DEVELOPMENT OF AN AUTOMATED ELECTROGUSTOMETER

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SUMMARY

In spite of electrogustometry having been in existence since the 1930s, there is no state of the art instrument to assess the electrogustometric threshold. A state of the art electrogustometer has been designed and constructed and tested for reliability and repeatability. This is based on embedded digital technology and is a semi-automatic, battery-powered portable instrument. Physical factors such as electrode area and stimulus duration affect the taste threshold but there are no recommended standards for these factors. Studies have been conducted to ascertain a recommended standard – a circular stainless steel electrode area of 28.5 mm^2 and a stimulus duration of 2 seconds.

While performing the test-retest assessment of the Sussex Electrogustometer, the new instrument, an anomaly was observed. Upon further investigation it was concluded that it was caused by alcohol consumed by a subject prior to the retest. Elaborate experiments were designed with the help of a neurologist and psychologist to understand the immediate effect of alcohol on taste for non-alcoholics. The results indicated an immediate improvement of taste for lower concentrations of alcohol and a delayed improvement for higher concentration. The studies were extended to

understand the immediate effect of anaesthetics and smoking on taste which showed that taste deteriorated as expected. The new machine was used successfully in the clinical environment by local doctors and a report on their findings has also been included within this thesis.

ACKNOWLEDGEMENT

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STATEMENT

I hereby declare that this thesis has not been and will not be, submitted in whole or in part to another University for the award of any other degree.

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CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Determining the way in which the sensory organs act and how the various sensory parameters can be measured have been a matter of interest for many centuries. Unlike vision and hearing, the sense of taste has not been vastly studied. Taste is principally measured by using chemicals or electric stimuli. Measurement of taste via chemogustometry involves application of chemical stimulants to the oral mucosa. The apparatus for this procedure is bulky and complicated and hence not commonly used. [1] The transduction of taste from its receptors on the oral mucosa to the centre of taste in the brain has been analysed using microscopy and techniques of cellular and molecular biology. [2] Measurement of taste using electric stimulus was first reported by Krarup in 1958 and this provided a simpler way to assess this sensory function. [3] This method of measuring taste is called electrogustometry.

Since the development and use of electrogustometry in the 1950s various electrogustometers have been developed, used and trialled. Electrogustometry essentially involves the application of controlled direct current stimuli to a specific region in the oral mucosa and assessing the subject's response thereof. [4] It, like chemogustometry, is a subjective test and psychophysical analysis of the subject's response is essential in determining the taste threshold. Most electrogustometers which have been developed are manually operated and an alternate forced-choice double-staircase algorithm is usually employed to determine the taste threshold. [4] Some computer controlled electrogustometers have also been developed to eliminate any operator bias in running this algorithm. [5] Nevertheless, despite these advances in electrogustometry, a stand-alone, automatic and portable electrogustometer is yet to be developed. Hence, electrogustometry is yet to become a common clinical tool.

The uses of electrogustometry include detection of middle-ear disease and tumours, assessment of taste loss caused by tonsillectomy, age, laryngomicrosurgery and diabetes and screening for subjects with Parkinson's disease, Bell's Palsy. In most cases of middle ear disease or tumours, which may be oncogenic, bilateral asymmetry in taste perception is commonly observed. The information is used to analyse the extent and region of damage of the neural pathway. [6 - 14]

The sense of smell augments the perception of taste to a great extent. When food, fluid or any foreign object is placed on the oral mucosa a somatosensory sense is also evoked. Gustation and the sense of touch on the oral mucosa are two different sensations so while measuring the gustometric function, the sense of touch must also be accounted for. The overall taste perceived is the summation of these three responses – gustometric response (the sensation of taste), olfactory response (the sensation of smell) and somatosensory response (the sensation of touch).

There are four principle types of taste – sweet, salt, sour and bitter. A fifth type of taste has recently been identified called umami. Chemogustometry can measure each of these types of tastes by application of various stimulants. However, the apparatus involved is bulky and the process not simple. This has limited the use of chemogustometry in the clinical setting. On the other hand, electrogustometry cannot differentiate the qualities of taste and only provides an overall quantified taste threshold, primarily that of sour taste. The information obtained from electrogustometry can be effectively used to determine various diseases and the integrity of the neural pathway as described in an earlier paragraph.

1.2 PROBLEM STATEMENT

Our laboratory was contacted by a local ENT registrar, Mr AD Morley, about investigating the use of electrogustometry in assessing middle ear diseases and tumours. He was looking to study this using the RION TR06, the current market standard for electrogustometers, as part of his MD studies. Upon assessment of this instrument it was recommended that a state of the art electrogustometer was required to be developed in order to reflect the advances in technology which would make electrogustometry a useful clinical tool.

A detailed study into electrogustometry was then carried out to understand the various electrogustometers that have been developed. Dr Ripley contacted Mr TR Bull, who had developed one of the earlier electrogustometers in the 1960s. He detailed its principles and donated the only machine to our laboratory for further studies. Contact was also made with Dr JA Stillman in New Zealand who has been studying electrogustometry since the 1990s. Mr Morley visited the Smell and Taste Research Centre in the University of Pennsylvania, USA to gain first-hand experience with RION TR06 and meet Dr R Doty, a pioneer in taste and smell studies.

After a detailed investigation, the salient features of a state of the art electrogustometer were defined. It was decided that the machine should be automatic, portable, stand alone, battery powered and simple to operate. Hence it was decided to employ embedded digital technology which would consume low power. One of the main criteria was that the machine needed to be completely isolated from the mains power supply.

Following the development of the Sussex Electrogustometer, the new state of the art machine, it was tested for reliability and repeatability. The RION TR06, which was made available by Mr Morley, was used as a standard with which to compare the new machine. Following further analysis of the literature it was noted that the physical parameters like duration of stimulus and surface area of the electrode used to apply this stimulus were different in different studies. The lack of a standard meant that the data could not be easily compared. A study was then carried out to determine the recommended standards for the duration of electrical stimulus and the area of electrode used to measure taste threshold.

While performing the re-test of one of the subjects during the assessment of the machine's repeatability, it was observed that there was a significant difference in taste threshold from the previous measurement. Upon further investigation into this anomaly it was noted that the subject had consumed alcohol immediately before the electrogustometric test. This led on to the next part of research and detailed studies were conducted to understand the immediate effect of alcohol on taste. The existing literature suggested that a lot of studies had been done to investigate the long term effects of alcohol and smoking on taste. None of these studies however commented on their immediate effect. Detailed experiments were conducted using alcohol, tobacco (in the form of cigarettes) and anaesthetics (in the form of oral sprays), in accordance with the ethical approval given by the Ethics Committee of the University of Sussex. Analysis of these results in collaboration with Prof Duka, a psychologist and Prof Kros, a neuro-biologist, showed the immediate effect of alcohol and smoking on taste which have been detailed in subsequent chapters.

The Sussex Electrogustometer was used in a clinical setting by a local consultant and his colleagues to understand how the machines were suited to clinical trials. A detailed report was provided which validated the design and clinical use of the Sussex Electrogustometer.

1.3 STRUCTURE

The second chapter of the thesis discusses the sense of taste, its histology, anatomy and neuro-biology. It explores the classical neural pathways and elaborates the different transduction mechanisms for different types of taste. This chapter details the anatomy of the taste buds – the principle taste receptors and explores the role of saliva in taste perception.

The third chapter discusses the measurement of taste using electrogustometry. This chapter lists and explains the various electrogustometers developed over the past fifty years and highlights the salient features of a state of the art electrogustometer. A table within this chapter compares the various electrogustometers developed.

The fourth chapter explains the design philosophy and construction of the Sussex Electrogustometer – a state of the art, stand alone, semi-automatic and portable electrogustometer based on embedded digital technology. It lists and elaborates the need and design of its constituent parts and also elaborates the design of the software embedded within the microcontroller. It also explores how a psychophysical analysis of the subject's behaviour can be carried out automatically by this machine. This chapter also notes the design of the various accessories of this machine – the electrodes, return path and feedback switch. The assembly, PCB design, power supply and enclosure design are also explained.

The fifth chapter of this thesis elaborates the studies conducted to assess the reliability and repeatability of the Sussex Electrogustometer. It is shown that the Sussex Electrogustometer has a high degree of reliability and repeatability when compared with the current market standard, the RION TR06, and when a test-retest assessment is done.

For electrogustometry to become a common clinical tool, a robust understanding of the physical constraints is necessary. The sixth chapter of this thesis elaborates studies conducted to determine the recommended standards for these physical constraints – the stimulus duration and electrode area.

The following chapter details the immediate effect of alcohol on taste. Various studies have previously been done to assess the long-term effect of alcohol on taste. However no studies have been done to understand the immediate effect of alcohol on taste in non-alcoholics. An elaborate study has been carried out to understand how alcohol affects the taste channels. The study designed with the help of a neurologist and psychologist explores the local and peripheral effect of alcohol on taste threshold.

The eighth chapter details the immediate effect of a depressant, tobacco, on taste threshold. As with alcohol, a lot of work has been done on taste thresholds of regular and heavy smokers. However, the immediate effect of smoking on an occasional smoker's taste threshold has not been investigated. This chapter elaborates the study conducted and the analysis of the results thereof.

The ninth chapter discusses the report from a local consultant about the clinical use of the Sussex Electrogustometer. The main aim of this project was to make a simple, portable, semi-automatic machine to determine electrogustometric taste threshold in the clinical setting. This chapter details the findings of the consultant and his colleagues who used this machine in the clinical setting.

The concluding chapter highlights the important aspects of each chapter and briefly discusses the scope of electrogustometry and the Sussex Electrogustometer and how electrogustometry is slowly becoming a common clinical tool.

CHAPTER 2

TASTE

2.1 INTRODUCTION

Taste is one of the five major senses of the human body, albeit, the least studied. This chapter details the physiology, histology, neurology and transduction of taste.

The entrance to the digestive system, the mouth or buccal cavity, forms an important part of the human body. It monitors the intake of food and fluid and contains the receptors for gustatory and somatosensory sensations. Taste buds act as the primary receptors for gustatory sensation. The structure and function of these taste buds will be elaborated in this chapter. 'Meissner corpuscles' and 'Krause end bulbs' are highly sensitive tactile receptors for evoking somatosensory responses. Merkel's touch receptors and other nerve endings for sensation of temperature also form part of the buccal cavity. [1]

This chapter details the various aspects of the process of ingestion pertaining to the sensation of taste, the role of saliva and details of the gustatory pathway. It explains the transduction of taste via nerves from taste buds to the brain. It also includes the analysis of different qualities of taste – salt, sour, bitter, sweet and umami.

2.2 DEVELOPMENT OF THE TASTE SYSTEM

Taste buds are specialized sensory cells residing in the complex gustatory epithelium. They are present on the tongue surface, soft palate, tonsils, pharynx and epiglottis. The tongue surface is formed of tissues with a rapidly renewing population. The life span of the final stage of differentiation of the epithelium is short. When the upper layer is removed, it needs to be quickly replaced. The source of stem cells for this is the basal layer which has a virtually infinite life span and continually differentiates to replace the lost upper epithelium. [15, 16] The replicator unit contains one stem cell surrounded by basal cells. This differentiates producing columns of raised protrusions called papillae. The growth of these cells depends on different factors like – nutrition, solubility and interaction via cell-cell communication. The surface of the tongue has various cell structures – the fungiform, filiform, foliate, conical and circumvalate papillae. The different patterns are also reflected in the molecular structure of these cells. The cytokeratin present in these cells are expressed differently in each type. [17]

The development and the regulation of the taste epithelia have been studied extensively. The embryonic epithelium is formed of two layers – a superficial periderm and a deep layer cell. The first type of taste cells that originate are the fungiform papillae. They appear as small protrusions on the tongue surface and cause the cells to elongate in the deep epithelial layer. The development of the papillae occurs at different times depending on the type of papillae. Generally most of the taste cells develop between nine and ten weeks of gestation. [17] The neural innervations of the tongue and gustatory system change and complicate with the synthesis of the taste system. The main sensory nerve branches extensively as its reaches the surface of the epithelium. The anatomy of the peripheral taste system will be elaborated later.

2.3 CLASSICAL GUSTATORY PATHWAYS

Various researches were carried out in the late 19th and early 20th century on gustatory pathways and they have now become the foundations of taste neurobiology. Two major approaches were noted: which cranial nerves are associated with the transduction of taste and how the perception of taste is altered by neurological diseases. Lewis and Dandy in 1930 published a detailed neurological and neuro-anatomical literature on gustatory pathways. [18] The sensory function of the facial nerve was described by J Ramsey Hunt in 1915. [19] This publication detailed the various branches of the facial nerve. The principle nerves responsible for the sensation of taste and the transduction of the resultant information are the cranial nerve VII (facial nerve), IX (glossopharyngeal nerve) and X (vagus nerve).

The facial nerve is a mixed nerve. The sensory ganglion is responsible for transduction of gustatory stimuli from the anterior two-thirds of the tongue. The nerve bundle passes through the stylomastoid foramen and geniculate ganglion and ends in the pons. The axons extend to the thalamus leading onto the gustatory areas of the cerebral cortex. It also contains axons from proprioreceptors in the muscles of the face and scalp. It also has extensions to the lachrymal, nasal, palantine and the saliva producing sublingual, sub maxillary and parotid glands. [20]

The glossopharyngeal nerve is also a mixed nerve. The sensory portion consists of axons from taste buds and somatic receptors on the posterior one-third of the tongue, from proprioreceptor in swallowing muscles supplied by the motor portion and from the stretch receptors in carotid sinus. The axons pass through the jugular foramen and end in the medulla. The motor portion is responsible for movement of the larynx during swallowing. [1]

The vagus nerve is also a mixed nerve. The sensory portion consists of a small number of taste buds in the epiglottis and pharynx, proprioreceptor of the muscles in the neck and throat. The axon passes through the jugular foramen and ends in the medulla oblongata. All the taste nerves meet in the gustatory nucleus of the medulla oblongata. [1]

The tongue receives its blood supply from the lingual artery which is a branch of the external carotid artery. The pink appearance of the tongue is because the epithelial layer is thinner than most other regions of the body and the arteries are closer to the surface of the tongue.

2.4 NEURAL ANATOMY OF THE GUSTATORY SYSTEM

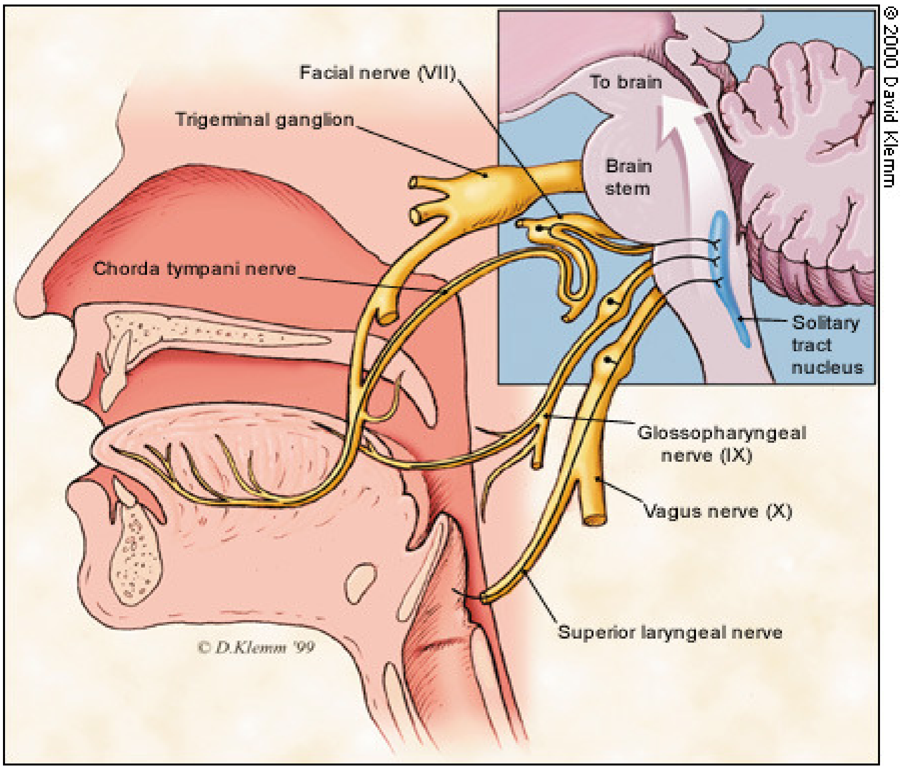

Fig 1: Neural anatomy of the peripheral taste pathways © David Klemm, 2000. [21]

The facial, glossopharyngeal and vagus nerve, different cranial nerves, innervate the taste receptor cells located in the oral mucosa. The facial nerve has two sensory sections related to gustation – the chordae tympani and the greater superficial petrosal. All the afferent nerves terminate in the rostral portion of the nucleus of the solitary tract of the brainstem. This is the first gustatory synapse. The other afferent nerves originating from the oral mucosa contain somatosensory perception. They are mainly carried by the trigeminal, the fifth cranial nerve, glossopharyngeal and vagus nerve. The central taste pathway begins in the medulla oblongata and projects rostrally in parallel with the general autonomic system. The facial nerve is primarily a motor nerve with small sensory attributes of gustation. The glossopharyngeal and vagus nerves are also mixed. They carry the gustatory and somatosensory sensations to the brain.

The nucleus of solitary tract (NST) is a heterogeneous collection of smaller nuclei that extend from the level of the spinal cord – medulla oblongata junction to the pons. The NST receives gustatory and somatosensory information. Another important nucleus of gustatory ganglion is the Parabrachial Nuclei (PbN). Electrophysiological analysis of PbN shows that it contains neurons that carry gustatory response from the tongue. The gustatory thalamus relay is located beyond these nuclei and relays the gustatory and somatosensory sensations.

Processing of gustatory response by the brain in relation to its sensory coding and taste discrimination is studied in context with the visceromotor integration. The ingestion of food depends on the analysis of its taste and other attributes like smell and somatosensory responses it evokes. The four principle types of taste, detailed later, are coded differently so that they can be analysed properly by the brain. Further coding of the gustatory information is done based on the degree of the taste. The gustatory neurons are narrowly tuned across the taste qualities. The resolution of the taste stimulus determines the sensitivity of the gustatory system and it varies from person to person. It can be trained if the food habits are changed. Taste information is coded depending on the intensity of each basic taste it contains. The labelled line hypothesis of neural coding involves the availability of different neurons to carry individual basic taste. However, the across-neuron pattern theory does not need different neurons to carry different basic taste responses. The gustatory information is also modulated at the synapse by the neurotransmitters.

2.5 THE ANATOMY OF THE PERIPHERAL TASTE SYSTEM

The peripheral taste system includes the taste buds on the lingual papillae and their distribution and innervations. The taste buds are clusters of columnar epithelial cells shaped like a bud, hence the name. They are spread throughout the oral cavity and the distribution varies from person to person. These taste buds are of different kinds and their spatial distribution maps the absorption and perception of different kinds of tastes. Taste receptor studies started from the times of Aristotle and in the mid-nineteenth century it was confirmed that the taste buds were responsible for the perception of gustatory stimuli. Since then various research has been carried out on the lingual papillae, the transduction of taste and the neural pathways of taste.

The mammalian papillae contain 50 columnar epithelial cells bundled together on the tongue surface. The taste buds are very similar in size ranging from 20-40 µm in diameter to 40-60 µm in length. [22] The neural activity in the gustatory axons is conducted to the central nervous system by cranial nerves.

The major types of papillae are valate, foliate and fungiform. The other papillae – filiform and conical do not contain taste buds. The valate papillae are present in a V shape across the root of the tongue. The foliate papillae consist of ridges between adjacent folds along the posterior margin of the tongue. The fungiform papillae are the easily identifiable pink elevations of about 0.5 mm diameter. They vary in appearance and are distributed over a large area of the tongue. There are approximately 4600 lingual taste buds per tongue. [23]

2.6 RECEPTOR MECHANISMS IN GUSTATION

There are four major kinds of taste qualitatively – sweet, bitter, sour and salt. This section will include the discussion of how each of these tastes is perceived.

Sweet

Sweet substances have varied and complicated structures. They can range from simple glucose – monosaccharides and sucrose – disaccharides to complicated carbohydrates, D and L amino acids, artificial sweeteners, chloroform, and plant proteins amongst others. Sweet taste is thought to be perceived by trans-membrane receptors coupled with G_s proteins and/or the amiloride blockable sodium ion channel. The sweet tastant activates the G proteins which in turn generate cAMP as the intracellular second messenger. cAMP decreases to generate phosphorylation of K^+ ions and taste cell hyperpolarisation. [1]

Bitter

There are two different receptor-mediated bitter transduction pathways. Bitter compounds bind to a trans-membrane receptor and activate it. This couples to a G_{14} protein which in turn activates phospholipase C to generate IP_3 which generates calcium ions from. This leads to the release of transmitters from the vesicles. The other transduction mechanism involves cell specific G protein gustaducin and phosphodieterase activation. Bitter substances activate opspin-like receptors which get bound to the gustaducin which will activate phosphodieterase to decrease levels of cAMP. This would lead to the phosphorylation of potassium channels and taste cell hyperpolarisation. [1]

Sour

The sour taste is attributed to the release of hydrogen or hydronium ions on the oral mucosa. This is often caused by the pH of the food or by electrolytic breakdown in the saliva. The hydrogen ion passes through the oral mucosa easily by diffusion and no gated channels or proteins are required for this transduction. The analysis of this taste is used to measure the threshold of taste using electrogustometry, a method involving application of current stimulus to the oral mucosa. Sour taste absorption does not involve phosphorylation and is solely based on ion gated channels making the process relatively simple. [1]

Salt

Salt taste is attributed to the sodium ions produced due to the ionisation of the tastant. This passes easily through the sodium channels without the need for proteins and/or phosphorylation. Thus the sour and salt tastes are easily perceived.

Transduction of stimulus perceived by the sensory organ or cell involves the transformation of energy in the stimulus to electrochemical energy required to transmit the neural impulse. The difference in intracellular potential caused by the presence of the stimulant is noted as a potential difference triggering the electrochemical impulse for the sensory neurons. [24, 25] The taste cells can be excited by electricity and have been previously studied at length. Different types of TTX-sensitive sodium ion channels, calcium and potassium channels are present in the oral mucosa able to trigger a neural response from the taste cells. The main constituent of salts is these ions which are electrolysed in presence of saliva. These channels are generally amiloride gated.

The chordae tympani, part of the facial nerve, contain two types of fibres. One type called the N fibre, sensitive only to sodium cation and another type is called the H fibre, sensitive to sodium and other cations. The N fibres are sensitive to amiloride and sodium cation likewise whereas the H fibres are more sensitive to sodium cation. [26] This alters the type of taste perceived at different locations depending on the nature of fibre present. The neural impulse is transmitted by the release of a synaptic transmitter caused by an exchange of sodium and potassium cations. With increase of sodium in the neural fibre potassium cations are lost causing depolarisation and giving rise to an action potential. This causes an influx of calcium cations which triggers the release of the neurotransmitter. [27]

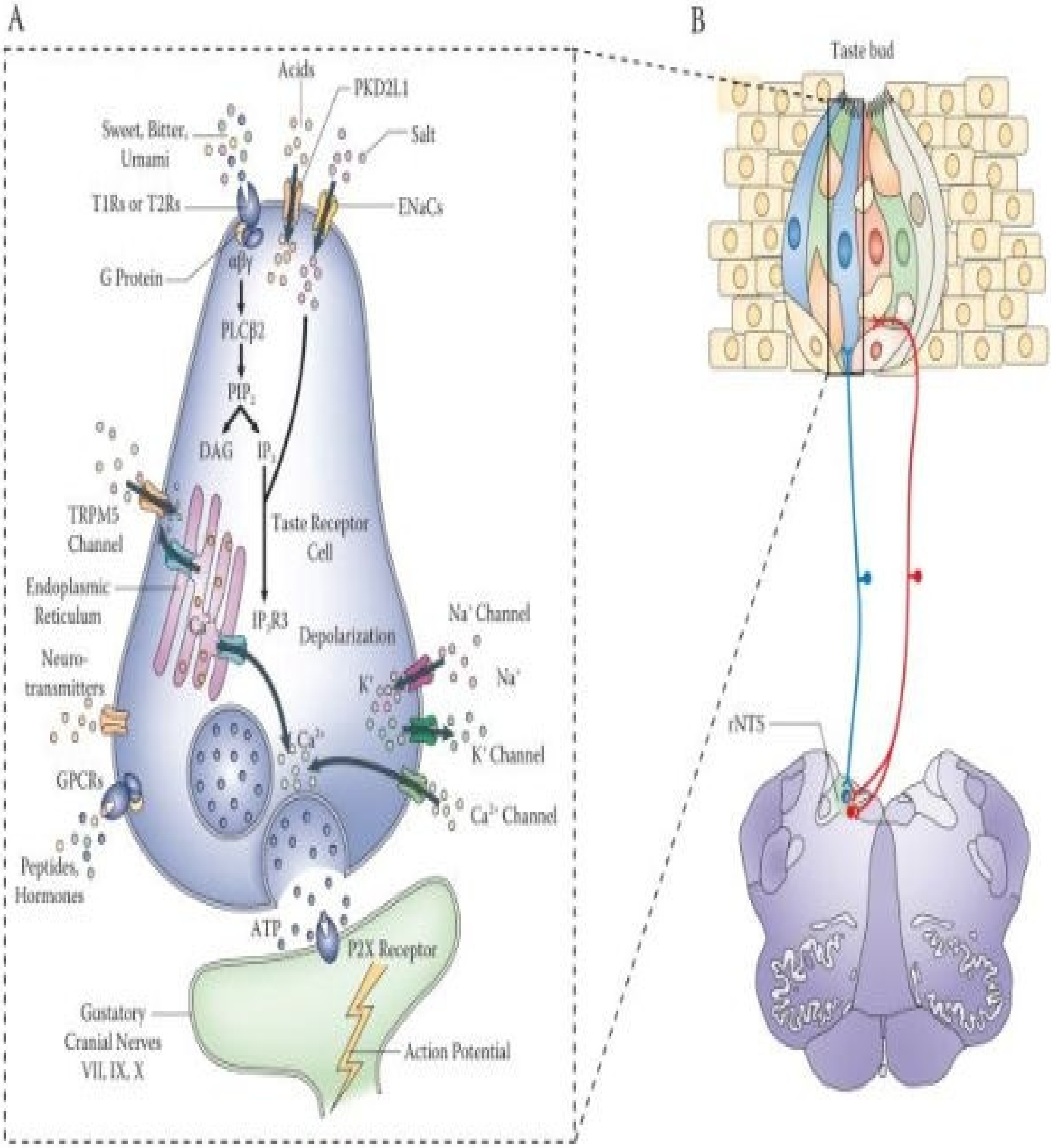

Fig 2: Transduction of taste [27]

2.7 GUSTATORY NEURAL CODING

The taste system is unique because the quality of the tastant needs to be determined, a quantitative analysis of the particular taste must be done and the resultant information must be processed in order to determine if there may be any physiological impact of ingesting that food. Thus the taste system must analyse and predict. Extensive learning is involved to train the taste system. A lot of it is inherited from ancestral systems and simple decisions like 'swallow' or 'expel' are easily made.

Evidence of four basic tastes has been found from patch-clamp tests. [28] The reason for segregating taste into four principle types are their unique receptor mechanisms, neural code, chemotopic organization, temporal properties, taste modifiers or suppressants and ethological consideration. Further studies have highlighted another distinct fifth type of taste called umami. It is elicited by monosodium-L-glutamate (MSG) and starchiness. [1]

The gustatory neurons are broadly tuned to taste stimuli. The breadth of tuning is an important parameter that impacts the information handling capacity of the neurons. The neurons carrying taste information are separately tuned to facilitate the decision making process of the brain.

2.8 SALIVA

Saliva is the main fluid secreted in the oral cavity and its function involves helping in mastication, first stage breakdown of food, prevention against any harmful microbes ingested through food, amongst others. Saliva is secreted mainly from three glands: parotid, submandibular and sublingual. It is also produced from other smaller glands –

labial, buccal, palantine and lingual glands. The saliva produced has only 10% of electrolyte including small concentrations of sodium and potassium ions. However, while travelling through the ducts the sodium ions are re-absorbed leaving the saliva rich in potassium. The saliva is also rich in proteins and peptides.

The functions of saliva are diverse. One of its main functions includes tissue permeability and lubrication. Saliva forms a thin aqueous layer of 0.07 – 0.10 mm on the surface of the oral mucosa. [29] The thin layer of saliva forms the first defence against harmful microbes and also protects the exposed enamel from harmful acids and sugars contained in food. The glyco-proteins with high molecular weight remain close to the tongue surface making it slippery and making the saliva highly viscous. Lubrication of the oral cavity by saliva helps in bolus formation, swallowing and speech.

Another principal function of the saliva is digestion. Amylase in saliva initiates the breakdown of starch in the oral cavity. Lipase present in the saliva helps digest fat. However, the biggest contribution of saliva in digestion is probably making the food more soluble to ease digestion at later stages. Many animals use saliva for grooming for its antiseptic qualities.

Saliva plays a very important part in taste perception. Dissolved food passes close to the taste buds which senses the gustatory information and relays it to the brain by means of nerves. The papilla grooves are deep and narrow which structurally does not help in tastant absorption. A pumping action is required in order to facilitate this absorption. The saliva secreted from the Ebner's cells located at the base of these grooves causes this necessary pumping action. The sodium and potassium ions in the saliva help in transduction of tastant information to the gustatory nerves. The pH of the food is also controlled before its introduction as a bolus to the oesophagus.

Saliva helps in acting as an electrolyte to evoke galvanic currents which can be used as a measure of taste transduction. Volta studied the effect of metals placed on the oral cavity stimulating taste buds. [30] Galvani placed two dissimilar metals on the oral mucosa to create galvanic currents, thereby stimulating the taste buds. [31] The magnitude of the current depends on the ionic content of the saliva, its flow rate and the metal in contact and its position in the electrochemical series.

2.9 MEASUREMENT OF TASTE

There are principally two ways of measuring taste – chemogustometry and electrogustometry. Chemogustometry involves application of chemical tastants to the oral mucosa whereas electrogustometry involves application of direct anodal current as stimuli to evoke gustatory response. Both these tests are essentially subjective. Chemogustometry needs the availability of different chemical tastants in various concentrations. It also needs a process of cleaning the oral mucosa prior to the application of a stimulus. This technique of measurement of taste can determine both quality and quantity of taste. However, chemogustometers are bulky and this limits the movement of the set up.

Chemogustometry includes the use of filter strips, cotton buds soaked in different stimulants and the use of pipettes to drop certain solutions onto certain areas. A number of devices have been developed to well-define the regions of the tongue. This method allows for a wide range of stimulants to be used and can help detect subtle changes in taste but is a slow process and not essentially practical for a clinical setting.

To overcome the disadvantages in chemogustometry and to make measurement of taste easy and common in a clinical setting, electrogustometry came into existence. With developments in electronics over the years a new state of the art machine is needed. The following chapter discusses electrogustometry. [1]

2.10 CONCLUSION

This chapter has detailed the physiological and neurological structure of the gustatory system. Insight was also given to some peripheral systems and organs. The next chapter will explain the process of measurement of taste – electrogustometry.

CHAPTER 3

ELECTROGUSTOMETRY

3.1 INTRODUCTION

Taste is one of the five major senses in our body albeit the least studied. In the previous chapter we discussed the physiology of taste including the different neural pathways. The histology of taste – the taste buds, structural and functional units of taste was also discussed. A brief overview of how taste is measured was also detailed in the previous chapter. Chemogustometry and electrogustometry, the two major techniques used to measure taste, were explained. In this chapter we will focus on Electrogustometry – its use and application. Electrogustometry has been in use since the 1950s. [3] This chapter will list and detail the principles of electrogustometry and discuss the various electrogustometers that have been made and clinically used. The salient features for a state of the art electrogustometer will be elaborated.

The sense of smell augments the perception of taste to a great extent. This thesis limits the analysis of taste to the sensation evoked by gustation. The basic tastes of sweet, salt, sour, bitter and umami are commonly perceived via the oral mucosa. Burning, soothing and tempering sensations have also been noted. Other than the sense of these basic tastes and sensations the trigeminal nerve responds to the sense of touch. When food, fluid or any foreign object is placed on the oral mucosa this sense is evoked. Gustation and the sense of touch on the oral mucosa are two different sensations so while measuring the gustometric function, the sense of touch must also be taken into account. The overall taste perceived is the summation of these three responses – gustometric response (the sensation of taste), olfactory response (the sensation of smell) and somatosensory response (the sensation of touch).

Electrogustometry, in its most common application, involves application of a regulated constant direct anodal current to the tongue as a stimulus to evoke gustatory potentials. This test is essentially subjective and the strength of the stimulus depends on the previous stimulus and the response of the subject. The electrogustometric threshold is said to have been reached when the minimum current level for which there is a positive response of gustatory sensation from the subject has been determined.

Knowledge of the taste function is used to study taste loss caused by age, tonsillectomy, laryngomicrosurgery, middle ear surgery and diabetes amongst others. The taste function has also been measured in subjects with cancer, Bell's palsy and Parkinson's disease. [7, 12, 13] The information has been used to analyse the extent and region of damage of the neural pathway.

The chordae tympani, an afferent taste nerve, passes through the middle ear. This route may be attributed to the way humans have evolved. [9, 28] Taste measurement before and after a surgical operation of the middle ear is useful. It helps detect any taste loss caused by the surgery having damaged the nerve. Electrogustometry is used to confirm bilateral symmetry or otherwise before and after the operation hence, a before and after test is essential. Bilateral asymmetry can be observed due to the presence of any tumours in the middle ear and this is an important diagnostic tool. [7, 8, 9, 10, 11, 13]

Measurement of taste using chemogustometry and electrogustometry is essentially subjective. Psychophysical elements and alterations of the test environment can easily skew the result. Thus, while using the electrogustometer for measuring taste threshold, added psychophysical checks and conformity of contributing factors must be put in place to avoid any skewness of the final result. [32, 33]

3.2 ELECTROGUSTOMETRY

The use of electric pulses to measure taste threshold was introduced before the 1950s. [3, 34] Electrogustometry is now a viable clinical tool to estimate taste function though yet to become commonly used. The taste function derived from electrogustometry is especially important in determining the integrity of the neural pathway. [35] Electrogustometry quantifies taste and measures the threshold of sensation of this. Chemogustometry on the other hand can help determine various taste types – like sweet, sour, bitter, salty and umami i.e. a more qualitative approach. The taste perceived in electrogustometry is sour metallic and is attributed to the absorption of the protons (or hydronium ions) liberated by the current stimulus. [36]

Since taste threshold measurement using electrogustometry is subjective, uniformity must be maintained in the way the subject is trained and the environment is set up. The difference between detection threshold and recognition threshold must be explained to the subject. The subject is asked to confirm the sensation only when he/she is sure about the perception. The effect of the trigeminal nerve detecting the sense of touch must also be minimised to avoid any bias. Hence the application of pressure on the electrode must be carefully controlled.

Adjukovic proposed that current density determines the taste threshold. Thus not only the current intensity but the size of the electrode is also instrumental in determining that threshold. [37, 38] The stimulus duration also affects the taste threshold. Thus standardisation of these physical factors must be achieved in order to compare taste threshold data. Studies are reported later in this thesis which ascertain and establish accurately the effect of such physical factors.

Electrogustometry has good test-retest reliability but training of the subject during measurement of electrogustometric threshold is essential to obtain a true response. [39] Before the start of the test, random stimuli are given to the subject so that they are trained to differentiate between gustatory evoked potential and any other senses, including somatosensory senses, which might be evoked. The training also familiarises the subject with the test environment and procedure. The compliance of the subject is assessed throughout the test by using null stimuli to account for various psychophysical factors.

Chemogustometry allows a qualitative analysis of the taste response. It involves a large instrumental set up involving different chemical tastants and filter papers. On the other hand, electrogustometry offers a quantitative approach and relatively smaller and portable instrumentation.

In electrogustometry weak anodal current stimuli is generally used to evoke a sour taste perception. [36, 40] Cathodal stimuli do not produce any significant recordable sensation hence the anodal current is used. [41] The stimulus is a constant direct current of predefined amplitude and duration. [5]

3.3 ELECTROGUSTOMETERS

Over the years various electrogustometers have been developed. Electrogustometry involves the application of a regulated direct current stimulus for a pre-defined duration to the oral mucosa at a specific location. The simplest application of this would be to place a battery on the tongue surface. This was studied by McClure and Lawless in 2006. They commented that this simple portable device may be used as a replacement of the conventional "taste meters". [42] Advances in technology have led to sophistication of the taste meters. One of the most common and widely used taste meters is the RION TR-06. The following sections describe and discuss the various electrogustometers that have been developed and trialled.

3.3.1 TR BULL MACHINE (Fig 3)

In the 1960s a very basic electrogustometer was designed by Mr TR Bull in the UK. It was a simple instrument with limited options and included very simple circuitry. It was not very widely used due to its limitations. A silver electrode of 0.5 cm diameter was used to apply the current stimuli. The current level was monitored using an analogue ammeter. The return path was formed by a thin metal disc held between the thumb and the forefinger. Tests using this machine led to the conclusion that 98% of people have their taste threshold between 10 and 55 µA. The threshold was observed to be lower at the tip of the tongue as compared to the rear end and the soft palate in accordance with other research. [43] The current stimulus was applied for duration of one second using a transistor controlled circuit. The circuit has low output impedance and is not very precise. Mr Bull has donated his machine to our laboratory for research purposes.

Fig 3: TR Bull machine

3.3.2 INDIAN ELECTROGUSTOMETER (Fig 4)

An electrogustometer was developed at the Indian Institute of Technology, Kanpur in 1965. The supply was from the 230 V mains AC voltage and the circuitry was based on silicon diodes. A stainless steel electrode of 5 mm diameter was used to apply the current pulse. The instrument had a range of 0 – 300 µA in steps of 3 µA or 10 µA. There are other control knobs on the front panel of the equipment – the threshold 'coarse knob' changes the threshold in steps of 5 µA and the threshold 'fine knob' changes it in steps of 1 µA. There is an option to power the device from a 120 V battery supply. The testing procedure for this machine involved training the subject with a 60 µA stimulus and then beginning the test from 0 µA and increasing it in steps of 5 µA till a distinct acidic taste was perceived. [44]

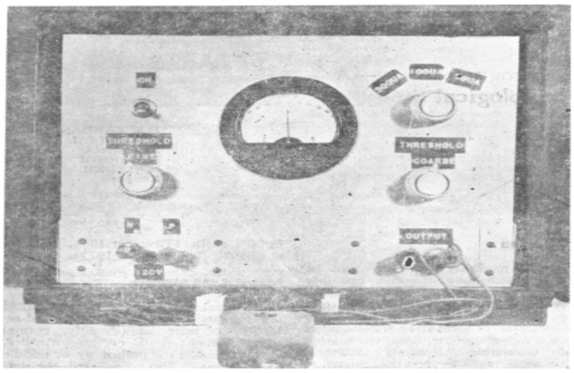

Fig 4: The Indian Electrogustometer

3.3.3 RION TR06 (Fig 5)

The RION TR06 is the most common instrument used to measure taste threshold by the application of electric stimulus. It is portable and has a current range from 4 µA to 400 µA. Constant direct current stimuli of predefined amplitude can be applied for pulse durations 0.5, 1.0, 1.5 and 2.0 s. There is also an option to apply this stimulus with user controlled duration. It is a safe and a battery-powered device.

As with other sensory systems, the taste mechanism has a logarithmic response and hence the RION TR06 has a current control which operates with logarithmic steps labelled as decibels. The scale is calibrated to make 0 db = 8 µA and hence the total range is -4 to +34 db according to the formula db = $\log_{10}[\mu A/8]$.

The application of a stimulus using the RION TR06 is essentially manual. The operator maintains a record on paper of the current stimulus applied to the subject and depending on this the amplitude of the next stimulus is determined. Sufficient time is given for the subject to recover but the recovery time is not essentially constant.

The RION TR-06 is the most widely used electrogustometer available currently. The current is applied using stainless steel electrodes. They are adjustable to be placed flat on the tongue surface. The return path is through a neck band with application of an electro-conductive gel to ensure good connectivity. The subject feeds back a positive response by a hand-held feedback switch which is noted by a flash of an LED. The pulse can be applied to the subject using a push button on the instrument or a foot pedal to suit the operator's convenience. The taste threshold is obtained by calculating the root mean square of the last five current values of the staircase.

The limitation of the RION TR06 is that it is not automated to control the alternate forced choice algorithm. Furthermore, there is no option for zero current level and hence all false pulses are produced by not pressing the output button. The RION TR06 does not use annuntiators to signal the occurrence of an event. It also does not provide a uniform environment for each stimulus by allowing variable recovery time and variable feedback time. A skilled operator is needed to operate this machine.

The RION TR06 is manufactured industrially by Sensonics Inc. [45]

Fig 5: RION TR-06

3.3.4 HALLE II (Fig 6)

The Halle II was developed in Germany and is similar to the RION TR06 in design and features. It has a range of -6dB to 40dB and applies electric stimuli for pulse duration of 500 ms. A high grade steel electrode is used for the application of the stimulus. The instrument is battery powered. A double staircase forced choice algorithm with random blank pulses is used by a skilled operator to find the taste threshold. [46]

Fig 6: The Halle II

3.3.5 PC ELECTROGUSTOMETER (Fig 7)

Following the successful testing of the HALLE II, the same laboratory in Germany developed an electrogustometer which was computer controlled. A constant current source was controlled by a computer. The forced-choice staircase algorithm was run using the computer which provided the calculated stimulus value in the range of 0.3 µA to 1000 µA using the parallel port. A printer was also used to track the test results. The software was written in Turbo-Pascal. [46]

Fig 7: The PC Electrogustometer

3.3.6 COMPUTER-CONTROLLED ELECTROGUSTOMETER

Loudon and Stillman developed a computer based electrogustometer to avoid human bias in the application of the current pulses. It was very similar to the PC electrogustometer developed in Germany. The computer ran the algorithm which was employed to determine the taste threshold. The pulse duration was also programmable using the computer. It was commented that the reliability of electrogustometry increased by using a computer. However, since it was a computer based device it was not easily portable and since it was connected to the mains power supply there was a potential safety problem. [5]

3.4 COMPARISION OF ELECTROGUSTOMETERS

The various electrogustometers available and manufactured are compared in the table below for reliability, portability, accuracy, speed, ease of operation and safety. Based on this comparison the salient features of the new instrument can be outlined.

Features	TR Bull Machine & The Indian Electrogustometer	RION TR06	Halle II	PC Electrogustometer
Reliability	Not reliable – Manually operated.	Not very reliable – Manually operated	Not very reliable – Manually operated.	Reliable – computer controlled
Portability	Easy	Easy	Easy	Difficult – as it is computer based.
Accuracy	Not very accurate as the control is analogue	Accurate	Accurate	Accurate
Speed	Average	Average – also depends on the skill of the operator	Fast	Fast
Ease of operation	Needs a skilled operator	Needs a skilled operator.	Needs a skilled operator	Easy
Safety	Safe	Safe	Safe	Machine is connected to the mains power supply

Table1: Comparison of Electrogustometers

3.5 SALIENT FEATURES OF A STATE OF THE ART ELECTROGUSTOMETER

With advances in technology it is now possible to make a new device that will incorporate the benefits of using a computer-controlled algorithm and be a stand-alone device. The requirement of a new electrogustometer is detailed as below:

1. **Stand alone and battery powered device**: the device should not be connected to the mains power supply in order to provide isolation and ensure subject safety.
2. **Automated**: the new device should have an automatic mode of operation which should be able to provide automatically controlled anodal stimuli in accordance with a pre-programmed alternate forced-choice double-staircase algorithm and subject feedback. The machine should also have a manually operated mode of operation to train the subject before the test.
3. **Portable**: the new machine should be portable to ensure it can be moved and set up easily in any environment. This will help electrogustometry become a common clinical tool.

3.6 CONCLUSION

This chapter discussed and compared various electrogustometers developed since the 1960s and highlighted the salient features of a state of the art electrogustometer. The next chapter will detail the structure, operation and functions of the Sussex Electrogustometer which has been designed to meet the criteria above.

CHAPTER 4

DESIGN AND CONSTRUCTION OF THE SUSSEX ELECTROGUSTOMETER

4.1 INTRODUCTION

The previous chapter has detailed measurement of taste using electrogustometry. It documented various types of electrogustometers, manual and computer controlled, which have been developed over the years and it listed the salient features of a state of the art electrogustometer. This chapter details the design philosophy, functional blocks, signal-detection strategy, enclosure design and operating principles of a new state of the art, semi-automated electrogustometer, based on an embedded digital system.

The Sussex Electrogustometer is a state of the art biomedical instrument used to measure electrogustometric threshold. It is flexible and easy to use. This machine is light, portable, robust, reliable, semi-automatic and battery powered. It is based on embedded digital technology, being controlled by a Peripheral Interface Controller (PIC). The Sussex Electrogustometer has two modes of operation – manual and automatic. The manual mode, used essentially to train subjects, can apply up to eight different current stimuli. The automatic mode employs an alternate forced-choice double-staircase algorithm to arrive at the electrogustometric threshold. Although this second mode is called "automatic", the machine does, of course, require the operator to set it up and to train the subject.

Psychophysical analysis of subjects' response is essential for all subjective tests. Electrogustometry is essentially subjective i.e. the result of a test depends on the subject's response to the given stimuli. Hence the signal-detection strategy must be carefully explained to the subject by means of the manual mode of operation. This chapter details various aspects of psychophysical analysis employed in arriving at the electrogustometric threshold.

4.2 DESIGN PHILOSOPHY

Electrogustometry involves the application of controlled and constant anodal direct current stimuli to the oral mucosa for a pre-defined duration to determine taste threshold. [3] This stimulus causes the perception of a sour-metallic gustatory sensation which may be attributed to the liberation of protons or hydronium ions. [41] The stimulus is applied to the surface of the tongue with a flexible stainless-steel circular electrode. A hand-held feedback switch indicates the subject's response. Whereas an instrument such as the RION TR06 requires the operator to act according to the subjects' response, the Sussex Electrogustometer is programmed to perform a staircase search for the electrogustometric taste threshold.

The electrogustometer has to produce constant anodal direct current stimuli. The electric current stimuli trigger the production of ions on the tongue surface which causes the perception of a sour metallic taste. The output is a constant current, not a constant voltage, to account for the variable body resistance between the tongue and neck. Previous research in electrogustometry has determined that a suitable range for an electrogustometer is 0 – 500 μA. [3,4,5] Experiments have also shown that the average body resistance between the tongue surface and neck is high, in the order of

10 to 100 kΩ, and variable, depending particularly on the water content of the region. The Sussex Electrogustometer needs to be designed to provide constant current stimuli in this range. In the automatic mode of operation, the current stimuli will be generated by the use of a programmed algorithm. The PIC calculates the required current as a digital number. Hence a Digital to Analogue Converter (DAC) is needed as an interface between the PIC and a voltage controlled current source. Previous research has also demonstrated that taste threshold depends on stimulus duration. [47] Hence, the Sussex Electrogustometer must have an adequate number of choices of stimulus duration in the range of 0.5 s to 2.5 s.

To ensure electrical safety, the instrument should be battery operated. Also, in order to drive a current up to 500 µA through a resistance of approximately 100 kΩ, a 50 V supply will be needed. This is quite high if it has to be sourced from a battery. A dc-dc converter has been employed by the Sussex Electrogustometer to generate such voltages to meet the demand.

The following sections detail the functional blocks of the Sussex Electrogustometer and their design and operation.

4.3 CURRENT SOURCE

The required output from an electrogustometer is controlled constant direct current stimuli. Hence a direct current source is required at the output end of the Sussex Electrogustometer.

An important design consideration for this current source is that the load for this machine is in tens of kilohms and variable. Body resistance between the tongue and neck can be as high as 10 kΩ to 100 kΩ. This load differs from subject to subject and for the same subject at different times depending particularly on the water content of the body. To supply the desired maximum direct current of 500 µA through this load a relatively high voltage of 50 V or more will be required. The electrogustometer has to be battery powered to ensure electrical safety. Hence such a high voltage demand needs to be addressed in the design of the power supply. A dc-dc converter module has been employed by the Sussex Electrogustometer to convert a 9 V battery output to up to 80 V as a worst case design.

4.3.1 Circuit Schematic:

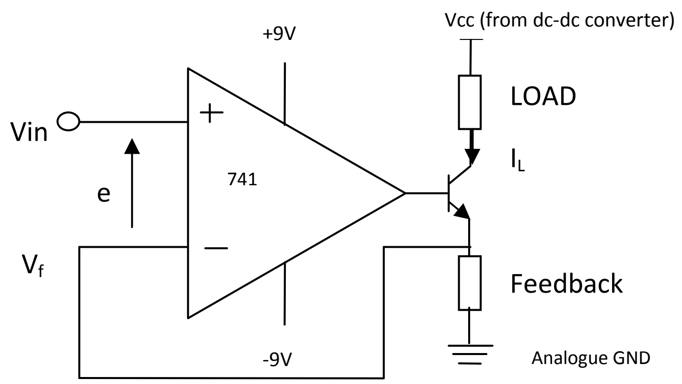

Fig 8: Circuit diagram of the constant current source

The operational amplifier is used in the trans-conductance mode with negative feedback. The amplifier operates using a supply of ± 9 V. In order to prevent saturation of the amplifier by the high voltage demand, the output is buffered with a high V_{ce} transistor. This allows the load resistance to be driven from the high V_{cc} which is provided by the dc-dc converter. The current flowing though the load is monitored by the feedback resistor in the emitter path of the transistor.

Because of the high gain of the operational amplifier, ‘e’ is almost equal to 0.

Hence,

$V_f = V_{in}$

$V_f = I_L R$

Hence,

$I_L = V_f / R$

Here, $V_f = V_{in} = 0$ to 5 V and the required current is 0 to 500 μA. Hence R = 10 kΩ.

Trans-conductance Gain of the Operational Amplifier = A/r_{out}

Where, A is the forward gain of the transistor and r_{out} is the output resistance of the operational amplifier.

Current Gain of the transistor = 100

Insertion loss between the operation amplifier and transistor $r_{out}/(r_{out} + \beta R)$

Hence, Forward Gain = $(A/r_{out}) \times (r_{out}/(r_{out} + \beta R)) \times \beta$

Reverse Gain = $V_f/I_L = R$

Hence, loop gain = $A \times B = (A\beta R) / (r_{out} + \beta R)$

Considering $A = 10^4$, $R = 10^4\ \Omega$, $\beta = 100$, $r_{out} = 100$

$L \approx 10^4$

r_{out} for transistor = $10^4\ \Omega$

Hence, output resistance of the current source is = L x $r_{out} \approx 10^8\ \Omega$

The output resistance of the current source is much greater than the body resistance. Hence, output current is independent of load.

4.3.2 Performance and testing:

The current source was tested for discrete and continuous response. The following observation was made when the demanded current was monitored for a high load.

Load	Demanded Current	Actual Current
100 kΩ	500 µA	498 µA

Table 2: Testing the current source

The slight loss in current is attributed to the finite output resistance of the circuit which appears to be 25 MΩ, not very far from the estimated 100 MΩ.

Also to monitor the continuous flow of current, the PIC was made to program a ramp pulse and the load current was monitored with respect to time. The output on the Cathode Ray Oscilloscope (CRO) clearly reflected the steady change in current with respect to voltage input.

4.3.3 DC-DC Converter:

To drive 500 µA through a 100 kΩ load more than 55 V will be needed, bearing in mind that there will be 5 V across resistor R and some voltage across the transistor as well as the 50 V across the load. Body resistance can be variable. This is a very high voltage to be sourced from a battery based power supply. To overcome this problem, a specially designed dc-dc converter is used which converts a 9 V battery voltage to 80 V.

The module was sourced from Hitek, series number GMA12-100PSI. The conversion in the module is carried out linearly, by use of the common magnetic method. The dc voltage is first converted to ac which is then transformed to the desired voltage level using a transformer or an inductor. This transformed ac voltage is now converted back to dc voltage. The supply to the dc-dc converter module is a 9 V regulated source. The output of the module is filtered from any ripple it might have to generate up to 80 V. This output is the collector supply voltage of the transistor.

4.4 DIGITAL TO ANALOGUE CONVERTER

An important part of the Sussex Electrogustometer is the **D**igital to **A**nalogue **C**onverter (DAC). As the name suggests, it converts a digital signal from the microcontroller into the analogue voltage needed by the voltage controlled current source. The AD7302 IC is used as the DAC in the Sussex Electrogustometer. This is an eight-bit, 20 pin DIP package IC with a range of 2.7 V to 5.5 V. It works with very low power with a maximum of 1 µA current absorption at 3.3 V. It is commonly used in portable battery powered instruments, programmable attenuators, programmable voltage and current sources and for digital gain and offset adjustment. The AD7302 is PIC compatible and the data is loaded to the registers on the rising edge of the active low chip select pin. The analogue output is available on two pins – A and B. The AD7302 has both internal and external reference capabilities. In the Sussex Electrogustometer, an external reference voltage of 5 V is used, carefully maintained by the use of a regulator.

The PIC produces an eight-bit output corresponding to the current level required and also sends a control signal to the DAC in form of the 'chip-select' instruction. The data bus of the DAC is refreshed on every rising edge at the chip select pin which is controlled by the PIC. Synchronous programming is essential to guarantee accurate functioning of the machine.

The DAC converts the input digital voltage to an equivalent analogue output voltage using the following formula:

$$V_0 = 2 \text{ x } V_{ref} \text{ x } (N/256)$$

where V_0 is the output voltage, V_{ref} is the reference voltage and N is the equivalent binary number corresponding to the eight-bit digital output from the microcontroller. The active low 'write' pin is connected to ground to keep the DAC switched on all the time for converting the data available on the DB0 – DB7 pins on resetting the chip select pin. The V_{dd}, REFIN (reference pin), and the active low LDAC are connected to 5 V. It is important that the digital and analogue grounds are separate. Dedicated analogue and digital ground lines must be present on the PCB to ensure their isolation. Depending on the selection of the A/B pin the analogue output is available on either of these pins. In the machine the A/B port is set to zero volts thus enabling pin A.

The IC diagram and other details of the AD7302 IC are provided in its datasheet.

4.5 DIGITAL PROCESSING UNIT

The Digital Processing Unit for this instrument comprises a PIC microcontroller, annuntiators – including an LED and buzzer, Liquid Crystal Display (LCD) and control switches. The Sussex Electrogustometer is a semi-automatic machine which employs the PIC to execute a forced-choice alternate double-staircase algorithm to arrive at the taste threshold. Apart from this, the PIC also controls a manual mode of operation which involves selection of the current stimulus by the operator, primarily used to train subjects. Control signals for the annuntiator, LCD and DAC are also provided by the PIC. The operation of the PIC is controlled by two push button control switches, the feedback switch from the subject and an inbuilt algorithm.

4.5.1 Peripheral Interface Controller (PIC)

A key component of the Sussex Electrogustometer's processing unit is the Peripheral Interface Controller (PIC). The function of this unit includes supporting the manual and automatic modes of operation, generating control signals for the DAC, LCD and annuntiator and receiving the subject's response to the stimuli through a feedback switch.

PICs were originally developed by General Instruments and are now marketed and manufactured by Microchip Technology. They are low cost, reprogrammable, low power and easy to program using Assembly or C Language. They are available in 28-pin or 40/44 pin packages. The PIC microcontroller chip used in the Sussex Electrogustometer is the PIC18f452. This was the latest one at the time of design. A 4 MHz crystal is used as a clock for the operation of the microcontroller. There are five ports available and their status can be controlled by the PORT, TRIS and LAT registers. PORT A is not used, PORT B is used as an output for the LCD data bus, PORT C is used as a control port, PORT D is used as an output for DAC and PORT E is not used.

The following sections detail the software and principle of operation of the two modes of operation and peripheral hardware of this instrument.

4.5.2 LCD

The LCD used in the Sussex Electrogustometer is a 16 x 2 alphanumeric LCD manufactured by Trident. The LCD module houses a LCD driver which controls the display and acts as an interface between the PIC and the LCD. By accessing the different registers in this controller the PIC can control the operation of the LCD. The Sussex Electrogustometer uses the LCD in a 5x8, two lines, cursor off, blink off and increment without shift mode.

4.5.3 Software

The PIC was programmed using Assembly language in the MPLAB IDE (Integrated Development Environment). The algorithm for the software has been detailed below.

Algorithm

1. ***Initialize the PIC:*** In this step the PIC is initialized. Most of the registers are cleared and deactivated so that there is no impediment to the progress of the staircase.

2. ***Activate the LCD:*** The LCD is first reset for 30 ms before it is set to operate in a two line, 5 x 8 display, increment without shift, cursor off and blink off mode.

3. ***Port Definition:*** Ports are set to their design default values by programming the PORT and TRIS registers. PORT B is set as output for the LCD data bus. PORT C is set as all outputs apart from pin four for the control bus. The control bus is detailed below. PORT D is set as the output for data bus for the DAC. The TRIS registers for each port are updated to reflect their input/output operation. Functions of the pins in the control port are listed below:

PC.0 - /WR control signal for DAC. (Active low)

PC.1 - Annuntiator

PC.2 - Roll push-button switch

PC.3 - Select push-button switch

PC.4 - Feedback switch input

PC.5,6,7 - Control bits for LCD

4. W***elcome message*** - "Sussex Electrogustometer" is displayed for 2 seconds.

5. ***Operation mode*****:** The Sussex Electrogustometer now presents the user with a choice of two operating modes – Manual and Automatic. Selection of either mode can be done by using the select and roll push button switches available on the front panel.

Manual Mode

The manual mode, used essentially to train the subject, can apply up to eight different stimuli for various durations. This mode presently employs current stimuli of 5, 25, 50, 100, 200, 300, 400, 500 µA on a linear scale which are expressed as -2.7, 3.3, 16.1, 21.9, 28.0, 31.5, 34 and 35.9 decibels in logarithmic units of current as expressed in the previous chapter. This logarithmic unit is same as that used in the RION TR06.

$db = \log_{10}[\mu A/8]$

These values may be changed by re-programming the PIC. The appropriate current level and stimulus duration are chosen by the user from a menu shown on the LCD by using select and roll button switches available on the front panel.

Automatic mode

The automatic mode of operation is based on an alternate forced-choice double-staircase algorithm. One staircase starts at 10 µA while the other starts at 40 µA as the approximate average for taste threshold is between 20 µA and 30 µA. The starting values of the staircase have an impact on the threshold value reached and the time needed for the test. Usually the test is more efficient and accurate if the starting value is close to the expected threshold. [48] Choices available for stimulus duration are presently 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 and 2.5 seconds. The automatic mode of operation has an initial step size of 20 µA. The algorithm comes to an end when there is a difference of 3 µA or less between the two staircases for at least three consecutive iterations. Different durations and starting values and step size of the staircase can be easily programmed.

The measurement of taste using electrogustometry is essentially subjective. The automatic mode of the Sussex Electrogustometer applies random blank stimuli to detect malingerers and to assess subject reliability. A score of false positive hits is maintained and is made available on the LCD. If this score gets to be too high the test is aborted, the subject is re-briefed and the test is repeated. The subject's response to a stimulus is recorded using a hand-held feedback switch, which is pressed when the subject senses a distinct sour-metallic taste. This active-high signal is directly fed back to the microcontroller, which updates the algorithm to generate the next stimulus. The magnitude of the step size halves every time the direction of the current function changes. The next section explains the staircase algorithm in more detail. The Sussex Electrogustometer is essentially an automatic machine with the need of manual intervention during training and set-up.

Staircase Algorithm

The staircase algorithm or methods of ups and downs is a commonly used algorithm to determine physiological thresholds. [47] This algorithm determines the threshold through applying stimuli above and below the threshold. The Sussex Electrogustometer employs an alternate forced-choice double-staircase algorithm.

A staircase algorithm starts with an arbitrary level chosen close to the expected threshold of a normal subject. The magnitude of the step size halves every time the direction of the current function changes. The automatic mode of operation has a minimum final step size of 1 µA. The threshold is reached when there is a difference of three micro-amperes or less between the two staircases for at least three consecutive iterations. The analysis however assumes that there is no psychophysical effect on the physiological response of the subject. [49] Since electrogustometry is a subjective experiment, psychophysical analysis of the subject's behaviour is required to validate the result.

The step size is an important aspect of the staircase. It determines how quickly the threshold is reached and how coarse the transitions are for the current function. The end of the staircase can be estimated when the stimuli reach an asymptotic level and remain there for a few iterations. The staircase method is very efficient and with proper approximations can arrive at the threshold with the application of very few stimuli. This algorithm however is not intelligent enough to prevent multiple prejudiced responses. To avoid interdependencies the use of double-staircase algorithms has been prescribed. [47] In this method two separate staircases run at alternate event cycles. The biasing of the staircase can be further reduced by application of blank pulses at random intervals, which could produce false-positive responses to screen for malingerers [48]. The automatic mode stops if there are more than five positive responses to blank stimuli.

The staircase algorithm employed in the Sussex Electrogustometer has been widely studied and tested for reliability and robustness. It is essential that the algorithm employed in determining the taste threshold be efficient and reliable. In order to evaluate the algorithm it was tested using Excel with an arbitrary value of taste threshold. So, Excel generated a stimulus value and in response a "Yes" or a "No" was entered. In the first test, the responses were those of a "perfect" subject. In the second test, one anomalous response was entered, in the third two and so on. The results of such simulations are tabulated below:

Anomalies during testing	Total number of steps required to reach taste threshold	Approximate test time in the automatic mode
0	8	< 2 minutes
1	12	2 minutes
2	16	2-3 minutes
3	24	3-4 minutes
4	32	4-5 minutes

Table 3: Simulation of the staircase

Another feature of a double staircase algorithm is the starting values of these staircases. If the values are equally spaced from the approximate threshold, the minimum numbers of steps are needed.

4.5.4 Psychophysical Analysis

A given stimulus does not always yield the same physiological response in the same environment. This is the principle reason why psychophysical analysis of the subject response is critical. If the stimulus is repeated a number of times it produces a distribution of results in the physiological dimension. Psychophysics studies the distribution in these responses. [50] In subjective tests where the result of the test depends directly on the subject response, a fail-safe procedure must be introduced to counter this dispersion in response.

Psychophysical responses may vary if the stimulus is univariate or binary or more than two. The following model shows how a single response, as is the case in the Sussex Electrogustometer, is processed in light of psychophysical analysis of physiological response.

Transduction Integration Judgement

S ----------------→ N ------------------→ P ----------------→ R

Here, S is the stimulus, N is the neural response, P is the perceived Intensity and R is the overall response. [46]

The sensitivity and specificity of the instrument are determined based on the detection strategy. In the Sussex Electrogustometer a positive response is required only if the subject is certain about the perceived gustatory response.

4.5.5 The False Test

A false test essentially involves a supply of zero level (blank) stimuli well disguised within a series of actual stimuli. The procedure of application of the stimulus to the subject should be exactly the same as that of any other stimuli giving the subject no way to distinguish between the normal stimuli and the false ones other than relying on the physiological response. This is essentially used to check for any possible psychophysical dispersion in subject response and/or to counter malingering.

In the Sussex Electrogustometer a false test has been built in to the staircase algorithm. Randomly, blank stimuli are presented to the subject. Depending on the subjects' response the false positive score is updated. This score indicates how many false positive responses have been made by the subject to the blank stimulus. If the score crosses five the staircase ends and the LCD shows that there has been a false positive error (displayed as "FP ERROR"). The subject is briefed about the test again and the test is repeated.

4.5.6 Timing

Timing is very essential for the sequential operation of the PIC. A crystal oscillator operating at 4 MHz is used to generate clock pulses. From previous practice and experience the following timings have been employed.

Physical Time delays

Pulse Duration: variable and user controlled.

Time to wait for response from the subject: up to three seconds.

Time to wait between consecutive pulses to allow the de-ionization of the hydronium ions on the tongue: up to three seconds.

Systemic Time delays

Time required for LCD to be reset: 30 ms

Time required for Command and Write Instruction for LCD: 1.53 ms

The systemic timings must be adhered to for the proper functioning of the machine. The physical timings may however be changed to suit needs. The microcontroller can also be clocked by RC or LC oscillators. Crystal oscillators are the most stable and reliable source of clock and hence have been used.

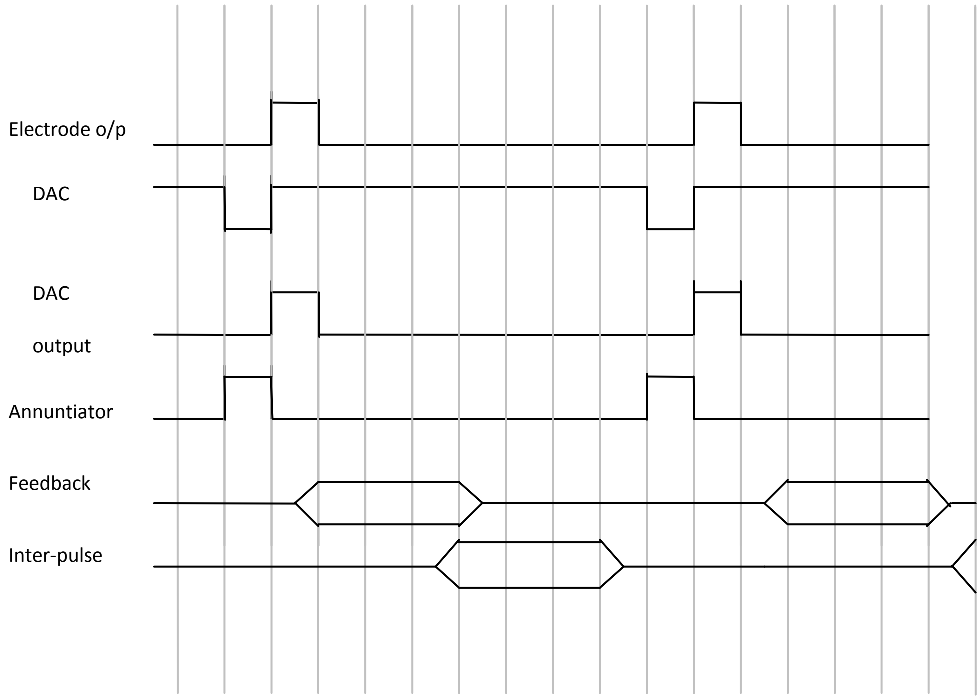

Fig 9: Timing diagram for the operation of the PIC microcontroller

4.5.7 Annuntiator

The Sussex Electrogustometer has a dual-mode annuntiator comprising an LED and a buzzer which deliver simultaneously optical and auditory warnings respectively. This is used to alert the subject before a stimulus is applied, to acknowledge a response from the subject and also to signal the end of the test.

Annuntiators play an important part in the test. If the annuntiators are too loud or bright, they might trigger false responses from the subject. Hence, the subject must be suitably trained, using the manual mode, before employing the automatic mode.

4.6 ELECTRODES & RETURN PATH

Stainless steel electrodes are used to apply the constant current stimulus to the tongue surface. Since the area of contact affects the taste threshold it is essential that the electrode tip is always in full contact with the tongue when the pulse is applied. The tongue does not always remain in the same position and can curl and twist. Hence the end of the electrode is designed to be flexible to ensure that the electrode is always in full contact with the tongue. The contact end of the electrode is circular and anodal current is applied through this. The shaft of the electrode is covered in a transparent plastic sheath to avoid any leakage of current.

The electric path is completed by connecting an electro-conductive pad on the neck. Conductive gel may be applied to enhance connectivity. The pads are similar to those used in electrocardiography.

4.7 PRINTED CIRCUIT BOARDS

The Sussex Electrogustometer has one printed circuit board (PCB) with different integrated circuits (ICs) and discrete components on it to achieve the complete functional and structural outcomes of the machine. The PCB supports the following components:

1. The PIC Microcontroller – 18f452
2. The DAC IC – AD7302
3. The OPAMP IC – 741
4. The DC-DC converter – GMA12-100PSI
5. TIP29 transistor

6. 4 MHz crystal
7. Two 15 pF ceramic capacitors
8. 1 kΩ resistor for the PIC
9. 10 kΩ control resistor
10. Ribbon cable connector for LCD module, switches and batteries.
11. A voltage regulator of 5 V for the supply to the PIC and reference voltage for DAC.

The PCB was designed manually using the Eagle software. The **E**asily **A**pplicable **G**raphical **L**ayout **E**ditor (EAGLE), version 5.6.0 for Windows was used. This software is developed and marketed by Cadsoft. The artwork was developed using this software which was then printed on transparencies to be transferred on to the PCB. The circuit board used was photo-resistant and sensitive to ultra-violet light. The artwork was placed on the PCB which was then exposed to UV light. The PCB was first cut to size and then the black protective tape was removed from the surface. The artwork was then placed on the copper side of the board. To ensure consistency in the artwork detailed inspection was done against light. The PCB was then placed with the artwork in the exposure unit for 2 – 8 minutes. The UV exposed PCB was then placed in the pre-heated developer solution tank (temperature approximately 25 – 30 °C). The developer is chemically balanced to give a consistent removal of resist. After the etching was completed the PCB was washed and left to dry. The PCB was then checked for continuity.

After this test, necessary holes were drilled using a mini laboratory hand drill. The components were then soldered onto the PCB. After successful testing of the prototype, further machine-made models were obtained with the PCBs masked. This provided a more robust, accurate and durable PCB. The PCB also houses connections for the control switches, main power supply and contrast for LCD. It also has connections for the leads for feedback and electrode output. The heat dissipation and weight distribution of individual ICs and the dc-dc converter have been carefully studied to place the components optimally on the board.

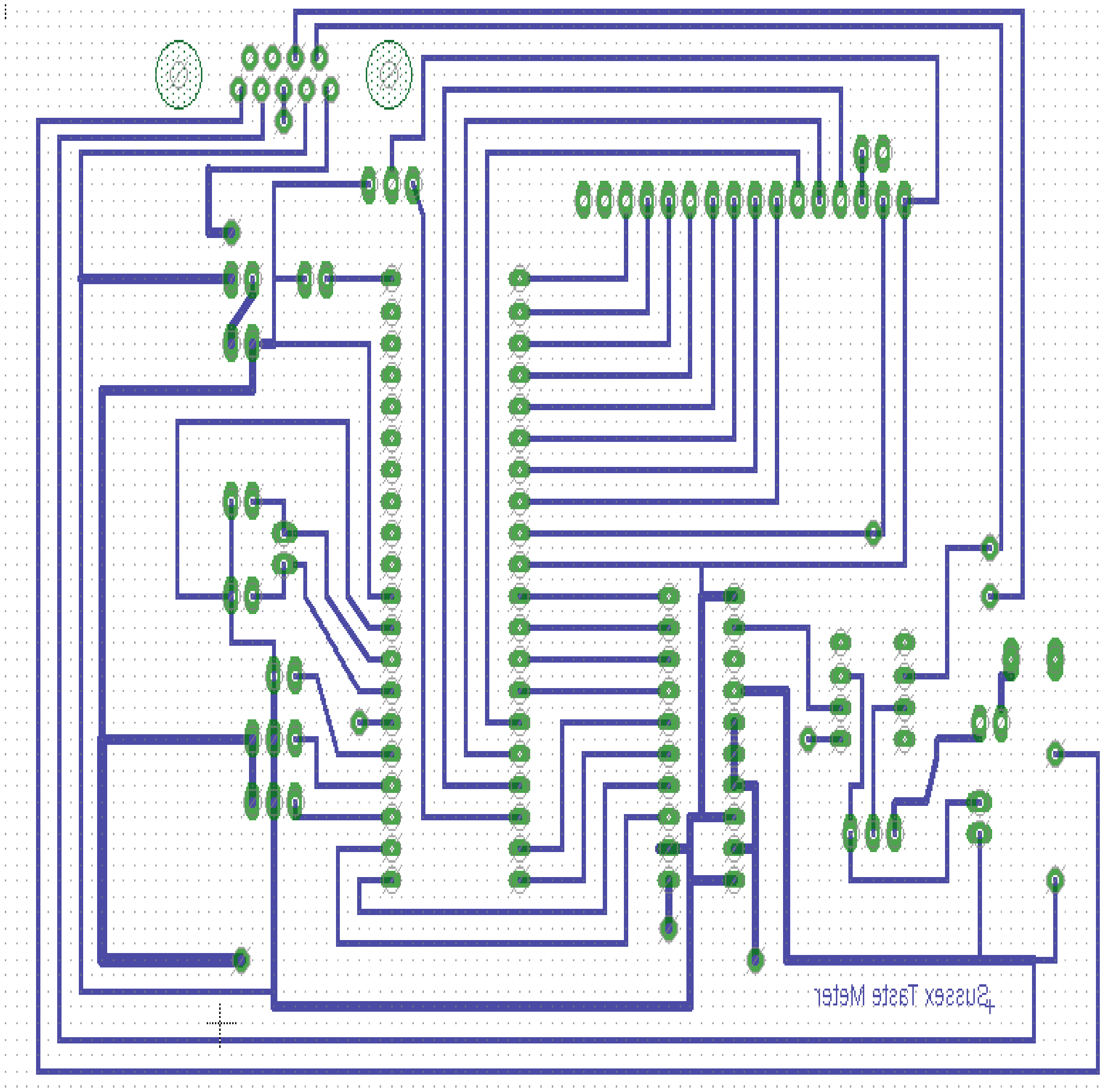

Fig 10: Artwork of the PCB for Sussex Electrogustometer

4.8 ASSEMBLY

The electronic circuits and components are housed in a simple plastic enclosure with an aluminium front panel. The front panel houses the LCD unit, start/stop switch, LED power-on indicator and the two selector switches. The potentiometer for LCD contrast and connector for the power supply are located at the rear of the enclosure. The electrode connector and feedback switch connector are located at the top. The different positioning of the various connectors on the machine has been designed to allow easy movement and flexibility to the user and subject. The weight of the instrument is approximately 430 gm. The heaviest component of the instrument is the dc-dc converter. The leads for the electrode and feedback switch are sufficiently long and flexible.

4.9 POWER SUPPLY

The Sussex Electrogustometer is completely battery powered to ensure electrical safety. The first prototype had a separate battery box which was connected to the main instrument using a DE - 9 sub-miniature connector and ribbon cable arrangement. The newer version of the instrument has the batteries inbuilt in the main enclosure. Power calculations for maximum rating have been carefully done to assess the battery life. The PIC takes a maximum of 1 W, the LCD module takes up to a maximum of 5.5 mW, the DAC takes up to 25 mW. Thus the digital circuitry takes about 1 W. One 9 V battery is used with a regulator of 5 V to provide the required voltage for the digital circuitry. The LCD unit needs a negative voltage of up to 1.5 V for its display. A potentiometer is used to alter the voltage if it is required to change the contrast. The analogue circuitry needs a positive and negative 9 V for the operation of the operational amplifier and a positive 9 V supply is also needed for the DC-DC converter.

A total of four 9 V PP3 batteries and a 1.5 V AA cell are used to power the Sussex Electrogustometer. A separate ground is maintained for the digital and analogue circuitry.

4.10 PICTURES OF THE SUSSEX ELECTROGUSTOMETER

Fig 11. Pictures of the Sussex Electrogustometer Prototype

4.11 CONCLUSION

The Sussex Electrogustometer offers an advance in automated electrogustometry and is aimed towards establishing electrogustometry as a common clinical tool. It is an ethically approved, battery powered, reliable, repeatable, robust, portable, semi-automatic electronic instrument used to measure electrogustometric taste threshold. It offers benefits of being automatic and also has a manual mode to allow complete user control on the level of current stimulus. The Sussex Electrogustometer is an automatic machine with the manual mode being used for set-up and training of the subject.

The next chapter describes how the prototype instrument was tested for reliability and repeatability.

CHAPTER 5

RELIABILITY AND REPEATABILITY OF THE SUSSEX ELECTROGUSTOMETER

5.1 INTRODUCTION

The previous chapter discussed and detailed the construction and assembly of a state of the art electrogustometer. It listed the various blocks of the Sussex Electrogustometer and elaborated the design philosophy. The previous chapter also listed the construction of the PCB and assembly of this new biomedical instrument. This chapter details work done on establishing the reliability and repeatability of the Sussex Electrogustometer.

The electrogustometer most commonly used is the RION TR06. This is a manually operated, stand alone, battery-powered device developed by Sensonics Inc [45]. The strengths of the RION TR06 include its speed, portability, simplicity – in application and interpretation, patient compliance and constant range of measurement. It is the first choice of clinicians. However, it is manually operated and hence subject to human error. With advances in electronics it is now possible to design and manufacture a semi-automated stand alone instrument for electrogustometry. Computer controlled devices have been trialled [4, 5]. However, particularly since they are not easily portable and are essentially connected to the mains power supply, the RION TR06 remains the current market standard for electrogustometry. In order to establish the reliability of the Sussex Electrogustometer the taste threshold obtained from using this is compared to those obtained using the RION TR06 for the same group of subjects. This experiment is detailed in this chapter. Furthermore, the Sussex Electrogustometer has also been tested for repeatability using an experiment reported in this chapter.

It is important to understand the reliability of taste threshold values obtained using electrogustometry. Lobb et al. reported that the reliability of the taste threshold increases with practice [51]. This implies that the manual mode of the Sussex Electrogustometer should be effectively used to train the subject so that a reliable taste threshold is obtained. Stillman et al. reported that despite the limitations of not being able to study the different types of taste, electrogustometry provides a reliable threshold [35]. Hence while conducting the experiments detailed in the following sections, the subject had been suitably trained using the manual mode of the Sussex Electrogustometer.

5.2 ASSESMENT

Two experiments were carried out to assess the reliability and repeatability of the Sussex Electrogustometer.

5.2.1 MATERIALS & METHOD

Subjects

Twenty healthy subjects were recruited from the students and staff at the University of Sussex. Nine of them were male and eleven were female of age range of 22 to 70 years, their mean age being 36.2 years.

Test Equipment

The Sussex Electrogustometer and the RION TR06 provided the electric stimuli.

Procedure

The taste thresholds of the 20 subjects were measured using the Sussex Electrogustometer, operating in its automatic mode, and the RION TR06 respectively. A circular stainless steel electrode of 28.5 mm^2 area was used in both the tests and was placed at 1.5 cm posterior to the tongue tip and 1.5 cm from the left margin of the tongue. The stimuli were applied for two seconds. The subjects were initially briefed about the instruments. The manual mode of the Sussex Electrogustometer was used to train them. After two weeks, the same set of subjects was tested again using the Sussex Electrogustometer.

5.2.2 RESULTS

The taste threshold results were compared (Fig 12) and a high degree of correlation ($r = 0.91$) between the Sussex Electrogustometer and the RION TR06 was observed. A high degree of correlation ($r = 0.94$) between the test-retest data of the Sussex Electrogustometer (Fig 13) was also observed.

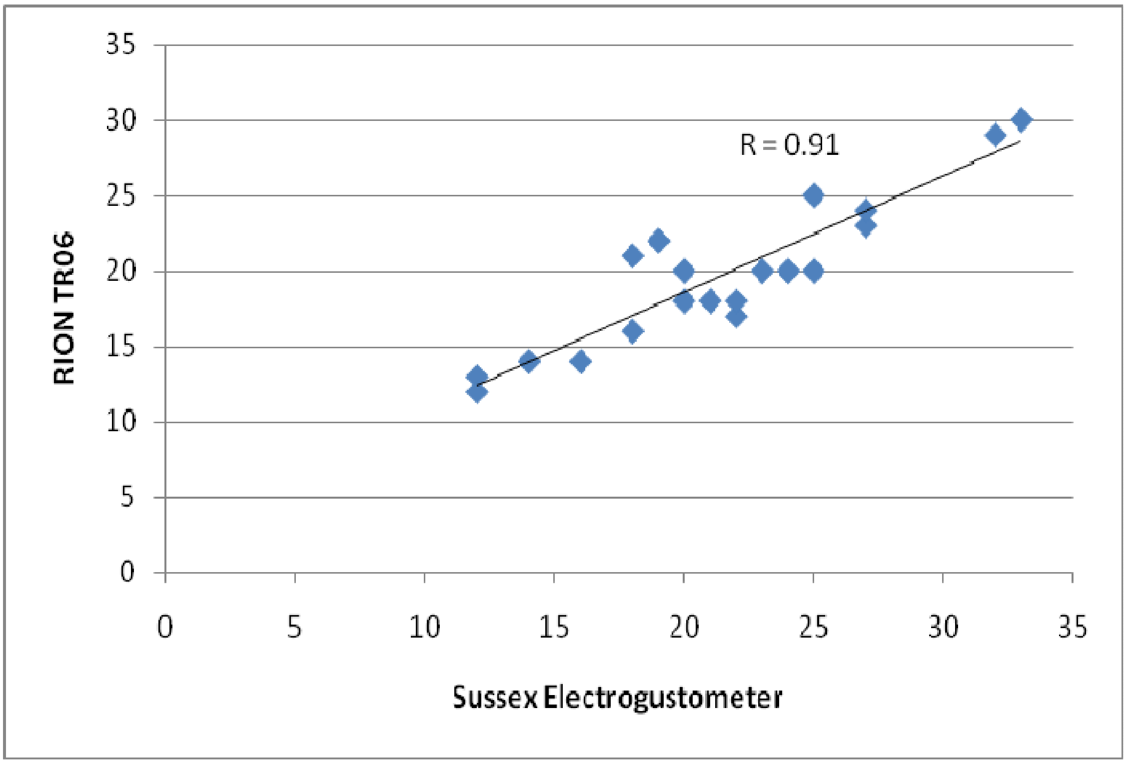

Fig 12: Reliability of the Sussex Electrogustometer

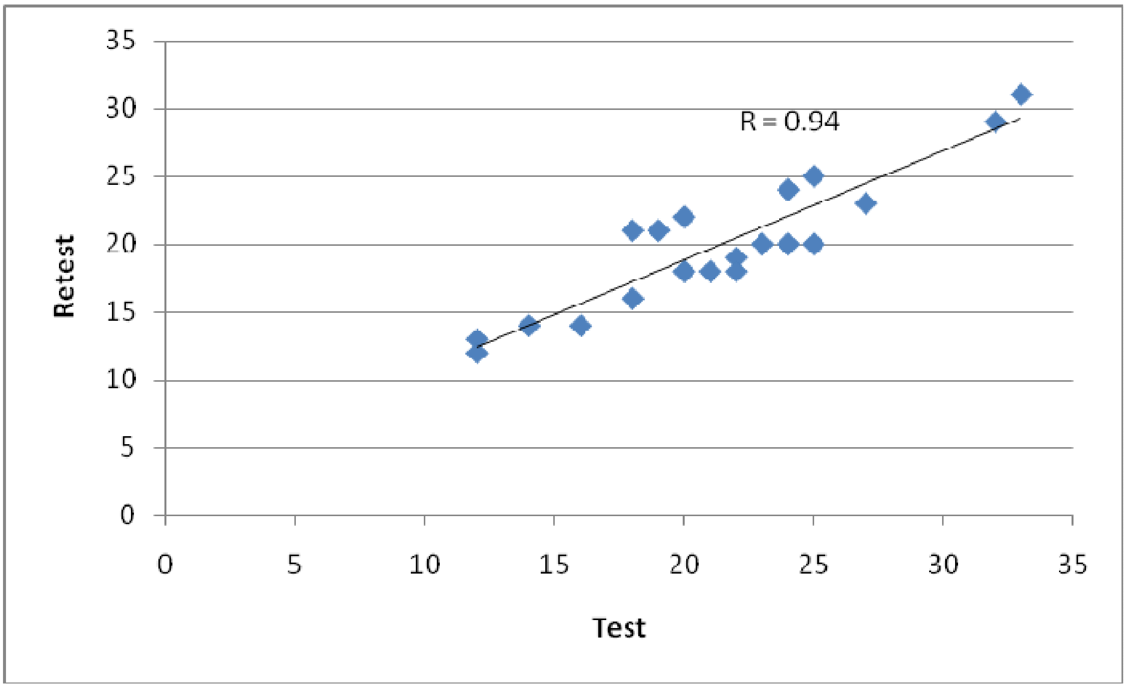

Fig 13: Repeatability of the Sussex Electrogustometer

5.2.3 DISCUSSION

The taste threshold data of the 20 subjects using the RION TR06 and the Sussex Electrogustometer show a high degree of correlation. The reliability of the RION TR06 has been extensively studied [52]. The high correlation hence establishes the reliability of the Sussex Electrogustometer. The test-retest data also show a high degree of correlation implying the repeatability of the Sussex Electrogustometer.

The Sussex Electrogustometer is the first semi-automated, battery-operated, stand alone electrogustometer. It is a microcontroller based device, with an inbuilt false test, operating in two modes. The test times are short: the machine arrives at the taste threshold after a few stimuli depending on the subject's response, using a pre-programmed double-staircase algorithm. The taste threshold also depends on factors such as stimulus duration and electrode area. The next chapter recommends a test procedure which will maximize the accuracy and reliability of electrogustometry.

5.3 CONCLUSION

The work done elaborated in this chapter confirms the reliability and repeatability of the Sussex Electrogustometer. This is essential for any biomedical instrument to be used in a clinical setting. With the high degrees of reliability, repeatability and electrical advances the Sussex Electrogustometer can be used as a new state of the art electrogustometer.

The next chapter discusses the effect of stimulus duration and electrode area on electrogustometric threshold.

CHAPTER 6

EFFECT OF STIMULUS DURATION AND ELECTRODE AREA ON TASTE THRESHOLD

6.1 INTRODUCTION

In the previous chapter we discussed the testing of a new state of the art electrogustometer called the Sussex Electrogustometer for reliability and repeatability. The block diagram of this machine was discussed and the individual blocks were studied at length in a previous chapter. The chapter also discussed the psychophysical considerations taken into account while designing the instrument. This chapter will deal with the study of physical constraints affecting the electrogustometric threshold. According to the literature various sizes of electrode have been used and there has been great variation in the stimulus duration. Recovery time for subjects has also not been standardised. For electrogustometry to become a common clinical tool a robust understanding of the physical constraints is necessary. This chapter details the physical factors affecting taste threshold.

In spite of electrogustometry having been in existence since the 1930s, there is no standard method to measure taste threshold. [34] Factors like stimulus duration and electrode area affect the subject's response and hence a control over the modality in which the stimulus is applied is important. A standard method of conducting the electrogustometric measurements will imply that results can be meaningfully compared.

This will also lead to the formation of a global database of electrogustometric threshold which would be a source for various statistical findings. Gender based, age based, race based standardisation of taste threshold can thereby be established.

6.2 EFFECT OF STIMULUS DURATION

Electrogustometric taste threshold depends on the quantified taste function of which it is a measure. However, physical constraints also affect the results of this subjective test. The main physical factors on which taste threshold depends are the duration for which the current stimulus is applied and the size of the electrode used. Hence spatial and temporal control of the stimulus is of prime importance to ensure standardisation of the examination. [47] To help standardise electrogustometry, an understanding of the effects of stimulation duration and electrode area on electrogustometric taste threshold is important. Bujas studied the effect of stimulus duration on taste threshold for one subject and concluded that it reached an asymptote at 1.0 s. [34] Fons and Osterhammel observed with three subjects that the taste threshold decreased with a pulse duration in the range of 2 to 150 ms and remained constant after that. [53] Stillman et al. commented that the taste threshold was slightly higher for 0.75 s pulse duration than 0.5 s. Nine subjects were involved in this study. [4] Loucks & Doty used the RION TR-06 to establish the taste threshold of twelve male and twelve female subjects with stimulus duration of 0.5 s, 1.0 s and 1.5 s, and found a minimum value at 1.0 second. The trend observed by them was inexplicably non-monotonic. [46] A further experiment using the Halle II, a computer controlled electrogustometer, showed that taste threshold remained unchanged with stimulus duration in the range of less than 0.75 s and greater than 2.0 s and decreased in the region between them. [45] An in-depth study is needed to establish the exact relationship between stimulus duration and taste threshold since none of these studies has produced a model to explain the results and some results are contradictory.

The sour metallic taste perceived in taste measurement using an electrogustometer is attributed to the liberation of protons or hydronium ions. [4] For a constant electrode size, the number of protons liberated will depend on the intensity of the pulse and the duration for which the current is applied. Thus, establishing the relationship between stimulus duration and taste threshold is essential to determine standardized testing parameters. Increased pulse duration would imply an increased liberation of protons on the oral mucosa thus increasing the intensity of the stimulus. However, this is not the case throughout the stimulus duration spectrum. After a certain value of pulse duration, its effect on taste threshold saturates as noted in some studies mentioned previously. This implies that the protons have a limited lifetime before they revert to being hydrogen. [41]

The available electrogustometers had a limitation on the time duration for which the stimulus could be applied. In the RION TR06, fixed values of 0.5, 1.0, 1.5, 2.0 and 2.5 seconds are available. Hence the observations made by these machines limited the analysis of the effect of stimulus duration. The RION TR06 also has a manual option for stimulus duration but this is not very reliable as it is subject to human error. The Sussex Electrogustometer provides a more elaborate range of choices for stimulus duration starting from 0.5 seconds up to 2.5 seconds at 0.25 seconds intervals. This allows a more refined analysis of the effect of stimulus duration on taste threshold. This range can also be easily altered by re-programming the machine.

6.3 EFFECT OF ELECTRODE AREA

The area of the circular electrode is also a contributing factor to the electrogustometric taste threshold. When a current stimulus is applied using an electrode, the effective area of the tongue on which the stimulus acts is slightly larger than the actual electrode size. [53] If the electrode area is too small, somatosensory responses are evoked along with gustatory response and hence it has been recommended that electrodes with very small areas should not be used. [54] The process for determining taste threshold involves application of stimuli both higher and lower than this threshold. Thus it is important to understand whether the gustatory response evoking factor is current intensity or current density. This can be determined by studying the effect of electrode area. Adjukovic concluded that gustatory response tends to increase with stimulation area for a fixed current intensity. This was, however, not noted for very small electrode areas. Thus he suggested that larger electrode areas are better. [37] Adjukovic, in another experiment, concluded that there is a power function relationship given by $I = 54.4\ A^{0.267}$, where I is the current intensity (µA) and A is the electrode area (mm^2). [38]

It has been commented that current density and not intensity affects taste threshold. [38] The spatial distribution of the tastant – the hydronium ions produced – causes gustatory sensation, which is recorded, and a threshold for the same is determined by the machine. The electrode surface in contact with the tongue is circular. Edged shapes, like a square, may cause polarisation of charges towards them, which will affect the uniformity in distribution of the stimuli. It is hence essential to have a uniform distribution of charged hydrogen ions over the oral mucosa. In electrogustometry taste is perceived by the absorption of positively charged hydronium ions by the oral mucosa. This is done by the ion-gated channels. Hence the distribution of these charged ions must be uniform in the area where this is applied. The lack of spatial uniformity may cause change in the signal detection strategy. The areas with different concentrations of stimuli will be perceived as separate stimuli. This will invoke a different signal detection strategy. Edged surfaces may also evoke somatosensory responses of touch, which may be mis-interpreted as a gustatory response. Hence it is important to have a circular electrode surface for electrogustometry.

The material of the electrode is also a determining factor for taste threshold. The conductivity of various materials differs and this may affect the actual current being applied to the tongue. The current will only vary from its set value when the total load resistance of the subject and electrode exceeds 200 kΩ. Stainless steel electrodes have been used in the Sussex Electrogustometer. Similar electrodes have also been used in the RION TR06. Stainless steel is a steel alloy with a minimum of 11% chromium. It does not stain, corrode or rust easily. It is also known as corrosion-resistant steel. This material is non-magnetic due to its crystalline structure. It is hard and not very brittle and can be produced in different shapes. The stainless steel electrode used in the Sussex Electrogustometer is specially designed to ensure that its circular front end completely touches the oral mucosa to ensure even current distribution.

6.4 EXPERIMENTS

In order to understand the effect of stimulus duration and electrode area on electrogustometric taste threshold, two experiments were conducted.

6.4.1 Material and Method

Twenty healthy subjects were recruited from the students at the University of Sussex. Nine of them were male and eleven were female of age range of 22 to 40, their mean age being 28.4. A brief medical history of the subjects was recorded prior to the test. No significant medical conditions were noted in any of the subjects which might suggest an abnormal electrogustometric taste threshold. A few subjects were mild consumers of alcohol and tobacco.

Test Equipment

The electric stimuli were produced by the Sussex Electrogustometer operating in the automatic mode.

Procedure

To determine the effect of stimulus duration on taste threshold: The hand-held stainless steel electrode of area 12.5 mm^2 was placed at 1.5 cm posterior to the tongue tip and 1.5 cm from the left margin of the tongue. The electrogustometric taste thresholds were measured for pulse durations of 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 and 2.5 seconds. The subjects had abstained from food or drink an hour before the test. Subjects were asked to repeat the test should there have been more than two false positive responses. The tests were repeated with electrodes of different sizes - 28.5 and 50 mm^2.

To determine the effect of electrode area on taste threshold: Taste threshold was measured using stainless steel electrodes of six sizes - 3.14, 12.5, 28.5, 50, 78.5 and 113 mm^2. The electrodes were positioned as described in the above section. The stimulus duration for this experiment was kept constant at two seconds.

6.4.2 RESULTS

Effect of stimulus duration on taste threshold:

Figure 14 shows the graph of the mean taste threshold of the 20 subjects with respect to stimulus duration. It shows very little variation in the range of 0.5 to 1.0 seconds. There is a monotonic decrease for durations of 1.0 to 2.0 seconds and no significant change in the range of 2.0 to 2.5 seconds. Similar results were obtained for the three different electrode areas.

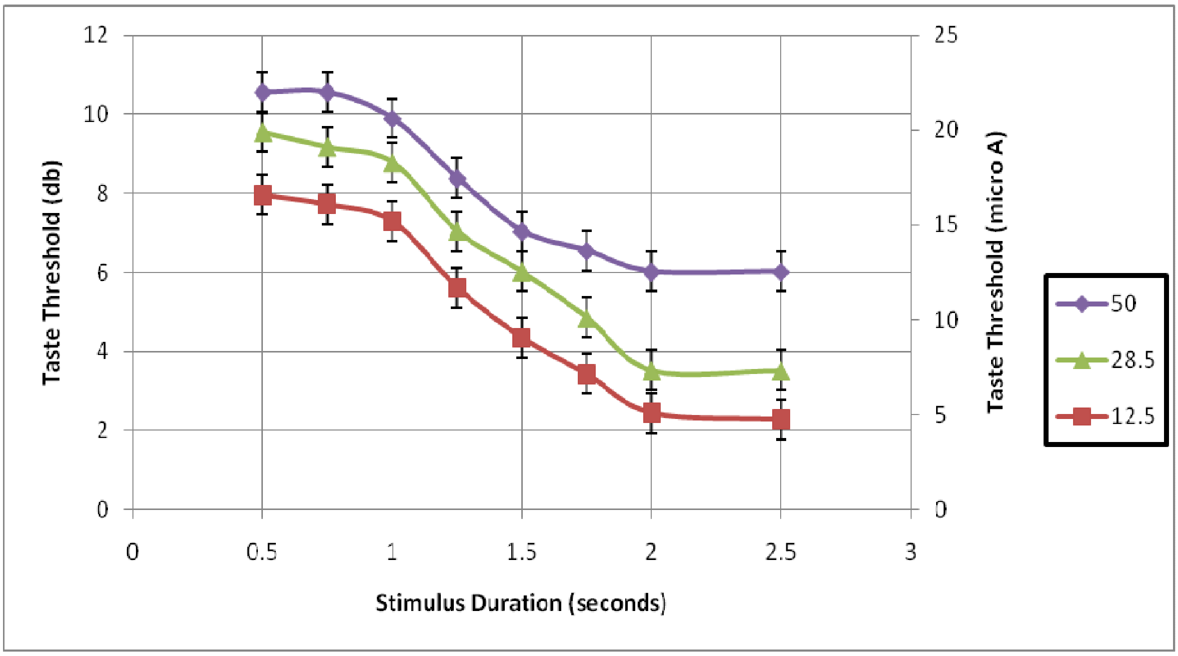

Fig 14: Effect of stimulus duration on taste threshold

Effect of Electrode Area on taste threshold:

Analysis of the mean taste threshold for the 20 subjects showed a generally linear increase with electrode radius as illustrated in Figure 15. The slight deviation for small electrode radius is probably due to somatosensory effects.

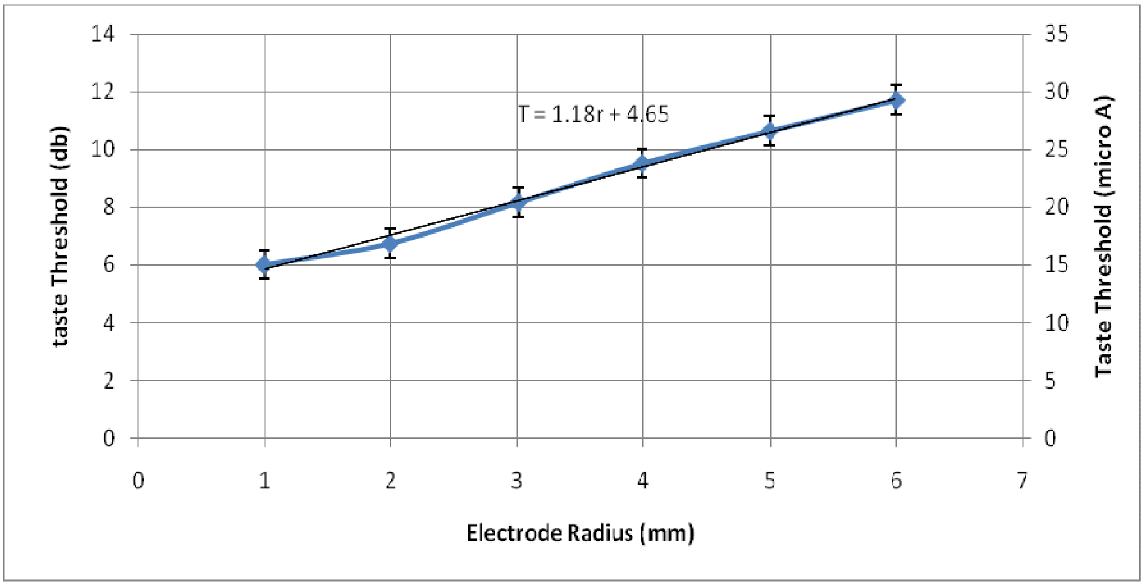

Fig 15: Effect of electrode radius on taste threshold

6.4.3 DISCUSSION

From the test results it may be concluded that the effect of stimulus duration on electrogustometric taste threshold is minimal for duration of up to one second. Both somatosensory and gustatory responses are evoked in this region. The additive effect of the two evoked responses constitute the overall subject response. With increase in stimulus duration from 0.5 s to 1.0 s, the gustatory response increases and the somatosensory response decreases and hence the total response remains almost the same. The somatosensory response may be attributed to the larger currents required for smaller stimulus durations. [38]

The taste threshold decreases linearly when the stimulus duration is greater than one second and less than two seconds. This decrease in threshold is due to the increased liberation of protons making the stimulus stronger. The somatosensory response is greatly diminished during this range of stimulus duration. This corresponds to the response observed by Marian in her experiment using the Halle II. [46]

The electrogustometric taste threshold for stimulus duration in the range of 2.0 to 2.5 seconds shows little variation. The current stimuli produce protons which evoke gustatory responses. These protons, however, have a finite lifetime after which they revert to being hydrogen. If the stimulus duration is greater than two seconds the lifetime of these protons is exceeded so their density tends to be constant. [38] Thus, the most suitable stimulus duration for electrogustometry is at least 2.0 seconds. When electrodes of different sizes were used, a similar trend was observed. This establishes that stimulus duration affects taste threshold independently of electrode area.

Electrogustometric taste function also depends on the size of electrode used to apply the current stimulus. Adjukovic commented that taste threshold depends on current density. [38] The current study has shown that taste threshold depends on electrode radius in a linear manner according to the equation, for r greater than 2 mm,

$$T = 1.18\, r + 4.65$$

where ‘T’ is the electrogustometric taste threshold and ‘r’ is the electrode radius. Thus the taste threshold depends on current density. For smaller electrodes, the somatosensory effects are more pronounced. An electrode size of 3 mm radius or 28.5 mm^2 area is recommended as a standard as smaller electrodes evoke somatosensory response whereas larger electrodes will lack precision of position.

6.5 CONCLUSION

This study has shown that taste threshold decreases with stimulus duration in the interval of 1.0 s to 2.0 s and remains relatively unaffected if the pulse duration is greater than 2.0 s and it is thus recommended that the stimulus duration to be used in electrogustometry should be at least 2.0 s. The study has also shown that taste threshold increases linearly with respect to electrode radius. When the electrode area is very small it evokes somatosensory response along with gustometric response. A large electrode area will require greater current levels and result in positional imprecision. As an overall compromise an electrode of 3 mm radius or 28.5 mm^2 area is thus recommended for use in electrogustometry.

The next chapter details the effect of alcohol on electrogustometric taste threshold. Alcohol consumption before food as an appetizer is quite common. It has been commented that alcohol consumption affects the smell and taste functions [55, 56, 57] Many studies have shown how the quality of taste deteriorates in alcoholics. [58] We have been unable to locate a study where electrogustometric taste threshold for normal subjects (non-alcoholics) has been studied. In the next chapter an elaborate study to understand the effect of alcohol on taste threshold has been detailed. The literature suggests that both electrogustometric and chemogustometric taste thresholds change in line with each other under the influence of alcohol. [59] Hence this study for non-alcoholics has been limited to electrogustometry. Ethical approval was obtained for this study as detailed in the Appendix 5 and the tests were carried out in a secure environment. A further study, involving the application of oral anaesthetics to non-alcoholics, was carried out to understand the modality in which alcohol affects taste threshold.

CHAPTER 7

THE EFFECT OF ALCOHOL ON TASTE THRESHOLD

7.1 INTRODUCTION

Following the construction of the Sussex Electrogustometer, it was tested successfully for reliability and repeatability. However, while performing the re-test of one of the subjects a significant difference in electrogustometric taste threshold was observed. Upon further investigation of this anomaly, it was concluded that this might have been caused by the consumption of alcohol by the subject before the re-test. A pilot study was conducted to observe the immediate effect of alcohol on electrogustometric threshold. This suggested that alcohol did indeed cause a change. A more elaborate study was then designed in collaboration with a psychologist and a neurologist to understand this effect.

The literature reports that alcohol affects the gustatory and olfactory response. In alcoholics these sensory perceptions are significantly diminished. [59] However, the immediate effect of alcohol in normal subjects has not been studied at length. This chapter deals with the understanding of the immediate effect of alcohol on electrogustometric threshold for a normal, non-alcoholic person.

Measurement of taste threshold is done by chemogustometry or electrogustometry; both of which are essentially subjective. [60] Alcohol may affect the responsivity of the subject and hence psychophysical factors must be carefully analysed and taken into account while determining taste thresholds after consumption of alcohol. [55] Wrobel et al. commented that alcohol affects the neurotransmitter-gated ion channels on the tongue surface which in turn affect the taste threshold. [58] No genetic effects of poor taste or smell functions have been observed [57]. Experiments by Lelievre et al. concluded that alcohol affects chemogustometric and electrogustometric thresholds in a similar way. [59]

Alcohol tastes sweet at lower strengths. As the concentration of alcohol increases, the taste changes to bitter and at higher concentrations a burning sensation is perceived. It has been noted that continued alcohol consumption affects the chemogustometric taste threshold for bitter solutions. [58] Deterioration in taste discrimination has also been observed in alcoholics.

Wrobel et al. conducted a study to assess the effects of acute and chronic exposure of alcohol on the taste response to Mono-sodium glutamate (MSG). The study again suggested that electrogustometric and chemogustometric taste threshold is significantly altered by continued alcohol consumption. [58] A similar study was done to assess the relationship between taste response to sweet solution and alcohol consumption by Wronski et al. The study involved alcoholics with and without parental alcoholic history and non-alcoholics. It was concluded that alcoholics with parental alcoholic history are more likely to have a greater affinity to sucrose. [62] Apart from these studies on different types of taste using chemogustometry, studies have also been done to assess the effect on taste threshold using electrogustometry. In a study carried out by Lelievre et al, 42 healthy subjects were randomly selected and their taste functions were assessed using electrogustometry and chemogustometry. Similar deterioration in taste was noted in alcoholics as compared to non-alcoholics using both these methods. A similar trend was observed for smokers. [59]

The aim of the study reported in this chapter is to determine the immediate effect of alcohol on electrogustometric threshold in a normal, non-alcoholic subject with no parental alcoholic history. In order to correlate the taste threshold to breath alcohol concentration a new function, called taste coefficient, has been defined. This is the reciprocal of the electrogustometric taste threshold in decibels.

Taste Coefficient = 1/Taste threshold

7.2 PHYSIOLOGY OF ALCOHOL DIGESTION

Alcohol affects the neural response of subjects. It has been observed by Tapert et al. that alcohol stimuli can cause atypical physiological, cognitive and neural response. [63] Up to 20% of the alcohol is directly absorbed by the walls of an empty stomach and reaches the brain via the blood stream. In the digestive tract alcohol is broken down by alcohol dehydrogenase enzyme. Women produce a far less amount of this enzyme and hence they are more likely to be affected by alcohol quickly. [16] The liver can only produce a fixed amount of this enzyme. The excess alcohol flows in the blood stream affecting the central nervous system including the taste processing centres. [64] However, the effect is not immediate. In spite of alcohol being present in the blood stream soon after its consumption it takes about 10-15 minutes before it can affect the brain significantly enough to alter the perception of taste.

7.3 PILOT STUDY

The repeatability experiment conducted using the Sussex Electrogustometer involved measuring electrogustometric threshold of subjects at two week intervals. During the retest of one of the subjects a significant difference in threshold was observed. This was thought to be due to consumption of alcohol before the test. Upon this observation a small pilot study was conducted to establish this cause.

Three students from the biomedical laboratory at the University of Sussex participated in this pilot study. Their electrogustometric threshold was measured before consumption of alcohol and after every fifteen minutes of the drink. 25 ml shots of Scotch whisky (Highland Park) were used. No food or drink was consumed by the participants for up to two hours before this test.

The results observed for the three participants are shown in the graph below.

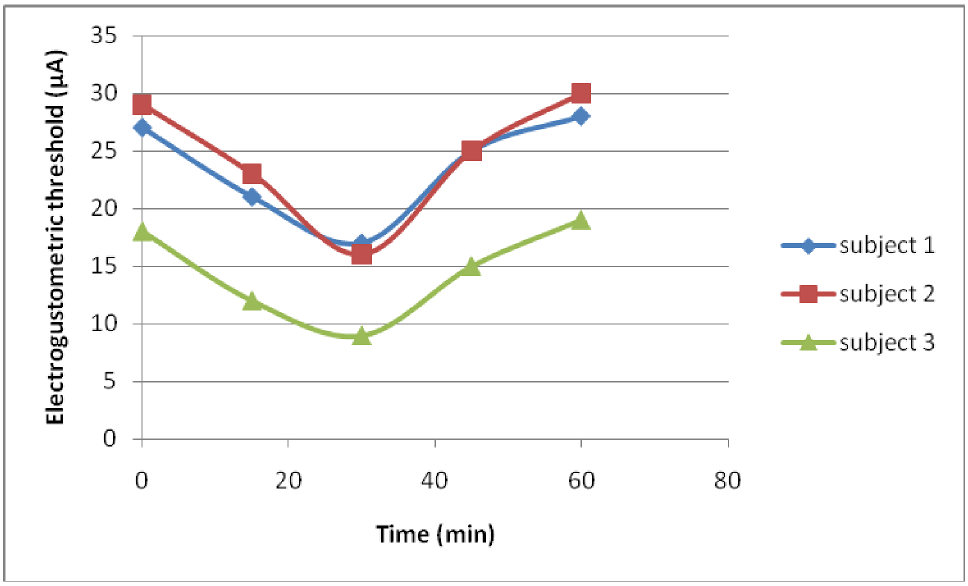

Fig 16: Pilot study to understand the effect of alcohol on electrogustometric threshold

All three subjects showed similar improvement of electrogustometric threshold which proved that the result observed for the subject during the repeatability experiment was not a singular event. This justified the need for a more elaborate and organised study into the understanding the immediete effect of alcohol on electrogustometric threshold. The literature suggests extensive work on understanding the effect of alcohol on taste for alcholics. This study would hence prove pioneering in this field. Collaborations were made with a psychologist and a neurologist to design an in-depth study and understand its findings.

7.4 EXPERIMENTS

7.4.1 Material and Method

Subjects: 16 healthy, normal (electrogustometric taste threshold less than 40 μA), non-alcoholic (up to 3-4 units of alcohol a week) university students with no parental alcoholic history were chosen for the study. On pre-experiment screening the subjects showed good bilateral symmetry of taste thresholds and their medical records did not suggest any apparent reason for loss of taste.

The study was carried out in accordance with the ethical approval given by the University of Sussex, School of Life Sciences Research Governance Committee. Each participant read and signed an informed consent form after the study procedure had been fully explained. (Attached in the Appendix 5)

Procedure

To understand the immediate effect of alcohol on electrogustometric threshold different tests were carried out. The participants were asked not to eat or drink anything one hour before any of these tests. All the tests were carried out in the monitored area of the

Experimental Psychology Laboratory at the University of Sussex in accordance to the recommendations of the ethics committee.

In the first experiment, the 16 subjects were asked to drink an alcohol-water mixture. The drink was prepared with the same amount of alcohol in varying concentrations diluted by distilled deionised water. The alcohol used was “Ethanol 90” and the concentration varied from 10% to 50% by volume in steps of ten. The electrogustometric taste thresholds and breath alcohol levels were noted just before the consumption of alcohol and at the 10^{th}, 20^{th}, 30^{th}, 40^{th} and 60^{th} minute thereafter. Each participant was asked to keep the drink on the oral mucosa for up to 20 seconds before consuming it. The drink was served in a clean disposable cup. This experiment was aimed at understanding the overall effect of alcohol on electrogustometric threshold and is referred later to as the alcohol ‘swallow’ experiment. The swallow experiment was repeated for different amounts of alcohol. Amounts of alcohol used in this experiment were 4 mg, 6 mg and 8 mg.

The second experiment involved the same participants being made subject to the same alcohol solutions. In this study, however, the participants were asked only to rinse their mouth with the test solution and not consume it. The electrogustometric taste thresholds were noted at similar time intervals as described in the swallow experiment. The drink was kept in the oral cavity for up to 20 seconds. This test was designed to analyse the local effect of alcohol on electrogustometric threshold and is also referred to later as the alcohol ‘rinse’ experiment.

The third experiment involved eight participants from the group. They were given the same alcohol-water solution through a tube that bypassed the oral mucosa. Their taste thresholds were noted before and after the application of the test solution at similar time intervals as previously mentioned. This test was designed to analyse the peripheral effect of alcohol on taste and is also referred later to as the alcohol 'bypass' experiment. In order to avoid the gag reflex the oral mucosa was anaesthetised. This test was done in an NHS clinic in Reading.

A further experiment was carried out with eight participants in the group to understand the effect of anaesthetics on taste threshold and compare the results with the effect of alcohol on taste. An oral anaesthetic, Covonia, was sprayed on the tongue and taste threshold was noted before the application of the spray and measured after five minutes and then at the 10^{th}, 20^{th}, 30^{th}, 45^{th} and 60^{th} minute thereafter.

In each of these experiments the Breath Alcohol Level (BAC) was measured each time the electrogustometric taste threshold was measured. The Sussex Electrogustometer was used in the automatic mode to assess the electrogustometric taste threshold.

7.4.2 RESULTS

Data were collected for the three sets of experiments with alcohol and the experiment with the anaesthetic spray and the following graphs were plotted. The taste coefficient (which is the reciprocal of taste threshold) is plotted against time. This measure has been employed to allow an easier comparison with Breath Alcohol Level since the respective graphs now have a very similar shape.

A) **Swallow experiment**

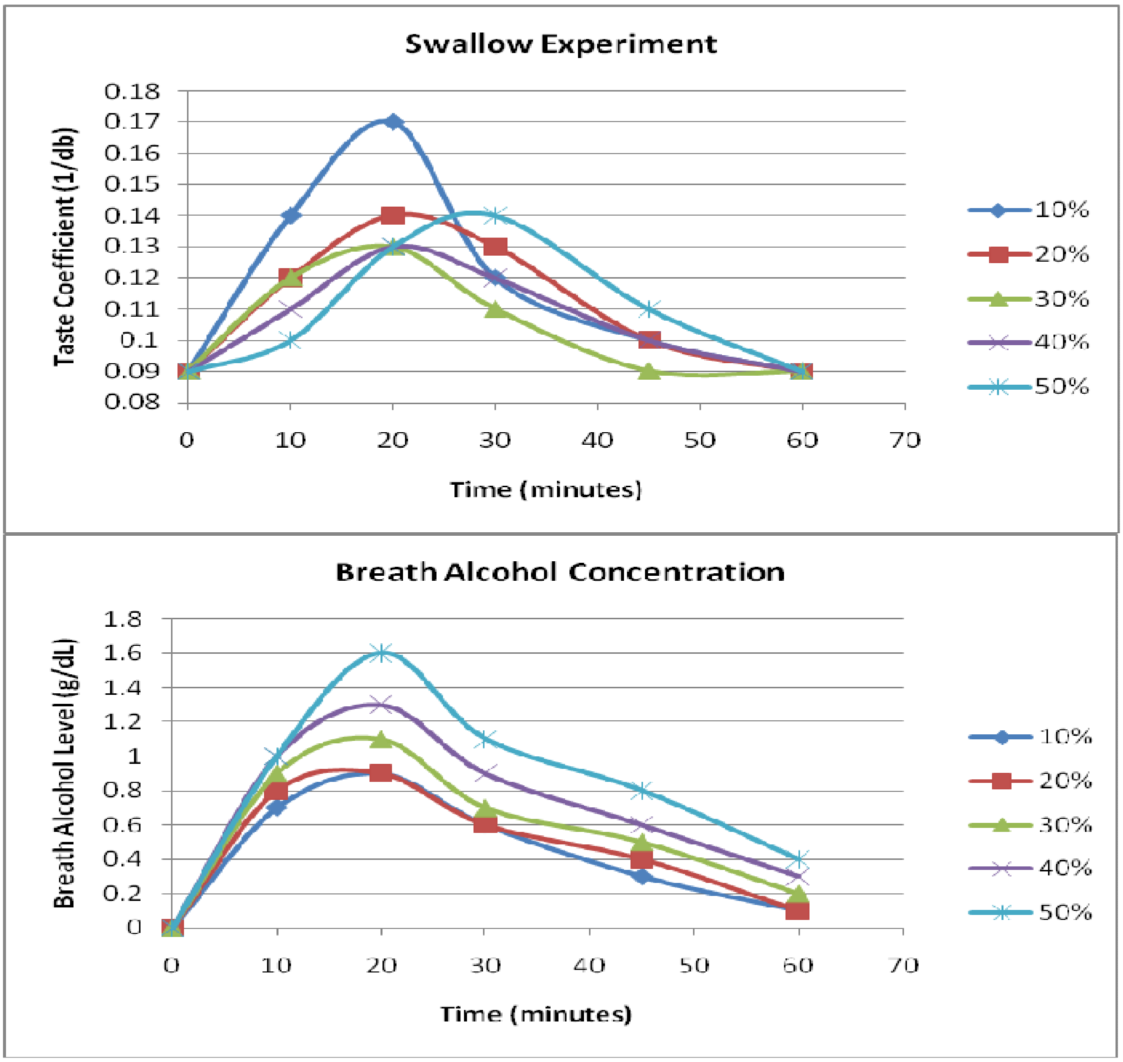

Fig 17: Swallow experiment result

The alcohol swallow experiment results, shown in the graph above, shows that consumption of alcohol affects taste function. The mixture with lower alcohol concentration affects taste to a greater extent as compared to the mixture with higher concentration. Also it can be inferred from the graph that the mixture with a higher alcohol concentration affects the taste function later than lower alcohol concentration mixture. The taste function reverts to its normal value within 30-45 minutes depending on the concentration of alcohol.

B) Rinse experiment

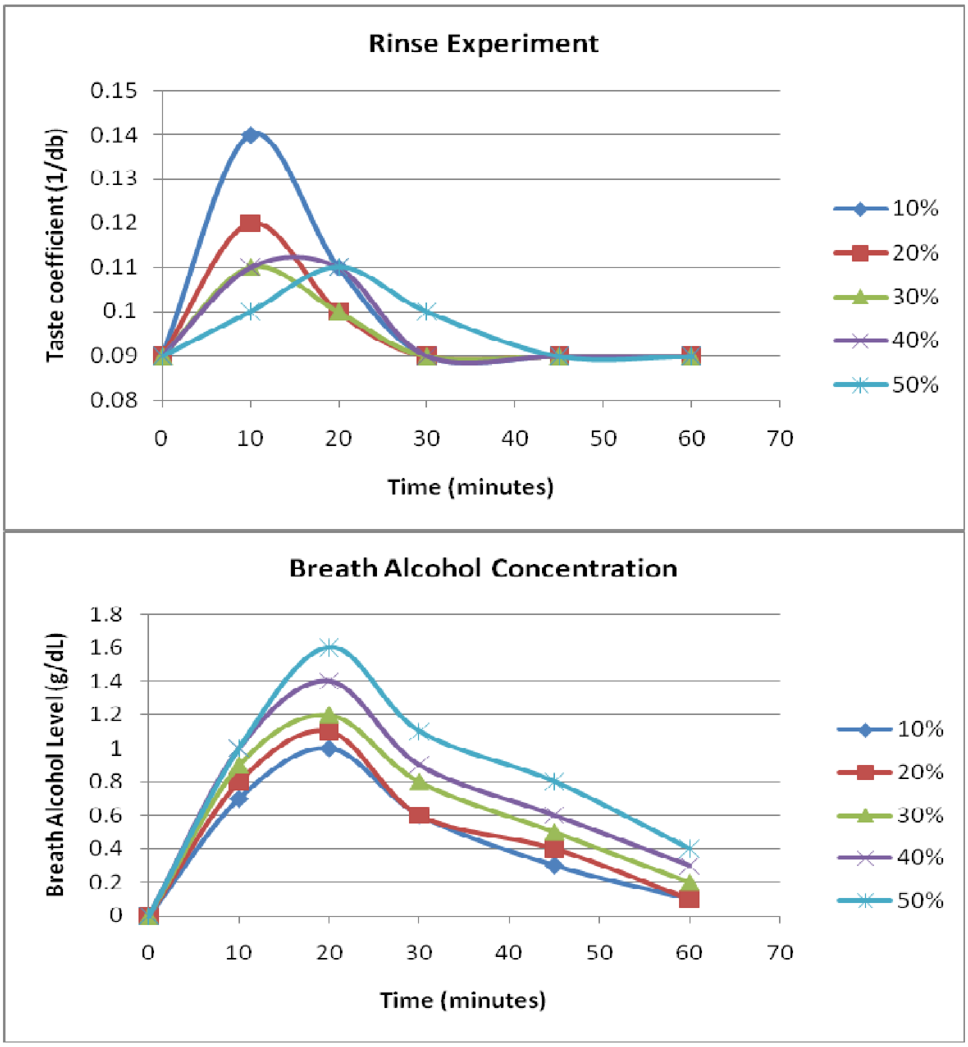

Fig 18: Rinse experiment result

The results of the rinse experiment detailed in the graph above shows that the taste function improves within 10-20 minutes of application of the alcohol-water mixture. The mixture with lower alcohol concentration improves the taste function to the greatest extent. The mixture with 50% alcohol concentration affects the taste function after about 20-25 minutes as compared to the 10% alcohol mixture which affects taste function within 10 minutes. The taste function reverts to its normal value within 30 minutes.

C) Bypass experiment

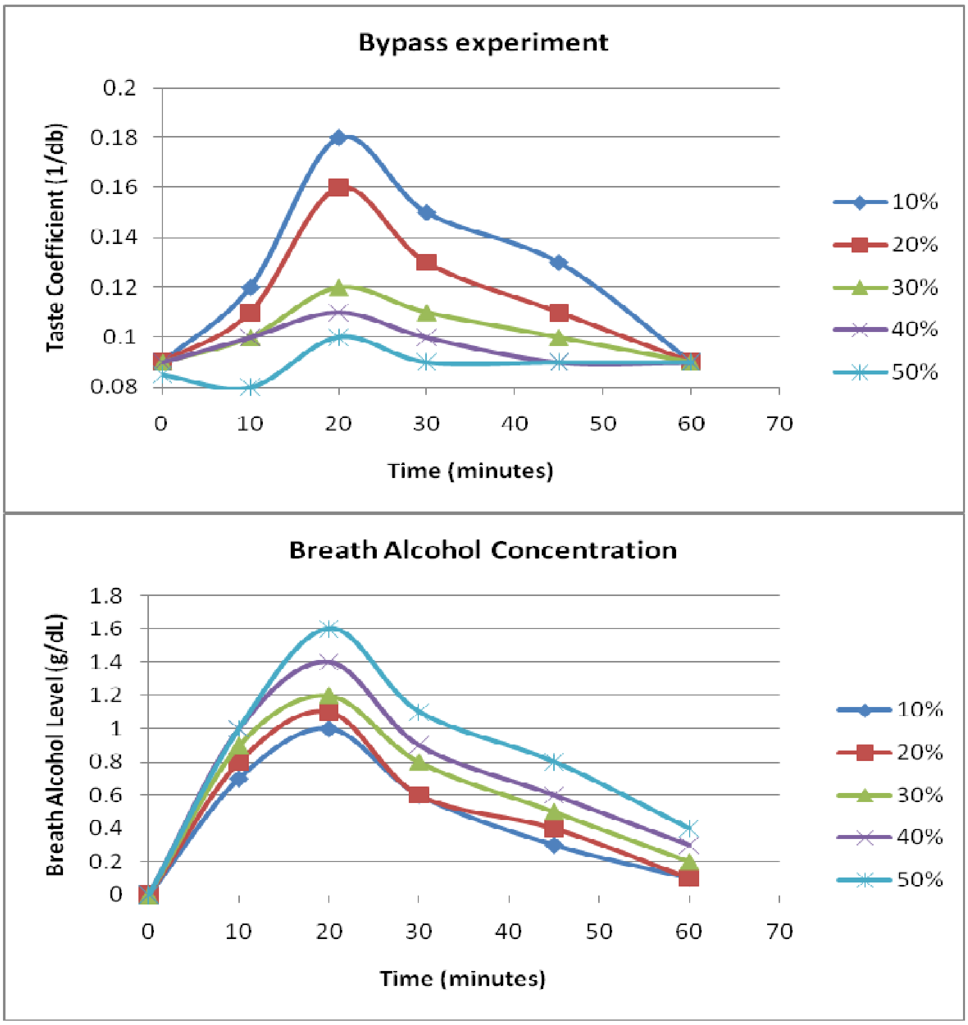

Fig 19: Bypass experiment result

The alcohol bypass experiment, desinged to understand the non-local effect of alcohol, shows that the taste function is affected significantly by the 50% alcohol mixture. The lower the concentration of alcohol, the less is the peripheral effect of alcohol on taste function. This effect is observed 20 minutes after the application of the alcohol – water mixture. The taste function reverts to its normal value within an hour for higher concentrations of alcohol (40% and 50%) and within 30 minutes for the lower concentrations (10%, 20% and 30%).

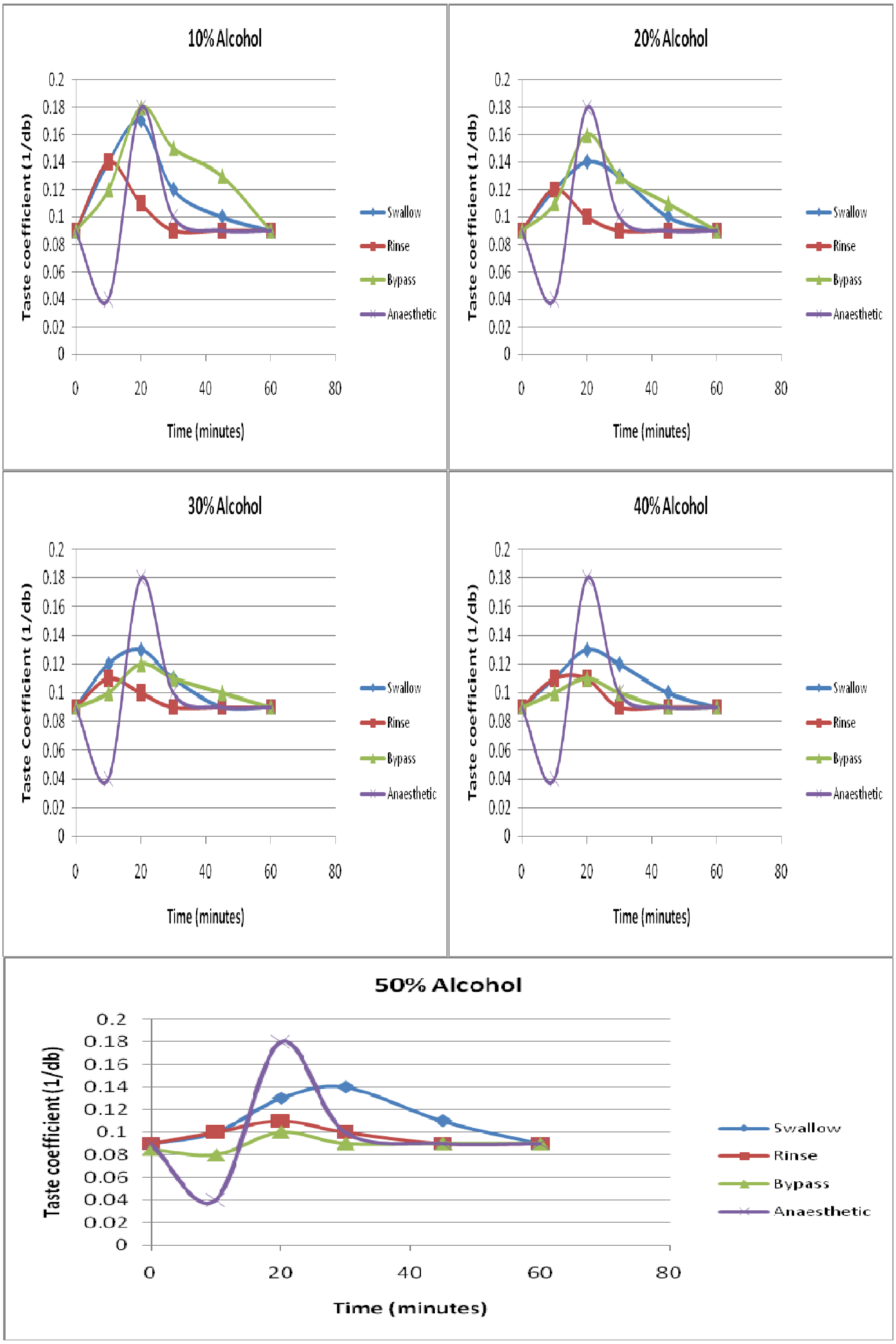

Fig 20: Effect of different concentrations of alcohol on taste threshold

The above set of graphs shows how different concentrations of alcohol affect the taste function. In the swallow experiment, the effect of the 10% alcohol mixture is the most prominent, whereas, in the bypass experiment, the 50% alcohol mixture's effect was notably higher. Also, from the graphs above it is evident that lower concentrations of alcohol affect taste function in the swallow and rinse experiments, whereas, the effect of higher concentration of alcohol is noted in the bypass experiment.

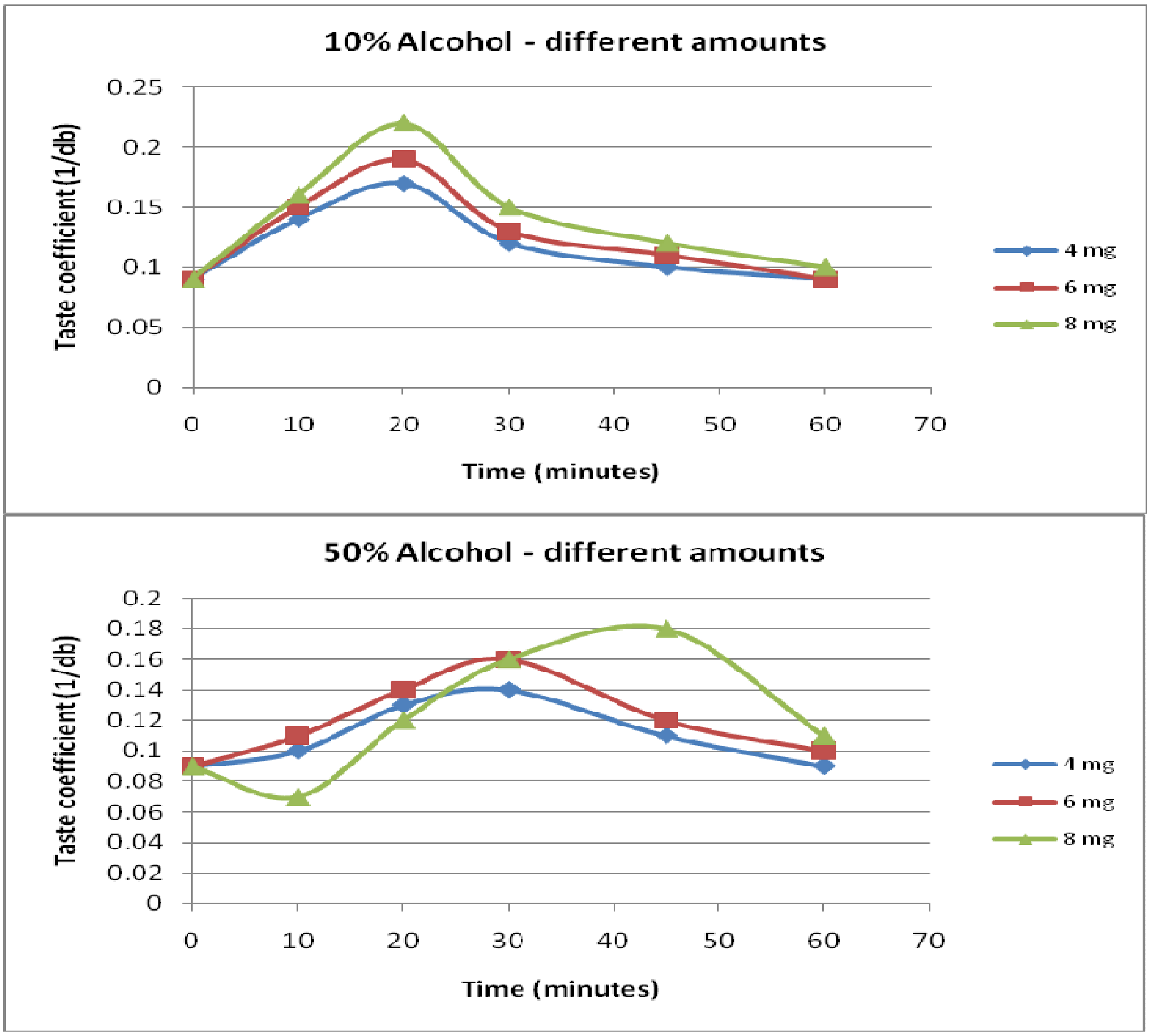

Fig 21: Effect of different amounts of 10% and 50% alcohol on tast coefficient

In the two graphs shown above, different amounts of alcohol (in weight) were used to study the effect on taste. In all the concentrations it was observed that higher amounts of alcohol cause the effect on taste function to be more pronounced. In the experiment with 8 mg of 50% alcohol, a distinct effect of the anaesthetic is observed, as discussed in the next graph.

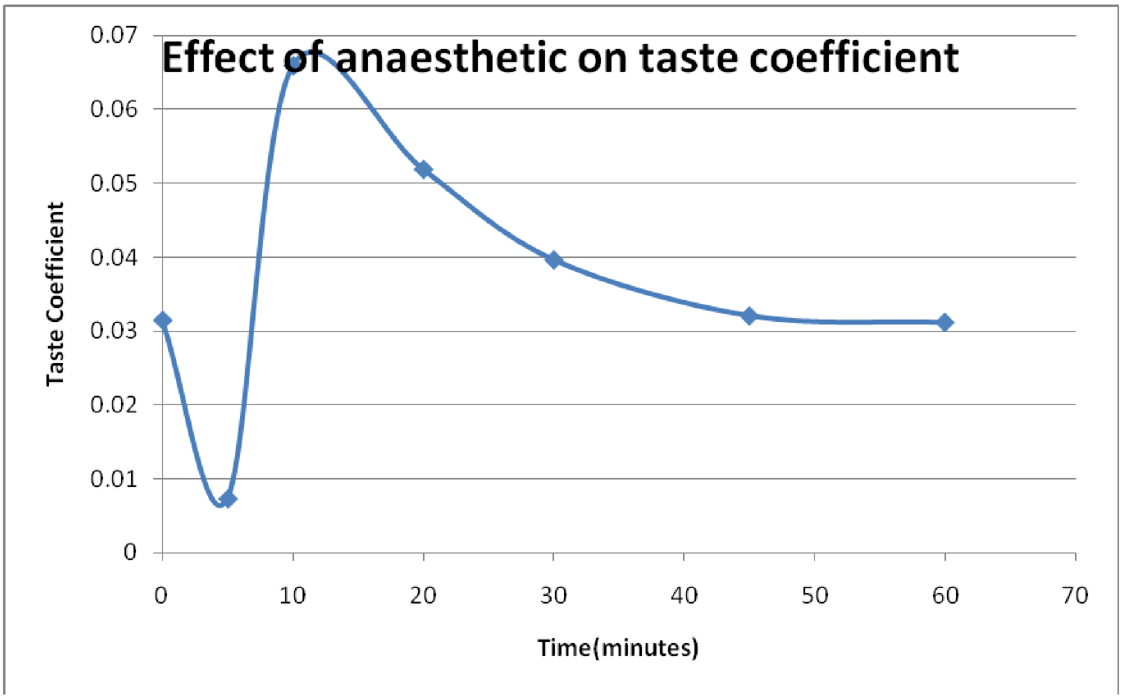

Fig 22: Effect of anaesthetics on taste

The graph above shows the effect of anaesthetic on taste function. Soon after the application of the anaesthetic, the taste function reduces. However, within five minutes it begins a recovery and overshoots the normal value. The taste function returns to the normal value within 30 – 40 minutes after the application of the anaesthetic.

7.4.3 DISCUSSION

The aperitif, an alcohol based drink, acts as a good appetizer and has been in use for many decades. However, no research has been done to directly relate how alcohol improves taste. It is widely known that continued alcohol consumption reduces sensory qualities including that of taste. However, this anomaly of taste sensation being improved immediately after consumption of a certain amount of alcohol has not been investigated.

Electrogustometry offers researchers an opportunity to quantify taste function and measure it with ease. In this study various experiments have been done to determine how alcohol consumption affects the immediate electrogustometric threshold. This study focussed on the immediate effect of alcohol on non-alcoholic, healthy subjects with no parental alcoholic history.

Alcohol mainly affects taste locally. In the alcohol rinse experiment it has been observed that the taste function improves within 10-20 minutes of consumption. Alcohol is easily absorbed by the blood vessels which flow very close to the surface of the tongue. This is instantly carried to the central nervous system via the blood altering the taste sensation. Reduced concentrations of alcohol are known to stimulate the nervous system quickly and a similar effect is noted in this study. [64] Higher concentrations of alcohol may have a mild anaesthetic effect on the oral mucosa and this is noted in the experiments conducted. High alcohol concentration desensitizes the tongue causing reduced response to stimuli.

The alcohol swallow experiment includes rinsing of the tongue with alcohol test solution and consuming it. This provides an overall explanation and elaborates the way in which alcohol affects taste. It has been noted earlier that alcohol can act as an anaesthetic locally and this is also shown in this part of the study. Higher concentrations diminish the sensitivity of the anterior region of the tongue where the electrogustometric measurement is done. An observation in the swallow experiment is that lower concentrations of alcohol improves taste perception to a greater extent as compared to a higher concentration of alcohol. This may be explained by the absorption of alcohol by the blood vessels near the lingual area and directly affecting the central nervous system quickly and not causing a marked anaesthetic effect.

The alcohol rinse experiment also reflects that lower concentrations of alcohol are easily absorbed by the blood vessels close to the surface of the tongue and immediately carried to the central nervous system affecting the taste function. Greater amounts of alcohol at higher concentration, as with the 8 mg alcohol in 50% concentration, cause a temporary anaesthetic effect.

The alcohol bypass experiment shows no or limited change in taste function for the first 15-20 minutes as the tongue recovers from the effect of the anaesthetics given to avoid gag reflex. In this study the mixture with greater concentration of alcohol is noted to alter taste more significantly. The digestive system can only process a certain amount of alcohol within a specified time. As the concentration of alcohol increases the amount of undigested alcohol also increases, in turn affecting the central nervous system and causing the taste function to be altered.

The immediate effect of alcohol on electrogustometric threshold can be detailed in the points below:

1. When alcohol is consumed a mild anesthetic effect is observed. This is more pronounced when the concentration of alcohol is higher.
2. Lower concentrations of alcohol are quickly absorbed by the blood vessels in the lingual area affecting the response of the central nervous system to taste stimuli.
3. Higher concentrations of alcohol are absorbed in the digestive tract and affect the brain later.

4. Alcohol cleans the surface of the oral mucosa increasing sensitivity to tastants.
5. Highly concentrated alcohol solution acts as an anesthetic.
6. Increased amounts of alcohol affect taste function in a similar way only to greater extents depending on the amount.

7.5 CONCLUSION

This chapter reports the study conducted to understand the immediate effect of alcohol on electrogustometric threshold. It can be commented that for non-alcoholic subjects with no parental alcoholic history the electrogustometric taste function is improved by the consumption of alcohol for a certain period of time. After this it returns to the normal state depending on the concentration of the alcohol. Alcohol also acts as an anaesthetic in higher concentrations reducing the taste coefficient. The next chapter discusses the effect of a depressant, smoking tobacco in the form of cigarettes, on electrogustometric taste function. Comparison is made to anaesthetics to understand the modality of this effect.

CHAPTER 8

THE EFEECT OF SMOKING ON TASTE THRESHOLD

8.1 INTRODUCTION

The immediate effect of alcohol on taste has been discussed in the previous chapter. This chapter discusses the effect of a depressant on taste threshold, smoking tobacco. It is often commented that taste function is significantly reduced by depressants. Tobacco constitutes an essential depressant and has been noted to alter the taste sensation significantly. [10, 66, 67] This chapter reports an experiment done to assess and compare the immediate effect of smoking the taste threshold of both smokers and non smokers.

Nicotine, an essential constituent of a cigarette, is an alkaloid which constitutes 0.6%-3.0% of dry tobacco. Its chemical formula is $C_{10}H_{14}N_2$. It is a hygroscopic, oily liquid miscible with water in its base form. It can easily penetrate the oral mucosa. Nicotine sublimes at low temperatures. Hence most of the nicotine in a cigarette is inhaled to cause the desired effects. It is one of the most addictive substances. It travels through the blood stream to the brain and thus affects the rest of the body. It is, however, processed very quickly. About 80% of the nicotine is broken down to cotinine by enzymes in the liver. It is also metabolised in the lungs and is filtered from the blood stream by the kidneys.

Nicotine changes the way in which the brain and body function. It causes an increased release of the hormone, adrenalin, causing rapid heartbeat, increased blood pressure and shallow breathing. Nicotine can also block the release of insulin, increasing the blood sugar level. It also increases the basal metabolic rate. Neural transmission is adversely affected by the presence of nicotine which causes the release of acetylcholine and heightens the activity in the cholinergic pathways throughout the brain. This, in turn, triggers the release of dopamine. The release of these neurotransmitters leaves the person in a more invigorated state. There are many harmful effects of smoking tobacco including cancer, heart diseases and strokes. [68, 69, 70]

Smoking affects the sensation of sweetness more than the other basic taste types. A study involving 27 people of whom some were smokers and some very light or occasional smokers was done in the Monell Chemical Senses Centre in Philadelphia. This study concluded that smokers were less sensitive to sweet substances as compared to light smokers. This effect was more pronounced in women as compared to men. [71] The work reported in this thesis is, however, limited to the study of the effect of smoking on the electrogustometric threshold: we did not employ chemogustometry.

Gustatory and olfactory sensory responses are often interchangeably described by people. Depressants cause both of these responses to diminish. Chronic smoking can cause significant alteration in the taste function and in most cases irreversibly. Pavlos et al. in their publication on the evaluation of young smokers and non-smokers using electrogustometry discuss how the taste threshold of smokers was significantly higher when compared to that of the control group of non-smokers. [66] Out of 62 subjects chosen from the Greek military forces, 34 were non-smokers and the remaining 28 were smokers. A statistically significant difference in the taste threshold of the two groups was noted. Two sets of information obtained by using electrogustometry and contact endoscopy provided useful information about taste buds and their functional ability following smoking. [66]

The effects of tobacco vary from person to person. It depends on how sensitive they are to smoking, how vulnerable the person is to the chemicals in tobacco, the number of cigarettes consumed in a day, the age when the person started smoking and so on. Taste transduction from the taste buds occurs in different afferent routes which are affected differently and hence smoking does not cause complete loss of taste. [76]

The effect of smoking on taste has been studied since the 1960s. However, most of the studies have been done by testing chronic smokers and their taste thresholds being compared to those of non-smokers used as a control group whereas the study reported in this thesis assesses the effect of smoking on taste threshold with respect to time. The study is focused on the immediate effect of smoking on the electrogustometric threshold.

8.2 EXPERIMENT

8.2.1 Material and Method

Subjects

Eight healthy subjects were recruited from the students at the University of Sussex. Three of them were female and five were male and their age range was 22 to 40, their mean age being 29.1. A brief medical history of each subject was recorded prior to the test. No significant medical conditions were noted in any of the subjects which might suggest an abnormal electrogustometric threshold apart from their smoking habits. Four subjects were regular smokers (at least 10 cigarettes in a day) whereas the other four were occasional smokers (less than 3 cigarettes a week).

8.2.2 Test Equipment

The electric stimuli were produced by the Sussex Electrogustometer operating in the automatic mode.

8.2.3 Procedure

The subjects were placed in two groups according to their smoking habits. Both of the groups were given the same brand of cigarette to smoke during the test to ensure similar contents of tobacco and nicotine. The subjects had been asked not to smoke or consume alcohol or sedatives for up to four hours before the test and not to eat or drink anything during and up to an hour before the test. Taste threshold was first measured for all the subjects. Following this each of them smoked a cigarette and then taste threshold was measured after five minutes and then at the 10^{th}, 20^{th}, 30^{th}, 40^{th} and 60^{th} minute.

8.2.4 RESULTS

The mean taste thresholds for the two groups were plotted on the same graph shown in figure 20. This graph also includes the response of the subjects to an anaesthetic spray as previously reported in chapter 7.

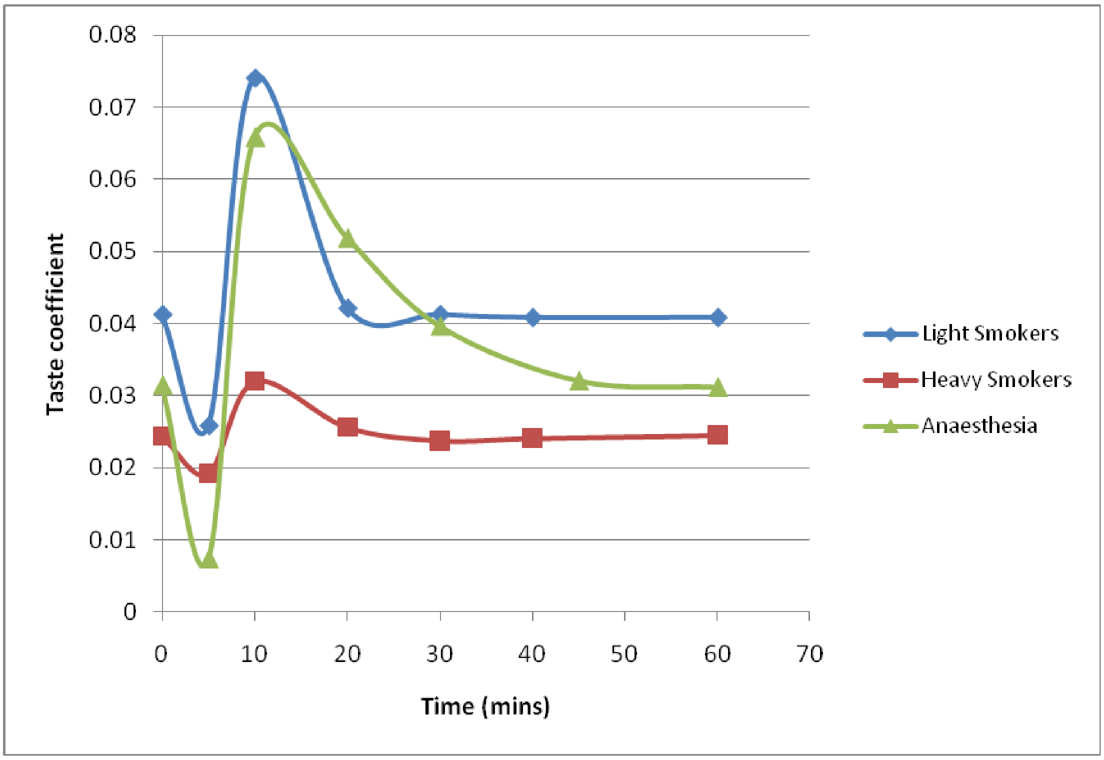

Fig 23: Effect of smoking and anaesthetics on electrogustometric threshold

A two tailed t-test was done on the data sets of the electrogustometric thresholds of the heavy and light smokers. This test is generally done to determine if there is any statistical similarity between two groups. The t value was 0.0037 indicating a statistically significant difference between the two groups. ($P < 0.05$)

8.2.5 DISCUSSION

The results indicate that the effect of smoking on the electrogustometric threshold of non-smokers and smokers is appreciably different. The study shows that there was a significant difference between the taste threshold of smokers and non-smokers both before and after the test, substantiating the chronic effect of smoking on the ability to taste. Minutes after consumption of the cigarette, the graphs show that the taste coefficient for both smokers and non-smokers was reduced. For non-smokers this reduction is much more pronounced. Like most systems in the human body, the taste function recovers and overshoots its normal threshold before returning to the normal value. The overshoot for non-smokers was equally pronounced. [77] Acute effects of smoking cause the release of the hormone adrenaline which causes increased sensitivity. This is notably more for non-smokers.

The recovery from smoking is much quicker than that from alcohol on taste as reported in the previous chapter. In essence the effect of smoking on taste is short lived as the taste function returns to the normal within 20 minutes and this correlates with the short half-life of nicotine. [70] Figure 20 shows that there is a great similarity between the effects on taste of smoking and of the anaesthetic. This is despite the fact that the anaesthetic acts primarily locally on the oral mucosa whereas the nicotine derived from smoking tends to affect the whole central nervous system.

8.3 CONCLUSION

The taste threshold is significantly altered due to the effects of smoking but whereas previous authors have reported on the chronic effects, this study has shown that the transient effects are significantly different too.

One of the aims of this project was to make a state of the art electrogustometer for clinical use. The next chapter details studies carried out by a local doctors using the Sussex Electrogustometer.

CHAPTER 9

CLINICAL APPLICATION OF ELECTROGUSTOMETRY

9.1 INTRODUCTION

The Sussex Electrogustometer has been studied to understand the immediate effect of alcohol and smoking on taste. The previous chapters detail the study conducted and results obtained. Electrogustometry has been in existence since the 1950s; however, it is not a common clinical tool. The Sussex Electrogustometer was designed to be a portable, simple, battery operated and semi-automatic machine to measure electrogustometric threshold. This chapter reports details of a clinical trial conducted at a local hospital using this machine.

Electrogustometry has been used to study taste loss caused by laryngomicrosurgery, tonsillectomy, middle ear surgery, diabetes etc. [7, 8, 9, 10, 11, 13] However, it is still not a viable tool to be used in clinics due to various limitations. The Sussex Electrogustometer was designed with a view of making electrogustometry a common clinical tool. Following its design and construction it was tested for reliability and repeatability reported in chapter five. In order to understand its performance in clinics suitable tests needed to be done. This chapter reports a study conducted by a local consultant and his colleagues using the Sussex Electrogustometer at the Royal Sussex County Hospital.

9.2 EXPERIMENT

The Sussex Electrogustometer was used by a local consultant and his registrar to assess the electrogustometric threshold of twenty subjects with various ailments. The studies were conducted at the Royal Sussex County Hospital.

9.2.1 SUBJECTS

Twenty subjects were chosen from the NHS patients at the Royal Sussex County Hospital. They were chosen from different clinics to get a larger range of ailments. The range included:

a) Supraglottitis
b) Leg Cellulitis
c) Bowel obstruction
d) Epistaxis
e) Back Pain
f) Fractured femur
g) Otitis externa
h) Nasal polyps
i) Snoring
j) Otitis media
k) Sinusitis
l) Nasal valve collapse
m) Wax impaction
n) Paradoxical vocal fold movement
o) Vertigo

p) Reflux

9.2.2 TEST EQUIPMENT

The Sussex Electrogustometer was first used in its manual mode to train the subject and then employed in its automatic mode to obtain the electrogustometric threshold.

9.2.3 PROCEDURE

The subjects were initially briefed as to why they were selected for the study. The procedure was then explained and trial stimuli were applied using the manual mode to train the subject on the response strategy. Once the subject was adequately trained, the automatic mode was employed to obtain the electrogustometric threshold. The test was carried out with stimulus duration of 2 seconds.

9.2.4 RESULTS

Hosp. No	**Diagnosis**	**Right**		**Left**	
		uA	dB	uA	dB
2033668	Supraglottitis	43	14.6	35	12.9
Observation	The subject showed high taste threshold indicating a potential loss of taste. Asymmetry was not noted.				
2336775	Leg cellulitis	1	-12.2	8	0
Observation	No evidence of taste loss observed.				
2022290	Bowel obstruction	8	0	1	-12.2
Observation	No evidence of taste loss observed.				
2408002	Epistaxis	14	4.7	8	0
Observation	No evidence of taste loss observed.				

N/A (Staff)	Nil	1	-12.2	1	-12.2
Observation	No evidence of taste loss observed.				
2044456	Back pain	3	-6.2	6	-2.7
Observation	No evidence of taste loss observed.				
3280141	Fractured NOF/femur/shoulder	61	17.6	29	11.3
Observation	Taste threshold significantly high and bilateral asymmetry observed. This may indicate underlying neural ailments.				
2188367	Otitis externa	10	1.7	1	-12.2
Observation	No evidence of taste loss observed.				
3220551	Nasal polyps	1	-12.2	18	0
2632816	Nasal polyps	63	17.9	53	16.4
Observation	Clear evidence of taste loss noted which is consistent with the diagnosis.				
2403133	Snoring	14	4.7	8	0
Observation	No evidence of taste loss observed.				
2385072	Otitis media	10	1.7	18	6.8
Observation	No evidence of taste loss observed.				
2700695	Sinusitis (with local anaesthetic)	49	15.7	39	13.8
Observation	Clear evidence of taste loss noted possibly due to anaesthesia.				
2817109	Nasal valve collapse	59	17.3	29	11.3
Observation	Clear evidence of taste loss noted which is consistent with the diagnosis.				
2750998	Supraglottitis	6	-2.7	8	0
3283591	Wax impaction	8	0	6	-2.7
Observation	No evidence of taste loss observed.				

2007306	Paradoxical vocal fold movement	57	17	18	6.8
Observation	Evidence of taste loss and bilaterla assemetry in line with the diagnosis.				
1284274	Vertigo	1	-12.2	8	0
Observation	No evidence of taste loss observed.				
3166457	Reflux	23	9.3	8	0
Observation	No evidence of taste loss observed.				
2126855	Otitis externa	10	1.7	12	3.3
Observation	No evidence of taste loss observed.				

Table 4: Clinical Study using the Sussex Electrogustometer

The results show that the Sussex Electrogustometer has been successfully used to measure electrogustometric threshold of up to twenty subjects with various ailments in a clinical environment.

9.2.5 DISCUSSION

The results suggest that electrogustometric threshold of the subjects can be easily measured using the Sussex Electrogustometer. A report provided by the doctor who carried out the study is detailed below:

The Sussex Taste meter was used on 20 volunteers. Following a short instructional course on how to use the machine, I found its setup and use very simple and the onscreen menus were very easy to navigate. Setting up the machine on a 'patient' was swift and required minimal time. The majority of testing sessions passed without problem and the immediate acquisition of results means its application in a clinic-based setting is easy to imagine.

There were a few minor practical and theoretical problems with the machine. Firstly, the tongue electrode seemed to be particularly sensitive to changes in angle, and if the patient moved the plate away from the flush position with the tongue, an immediate reduction in thresholds was seen. As the tip of the electrode was very mobile on its hinge, this meant that the position of the electrode on the patient's tongue had to be closely scrutinized. There was also a lag on the patient response pad meaning that frequently correct pressing of the button was not registered but required the tester to verbally prompt the patient to repeat their button press.

A slightly more theoretical problem is what the nerve is actually stimulating. Some patients reported quite a metallic taste when stimulated, whereas others felt that it produced little taste sensation but more of an electrical stimulation. The question from a taste point of view would therefore be whether the taste meter is stimulating the chorda tympani (special sensory – taste) or in fact the lingual nerve (somatic sensory) and more studies may have to be done to elucidate this. However, overall the device was simple, easy to use, produced no complications and should be developed further to enable it to be incorporated into the clinic.

Following this report a minor adjustment to the response time was made to reflect the suggestions made.

9.3 CONCLUSION

The study detailed in this chapter is the first step in establishing the Sussex Electrogustometer as a common clinical tool. It has been easy to use and with a few modifications, which have already been done, will make electrogustometry a viable clinical tool.

CHAPTER 10

CONCLUSION

Electrogustometry is now slowly becoming a common clinical tool. The successful design, manufacture and testing of the Sussex Electrogustometer has helped doctors use electrogustometers in clinics. Since the 1950s a few electrogustometers have been developed and used. However, none of them is a state of the art, battery powered, simple and reliable instrument. Following the use of the Sussex Electrogustometer at the Royal Sussex County hospital, the awareness of electrogustometry is increasing. Our laboratory has already been contacted by doctors to do further research with the machine.

Various diseases like Bell's palsy, Parkinson's disease etc can be screened using electrogustometry. It can also help identify early signs of diabetes and there is a scope of further research into studying its prognosis. Prevention of diabetes is a topic of great interest and electrogustometers may be employed to analyse the deterioration of taste threshold for subjects with parental diabetic history. With this data, preventive measures for potential diabetes patients may be explored.

The perception of taste, as explained earlier in this thesis, does not solely depend on sensation of taste via the taste buds. It is augmented by the sense of smell, olfactory sense, and the sense of touch on the surface of the tongue referred to as the somatosensory sense. Understanding the extent of the effect of olfactory and somatosensory senses in perception of taste may be carried out using electrogustometry and olfactometers.

Further practical application of electrogustometry lies in the tasting industry. Wine and food tasting may potentially be standardised and made objective with a complete knowledge of taste threshold. The cause of dysgeusia can also be analysed using electrogustometry.

10. 1 SUMMARY

This thesis started with an introduction to taste and various methods of measuring its threshold. The following chapter explored electrogustometry and various electrogustometers. The salient features of a state of the art electrogustometer were also described. Chapter Four described the design, construction and fabrication of the Sussex Electrogustometer, a state of the art machine designed based on the salient features discussed in Chapter Three. Following the successful manufacture of the Sussex Electrogustometer, it was tested for reliability and repeatability.

Upon reviewing the literature available for electrogustometry it was noted that there was no recommended standard values for physical constraints, stimulus duration or electrode area, that determine the taste threshold. Hence it was not possible to compare data collected for different experiments. Studies described in Chapter Six conclude recommended standard stimulus duration of 2 seconds and an electrode area of 28.5 mm^2.

The accidental observation of the immediate effect of alcohol on taste during the repeatability testing of the Sussex Electrogustometer led on to the next section of studies reported in this thesis. Collaborations were made with a local psychologist and neurologist to determine the best way to understand how alcohol effects taste immediately after its consumption in non-alcoholics. This study has been reported in

Chapter Seven. This was extended further into understanding the immediate effect of smoking on taste reported in Chapter Eight.

The main purpose of the research was to make an electrogustometer which would make electrogustometry a common clinical tool. The Sussex Electrogustometer was tested by a local consultant and his associate at the Royal Sussex County Hospital. The detailed report is discussed in Chapter Nine. The studies proved that the new machine was easy to use in the clinical setting and could help electrogustometry become a common clinical tool.

10.2 FURTHER RESEARCH

The project was conceived with a view of making Brighton the taste centre of the UK. A study done by a local registrar, Mr AD Morley, concluded that a state of the art machine was required that could be used to measure electrogustometric thresholds in the clinical environment. As part of this project the Sussex Electrogustometer was designed and manufactured. It was tested in the clinical environment by a local consultant, details of which are provided in Chapter 9. As part of his MD, Mr Morley had conducted detailed taste studies for up to 400 patients at the Royal Sussex County Hospital and other clinics in East Sussex. However, due to unforeseen reasons he has been unable to conduct a similar study with the Sussex Electrogustometer. Further clinical research can be done with a consultant who can carry out further studies using the Sussex Electrogustometer.

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APPENDIX 1

THE SOFTWARE CODE FOR THE DOUBLE STAIRCASE ALGORITHM

```
; HEADER DOCUMENTATION
; this is the code for double alternate forced choice staircase
; pic port configuration:
; portA : not used
; portB : LCD data bus
; portC : PC.0 : WR for DAC
;               PC.1 : LED for end of staircase
;               PC.2 : LED and BUZZER for pulse to electrode
;               PC.3 : LED for improper current flow detect
;               PC.4 : feedback
;               PC.5 : RS for LCD
;               PC.6 : R/W for LCD
;               PC.7 : E for LCD
; portD : electrode output
; portE : not used
; Files required : P18F452.INC
;***********************************************************************
        LIST P=18F452                   ;directive to define processor
        #include <P18F452.INC>          ;processor specific variable definitions
;***********************************************************************;Configuration bits
; The __CONFIG directive defines configuration data within the .ASM file.
; The labels following the directive are defined in the P18F452.INC file.
; The PIC18FXX2 Data Sheet explains the functions of the configuration bits.
```

```
    __CONFIG    _CONFIG1H, _OSCS_OFF_1H & _HS_OSC_1H
    __CONFIG    _CONFIG2L, _BOR_OFF_2L & _PWRT_OFF_2L
    __CONFIG    _CONFIG2H, _WDT_OFF_2H
    __CONFIG    _CONFIG3H, _CCP2MX_OFF_3H
    __CONFIG    _CONFIG4L, _STVR_OFF_4L & _LVP_OFF_4L & _DEBUG_OFF_4L
    __CONFIG    _CONFIG5L, _CP0_OFF_5L & _CP1_OFF_5L & _CP2_OFF_5L & _CP3_OFF_5L
    __CONFIG    _CONFIG5H, _CPB_OFF_5H & _CPD_OFF_5H
    __CONFIG    _CONFIG6L, _WRT0_OFF_6L & _WRT1_OFF_6L & _WRT2_OFF_6L & _WRT3_OFF_6L
    __CONFIG    _CONFIG6H, _WRTC_OFF_6H & _WRTB_OFF_6H & _WRTD_OFF_6H
    __CONFIG    _CONFIG7L, _EBTR0_OFF_7L & _EBTR1_OFF_7L & _EBTR2_OFF_7L & _EBTR3_OFF_7L
    __CONFIG    _CONFIG7H, _EBTRB_OFF_7H
;**********************************************************************;
;Variable definitions
; These variables are only needed if low priority interrupts are used.
        CBLOCK    0x080
        STATUS_TEMP
        BSR_TEMP
        WREG_TEMP
        DELAY1
        DELAY2
        DISPLAY
        UTH_W
        HU_W
        COUNT_H
        COUNT_L
        COUNT_RESPONSE1
```

```
COUNT_RESPONSE2
COUNT_RESPONSE3
PREVIOUS_RESPONSE_U
PREVIOUS_RESPONSE_L
LTH_W
HL_W
FP_COUNT
FPSCORE
ENDC
CBLOCK      0x000
EXAMPLE             ;example of a variable in access RAM
ENDC
;**********************************************************************;EEPROM data
; Data to be programmed into the Data EEPROM is defined here
ORG   0xf00000
DE    "Test Data",0,1,2,3,4,5
;**********************************************************************;Reset vector
; This code will start executing when a reset occurs.
ORG   0x0000
goto  Main          ;go to start of main code
;********************************************************************** Main:
movlw 0x00
movwf SSPCON1, 0
movwf TXSTA,0
movwf OSCCON,0
bcf         RCON,7
movlw 0x00
movwf EECON1,0
```

```
movwf INTCON,0; disabled all external interrupts
movlw        b'10000000'
movwf INTCON2,0 ; disabled all interrupts
movlw 0x00
movwf INTCON3,0 ; disabled external interrupts
movwf PIR1,0
movwf PIR2,0
movwf PIE1,0
movwf PIE2,0
movwf IPR1,0
movwf IPR2,0
movwf ADCON0,0
movwf ADCON1,0
movwf T0CON, 0; timer 0 is disabled
movwf TMR0L, 0
movwf TMR0H, 0
movwf T1CON, 0; timer 1 is disabled
movwf TMR1L, 0
movwf TMR1H, 0
movwf T2CON, 0; timer 2 is disabled
movwf T3CON, 0; timer 3 is disabled
movwf TMR3L, 0
movwf TMR3H, 0
movwf CCP1CON, 0;
movwf CCPR1L, 0;
movwf CCPR1H, 0;
movwf CCPR2L, 0;
movwf CCPR2H, 0;
movwf CCP2CON, 0;
movwf SSPSTAT, 0
```

```
movwf SSPCON2,0

movwf RCSTA,0

movwf SPBRG,0

movwf ADRESH,0

movwf ADRESL,0

movwf LVDCON,0;

movwf WDTCON,0

;*******************************************************************

; presetting ports : PORTB - LCD DATA BUS, PORTC - CONTROL BUS, PORTD - ELECTRODE DATA BUS

MOVLW 0x00

MOVWF PORTA,0

MOVWF PORTB,0

MOVWF PORTD,0

MOVWF PORTE,0

MOVWF TRISB,0

MOVWF TRISD,0

MOVWF TRISE,0

MOVWF TRISA,0

MOVLW 0X01

MOVWF PORTC,0

MOVLW 0X10

MOVWF TRISC,0

;********************************************************************

; DECLARE THE INITIAL VALUES OF THE VARIABLES AND CONTROL PORTS

MOVLW 0X27

MOVWF UTH_W; 40 MICRO AMPS

MOVLW 0X10

MOVWF LTH_W; 10 MICRO AMPS -- the start values are close to the said normal to avoid any biasing
```

```
        MOVLW 0X0D
        MOVWF HU_W,1; H (STEP SIZE) = 20 decimal units of MircoA
        MOVLW 0X0D
        MOVWF HL_W,1 ; H (STEP SIZE) = 20 decimal units of MircoA
        MOVLW 0X01
        MOVWF PREVIOUS_RESPONSE_U
        MOVLW 0X02
        MOVWF PREVIOUS_RESPONSE_L
        MOVLW 0X03
        MOVWF COUNT_H,1
        MOVWF COUNT_L,1
        MOVWF FP_COUNT
        MOVLW 0X00
        MOVWF FPSCORE
;**********************************************************************
; initialise LCD
        MOVLW 0XFF
        MOVWF DELAY1
        MOVWF DELAY2
; WAIT FOR 30ms AFTER POWER UP
BACK1:      DECFSZ DELAY1,1
            GOTO BACK1
            DECFSZ DELAY2,1
            GOTO BACK1
; SET THE FUNCTION SET -- 2 LINE MODE AND 5*8 DISPLAY
        MOVLW 0X38
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
```

```
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
; SET DISPLAY ON/OFF CONTROL -- DISPLAY ON, CURSOR OFF, CURSOR BLINK ON
        MOVLW 0X0C
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
; DISPLAY CLEAR
        MOVLW 0X01
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY1.53
        CALL CONTROL_RESET
; ENTRY MODE SET -- INCREMENT WITHOUT ENTIRE SHIFT
        MOVLW 0X06
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
; SET GGRAM ADDRESS START AS 00
        MOVLW 0X40
```

```
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
; SET DDRAM ADDRESS START AS 00
        MOVLW 0X80
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
;*****************************************************************
;WRITE SUSSEX TASTE METER:
        CALL WELCOME
        CALL TT
        CALL DISPLAYFP
; SET DDRAM ADDRESS START AS 03 ie. ROW 1 COLUMN 4
        MOVLW 0X83
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
UPPER_STAIRCASE:
        MOVLW 0X09
        MOVWF PORTC,0
```

```
        BCF STATUS,4
        BCF STATUS,2
        MOVLW 0XFF
        MOVWF COUNT_RESPONSE1
        MOVWF COUNT_RESPONSE2
        MOVLW 0X05
        MOVWF COUNT_RESPONSE3
        MOVFF UTH_W,DISPLAY
        CALL DYNAMICDISPLAY
        MOVFF UTH_W,PORTD
        MOVLW 0X00
        MOVWF PORTC,0
        NOP
        NOP
        MOVLW 0X01
        MOVWF PORTC,0; TOGGLE WR FOR DAC
BACK_A:     ; ---0.5 sec pulse duration
        NOP
        DECFSZ COUNT_RESPONSE1
        GOTO BACK_A
        DECFSZ COUNT_RESPONSE2
        GOTO BACK_A
        DECFSZ COUNT_RESPONSE3
        GOTO BACK_A
        MOVLW 0X00
        MOVWF PORTD,0
        NOP
        MOVLW 0X00
        MOVWF PORTC,0
        NOP
```

```
        MOVLW 0X01
        MOVWF PORTC,0; TOGGLE WR FOR DAC
        GOTO LOOP_WAIT_FOR_RESPONSE
LOOP_WAIT_FOR_RESPONSE:  ; WAIT FOR 3 SECONDS FOR RESPONSE
        MOVLW 0XFF
        MOVWF COUNT_RESPONSE1
        MOVWF COUNT_RESPONSE2
        MOVLW 0X0A
        MOVWF COUNT_RESPONSE3
BACK_1:         NOP
                NOP
                MOVLW 0X11
                SUBWF PORTC,0,0
                BZ RESPONSE_YES_U
                DECFSZ COUNT_RESPONSE1,1,1
                GOTO BACK_1
                DECFSZ COUNT_RESPONSE2,1,1
                GOTO BACK_1
                DECFSZ COUNT_RESPONSE3,1,1
                GOTO BACK_1
                GOTO RESPONSE_NO_U
RESPONSE_YES_U:
                BCF STATUS,4
                BCF STATUS,2
                BCF STATUS,0
                CALL DEBOUNCE_UPPER
                BCF STATUS,4
                BCF STATUS,2
                BCF STATUS,0
                MOVLW 0X01
```

```
                    SUBWF PREVIOUS_RESPONSE_U
                    BZ SAME_YES_U
                    GOTO DIFFERENT_YES_U
RESPONSE_NO_U:
                    BCF STATUS,4
                    BCF STATUS,2
                    BCF STATUS,0
                    MOVLW 0X02
                    SUBWF PREVIOUS_RESPONSE_U
                    BZ SAME_NO_U
                    GOTO DIFFERENT_NO_U
SAME_YES_U:; uth_w = uth_w - hu_w
          BCF STATUS,0
          BCF STATUS,2
          BCF STATUS,4
          MOVF HU_W,0
          SUBWF UTH_W,1
          BN LOWER_LIMIT_REACHED
          MOVLW 0X01
          MOVWF PREVIOUS_RESPONSE_U,1
          MOVFF STATUS,WREG
          GOTO CONDITIONS_L1
DIFFERENT_YES_U:; hu_w = hu_w/2; uth_w = uth_w - hu_w
          BCF STATUS,0
          BCF STATUS,4
          RRCF HU_W,1,1
          MOVF HU_W,0
          SUBWF UTH_W,1
          BN LOWER_LIMIT_REACHED
          MOVLW 0X01
```

```
        MOVWF PREVIOUS_RESPONSE_U,1
        MOVFF STATUS,WREG
        GOTO CONDITIONS_L1
SAME_NO_U:; uth_w = uth_w + hu_w
        BCF STATUS,0
        MOVF HU_W,0
        ADDWF UTH_W,1
        BC UPPER_LIMIT_REACHED
        MOVLW 0X02
        MOVWF PREVIOUS_RESPONSE_U,1
        MOVFF STATUS,WREG
        GOTO CONDITIONS_L1
DIFFERENT_NO_U:; hu_w = hu_w/2, uth_w = uth_w + hu_w
        BCF STATUS,0
        RRCF HU_W,1,1
        MOVF HU_W,0
        ADDWF UTH_W,1
        BC UPPER_LIMIT_REACHED
        MOVLW 0X02
        MOVWF PREVIOUS_RESPONSE_U,1
        MOVFF STATUS,WREG
        GOTO CONDITIONS_L1
CONDITIONS_L1:
        MOVLW 0X01
        SUBWF HU_W,0
        BZ NEXT_1
        BN NEXT_1
        BCF STATUS,4
        BCF STATUS,2
        GOTO LOOP_DELAY_LOWER_L1
```

```
NEXT_1:; process if hu_w = 0X01
        DECFSZ COUNT_H,1,1
        MOVFF COUNT_H,WREG
        SUBLW 0X01
        BZ LOOP_END_3
        BN LOOP_END_3
        GOTO LOOP_DELAY_LOWER_L1
LOOP_DELAY_LOWER_L1:
        MOVLW 0XFF
        MOVWF COUNT_RESPONSE1
        MOVWF COUNT_RESPONSE2
        MOVLW 0X0A
        MOVWF COUNT_RESPONSE3
BACK_DELAY_LOWER_L1:NOP
        NOP
        NOP
        DECFSZ COUNT_RESPONSE1
        GOTO BACK_DELAY_LOWER_L1
        DECFSZ COUNT_RESPONSE2
        GOTO BACK_DELAY_LOWER_L1
        DECFSZ COUNT_RESPONSE3
        GOTO BACK_DELAY_LOWER_L1
        GOTO FP_CHECK
LOOP_END_3:
        NOP
        GOTO END_ALL

FP_CHECK: DECFSZ FP_COUNT,1,1
        BZ FP_TEST
        GOTO LOWER_STAIRCASE
```

```
;*********************************************************88
LOWER_LIMIT_REACHED:
        BCF STATUS, 4
        MOVLW 0X00
        MOVWF UTH_W
        MOVWF LTH_W
        CALL DISPLAY_LOWER_LIMIT_REACHED
        GOTO END_ALL
;******************************************************************
****
UPPER_LIMIT_REACHED:
        BCF STATUS,0
        MOVLW 0XFF
        MOVWF UTH_W
        MOVWF LTH_W
        CALL DISPLAY_UPPER_LIMIT_REACHED
        GOTO END_ALL
;******************************************************************
**** FP_TEST:
        MOVLW 0X09
        MOVWF PORTC,0
        BCF STATUS,4
        BCF STATUS,2
        MOVLW 0XFF
        MOVWF COUNT_RESPONSE1
        MOVWF COUNT_RESPONSE2
        MOVLW 0X05
        MOVWF COUNT_RESPONSE3
        MOVFF LTH_W,DISPLAY
        CALL DYNAMICDISPLAY
        MOVLW 0X00
```

```
        MOVFF WREG, PORTD
        MOVLW 0X00
        MOVWF PORTC,0
        NOP
        NOP
        MOVLW 0X01
        MOVWF PORTC,0
        NOP
BACK_FP_L2: NOP
        DECFSZ COUNT_RESPONSE1
                GOTO BACK_FP_L2
                DECFSZ COUNT_RESPONSE2
                GOTO BACK_FP_L2
                DECFSZ COUNT_RESPONSE3
                GOTO BACK_FP_L2
                MOVLW 0X00
                MOVWF PORTD,0
                NOP
                MOVLW 0X00
                MOVWF PORTC,0
                NOP
                MOVLW 0X01
                MOVWF PORTC,0
                GOTO LOOP_WAIT_FOR_RESPONSE_FP
LOOP_WAIT_FOR_RESPONSE_FP:
        MOVLW 0XFF
        MOVWF COUNT_RESPONSE1
        MOVWF COUNT_RESPONSE2
        MOVLW 0X0A
        MOVWF COUNT_RESPONSE3
```

```
BACK_FP2_L2:        NOP
                    NOP
                    MOVLW 0X11;
                    SUBWF PORTC,0,0
                    BZ LOOP_YES_FP
                    DECFSZ COUNT_RESPONSE1
                    GOTO BACK_FP2_L2
                    DECFSZ COUNT_RESPONSE2
                    GOTO BACK_FP2_L2
                    DECFSZ COUNT_RESPONSE3
                    GOTO BACK_FP2_L2
                    GOTO LOOP_NO_FP
LOOP_YES_FP: MOVLW 0X01
        ADDWF FPSCORE,1
        CALL DISPLAYFPSCORE
LOOP_NO_FP: CALL DISPLAYFPSCORE
        GOTO LOWER_STAIRCASE
;*********************************************************************
**** LOWER_STAIRCASE:
        MOVLW 0X09
        MOVWF PORTC,0
        BCF STATUS,4
        BCF STATUS,2
        MOVLW 0XFF
        MOVWF COUNT_RESPONSE1
        MOVWF COUNT_RESPONSE2
        MOVLW 0X05
        MOVWF COUNT_RESPONSE3
        MOVFF LTH_W,DISPLAY
        CALL DYNAMICDISPLAY
```

```
        MOVFF LTH_W, PORTD
        MOVLW 0X00
        MOVWF PORTC,0
        NOP
        NOP
        MOVLW 0X01
        MOVWF PORTC,0
        NOP
BACK_11_L2: NOP
        DECFSZ COUNT_RESPONSE1
                GOTO BACK_11_L2
                DECFSZ COUNT_RESPONSE2
                GOTO BACK_11_L2
                DECFSZ COUNT_RESPONSE3
                GOTO BACK_11_L2
                MOVLW 0X00
                MOVWF PORTD,0
                NOP
                MOVLW 0X00
                MOVWF PORTC,0
                NOP
                MOVLW 0X01
                MOVWF PORTC,0
                GOTO LOOP_LOWERWAIT_FOR_RESPONSE_L2
LOOP_LOWERWAIT_FOR_RESPONSE_L2:
        MOVLW 0XFF
        MOVWF COUNT_RESPONSE1
        MOVWF COUNT_RESPONSE2
        MOVLW 0X0A
        MOVWF COUNT_RESPONSE3
```

```
BACK_12_L2:     NOP
                NOP
                MOVLW 0X11;
                SUBWF PORTC,0,0
                BZ LOOP_YES_LOWER
                DECFSZ COUNT_RESPONSE1
                GOTO BACK_12_L2
                DECFSZ COUNT_RESPONSE2
                GOTO BACK_12_L2
                DECFSZ COUNT_RESPONSE3
                GOTO BACK_12_L2
                GOTO LOOP_NO_LOWER
LOOP_YES_LOWER:
                BCF STATUS,4
                BCF STATUS,2
                BCF STATUS,0
                CALL DEBOUNCE_LOWER
                BCF STATUS,4
                BCF STATUS,2
                BCF STATUS,0
                MOVLW 0X01
                SUBWF PREVIOUS_RESPONSE_U
                BZ LOOP_SAME_YES_LOWER
                GOTO LOOP_DIFFERENT_YES_LOWER
LOOP_SAME_YES_LOWER:; LTH_W = LTH_W - HL_W
        BCF STATUS,0
        BCF STATUS,4
        MOVF HL_W,0
        SUBWF LTH_W,1,1
        BN LOWER_LIMIT_REACHED_LOWER
```

```
        MOVLW 0X01
        MOVWF PREVIOUS_RESPONSE_L,1
        MOVFF STATUS,WREG
        GOTO LOOP_CONDITIONS_L2
LOOP_DIFFERENT_YES_LOWER:
; HL_W = HL_W/2, LTH_W = LTH_W - HL_W
        RRCF HL_W,1,1
        MOVF HL_W,0
        SUBWF LTH_W,1,1
        BN LOWER_LIMIT_REACHED_LOWER
        MOVLW 0X01
        MOVWF PREVIOUS_RESPONSE_L,1
        MOVFF STATUS,WREG
        GOTO LOOP_CONDITIONS_L2
LOOP_NO_LOWER:
        MOVF PREVIOUS_RESPONSE_L,0
        SUBLW 0X02
        BZ LOOP_SAME_NO_LOWER
        GOTO LOOP_DIFFERENT_NO_LOWER
LOOP_SAME_NO_LOWER: ; LTH_W = LTH_W + HL_W
        BCF STATUS,0
        MOVF HL_W,0
        ADDWF LTH_W,1,1
        BC UPPER_LIMIT_REACHED_LOWER
        MOVLW 0X02
        MOVWF PREVIOUS_RESPONSE_L,1
        MOVFF STATUS,WREG
        GOTO LOOP_CONDITIONS_L2
LOOP_DIFFERENT_NO_LOWER: ; HL_W = HL_W/2; LTH_W = LTH_W + HL_W
        BCF STATUS,0
```

```
        RRCF HL_W,1,1
        MOVF HL_W,0
        ADDWF   LTH_W,1,1
        BC UPPER_LIMIT_REACHED_LOWER
        MOVLW 0X02
        MOVWF PREVIOUS_RESPONSE_L,1
        MOVFF STATUS,WREG
        GOTO LOOP_CONDITIONS_L2
        LOOP_CONDITIONS_L2:
        MOVLW 0X01
        SUBWF HL_W,0
        BZ NEXT_4
        BN NEXT_4
        BCF STATUS,4
        BCF STATUS,2
        GOTO DELAY_LOOP_UPPER
NEXT_4:
        DECFSZ COUNT_L,1,1
        MOVFF COUNT_L,WREG
        SUBLW 0X01
        BZ LOOP_END_4
        BN LOOP_END_4
        GOTO DELAY_LOOP_UPPER
DELAY_LOOP_UPPER:
        MOVLW 0XFF
        MOVWF COUNT_RESPONSE1
        MOVWF COUNT_RESPONSE2
        MOVLW 0X0A
        MOVWF COUNT_RESPONSE3
BACK_DELAY_1_L2:
```

```
        DECFSZ COUNT_RESPONSE1,1,1
        GOTO BACK_DELAY_1_L2
        DECFSZ COUNT_RESPONSE2,1,1
        GOTO BACK_DELAY_1_L2
        DECFSZ COUNT_RESPONSE3,1,1
        GOTO BACK_DELAY_1_L2
        MOVLW 0X01
        MOVWF PORTC
        GOTO UPPER_STAIRCASE
LOOP_END_4:
        GOTO END_ALL;
;********************************************
UPPER_LIMIT_REACHED_LOWER:
        GOTO UPPER_LIMIT_REACHED
LOWER_LIMIT_REACHED_LOWER:
        GOTO LOWER_LIMIT_REACHED
END_ALL:
        MOVFF UTH_W,WREG
        CALL DYNAMICDISPLAY
        MOVLW 0X11
        SUBWF PORTC,0,0
        MOVFF LTH_W,WREG
        CALL DYNAMICDISPLAY
        MOVLW 0X03
        MOVWF PORTC,0
        GOTO END_ALL
;****************************************************************

WELCOME:
; SET DDRAM ADDRESS START AS 05
```

```
        MOVLW 0X85
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
; S
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X53
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; U
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X55
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; S
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X53
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; S
        MOVLW 0X21
```

```
        MOVWF PORTC,0
        MOVLW 0X53
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; E
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X45
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; X
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X58
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; SET DDRAM ADDRESS START AS 43
        MOVLW 0XC3
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
; T
        MOVLW 0X21
        MOVWF PORTC,0
```

```
        MOVLW 0X54
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; A
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X41
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; S
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X53
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; T
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X54
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; E
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X45
        MOVWF PORTB,0
```

```
        NOP
        CALL REDO_WRITE
;
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X20
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; M
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X4D
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; E
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X45
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; T
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X54
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
```

```
; E
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X45
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; R
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X52
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
;NOP FOR 2 SECS
        MOVLW 0XF0
        MOVWF COUNT_RESPONSE1
        MOVWF COUNT_RESPONSE2
        MOVLW 0X09
        MOVWF COUNT_RESPONSE3
BACK_WELCOME:  NOP
                        DECFSZ COUNT_RESPONSE1,1,1
                        GOTO BACK_WELCOME
                        DECFSZ COUNT_RESPONSE2,1,1
                        GOTO BACK_WELCOME
                        DECFSZ COUNT_RESPONSE3,1,1
                        GOTO BACK_WELCOME
; DISPLAY CLEAR
        MOVLW 0X01
        MOVWF PORTB,0
```

```
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY1.53
        CALL CONTROL_RESET
        RETURN
;******************************************************************************
****;TT:
; SET DDRAM ADDRESS START AS 00
        MOVLW 0X80
        MOVWF PORTB,0
        NOP
        MOVLW 0X82
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
;T
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X54
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
;T
        MOVLW 0X54
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
;******************************************************************************
****
DYNAMICDISPLAY:
```

```
        MOVLW 0X00
        SUBWF DISPLAY,0
        BZ DISPLAY000
        GOTO NEXT2
DISPLAY000:
        CALL ADDRESS_HUNDREDS
        CALL DISPLAY0
        CALL ADDRESS_TENS
        CALL DISPLAY0
        CALL ADDRESS_UNITS
        CALL DISPLAY0
        RETURN
NEXT2:  MOVLW 0X01
        SUBWF DISPLAY,0
        BZ DISPLAY001
        GOTO NEXT3
DISPLAY001:
        CALL ADDRESS_HUNDREDS
        CALL DISPLAY0
        CALL ADDRESS_TENS
        CALL DISPLAY0
        CALL ADDRESS_UNITS
        CALL DISPLAY2
        RETURN
; *** codes omitted – repetition till below ***;
NEXT256:        MOVLW 0XFF
        SUBWF DISPLAY,0
        BZ DISPLAY0FF
DISPLAY0FF:
        CALL ADDRESS_HUNDREDS
```

```
        CALL DISPLAY4
        CALL ADDRESS_TENS
        CALL DISPLAY0
        CALL ADDRESS_UNITS
        CALL DISPLAY0
        RETURN
;*****************************************************************
DISPLAY0:;0
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X30
        MOVWF PORTB,0
        NOP
        MOVLW 0XA1
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        MOVLW 0X21
        MOVWF PORTC,0
        RETURN

DISPLAY1:;1
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X31
        MOVWF PORTB,0
        NOP
        MOVLW 0XA1
        MOVWF PORTC,0
        CALL DELAY39
```

```
        CALL CONTROL_RESET
        MOVLW 0X21
        MOVWF PORTC,0
        RETURN
DISPLAY2:;2
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X32
        MOVWF PORTB,0
        NOP
        MOVLW 0XA1
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        MOVLW 0X21
        MOVWF PORTC,0
        RETURN
DISPLAY3:;3
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X33
        MOVWF PORTB,0
        NOP
        MOVLW 0XA1
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        MOVLW 0X21
        MOVWF PORTC,0
        RETURN
```

```
DISPLAY4:;4
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X34
        MOVWF PORTB,0
        NOP
        MOVLW 0XA1
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        MOVLW 0X21
        MOVWF PORTC,0
        RETURN
DISPLAY5:;5
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X35
        MOVWF PORTB,0
        NOP
        MOVLW 0XA1
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        MOVLW 0X21
        MOVWF PORTC,0
        RETURN
DISPLAY6:;6
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X36
```

```
        MOVWF PORTB,0
        NOP
        MOVLW 0XA1
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        MOVLW 0X21
        MOVWF PORTC,0
        RETURN
DISPLAY7:;7
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X37
        MOVWF PORTB,0
        NOP
        MOVLW 0XA1
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        MOVLW 0X21
        MOVWF PORTC,0
        RETURN
DISPLAY8:;8
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X38
        MOVWF PORTB,0
        NOP
        MOVLW 0XA1
        MOVWF PORTC,0
```

```
        CALL DELAY39
        CALL CONTROL_RESET
        MOVLW 0X21
        MOVWF PORTC,0
        RETURN
DISPLAY9:;9
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X39
        MOVWF PORTB,0
        NOP
        MOVLW 0XA1
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        MOVLW 0X21
        MOVWF PORTC,0
        RETURN
;*****************************************************************
; SET DDRAM ADDRESS START AS ROW 2 COLUMN 2
ADDRESS_HUNDREDS:
        CALL CONTROL_RESET
        MOVLW 0X83
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        RETURN
```

```
; SET DDRAM ADDRESS START AS R2, C3
ADDRESS_TENS:
        CALL CONTROL_RESET
        MOVLW 0X84
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        RETURN
; SET DDRAM ADDRESS START AS R2, C4
ADDRESS_UNITS:
        CALL CONTROL_RESET
        MOVLW 0X85
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        RETURN
;*****************************************************************
DEBOUNCE_UPPER:
        MOVLW 0X05
        MOVWF COUNT_RESPONSE1
CHECK_AGAIN_U:  MOVLW 0X11
        SUBWF PORTC,0
        BZ TRUE_RESPONSE_U
        GOTO FALSE_RESPONSE_U
```

```
TRUE_RESPONSE_U:
        MOVLW 0X01
        SUBWF COUNT_RESPONSE1,1
        BZ END_DEBOUNCE_U
        GOTO CHECK_AGAIN_U
END_DEBOUNCE_U:
        RETURN
FALSE_RESPONSE_U:
        GOTO RESPONSE_NO_U
DEBOUNCE_LOWER:
        MOVLW 0X05
        MOVWF COUNT_RESPONSE1
CHECK_AGAIN_L: MOVLW 0X11
        SUBWF PORTC,0
        BZ TRUE_RESPONSE_L
        GOTO FALSE_RESPONSE_L
TRUE_RESPONSE_L:
        MOVLW 0X01
        SUBWF COUNT_RESPONSE1,1
        BZ END_DEBOUNCE_L
        GOTO CHECK_AGAIN_L
END_DEBOUNCE_L:
        RETURN
FALSE_RESPONSE_L:
        GOTO LOOP_NO_LOWER
DELAY39:
        MOVLW 0XDF
        MOVWF DELAY1
BACK2:  DECFSZ DELAY1,1
        GOTO BACK2
```

```
        RETURN
DELAY1.53:
        MOVLW 0XFF
        MOVWF DELAY1
        MOVLW 0XFF
        MOVWF DELAY2
BACK3: NOP
        DECFSZ DELAY1,1
        GOTO BACK3
        DECFSZ DELAY2,1
        GOTO BACK3
        RETURN
CONTROL_RESET:
        MOVLW 0X01
        MOVWF PORTC
        RETURN
REDO_WRITE:
        MOVLW 0XA0
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        MOVLW 0X20
        MOVWF PORTC,0
        RETURN
DISPLAY_UPPER_LIMIT_REACHED:; SET DDRAM ADDRESS START AS 02
        MOVLW 0X82
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
```

```
        CALL DELAY39
        CALL CONTROL_RESET
; U
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X55
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; P
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X50
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; P
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X50
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; E
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X45
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
```

```
; R
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X52
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
;
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X20
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; L
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X4C
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; I
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X49
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; M
        MOVLW 0X21
```

```
        MOVWF PORTC,0
        MOVLW 0X4D
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; I
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X49
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; T
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X54
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
LOOPING_U: NOP
        GOTO LOOPING_U
        RETURN
DISPLAY_LOWER_LIMIT_REACHED:    ; SET DDRAM ADDRESS START AS 02
        MOVLW 0X82
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
```

```
; L
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X4C
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; O
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X4F
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; W
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X57
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; E
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X45
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; R
        MOVLW 0X21
```

```
        MOVWF PORTC,0
        MOVLW 0X52
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
;
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X20
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; L
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X4C
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; I
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X49
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; M
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X4D
```

```
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; I
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X49
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; T
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X54
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
LOOPING_L: NOP
        GOTO LOOPING_L
        RETURN
DISPLAYFP:; SET DDRAM ADDRESS START AS R2, C2
        MOVLW 0XC2
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
; F
        MOVLW 0X21
```

```
        MOVWF PORTC,0
        MOVLW 0X46
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; P
        MOVLW 0X50
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
 DISPLAYFPSCORE:; SET DDRAM ADDRESS START AS R2, C5
        MOVLW 0XC5
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET

        MOVLW 0X00
        SUBWF FPSCORE,0
        BZ FPDISPLAY00
        GOTO NEXT2FP
FPDISPLAY00:
        CALL ADDRESS_TENS_FP
        CALL DISPLAY0
        CALL ADDRESS_UNITS_FP
        CALL DISPLAY0
        RETURN
NEXT2FP:    MOVLW 0X01
```

```
        SUBWF FPSCORE,0
        BZ FPDISPLAY01
        GOTO NEXT3FP
FPDISPLAY01:
        CALL ADDRESS_TENS_FP
        CALL DISPLAY0
        CALL ADDRESS_UNITS_FP
        CALL DISPLAY1
        RETURN
NEXT3FP:    MOVLW 0X02
        SUBWF FPSCORE,0
        BZ FPDISPLAY02
        GOTO NEXT4FP
FPDISPLAY02:
        CALL ADDRESS_TENS_FP
        CALL DISPLAY0
        CALL ADDRESS_UNITS_FP
        CALL DISPLAY2
        RETURN
NEXT4FP: MOVLW 0X03
        SUBWF FPSCORE,0
        BZ FPDISPLAY03
        GOTO NEXT5FP
FPDISPLAY03:
        CALL ADDRESS_TENS_FP
        CALL DISPLAY0
        CALL ADDRESS_UNITS_FP
        CALL DISPLAY3
        RETURN
NEXT5FP: MOVLW 0X04
```

```
        SUBWF FPSCORE,0
        BZ FPDISPLAY04
        GOTO NEXT6FP
FPDISPLAY04:
        CALL ADDRESS_TENS_FP
        CALL DISPLAY0
        CALL ADDRESS_UNITS_FP
        CALL DISPLAY4
        RETURN
NEXT6FP: MOVLW 0X05
        SUBWF FPSCORE,0
        BZ FPDISPLAY05
        GOTO NEXT7FP
FPDISPLAY05:
        CALL ADDRESS_TENS_FP
        CALL DISPLAY0
        CALL ADDRESS_UNITS_FP
        CALL DISPLAY5
        RETURN
NEXT7FP: CALL DISPLAY_FP_ERROR
        RETURN
ADDRESS_TENS_FP:
        CALL CONTROL_RESET
        MOVLW 0XC4
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
```

```
        RETURN
ADDRESS_UNITS_FP:
        CALL CONTROL_RESET
        MOVLW 0XC5
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
        RETURN
DISPLAY_FP_ERROR:; SET DDRAM ADDRESS START AS R2, C5
        MOVLW 0XC5
        MOVWF PORTB,0
        NOP
        MOVLW 0X81
        MOVWF PORTC,0
        CALL DELAY39
        CALL CONTROL_RESET
; E
        MOVLW 0X21
        MOVWF PORTC,0
        MOVLW 0X45
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; R
        MOVLW 0X52
        MOVWF PORTB,0
        NOP
```

```
        CALL REDO_WRITE
; R
        MOVLW 0X52
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; O
        MOVLW 0X4F
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
; R
        MOVLW 0X52
        MOVWF PORTB,0
        NOP
        CALL REDO_WRITE
        GOTO DISPLAY_FP_ERROR
        RETURN
END
```

APPENDIX 2

EFFECT OF FOOD ON ELECTROGUSTOMETRIC THRESHOLD

INTRODUCTION

Food forms one of the major tastants in normal day to day life. It is important to understand how this effects the electrogustometric threshold. It is known that different kinds of cuisines effects taste differently - some are hot, some starchy etc. Electrogustometry provides researchers a tool to quantify taste. This quantified taste allows comparison of data on a level plane. In stead of differentiating food based on its quality, electrogustometry offers the unique option to differentiate food based on quantity. This chapter discusses a small experiment conducted to understand how the electrogustometric thresholds were affected by consumption of different kinds of food.

A study conducted by Sardana et al. concluded that people habituated to an Indian diet have and average electrogustometric threshold of approximately 30.2 μA . This study was carried out in India between 1965 and 1972 and involved more than 300 participants. This study also reported that the average taste threshold for smokers were higher than that of non-smokers.

Taste studies conducted across the world with various electrogustometers report different average taste threshold. This can be attributed to the different stimulus duration and electrodes used. Dietary habits of the subject are also key in the different threshold levels measured. As summarised by Sardana et al. the normal electrogustometric thresholds measured by various machines were as detailed in the table below.

Study group	Average electrogustometric threshold (μA)
Krarup	5.75 – 300
Bull	35
Peries & Miles	30
Sardana	30.2

Table 4: Electrogustometric threshold measured by various machines

It would be interesting to study the immediate effect of certain cuisines on a population not used to such diets. The study reported in this chapter details the effect of Chinese and Indian food on three British subjects.

EXPERIMENT

10.2.1 SUBJECTS

Three students from the University of Sussex volunteered to participate in this study.

10.2.2 TEST EQUIPMENT

The Sussex Electrogustometer was used in its automatic mode to determine the electrogustometric threshold.

10.2.3 PROCEDURE

Electrogustometric threshold was measured for the three subjects after which they were given a Chinese meal sourced from a local restaurant. The subjects had not consumed any food or drink for up to two hours before the test. Taste threshold was measured for up to an hour after the consumption of the food at fifteen minute intervals. The following day the test was repeated using Indian food sourced from a local restaurant.

10.2.4 RESULTS

The average electrogustometric thresholds of the three participants were plotted in the graph below.

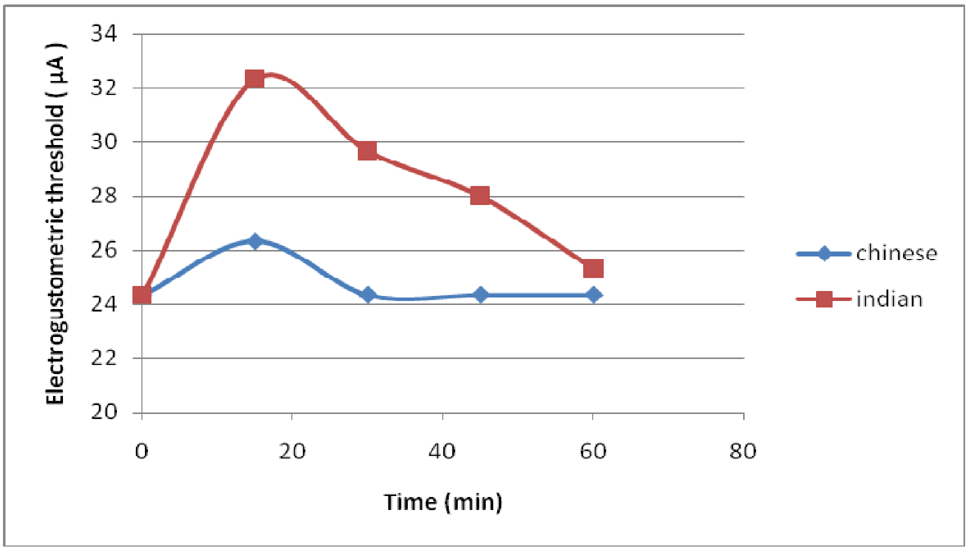

Fig 24: The effect of Indian and Chinese food on electrogustometric threshold

10.2.4 DISCUSSION

From the above result it is apparent that Indian food affects the taste threshold more as compared to Chinese food. It is important to note that the subjects were acclimatised to British food and hence both these diets were equally foreign. Indian food is spicy and affected the taste threshold more than the Chinese food. Monosodium glutamate is found in Chinese food and is said to alter taste slightly.

CONCLUSION

The study reported in this chapter concludes that the immediate effect of Indian food on electrogustometric threshold is greater than Chinese food.

First Experiences with the Sussex Electrogustometer

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Abstract

In spite of electrogustometry having being practiced since the 1950s, it is not a commonly used clinical tool. Various factors such as lack of standardisation in procedure have inhibited the growth of this technique of assessing the human taste function. However, with advancements in technology, a state of the art, semi-automated, battery-powered stand-alone electrogustometer has been designed and tested successfully at the University of Sussex. The Sussex Electrogustometer has been compared with the RION TR06, the current market standard, for reliability and repeatability. A high degree of correlation of 0.94 was observed in the taste threshold of 20 normal subjects measured using both the RION TR06 and the Sussex Electrogustometer. Further studies were carried out to study successfully the repeatability of the Sussex Electrogustometer. The test-retest data for the machine also showed a high degree of correlation of 0.91. We are confident that the Sussex Electrogustometer will be a viable instrument in the clinical environment and make electrogustometry a common clinical tool.

Keywords: Taste, electrogustometry.

INTRODUCTION

Taste is one of the least studied senses. The minimum amount of stimulus required to arouse a gustatory response is called the taste threshold. There are two principal methods to measure this – chemogustometry and electrogustometry. Chemogustometry involves the application of chemical tastants of varying strengths to the oral mucosa. The tastant can either rinse the whole of the oral mucosa or can be applied to a part of it using filter paper or tablets. [1, 2, 3, 4] Electrogustometry involves application of a pulse of regulated constant direct anodal current to the oral mucosa for a predefined duration as the stimulus to evoke gustatory potentials. The electrogustometric taste threshold is said to have been reached when the minimum current level for which there is a positive response of gustatory sensation from the subject has been determined. [5] Both chemogustometry and electrogustometry are essentially subjective tests and hence psychophysical analysis of a subject's behaviour is important. Knowledge of the taste function is used to study taste loss caused by, for instance, age, tonsillectomy, laryngomicrosurgery, middle ear surgery, Bell's palsy and diabetes. [6, 7, 8, 9]

Electrical stimuli have been used to measure taste threshold since the 1950s. [5, 10] Electrogustometry is now fast becoming an established clinical tool, but there is a need to standardise test procedure and automate wherever possible. The taste function derived from electrogustometry is especially important in determining the integrity of the neural pathway. [11] Electrogustometry quantifies taste and measures the threshold of this sensation. This is independent of the quality or nature of the taste. Chemogustometry on the other hand can help determine various taste types – such as sweet, sour, bitter, salty and umami i.e. a more qualitative approach. The taste perceived in electrogustometry is sour metallic and has been attributed to the absorption of the protons liberated by the electric stimuli. [12] However, this perception of sour taste has been reviewed and no direct relation between the sour taste and electrical stimulus could be determined. [13]

The electrogustometer most commonly in use is the RION TR06. This is a manually operated, stand-alone, battery-powered device developed by Sensonics Inc. [17] The strengths of the RION TR06 include its speed, portability, simplicity – in application and interpretation, patient compliance and constant range of measurement. It is the first choice of clinicians to measure electrogustometric taste threshold. However it is manually operated and hence subject to human error. With advances in electronics it is now possible to design and manufacture a semi-automated stand-alone instrument for electrogustometry. Computer controlled devices have been trialled. [14, 15] However, since they are not easily portable and are essentially connected to the mains power supply, the RION TR06 remains the current market standard for electrogustometry.

The Sussex Electrogustometer is a stand alone, battery-powered semi-automatic device used to measure taste threshold. It has two modes of operation - manual and automatic. The manual mode is commonly used to train the subject and to offer any specific current stimulus if needed during a study. A pre-programmed alternate forced-choice double-staircase algorithm is used to determine the magnitude of the current stimulus in the automatic mode. The current stimulus is applied for a fixed duration which is pre-defined by the user. The automatic mode also provides random blank stimuli (false positives) to check for subject reliability and to screen for malingerers. LED and buzzer annunciators are used to alert the subject and users to different events detailed later. The Sussex Electrogustometer can provide a constant anodal current stimulus from 0 μA to 500 μA in steps of 1 μA. A study was carried out to determine the reliability and repeatability of this new machine.

DETAILS OF THE SUSSEX ELECTROGUSTOMETER

The Sussex Electrogustometer is a microprocessor-based, semi-automatic instrument designed to determine electrogustometric threshold by application of constant regulated direct current stimuli. The main functional blocks of the machine are a digital processing unit incorporating control switches and a liquid crystal display (LCD), a digital-to-analogue converter (DAC) and a constant current source. The processing unit is a Peripheral Interface Controller (PIC). As mentioned earlier, the Sussex electrogustometer operates in two modes – manual and automatic. The manual mode, used essentially to train the subject, can apply up to eight different electric current stimuli for various durations. This mode can presently generate current stimuli of 5, 25, 50, 100, 200, 300, 400, 500 µA on a linear scale which are expressed as -2.7, 3.3, 16.1, 21.9, 28.0, 31.5, 34 and 35.9 decibels in logarithmic units. These stimuli can be applied to the subject for eight different durations 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 and 2.5 seconds. The appropriate current level and stimulus duration are chosen by the user from a menu shown on the LCD by using button switches available on the front panel. The automatic mode of operation is based on an alternate forced-choice double-staircase algorithm. One staircase starts at 10 µA while the other starts at 40 µA. The automatic mode of operation has an initial step size of 20 µA which reduces to 1 µA. The algorithm comes to an end when there is a difference of 3 µA or less between the two staircases for at least three consecutive iterations. The current stimuli strengths in the manual mode, the stimulus duration, step size and starting values of the staircase in the automatic mode can be easily changed by reprogramming the PIC.

The PIC produces an eight-bit digital output, which is converted to an equivalent voltage by the DAC. This analogue voltage is used as an input by the voltage-controlled constant-current source. The microcontroller also produces various control signals for the LCD, annuntiator and DAC. The reference voltage for the DAC is accurately maintained at 5 V by a regulator.

The measurement of taste using electrogustometry is essentially subjective. The automatic mode of the Sussex electrogustometer applies occasional random blank stimuli to detect malingerers and to assess subject reliability. A score of false positive hits is maintained and is made available on the LCD. If this score gets to be too high the subject is re-briefed and the test is repeated. The subject response to applied stimulus is recorded using a hand held feedback switch, which is pressed when the subject feels a distinct sour-metallic taste. This active-high signal is directly fed back to the microcontroller, which updates the algorithm to generate the next stimulus. The magnitude of the step size halves every time the direction of the current function changes. The Sussex Electrogustometer also has different annuntiators. LED and buzzer based annuntiators are used to alert the subject before a stimulus is applied. The end of the test is also signalled using the LED based annuntiator. The 16 rows, 2 columns LCD displays variously the mode of operation, current stimulus applied to the subject in both decibels and microamperes plus the false positive score. It also shows the different stimulus duration and mode of operation options and notifies the end of the test.

The output end of this instrument is a voltage-controlled constant-current source. This is an operational amplifier based device with a transistor buffer. The Sussex Electrogustometer has very high output impedance of about 25 MΩ and can generate up to 500 µA in steps of 1 µA. The circular electrode used to apply the stimulus is a stainless steel and flexible device. It is specially designed to ensure that it remains flat on the tongue surface at most times. The electrical return path is provided by a small connector pad similar to the ones used in electrocardiography, applied to the neck area with electro-conductive gel to ensure good electrical conductivity.

OPERATING PROCEDURE:

1. The electrode, return path and feedback switch are connected to the Sussex Electrogustometer and then the power switch is turned on.
2. The LCD Displays "Sussex Taste Meter".
3. After two seconds, the LCD shows the operation modes – manual and automatic. The roll push button switch is used to switch between the modes and the select push button is used to select either of the two modes.
4. It is recommended that the manual mode be selected first. This will help train the subject. The roll push button can be pressed to see various current and stimulus duration options. The subject is then briefed about the signal detection strategy and the return path and electrodes are placed in their positions. The hand-held feedback switch is also given to the subject. The necessary selection is done and the required current stimulus is applied to the subject. The subject gives a response based on the signal detection strategy explained.
5. After the subject has been trained, suitable selection is made to roll over to the automatic mode of operation.
6. When the machine enters the automatic mode of operation, the user cannot control the value of current stimulus. A pre-defined alternate forced-choice double-staircase algorithm determines this. The user can, however, choose the duration for which the stimulus is to be applied. A set of choices, detailed previously, is made available before the start of this mode. Selection of a particular duration is made with the select and roll push buttons on the front panel.
7. Before the current stimulus is applied to the subject, a LED and buzzer annuntiator are triggered. This alerts the subject. If a distinct sour-metallic taste is perceived, the

subject presses his hand held feedback switch. If there is no such sensation, the feedback switch is not pressed. The machine waits for up to three seconds in the automatic mode before applying the next stimulus. The buzzer is sounded twice as an acknowledgement of a positive response.

8. When the staircase has been completed, the LED and buzzer are turned on for a continued period and the LCD displays the end of the test as "END".
9. The taste threshold value and false positive score shown on the LCD are then recorded.
10. If the false positive score is too high the subject is re-briefed and the test is repeated.

Two experiments were carried out to assess the reliability and repeatability of the Sussex Electrogustometer.

MATERIALS & METHOD

Subjects

Twenty healthy subjects were recruited from the students and staff at the University of Sussex. Nine of them were male and eleven were female of age range of 22 to 70 years, their mean age being 36.2 years.

Test Equipment

The Sussex Electrogustometer and the RION TR06 provided the electric stimuli.

Procedure

The taste thresholds of the 20 subjects were measured using the Sussex Electrogustometer, operating in its automatic mode, and the RION TR06 respectively. A circular stainless steel electrode of 28.5 mm^2 area was used in both the tests and was placed at 1.5 cm posterior to the tongue tip and 1.5 cm from the left margin of the tongue. The stimulus was applied for two seconds. The subjects were initially briefed about the instruments. The manual mode of the Sussex Electrogustometer was used to train them. After two weeks, the same set of subjects was tested again using the Sussex Electrogustometer.

RESULTS

The taste threshold results were compared (Fig 1) and a high degree of correlation (r = 0.94) between the Sussex Electrogustometer and the RION TR06 was observed. A high degree of correlation (r = 0.91) between the test/retest data of the Sussex Electrogustometer (Fig 2) was also observed.

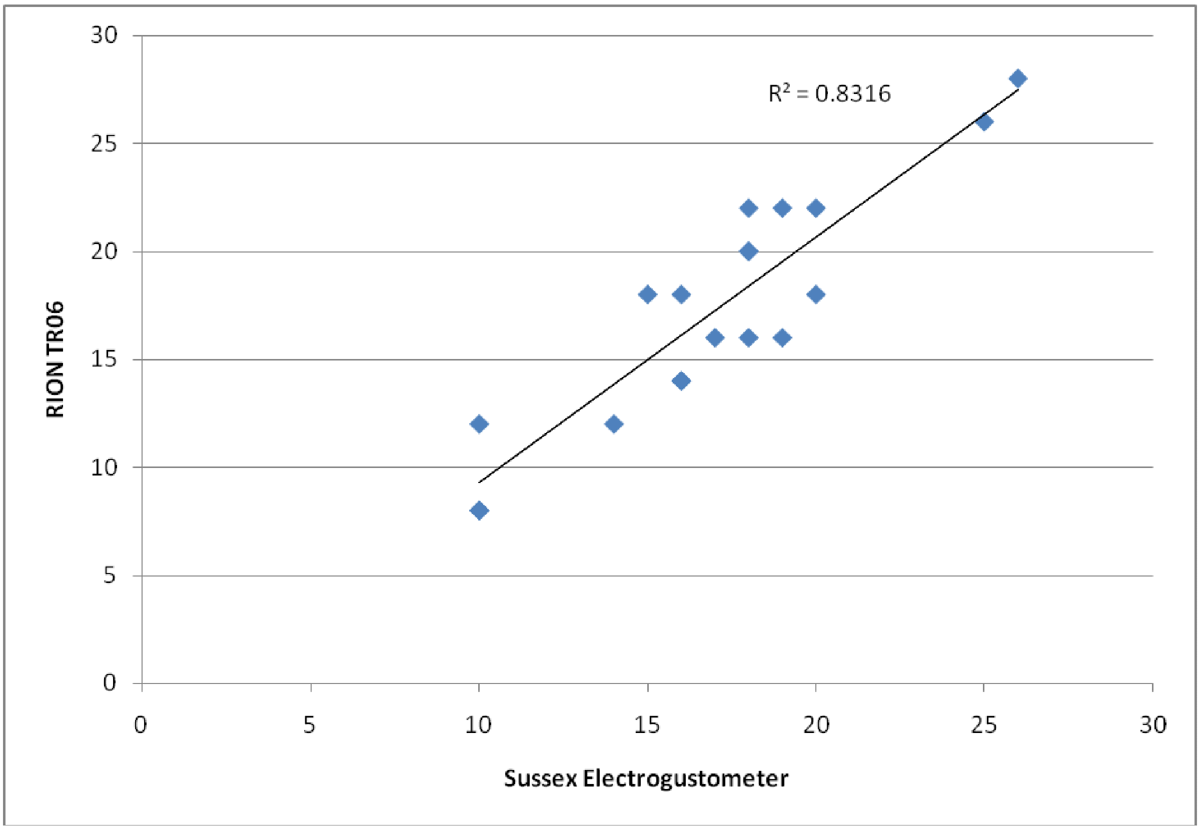

Figure 1: Comparison of RION TR06 and Sussex Electrogustometer

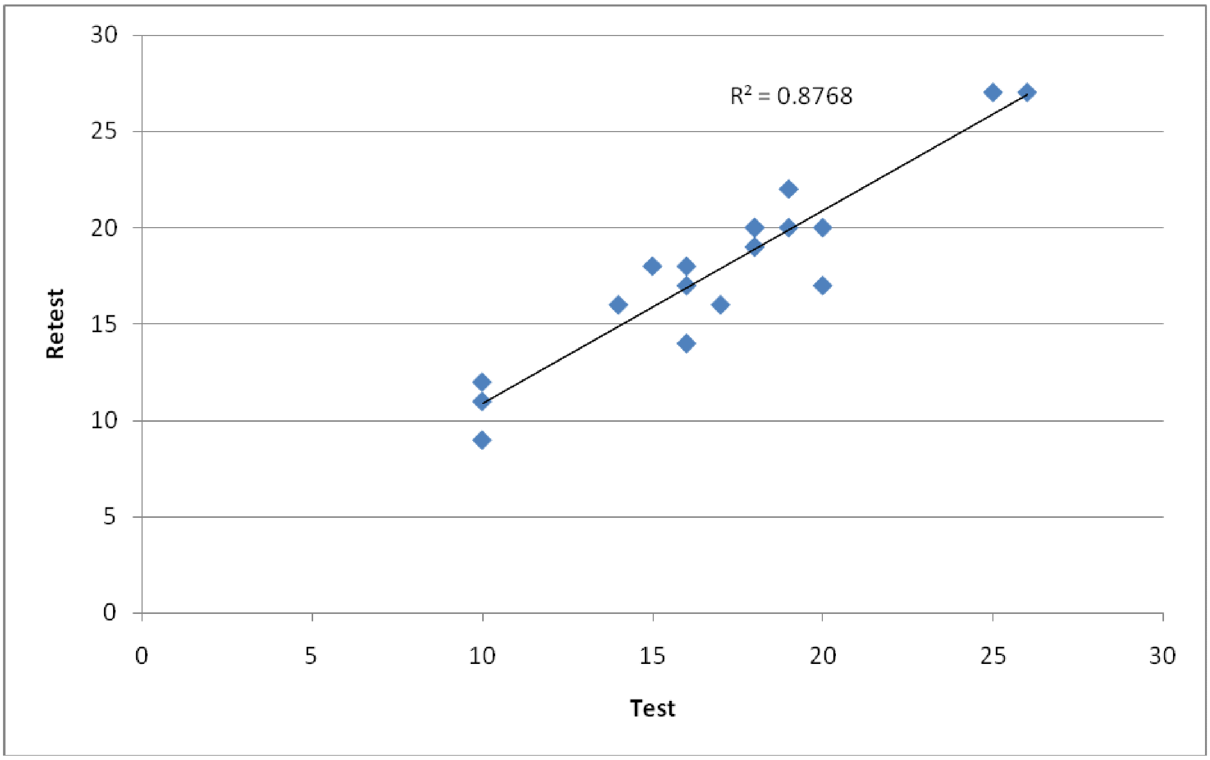

Figure 2: Test Re-test for Sussex Electrogustometers

DISCUSSION

The taste threshold data of the 20 subjects using the RION TR06 and the Sussex Electrogustometer show a high degree of correlation. The reliability of the RION TR06 has been extensively studied. [16] The high correlation hence establishes the reliability of the Sussex Electrogustometer. The test/retest data also show a high degree of correlation implying the repeatability of the Sussex Electrogustometer.

The Sussex Electrogustometer is the first semi-automated, battery-operated, stand alone electrogustometer. It is a microcontroller based device with an inbuilt false positive test operating in two modes. The test times are short: the machine arrives at the taste threshold after a few stimuli depending on the subject's response, using a pre-programmed alternate forced-choice double-staircase algorithm. The taste threshold also depends on factors such as stimulus duration and electrode area. We are working towards a recommended test procedure which will maximise the accuracy and reliability of electrogustometry.

CONCLUSION

The Sussex Electrogustometer offers an advance in automated electrogustometry and is aimed towards establishing electrogustometry as a common clinical tool.

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The Effect of Stimulus Duration and Electrode Area on Electrogustometric Taste Threshold

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Abstract

In spite of electrogustometry having been in existence since the 1930s, there is no standard method to measure taste threshold. Factors like stimulus duration and area of electrode affect the subject's response and hence a control over the modality in which the stimulus is applied is important. Electric current stimuli of varying durations were applied to 20 subjects using the Sussex Electrogustometer. [1] The stimulus durations used were in the range of 0.5 to 2.5 seconds. Hand-held stainless steel electrodes of three sizes were used. (12.5, 28.5 and 50 mm^2) It was observed that there is little variation in taste threshold with stimulus duration in the ranges of 0.5 to 1.0 and 2.0 to 2.5 seconds, irrespective of electrode area. The taste threshold function decreases monotonically with durations of 1.0 to 2.0 seconds. Given that small durations imply large currents and large currents evoke somatosensory responses, a stimulus duration of at least two seconds is recommended.

A further experiment was done to determine the effect of electrode area on electrogustometric taste threshold. Six circular electrodes of different sizes in the range 3.14 to 113 mm^2 were used to measure the taste threshold of the 20 subjects with stimuli of two seconds duration. The results

indicate a linear relationship between taste threshold and electrode radius. Since small electrodes evoke somatosensory response, an electrode of size at least 3 mm radius, 28.5 mm^2 area is recommended.

Keywords: Taste, electrogustometry, stimulus duration, electrode area

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INTRODUCTION

The application of an electric stimulus to evoke gustatory response and the measurement of the threshold of such a response is called electrogustometry and a logarithmic scale is employed to mirror the typical human response. Electrogustometric taste threshold depends on the quantified taste function of which it is a measure. However, physical constraints also affect the results of this subjective test. The main physical factors on which taste threshold depends are the duration for which the current stimulus is applied and the size of the electrode used. Hence spatial and temporal control of the stimulus is of prime importance to ensure standardisation of the examination. [2] To help standardise electrogustometry, an understanding of the effects of stimulation duration and electrode area on taste threshold is important.

Bujas studied the effect of stimulus duration on taste threshold for one subject and concluded that it reached an asymptote at 1.0 s. [3] Fons and Osterhammel observed with three subjects that the taste threshold decreased with a pulse duration in the range of 2 to 150 ms and remained constant after that. [4] Stillman et al commented that the taste threshold was higher for 0.75 s pulse duration than 0.5 s. Nine subjects were involved in this study. [5] Loucks & Doty used the RION TR-06 to establish the taste threshold of twelve male and twelve female subjects with stimulus duration of 0.5 s, 1.0 s and 1.5 s, and found a minimum value at 1.0 second. The trend observed by them was inexplicably non-monotonic. [2] A further experiment using the Halle II, a computer controlled electrogustometer, showed that taste threshold remained unchanged with stimulus duration in the range of less than 0.75 s and greater than 2.0 s and decreased in the region between them. [6] An in-depth study is needed to establish the exact relationship between stimulus duration and taste threshold since none of these studies has produced a model to explain the results and some results are contradictory.

The sour metallic taste perceived in taste measurement using an electrogustometer may be attributed to the liberation of protons from the electrode on the tongue surface. [5] For a constant electrode size, the number of protons liberated will depend on the intensity of the pulse and the duration for which the current is applied. Thus, establishing the relationship between stimulus duration and taste threshold is essential to determine standardized testing parameters. Increased pulse duration would imply an increased liberation of protons on the oral mucosa thus increasing the intensity of the stimulus. However, this is not the case throughout the stimulus duration spectrum. After a certain value of pulse duration, its effect on taste threshold saturates as noted in some studies mentioned previously. This implies that the protons have a limited lifetime before they revert to being hydrogen. [7]

The area of the circular electrode is also a contributing factor to the electrogustometric taste threshold. When a current stimulus is applied using an electrode, the effective area of the tongue on which the stimulus acts is slightly larger than the actual electrode size. [2] If the electrode area is too small, somatosensory responses are evoked along with gustatory response and hence it has been recommended that electrodes with very small areas should not be used. [8] The process for determining taste threshold involves application of stimuli both higher and lower than this threshold. Thus it is important to understand whether the gustatory response evoking factor is current intensity or current density. This can be determined by studying the effect of electrode area. Ajdukovic concluded that gustatory response tends to increase with stimulation area for a fixed current intensity. This was, however, not noted for very small electrode areas. Thus he suggested that larger electrode areas are better. [9] Adjukovic, in another experiment, concluded that there is a power function relationship given by $I = 54.4\ A^{0.267}$, where I is the current intensity (µA) and A is the electrode area (mm^2). [10]

The conflicting observations by previous authors suggested that a further study is required to determine the effect of stimulus duration and electrode area on electrogustometric taste threshold. We used the Sussex Electrogustometer, a newly developed, semi-automatic machine developed at the University of Sussex. This machine is battery operated and RoHS compliant. Its automatic mode produces constant current stimuli of pre-determined values using a double- staircase algorithm. A buzzer and warning lamp are used as annunciators to alert the subject and shortly afterwards the current pulse is applied. If the subject senses any taste, he presses a hand-held feedback switch. Blank stimuli are also applied randomly to determine subject reliability and to screen for malingerers. A manual mode of operation is available for subject training. [1]

MATERIALS & METHOD

Subjects: Twenty healthy subjects were recruited from the students at the University of Sussex. Nine of them were male and eleven were female of age range of 22 to 40, their mean age being 28.4. A brief medical history of the subjects was recorded prior to the test. No significant medical conditions were noted in any of the subjects which might suggest an abnormal electrogustometric taste threshold. A few subjects were mild consumers of alcohol and tobacco.

Test Equipment: The electric stimuli were produced by the Sussex Electrogustometer operating in the Automatic mode.

Procedure:

To determine the effect of stimulus duration on taste threshold: The hand-held stainless steel electrode of area 12.5 mm^2 was placed at 1.5 cm posterior to the tongue tip and 1.5 cm from the left margin of the tongue. The electrogustometric taste thresholds were measured for pulse durations of 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0 and 2.5 seconds. The subjects had abstained from food or drink an hour before the test. Subjects were asked to repeat the test should there have been more than two false positives. The tests were repeated with electrodes of different sizes - 28.5 and 50 mm^2.

To determine the effect of electrode area on taste threshold: Taste threshold was measured using stainless steel electrodes of six sizes - 3.14, 12.5, 28.5, 50, 78.5 and 113 mm^2. The electrodes were positioned as described in the above section. The stimulus duration for this experiment was two seconds.

RESULTS

Effect of stimulus duration on taste threshold:

Figure 1 shows the graph of the mean taste threshold of the 20 subjects with respect to stimulus duration. It shows very little variation in the range of 0.5 to 1.0 seconds. There is a monotonic decrease for durations of 1.0 to 2.0 seconds and no significant change in the range of 2.0 to 2.5 seconds. Similar results were obtained for the three different electrode areas.

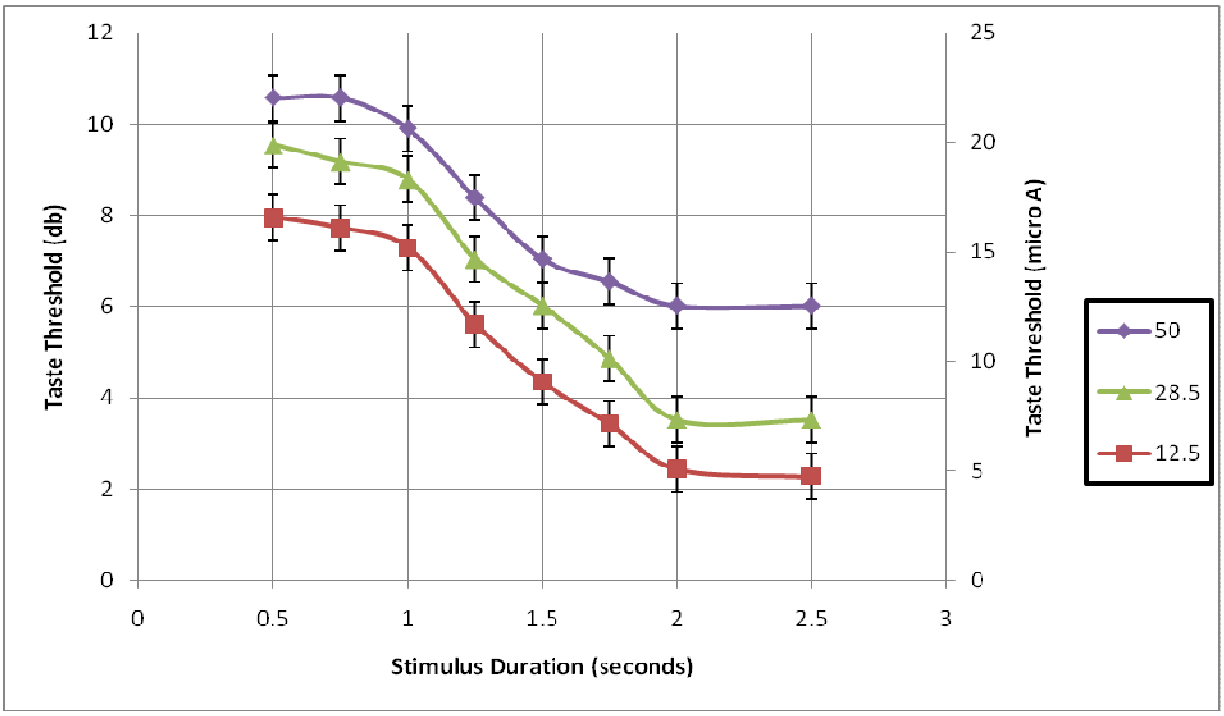

Fig 1. Effect of stimulus duration on taste threshold

Effect of Electrode Area on taste threshold:

Analysis of the mean taste threshold for the 20 subjects showed a generally linear increase with electrode radius as illustrated in Figure 2. The slight deviation for small electrode radius is probably due to somatosensory effects.

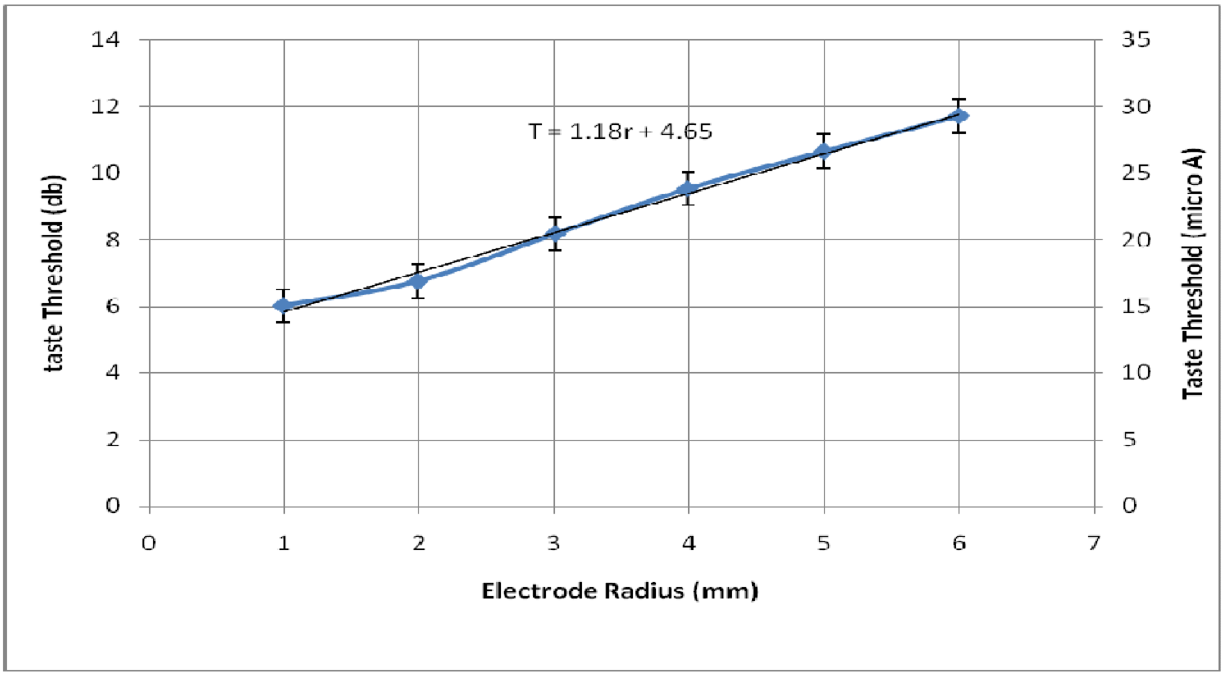

Fig 2. Effect of electrode radius on taste threshold.

DISCUSSION

From the test results it may be concluded that the effect of stimulus duration on electrogustometric taste threshold is minimal for durations of up to one second. Both somatosensory and gustatory responses are evoked in this region. The additive effect of the two evoked responses constitute the overall subject response. With increase in stimulus duration from 0.5 s to 1.0 s, the gustatory response increases and the somatosensory response decreases and hence the total response remains almost the same. As mentioned earlier, the somatosensory response may be attributed to the larger currents required for smaller stimulus durations. [10]

The taste threshold decreases linearly when the stimulus duration is greater than one second and less than two seconds. This decrease in threshold is due to the increased liberation of protons making the stimulus stronger. The somatosensory response is greatly diminished during this range of stimulus duration. This corresponds to the response observed by Marian in her experiment using the Halle II. [6]

The electrogustometric taste threshold for stimulus duration in the range of 2.0 to 2.5 seconds shows little variation. The current stimuli produce protons which evoke gustatory responses. These protons, however, have a finite lifetime after which they revert to hydrogen. If the stimulus duration is greater than two seconds the lifetime of these protons is exceeded so their density tends to be constant. [10] Thus, the most suitable stimulus duration for electrogustometry is at least 2.0 seconds. When electrodes of different sizes were used, a similar trend was observed. This establishes that stimulus duration affects taste threshold independently of electrode area.

Electrogustometric taste function also depends on the size of electrode used to apply the current stimulus. Adjukovic commented that taste threshold depends on current density. [10] The current study has shown that taste threshold depends on electrode radius in a linear manner according to the equation, for r greater than 2 mm, $T = 1.18\ r + 4.65$, where T is the taste threshold and r is the electrode radius in mm. Thus the taste threshold depends on current density. For smaller electrodes, the somatosensory effects are more pronounced. An electrode size of 3 mm radius or 28.5 mm^2 area is recommended as smaller electrodes evoke somatosensory response whereas larger electrodes will lack precision of position.

CONCLUSION

This study has shown that taste threshold decreases with stimulus duration in the interval of 1.0 s to 2.0 s and remains relatively unaffected if the pulse duration is greater than 2.0 s and we recommend that the stimulus duration to be used in electrogustometry should be at least 2.0 s. The study has also shown that taste threshold increases linearly with respect to electrode radius.

When the electrode area is very small it evokes somatosensory response along with gustometric response. A large electrode area will require greater current levels and result in positional imprecision. An electrode of 3 mm radius or 28.5 mm^2 area is recommended for use in electrogustometry.

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School of Life Sciences Research Governance Committee

CERTIFICATE OF APPROVAL

Title of Project	Study of the effect of alcohol on electrogustometric taste threshold
Principal Investigator	**Dr. Lionel G Ripley**
Student	**Anirban Banerjee**
Collaborators	**Prof. Theodora Duka**
Duration of approval (not greater than 4 years)	12 months

This project has been given ethical approval by the School of Life Sciences Research Governance Committee.

NB. If the actual project start date is delayed beyond 12 months of the expected start date, this Certificate of Approval will lapse and the project will need to be reviewed again to take account of changed circumstances such as legislation, sponsor requirements and University procedures.

Please note and follow the requirements for approved submissions:
Amendments to protocol.

- Any changes or amendments to approved protocols must be submitted to the committee for authorisation prior to implementation.

Feedback regarding the status and conduct of approved projects

- Any incidents with ethical implications that occur during the implementation of the project must be reported immediately to the Chair of the committee.

The principal investigator is required to provide a brief annual written statement to the committee, indicating the status and conduct of the approved project. These reports will be reviewed at the annual meeting of the committee. A statement by the Principal Investigator to the Committee indicating the status and conduct of the approved project will be required on the following date(s):

December 2009, December 2010……………………………………………………….

Signed: ………….. …Jennifer Rusted………………..
Chair of the Research Governance Committee

Date: …………7th April 2009…………….

Subject Information

Title
The Effect of Alcohol on Electrogustometric Taste Threshold

Conducted in the Engineering Laboratory, by Mr Anirban Banerjee
Investigators: Mr Anirban Banerjee, Dr Lionel G Ripley and Prof. Thoedora Duka

The aims of the study

This study is going to investigate the effects of alcohol on taste threshold.

Outline of experimental sessions

You will be asked to attend the labs in the Department of Engineering, University of Sussex. There will be up to 3 sessions a week and will take place early afternoon.
At the beginning of the session you will be asked to complete an information sheet and will be asked to consume a dosage of absolute alcohol of up to 50ml in volume (4gm to 40gm). Your taste threshold will then be measured using an electrogustometer for one hour in 10min intervals.

The amounts of alcohol you will be asked to drink

When you decide to participate in this study you should be prepared to consume an amount of alcohol that is equivalent to about 4 units (i.e., 2 pints of lager or 4 small glasses of wine) at the beginning of each experimental session. The total volume of the drink will be 50ml and will vary in alcohol concentration to 10% to 40%.

At the end of each test session we will measure your breath alcohol concentration (BAC), and you will be asked to remain in the waiting room of the laboratory until BAC levels have fallen to half of the limit below which you are legally allowed to drive (the legal limit it 0.08%; we will ask you to wait until levels have fallen to 0.04% BAC). How quickly these levels are reached varies considerably between individuals, but most participants should be able to leave the laboratory after 1 1/2 hours from the start of testing.

Anirban Banerjee
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Since we think that a BAC below 0.04% is still quite high we also require that **you agree to not drive a car or ride a motorbike or push-bike for at least two hours after completion of each test session**.

What is required to participate in the study

In order to participate in the study you need to fulfil the following requirements:

- You need to be between 18 and 40 years old.
- You need to feel a medical questionnaire
- You need to be able to give us an estimate of your average weekly alcohol consumption.

What you should avoid doing before test sessions

If you decide to participate we would like you to avoid the following:

- Drinking alcohol for at least 12 hours before each test session.
- Taking illicit drugs for one week before each test session.
- Taking sleeping pills for at least 48 hours before each test session.
- Eating a high-fat breakfast or lunch before each test session.
- Not to eat or drink anything for 1 hour before the start of each session.

Informed consent

University procedures require that you sign the consent form overleaf stating that the purposes and procedures of the study have been explained to you. **Please understand that you are free to withdraw from the study at any time.**

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Measurement of Taste

Taste forms one of the 5 major senses in the human body. It is one of the least studied senses. Taste is measured either by use of chemicals or electric pulses. Measurement of taste threshold using electric pulses is called '*Electrogustometry*'. The Sussex Taste Meter is a state-of-the-art electrogustometer. The aim of this study was to determine how electrogustometric taste threshold varies with the consumption of alcohol in a normal, non-alcoholic subject over time.

The Sussex Taste Meter measures your taste by the application controlled current through the tongue surface. A stainless steel electrode is used to apply this stimulus on the tongue surface. The magnitude of current is <500 µA. This level of current is very small and harmless. The equipment is battery powered and safe. This test is subjective and hence depends upon your response to the applied stimulus. It has been commented that heavy alcohol consumption may affect taste and smell function. It is important to understand the effect of alcohol on human taste.

If you would like to participate in the study please complete the form overleaf and return *as soon as possible* in the stamped addressed envelope enclosed.

Should you want any further information please do not hesitate to contact me.

Thanking you in anticipation.

Yours truly,

Anirban Banerjee
Postgraduate Research Student
Department of Engineering & Design
University of Sussex

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VOLUNTEER INFORMED CONSENT FORM

I have read and had explained to me the attached information sheet of which I retain a copy. The nature and purpose of the testing of alcohol administration has been explained to me by one of the investigators. I am aware that I have the right to withdraw from the experiment at any time.

I undertake to:

1. Refrain from drinking alcohol for at least 12 hours before each test session.
2. Refrain from using illicit drugs for at least 1 week before test sessions.
3. Refrain from using sleeping pills for 48 hours before test sessions.
4. Eating or drinking anything for 1 hour before the start of each session.

I give my consent for the study directors to contact my general practitioner to assess my general level of health. I understand that giving this authorisation does not commit me to participation in the study and that I am free do withdraw at any time.

Name:..

Date of birth:...

Address:...

....

Phone number:...

Signed:..

Date:..

Witnessed:..

Details of General Practitioner:

Name:

Address:

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