nucleus Posterior nucleus
Forebrain- Subthalamic	Zona Incerta
Forebrain - Hypothalamic	Lateral nucleus Paraventricular nucleus
Forebrain – General	Insular Cortex Lateral Central Amygdala nucleus Cingulate Cortex Primary and Secondary somatosensory cortex.

3. Aims and Hypotheses

1. To characterize the origin and neurochemical phenotypes of afferent inputs projecting to the nTS and the Pa5 using conventional retrograde neuroanatomical tracing and immunohistochemistry. It is hypothesized that distinct subsets of afferent neurons will have organized termination patterns in these two discrete brainstem nuclei.

2. To characterize the airway specific ascending projections from the nTS and Pa5 to subcortical and cortical structures using a conditional herpes viral transneuronal anterograde tracing system. It is

hypothesized that the nTS and Pa5 will have organized and specific projections to the brain. It is predicted that these regions will differ between these two brainstem nuclei.

3. To determine the function of the Pa5 in regulating tracheal-induced respiratory reflexes by modulating its activity with microinjections of drugs in anaesthetized animals. It is hypothesized inhibition of the Pa5 will modify vagal reflex mediated changes in breathing pattern.

4. Methods and Experimental Plan

4.1 Assessment of the Origin of Afferent Inputs to the Nucleus of the Solitary Tract (nTS) and the Paratrigeminal Nucleus (Pa5) with Retrograde Neuroanatomical Tracing using fluorescent-conjugated Cholera Toxin Subunit B (CTb)

Experiment one will address the first aim by tracing the extrathoracic neuronal connections from the vagal ganglia to the nTS and the Pa5. These neurons will be traced by the retrograde neuronal tracer Cholera Toxin-beta subunit (CTb) labeled with a 594 (red) or 488 (green) fluorescent marker (Dederen et al., 1994). These fluorescent CTb constructs will be directly injected into the nTS and Pa5 via recovery surgeries on Sprague Dawley rats (Armstrong and Hopkins, 1998). Briefly rats (150-200g) will be anaesthetized with isoflurane (2-3% in 100% Oxygen) and placed in a stereotaxic frame (McGovern et al., 2012a). The skull will then be exposed by a midline incision and the muscles overlying the atlanto-occipital membrane will be retracted (Futuro Neto et al., 2011). A small window will be made in the atlanto-occipital membrane in order to view obex, which is a landmark on the dorsal surface of the brain (Armstrong and Hopkins, 1998; Futuro Neto et al., 2011).

Using a micromanipulator, a pulled glass micropipette (~20μm tip diameter), containing 594-CTb, will be positioned over obex and the following stereotaxic co-ordinates will be used to position the micropipette into the region of the dorsolateral nTS (Armstrong and Hopkins, 1998). The stereotaxic coordinates used will be 0.5mm anterior-posterior (AP), 0.5 medial-lateral (ML) and 0.3/0.5dorsal-

ventral (DV) (Paxinos and Watson, 1986; van Bockstaele et al., 1999). At these co-ordinates 150-200nl of 594-CTb will be injected into the nTS using a pressure driven nanoinjector and then the needle will be left in the injection site for 30 seconds to one minute to avoid the tracer spreading (Dederen et al., 1994; Ma et al., 2005; McGovern et al., 2012a; Saxon and Hopkins, 2006). This procedure will be repeated to inject 488-CTb into the Pa5, this time using stereotaxic co-ordinates of 2mm AP, 2.8mm ML and 0.3mm DV in reference to obex (Armstrong and Hopkins, 1998; Paxinos and Watson 1986). These injections will be made unilaterally in the rats in order to assess the levels of contralateral versus ipsilateral projections from the brainstem nuclei to the vagal ganglia. Following these surgeries the incision will be sutured and the rats will be allowed to recover for seven days (McGovern et al., 2012a; Saxon and Hopkins, 2006).

4.1.1 Dissection and Sectioning

After this recovery period the rats will be euthanized with an overdose of sodium pentobarbital and perfused fixed with 5% sucrose and 4% paraformaldehyde (PFA) in phosphate buffer solution (PBS). Once the rats are fixed, dissection of the vagal ganglia, brainstem and brain will be conducted and these structures will be post-fixed in 4% PFA and then stored in 20% sucrose for cryoprotection. After storage these structures will be frozen in Optimal Cutting Temperature compound (OCT) before being sectioned using a cryostat. Sections will be made at 50µm for the brain and brainstem and 14µm for the vagal ganglia. These sections will be mounted onto gelatin coated slides for screening under the fluorescent microscope (McGovern et al., 2012a; McGovern et al., 2012b). Screening these sections will enable us to determine if the nTS and Pa5 are innervated equally by neurons of both the jugular and nodose ganglia or if there is specificity in the connections from the vagal ganglia to these brainstem nuclei.

4.1.2 Characterization of airway sensory neurons in the Nucleus of the Solitary Tract (nTS) and the Paratrigeminal Nucleus (Pa5)

After tracing the neurons they will be characterized into the two types of airway neurons based on their immunohistochemical profile and somal size (Kubin et al., 2006). Mechanosensory neurons (A-fibers) are medium to large in somal diameter, myelinated and do not express neuropeptides such as substance P and CGRP, while chemosensory neurons (C-fibers) are small in somal diameter, unmyelinated and typically express neuropeptides (Kubin et al., 2006). Given these traits we can perform immunohistochemistry with primary antibodies for neurofilament proteins (expressed in myelinated neurons), and the neuropeptides substance P and calcitonin-gene related peptide (CGRP) to characterize the neurons traced from the vagal ganglia to the nTS and the Pa5 (Table 1) (Masliukou et al., 2014; Myohanen et al., 2008). The primary antibodies will be applied to the sections of the brainstem and vagal ganglia and then left to incubate for 24 hours at room temperature (Masliukou et al., 2014; Xu et al., 2012). After incubation the primary antibody solution will be removed and the sections will be washed with PBS followed by the application of the fluorescent secondary antibodies (Table 2) (Xu et al., 2012). The first secondary antibody is a biotinylated antibody and the second is AMCA avidin, which will allow for fluorescence. Both of these antibodies will be incubated for one hour at room temperature and then wasched with PBS between incubations (Masliukou et al., 2014; Xu et al., 2012). These will be allowed to dry overnight before conducting fluorescent microscopy. Negative control experiments, excluding primary antibody application, will be run in parallel (Jeffry et al., 2009).

Table 1: Primary Antibodies for Immunohistochemistry

Antibody Name	Type	Host	Supplier	Dilution
Neurofilament 160KD	Polyclonal	Rabbit	Abcam	1:5000
Anti-substance P Antibody	Polyclonal	Guinea Pig	Abcam	1:1800
Anti-CGRP Antibody	Polyclonal	Goat	Abcam	1:500

Table 2: Secondary Antibodies for Immunohistochemistry

Antibody Name	Host	Supplier	Dilution
Anti-rabbit IgG biotinylated	Goat	Vector Labs	1:500
Anti-guinea-pig IgG biotinylated	Goat	Vector Labs	1:500
Anti-goat IgG biotinylated	Donkey	Vector Labs	1:500

4. 2 Assessment of the second order sensory neuron projections from the Nucleus of the Solitary Tract (nTS) and the Paratrigeminal Nucleus (Pa5) to the brain

4.2.1 Fluorescent HSV-1 H129

Experiment two will address the second aim by tracing and characterizing the ascending projections from the nTS and the Pa5 to the brain. This tracing will be performed using an anterograde transynaptic neuronal tracer HSV-1 H129 (McGovern et al., 2012b). Since HSV-1 H129 is a transynaptic tracer it will travel from its injection site, in the airways, to the brain specifically along airway sensory neurons (McGovern et al., 2012b). Recently the Mazzone Lab constructed a novel HSV-1 H129 strain that has the capability of causing infected cells to fluoresce (McGovern et al., 2012b). This was enabled by inserting an Enhanced Green Fluorescent Protein (EGFP) cassette into an intergenic region of the HSV-1 H129 genome (McGovern et al., 2012b). The Mazzone Lab showed that this novel anterograde neuronal tracer is stable as it retains its replication properties and neuroinvasiveness (McGovern et al., 2012b).

4.2.2 Cre-Recombinant HSV-1 H129

This method has been further enhanced by generating a HSV-1 H129 recombinant virus that switches from expressing EGFP to td-Tomato in the presence of Cre-recombinase. In brief, this virus has an

EGFP cassette, which is flanked by LoxP sites, and downstream of this is a fluorescent td-tomato cassette (see Figure 2). Thus, in the presence of Cre, the green cassette is permanently excised from the genome and the td-tomato cassette is moved into frame such that the virus switches from expressing green to red fluorescence in infected cells. Cre can be expressed in a brain region of interest by microinjecting a viral vector (e.g adeno-associated viral vector (AAV) or similar) that expresses Cre under the control of a synpasin promoter (Card et al., 2011; Kugler et al., 2003). This will induce Cre expression in neurons only in the region of the microinjection. The subsequent injection of the Cre-inducible HSV-1 H129 virus into the airways will allow projections passing through Cre-expressing neurons in the brain to be differentiation from all other relay nuclei (as the virus will express red fluorescence in all synaptically connected neurons).This system has been developed and validated in the Mazzone Lab (McGovern et al., unpublished observations).

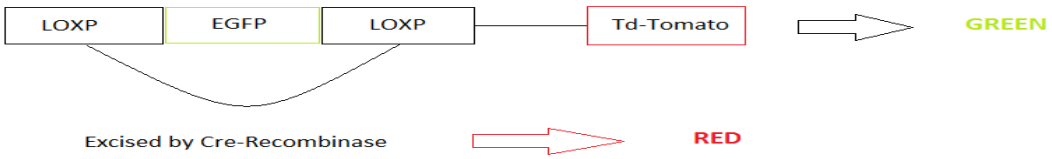

Figure 2: Cre-dependent Herpes Simplex Virus 1 strain H129 (HSV-1 H129). This fluorescent construct expresses Enhanced Green Fluorescent Protein (EGFP) until it contacts Cre-recombinase, which causes it to switch to red fluorescence.

4.2.3 Cre-Recombinant HSV-1 H129 injections

Recovery surgeries will be performed to firstly inject an AAV-Cre into either the nTS or Pa5, followed four weeks later by injections of the Cre-inducible HSV-1 H129 neuronal tracer into the airways (McGovern et al., 2012a). This will be followed by fixation, perfusion, dissection, sectioning of the

brain, brainstem and vagal ganglia and screening under the fluorescent microscope, as described in experiment one. Screening the brain, brainstem and vagal ganglia sections in this experiment will allow us to determine where the airway specific nTS and Pa5 pathways project to in the brain.

4.3 Identifying the role of the Paratrigeminal Nucleus (Pa5) in respiration

4.3.1 Anaesthetized surgery for upper airway electrical stimulation

Experiment three will address the third aim by testing the respiratory function of the Pa5 in reflex responses evoked by stimulating the upper airways. Under urethane anesthesia rats will firstly have their carotid artery and trachea cannulated to allow us to monitor their blood pressure measure spontaneous respiration, respectively (Canning et al., 2004). A small incision will be made in the trachea above the level of the tracheal cannula and a custom made bipolar stimulating electrode will be placed on the tracheal mucosa for stimulating airway sensory nerve terminals and evoking reflex changes in breathing (Canning et al., 2004). Rats will be placed in a stereotaxic frame and their brainstems exposed for microinjections of muscimol (a GABA antagonist what will induce neuronal inhibition) into the Pa5 (2mm AP, 2.8mm ML and 0.3mm DV) (Armstrong and Hopkins, 1998; Paxinos and Watson 1986; Takemura et al., 2000).

4.3.2 Monitoring respiratory reflexes

To assess the function of the Pa5 we will monitor respiration to detect any changes under varying treatment conditions. Studies have shown that tracheal stimulation, for example by distilled water, causes a large number of respiratory reflexes (Nishino et al., 1988). In the rat, this is characteristically a slowing of respiration and eventual apnea at strong stimulus intensities (Subramanian et al., 2007). As a result these can be used as behavioural outputs to directly observe the function of the Pa5. Increasing stimulus frequencies at a constant stimulus intensity will be used to generate a frequency-

response relationship for respiratory slowing in the absence and presence of muscimol in the Pa5. Following this respiratory function test the rats will be perfused and their brainstems will be dissected for sectioning and screening under the microscope, as described in experiment one. This step is to ensure that injections are confined to the Pa5.

4.4 Data analysis

Immunohistochemistry in vagal ganglia will be quantified by counting the fluorescent neurons in the section and calculating these counts as a percentage of all identifiable neurons in the area of interest (McGovern et al., 2012a). In addition, the somal size distribution of immunostained and retrogradely labelled cells will be quantified and compared between brainstem injection sites. For anterograde CTb labeling within the brain, the label density will be categorized on a 4 point scale, where 0 is no labelling, 1 is sparse labelling, 2 is moderate labelling and 3 is dense labelling (Agster and Burwell, 2009). These scores will be relative to the most intensely labeled region in each animal to standardize between experiments. This data will be collated in histograms to show the label density for anterograde fibers from the nTS and Pa5 to higher brain regions. In turn, these tracing data sets will be analyzed by a paired Students T-test or analysis of variance (ANOVA). For the functional experiments, respiration will be measured in order to compare to baseline respiration with the respiration following inhibition of the Pa5 (Nishino et al., 1988). These results will be analyzed by an ANOVA.

Finally, all data sets will be presented as mean ± standard error, the significance value will stand at $P<0.05$ and Graphpad Prism software will be used to perform all analyses (McCulloch and Panneton, 1997).

Table 3: Expected Experimental Timeline

Task	Time
Experiment 1	March - May
Experiment 2	April – July
Experiment 3	May - August
Thesis Writing	April - October

5. Expected Outcomes and Significance

5.1 Expectations of aim one and experiment one

It is expected that experiment one will identify the specific termination pattern of the jugular and nodose ganglia within the nTS and the Pa5 in the brainstem. We expect to find that the jugular ganglia will project largely to the Pa5 while the nodose ganglia will favour the nTS. This is because we suspect that the Pa5 is primarily involved in the conscious perception of nociception and in turn will relay extrathoracic information from chemosensors, which are the only type of sensory neuron found in the jugular ganglion (Riccio et al., 1996). Alternately, it is predicted that the nodose ganglia will project to the nTS because we believe that this nucleus may be less involved in pathways responsible for the conscious perception of airways sensations, but rather is more involved in relaying more classical viscerosensory information to the brain. This is suggested because a study by Tsai and Davenport (2014) has shown that the vagus nerve transmits mechanical information to the nTS for important modulations such as respiratory load compensation. Load compensation occurs, following mechanical challenges to respiration, in order to maintain ventilation (Tsai and Davenport, 2014). Hence, it is likely that the nTS will receive a mix of chemosensors and mechanosensors from the nodose ganglia to allow for critical reflexes and emotive sensory processing (Riccio et al., 1996).

5.2 Expectations of aim two and experiment two

It is expected that experiment two will trace the projections of the nTS and Pa5 to their specific subcortical and cortical termination sites. Since we expect the Pa5 to be involved in nociception we to expect that it will project to higher brain regions necessary for perceptual noxious sensation and/or nociceptive modulation. Studies have identified brain regions involved in both pain and airway sensations. These studies suggest multiple ascending circuits transmit noxious information to the brain, including one that projects from second order neurons in the spinal cord or brainstem, through medial thalamic nuclei and onto the cingulate and insula cortex, and a second that projects via later thalamic nuclei and onto the primary sensory cortex (Hadjipavlou et al., 2006; Wang et al., 2004). There are also projections into pontine, hypothalamic and subthalamic nuclei, and the amygdala that play important roles in regulating sensory processing (Hadjipavlou et al., 2006). Although, specific patterns of projections from the nTS and Pa5 are difficult to predict, one major outcome expected is that the Pa5 projections will form a substantial component of the lateral pathway to the somatosensory cortex (Wang et al., 2004). We also believe that Pa5 projections will contribute to circuitry that resembles a descending inhibitory pathway that has been described for spinal nociceptive circuits (Pagano et al., 2012).

5.3 Expectations of aim three and experiment three

Previous studies have shown that modulation of neurotransmission in the nTS alters basal respiration and respiratory responses evoked by the stimulation of vagal afferent fibres (Subramanian et al., 2007). Experiment three is expected to provide insight into whether the Pa5 plays a similar functional role in regulating baseline respiration or evoked respiratory-reflexes. We believe that basal respiration will change when the Pa5 is excited, and that reflex changes in breathing pattern evoked by tracheal sensory nerve stimulation will be reduced when neurotransmission in the Pa5 is inhibited.

5. 4 Significance

In summary, these experiments will provide important insights into the brain regions involved in sensing airway irritation. The neural circuitry of respiration is important to study because updated therapeutics are required to treat an extensive number of patients with respiratory disease and discomforts, such as dyspnea and excessive cough (Davenport and Vovk, 2009). Protective airway reflexes are essential and inhibiting these reflexes can result in death due to aspiration pneumonia, a common cause of mortality in patients with neurological disorders (Marik, 2001). Thus targeting the abnormal sensations that become severe and excessive in patients with respiratory disease might be a better approach. In most patients, this means reducing sensory inputs to the brain to relieve sensations that contribute to disease morbidity. However, in some patients with very severe respiratory disease there may be a paradoxical loss of respiratory sensations that can consequently increase mortality due to patients not adequately medicating (Davenport and Vovk, 2009). Therefore, if the proposed experiments find that the Pa5 is involved specifically in airway nociception, while the nTS is involved in critical respiratory reflexes, then the Pa5 will be highlighted as a potential therapeutic target that would allow us to modulate sensations without abolishing the necessary reflexes.

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