Analysis and Correlation Study of Human Masseter Muscle with EMG, Ultrasonography & 3D Imaging

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1 LITERATURE REVIEW

1.1 INTRODUCTION

Understanding of the craniofacial hard tissues, particularly the maxillary and mandibular skeletal and dental bases, is comprehensive with many dentally oriented studies reported in the literature. However, specific data from the facial soft tissues, such as the muscles of mastication and their function, are comparatively few. Several reasons might explain this scarcity. Firstly, the study of real-time function in human subjects needs precise measuring devices which can be economically restrictive. Secondly, the soft tissues are very dynamic with multi-factorial influences from the four different dimensions- sagittal, transverse, vertical and time.

The masseter muscle is believed to play a significant role in determining the underlying facial skeletal structure and recording the muscle form and function is relevant to understanding growth, development and orthodontic treatment effects upon the craniofacial complex.

The aim of this review is to present the masseter muscle physiology with reference to EMG studies and our current understanding of the masseter muscle spindles. The second part of this review (Chapter 2) explores different imaging technologies which have enhanced our understanding of the morphology and dynamics of the masseter. Finally, alternative methods of investigation which can be employed, such as mathematical models, will also be briefly discussed.

1.2 THE MASSETER MUSCLE

The human masticatory system has been extensively studied from anatomical, physiological and histological aspects. The masseter muscle, in particular, as one of the major jaw elevators of the masticatory system, is a skeletal muscle that responds to cortical input. However, there is a fundamental difference between the masticatory muscles and the skeletal muscles from other parts of the body due to embryological differences. Masticatory muscles arise from neural crest cells whereas skeletal muscles are derived from mesoderm. Another important distinction is that masticatory muscles are endowed with “superfast myosin” with subsequent high ATP-ase activity making them highly efficient in generating forces in short and quick succession[9].

The masseter is a complex jaw elevator muscle with a multi-pennate structure. As one of the major masticatory muscles, it provides force and precision for chewing. Moreover, it is involved in speech, facial expressions and proprioception[10-13]. Recent studies have analysed the masseter muscle with computerized tomography (CT scanning), magnetic resonance imaging (MRI) electromyography (EMG), ultrasonography (US) and 3D imaging[14-29].

1.2.1 GROSS ANATOMY OF THE MASSETER MUSCLE

Historically, the most explicit account of the masseter muscle is provided by Schumacher (1961) in Hannam[30] along with functional organization of the human masseter muscle in Gaudy *et al*.[31]. The masseter muscle is multipennate (feather-like, Latin.) with superficial, intermediate and deep fibres[32]. Pennation is also found in the medial pterygoid muscle and provides mechanical advantage with enhanced power production in spatial orientation. Sechnenspiegel (German terminology) are internal aponeuroses aligned para-sagittally which form septa between the masseter muscle groups and extend from the zygomatic arch to the mandibular border. There are generally four groups, Sechnenspiegel I-IV (Ebert, 1939,;Schumacher, 1961) in Hannam[30] in which these septa are orientated in three dimensions relative to the position of the zygomatic arch[33].

The range of movement is proportional to the length of the fibres and the product of the cosines of pennation angles which are generally at 20°[28],[27]. Details of mechanical efficiency, contraction dynamics and pressure tension have been extensively studied[34, 35] and are beyond the scope of this review.

The human masseter muscle is known to have two or three heads originating from the zygomatic arch[36]. The muscle has its insertions into the angle of the mandible and posterior border of the ramus.

1.2.2 SUPERFICIAL MASSETER

The superficial part, which comprises a thick aponeurosis along with a multivariate aponeurosis, originates from the anterior two thirds of the zygomatic arch approximating the zygomatic process. The muscle traverses obliquely and is inserted into the angle of the mandible anteriorly to the ascending ramus and the gonial angle.

1.2.3 INTERMEDIATE MASSETER

The intermediate masseter arises from the central and medial third of the zygomatic arch from the lower border approximately posterior third and inserted into the central part of the ascending border of the ramus of the mandible.

1.2.4 DEEP MASSETER

The deep masseter is embedded into the depth of the superficial and intermediate parts of the muscle, arises from the inner-most surface of the zygomatic arch and inserts into the upper parts of the ascending ramus close to the level of the coronoid process.

1.2.5 FUNCTION

The varying anatomical orientation of the muscle is translated into the differences in task potentials. The activation of superficial fibres assists in elevation, protrusion and contralateral movements. However, the deep and intermediate fibres produce elevation, retrusion and ipsilateral movements[30]

1.2.6 NERVE SUPPLY

The fifth cranial nerve (trigeminal) is a mixed motor and sensory nerve which carries the main motor supply to the muscles of mastication via the mandibular division. The large sensory and small motor root arise from the brainstem from the mid-lateral portion of the pons and exits through the foramen ovale into the infra-temporal fossa[37]. The large, unipolar cell bodies of the motor neurones are found in the trigeminal mesencephalic nucleus in the mid pons and consist of both α and γ motoneurons[38]. The axons of the afferent fibres bypass the trigeminal ganglion and enter the pons through the motor rather than the sensory root of the trigeminal nerve.

1.2.7 CONTROL OF MASTICATION

Motoneurons in the muscles of mastication are activated and modulated by three delivery centres: the (a) motor cortex, (b) central pattern generator (CPG) and (c) peripheral input from muscle spindles, periodontal mechanoreceptors (PMR) and to lesser extent, the skin and tendon receptors of temporomandibular joint. The motor cortex is responsible for the conscious initiation and termination of mastication and motor-evoked potentials are examined during voluntary contractions[1, 39].

The CPG is a group of cells located in the median bulbar reticular formation between the motor root of the trigeminal nerve and the inferior olive[40]. In the opening cycle of mastication, the CPG excites the motor neurons in the depressors while simultaneously inhibits the elevators. It sets the rhythm for mastication[41].

Finally, peripheral feedback is provided by the PMR and the muscle spindles in the elevator masticatory muscles. Most of the work which illustrates the importance of peripheral feedback is derived from animal studies[42, 43] and concentrates around PMR[40, 44-46].

The complex peripheral feedback from the motoneurons is illustrated (Fig 1.1) in the wiring diagram[3] which reflects work from two different algorithms reflex work and simulated chewing work. The wiring diagram illustrates the complexity and finesse for the control of mastication.

1.2.1 THE MOTOR UNITS AND MUSCLE FIBRE TYPES

The smallest functional compartment of the motor system is the motor unit which comprises a bundle of skeletal muscle fibres, a single motoneuron and motor axon. The motor unit is a basic functional contractile unit and in the masseter muscle its innervation ratio is 1:640, providing adequate control and efficiency with force generation.

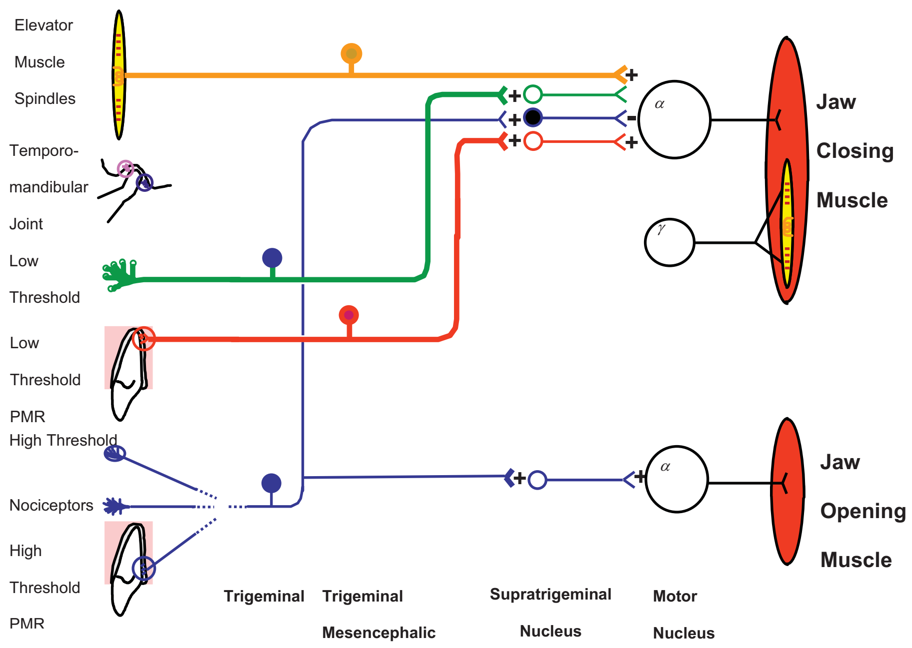


Fig 1.1 : Wiring diagram of the human masticatory system. Position of the cell bodies of the neurons and motoneurons have been deduced from animal studies. In the human model, this represents masticatory muscle as estimated under static conditions only[3] (Kindly provided by Prof KS Türker).

There are three types of muscle fibre types; the slow (S), fast fatiguing (FF) and fatigue resistant (FR). Histochemically, they are distinct in the number of mitochondria present and biochemical processes with ATPase production. Therefore, there is a range of force levels with high forces generated for brief moments and low forces and postural control sustained over a period of time[47, 48]. The muscle fibre type and its distribution is different in the various muscles of mastication. Our understanding of the myosine heavy chains and their distribution in elevator muscles comes from recent studies[49, 50]. Peripheral sensory feedback is vital for protecting orofacial integrity as a high level of force is generated with increased occlusal resistance in dentate individuals. The two most important peripheral sensory inputs come from

1. Muscle spindles
2. Periodontal Mechanoreceptors (PMR's)

1.2.2 MUSCLE SPINDLES

Muscle spindles are specialized sensory receptors present in abundance in the masseter muscle. The muscle spindles are sensitive to length changes and reside in the intra-fusal fibres. The intra-fusal fibres comprise central non-contractile and terminal contractile elements.

Proprioception from the masseter muscle is possible through muscle spindles acting as specialized stretch receptors. The mesencephalic nucleus is unique with peripheral afferent nerve cell bodies located in the central nervous system[51]. A monosynaptic reflex arc is present between the contralateral branch of the mesencephalic nucleus and the motor nucleus of the trigeminal nerve. The jaw closing muscles (masseter, temporalis and medial pterygoid) are rich in muscle spindles (Table 1.1)[52]The elevator muscles have up to 36 intrafusal fibres per spindle which is regarded as the highest density in the human body. This provides a strong proprioception in the jaws[53]. This is in stark contrast to the masseter muscle which has the second highest proportion of muscle spindles[54]. Interestingly, the digastric muscle, like the other jaw depressors, does not have spindles[55, 56].

Elevator	Muscle spindle population		Total
Temporalis	208 (Horizontal fibres)	134 (Vertical Fibres)	342
Masseter	91 (superficial)	23 (deep)	114
Medial Pterygoid	59		59
Lateral Pterygoid	6		6

Table 1.1 Muscle spindle population in various elevator muscles of mastication[52]

Uniquely, the muscle spindles have a double afferent innervation. These are:

a) Group Ia myelinated afferent (primary) fibres which are large (12-20μm diameter) and terminate as annulo-spiral endings in the centre of intrafusal fibres.

b) Group II myelinated efferent (secondary) fibres which are small (4-12μm diameter) and end on either side of the central region as a spray.

Each muscle spindle has its own afferent and efferent nerve supply. The intrafusal fibres are innervated by the gamma motor neurons (γ) while the extrafusal fibres are supplied by alpha motor neurons (α). Two types of afferent sensory nerve endings are present in the masseter muscle; primary (annulo-spiral) and secondary (flower-spray). Primary endings are wrapped around the central portion and are sensitive to stretch and the speed at which stretch occurs. The secondary endings are found on the terminal ends of the fibre and are activated by stretch only.

It is important to appreciate that elevator muscle movements result from central co-activation of both α and γ motor neurones; hence, a and γ co-activation[57]. A comprehensive overview has been presented[58] for the neuro-anatomical description of the masticatory reflexes.

This, along with peripheral input from the periodontal mechanoreceptors (PMR's), makes the modulation process impeccably precise even during high masticatory force application. As soon as the resistance ends, the occlusion is prevented from high impact that can be damaging to the oro-dental structures.

The masseter muscle spindles provide large force generation and postural control, although the role of the spindles in static and dynamic conditions is controversial [59]. Some studies show no activity at rest[60],[61] while others have conclusively found action during rest [62]. An important study [63] deduced that muscle spindles contribute to stabilization of the mandible during body movements[64]. The visco-elastic nature of the masseter muscle maintains posture control while the intermaxillary distance is maintained during walking, running or jogging by stretch reflex fine tuning of the muscle length[47, 65].

During mastication, forces are precisely controlled with the peripheral feedback arising from muscle spindles, PMRs and peripheral receptors [reviewed in Türker, 2002][3]. In animal experiments where muscle spindles were destroyed, Morimoto and Nagashima[66]convincingly concluded that jaw elevators lost facilitation completely, providing unequivocal evidence for the significance of muscle spindle response.

1.2.3 REFLEXES

Generally, the reflexes in the masticatory region are classified according to the stimulus that evokes the reflex[3]; for example mechanical, electrical and magnetic. Other historical nomenclatures use the term jaw-jerk reflex, jaw opening reflex or unloading reflex.

1.2.4 STRETCH REFLEXES

Exciting homonymous motoneurons from a muscle spindle afferent results in reflex activation commonly referred to as the spindle reflex. The spindle reflexes can be elicited with mechanical stimulation (Stretch reflex) or electrical stimulation (H-reflex). This mechanical method was introduced in the field of neurophysiology and successfully produced a stretch reflex in participating subjects[67]. In the current study, every effort was made to prevent artifacts and noise during the process which is a limitation of mechanical stimulation.

There is a range of reflexes that can be stimulated with tooth tapping. For example, there is a jaw opening reflex in which jaw closing muscles are inhibited and this normally occurs when tooth contacts are made during the masticatory cycle[68]. Jaw muscles can be stretched and spindles activated by chin taps, creating the jaw jerk reflex[69]. When skin or muscle groups are stimulated several receptors fire simultaneously making the study of single entities and spindle responses difficult. However, the orthogonal tooth tapping of incisors in a human model can evoke reflexes in PMR and spindles alone[70]. The stretch reflex in the masticatory system is very rapid with activation of elevators within 7-8ms. This enables quick reaction to change within the masticatory apparatus.

1.2.5 UNLOADING REFLEX

The highly specialized sensory muscle spindles respond to increasing bolus resistance during mastication[58] with alpha-gamma coactivation. Shortening and the emission of cortical signals occurs, further contracting the muscle fibres to overcome the resistance. However, as soon as the bolus has been incised the spindle stretch ends and spindles become slack and cease contraction like a shock absorber with no further action potentials sent via Ia fibres. This halts any further contraction from the muscles, preventing the jarring effect threatening the integrity of the oro-facial region. Moreover, the unloading reflex, along with activation of depressor masticatory muscles, assists in fine tuning and modulating the activity of elevators. This is further refined by peripheral sensory input.

1.2.6 PERIODONTAL MECHANORECEPTORS (PMR'S)

The PMR's are an essential part of the peripheral sensory modulation of mastication. PMR's encircle the root of the tooth and their distribution varies from being highly dense in the apical region[71] to being least dense in the cervical region. There are two types of receptors; the slowly adapting (SA) and the rapidly adapting (RA). The distribution varies as SA type receptors are found in the apical third of the ligament.

As with most physiological findings, our basic understanding is derived from animal models. Likewise, Ness[71] brought insight into the role of the periodontal mechanoreceptors in the rabbit model. The slowly adapting (SA) and the rapidly adapting (RA) distribution varies as SA type receptors are found in the apical third of the ligament. Conduction velocities for the PMR's have been calculated to lie in the range of 25-80 m/s. Most importantly, cell bodies for PMR lie in both the trigeminal ganglion and the trigeminal mesencephalic nucleus. Reports suggest that mechanical stimulation of teeth may excite interneurones in the supra-trigeminal nucleus[54].

However, our current understanding for PMRs in humans has been due to extensive work done by Trulsson in Sweden with receptive fields in periodontal afferents and directional sensitivity[45]. PMR have many functions including excitation following slight displacements, providing directional stability, indirectly protecting soft tissues intra-orally like the tongue and cheek, sustaining high masticatory force level [41] and assisting the teeth to hold the bolus prior to occlusion[72]. A recent study shows the PMR bolus holding mechanism is reduced significantly when alveolar bone loss occurs[46, 73]. Directional stability is provided by periodontal receptors located in the mesial and labial of the tooth with cell bodies present in the trigeminal mesencephalic nucleus[44].

Mechanical stimulation of the tooth triggers a response in the PMR. This model allows relatively non-invasive research in a human subject via an anaesthetic block method. Furthermore, recent work has shown that PMR's are an essential part of the peripheral sensory modulation for mastication[3].

1.2.7 MODULATION OF REFLEXES OF PMR ORIGIN

All work done so far in the human model has exhibited the reflexes from PMR's in a static state. Researchers are convinced[74] that there is modulation, just like in the limbs, through the spindles and peripheral receptors working jointly to provide smooth movement in the jaws. However, for this to be demonstrated, a highly standardized protocol utilizing precise stimulus application in conscious human subjects during mastication is required.

1.2.8 IMAGES FOR DETERMINING MASSETER MUSCLE DIMENSIONS

With new imaging techniques, the current trend is to study masseter muscle changes in real time. Ultrasonography has shown differences in muscle thickness in different parts of the masseter due to the unique pennation orientation[8]. Similarly, MRI has been used to assess muscle volume, length and area in asymmetry cases[75]. Another MRI study has tried to find the exact location of change in pennation orientation[76] within the masseter muscle. In a recent study, Higashino[77] has tried to superimpose MRI of the masseter images over cephalograms. The synthesized image showed the masseter images had a close correlation to skeletal variables derived from the cephalometric analysis; for example, the mandibular plane angle. Moreover, they found that volume was closely related to gonial angle and mandibular plane angle. The work concluded that such synthesized images could provide valuable information regarding vertical and antero-posterior mandibular development. However, it still incurs two sets of investigations. Again, 3D CT scans have been used to determine the relationship between masseter muscle morphology, ramus dimensions and occlusal forces[78].

1.3 EMG STUDIES

Our understanding of the neurophysiology of the muscles of mastication has increased substantially in the past 60 years. With the introduction of electrophysiological tools in the 1950's[79], an array of experimental protocols have become available around the globe. Although the majority of studies have used animal models due to the invasiveness of the procedures, results from such studies still provide valuable data. The earliest EMG studies reported on muscles of mastication during basic mandibular movements were from Moyers (1950), Carlsöö(1952), Göpfert & Göpfert (1955) in Moller[80]. These preliminary studies improved our fundamental understanding of the complex masticatory system. Other work of historical interest is from Perry and Harris[81] and Ahlgren[82].

In the human model, indirect methods are employed and are preferably, non-invasive. Surface electromyography (SEMG) is a tool that is used extensively to explore the neural circuitry. Its limitations are discussed later but is considered user-friendly, non-invasive and has extensive applications in neurophysiology.

EMG registers signals of muscle contractility through action potentials delivered by the motoneurones. Highly refined bipolar surface electrodes are sensitive to these electrical signals and, once amplified, are visible as the EMG recordings.

1.3.1 DEVELOPMENT OF DEVICES

The first chewing apparatus was described by Moller[80]. It was a landmark achievement in which bipolar recordings could be saved on photographic oscilloscopes rather than printed by inkwriters. Therefore, several experiments were designed to enhance our understanding of muscle activity during chewing, postural activities, full effort clenching, swallowing patterns and even facial morphology [80]. This innovative apparatus only required an EMG recorder with electrodes. The electrical activity was recorded on three channels. Although an advanced methodology for its time, Moller's apparatus had the serious limitation of un-standardized positioning of the subject. This produced a lack of reproducibility, albeit the reproducibility factor poses a challenge even with contemporary advancements. Other methodological issues arose from interference from neighbouring potentials, rejection of common voltage, distortion of amplitude and uneven distribution of electrical activity [80]. Over time, several global studies have been conducted which have improved designs and chewing devices[83, 84] leading to an enhanced understanding of the reflexes in the oro-facial regions.

The periodontal-masseteric reflex was first described by Goldberg[67] highlighting the importance of not only PMR but also the role of the masseter muscle and central connections, due to the short latency. This study concluded that there was a central connection located in the mesencephalic nucleus that was responsible for the excitatory masseteric reflex evoked by PMR and gingival receptors. One can appreciate that, even with a very simplistic methodology and manual tapping of the teeth, Goldberg was able to deduce these important findings.

Mechanical stimulation has been further refined in subsequent studies with differential force stimulation and variable rise times[85] that refine our current understanding of the periodontal-masseteric reflex system. The mechanical stimulation can be directed orthogonally or axially depending on the PMR recruitment response required[44]. Generally, axial stimulation is closely mirrored by the physiological stimulation that would occur during normal mastication. However, the argument to use orthogonal stimulation is its ability to consistently keep the mechanical stimulus in contact with the labial surface of the tooth being tested, particularly in dynamic experiment paradigms. Sowman in 2007[86] has criticized and suggested that orthogonal stimulation would negate the extent of biomechanical coupling for modulation.

The experimental device used in the current study was developed by Türker *et al.* [7]. Details given in Methods (Chapter 4) overcame previous reported limitations. Most importantly, standardization of the variables such as position, stimulations and static and dynamic experiments for humans was achieved. The device's ability to simulate natural chewing process and record EMG on over 8 channels was an added benefit. Bite plates were constructed with a titanium shaft coupled to a linear motor. Moreover, resistance to chewing could be injected into the system through appropriate computer programming. The device had the capacity to study several different modes of activity such as *normal chewing mode, position hold mode, no force mode, fixed force mode.* Overall, freedom of the lower jaw to move in the sagittal plane whilst permitting all natural movements including rotation was unique to the system.

The PID (proportional integral derivative) compensator has a high speed microprocessor that can control forces on the lower bite plate and measure force levels by strain gauges located in the motor lever arm. Predetermined force levels assist in simulating the resistance that a food bolus might produce by standardizing the force levels.

Therefore, this device surpasses previous attempts at standardization and recording reflexes both in static and dynamic modes with a high degree of precision and has subsequently, led to important publications[70, 74, 87-91].

1.3.2 EMG STUDIES AND SIGNIFICANCE IN ORTHODONTICS

Work in this field has resulted in few conclusive findings. A canonical correlation analysis between facial morphology, age, gender and EMG during rest and contraction has not found a statistically significant correlation[92]. EMG and bite force have been studied extensively[80, 93, 94] with consistent findings of reduced force levels in dolichofacial patterns.

Different vertical facial types, both in adults and children, produce differences in EMG responses recorded over the course of a day[95]. Masseter muscle EMG activity was found to be consistently longer in short vertical dimension facial types as compared to high mandibular plane angle individuals. The variables of bite force, muscle efficiency and mechanical advantage in children with vertical growth patterns[96] were negatively correlated with muscle efficiency and vertical proportions.

Morimitsu *et al.* documented recordings of EMG from masseter muscles which showed positive correlations with linear cephalometric measurements; particularly, the muscle activity was significantly related to mandibular dimensions such as (Co-Go) and body length (Go- Me)[97]. In fact, the muscle activity increased with decrease in vertical proportions such as mandibular plane angle. Furthermore, they compared the muscle activities with anterior-posterior skeletal base relationships indicated by SNA and SNB variables. Morimitsu *et al.* strongly advocated the use of EMG for examining masticatory activity, but one could argue that the reproducibility with electrode placement at subsequent visits may prove to be a challenge[98].

A comprehensive study by Tuxen *et al.*[99] compared masseter muscle fibre types along with function from EMG and facial morphology. They concluded that even with intensive analysis of different dimensions, linear regression analyses failed to show any significant association highlighting, once again, the complexity of the craniofacial region.

A recent publication[29] has utilized EMG to analyse masseter muscle differences in individuals who brux in their sleep. The authors believe their system can help diagnose different types of bruxism and is aptly termed the innovative bruxism analysing system. Once again, the literature supports the use of EMG as a useful mode of investigation.

1.3.3 LIMITATIONS & METHODOLOGICAL ISSUES

EMG has provided insight into the neuromuscular system but there are inherent limitations which must be minimized in order to derive meaningful data. This has been reviewed thoroughly[3]. EMG accuracy may be enhanced by the use of intramuscular electrodes compared with SEMG (surface EMG) electrodes. However, SEMG electrodes with built-in amplifiers can assist in reducing cross-talk and movement artifact. Passive surface electrodes require skin preparation and monitoring of resistance below 10Ω for clear data recording. Contemporary SEMG involves the use of bipolar electrodes, which suppress noise. The amplification should counter distortion, preferably 10 x higher than the electrode-to-skin electrical resistance. The filter level is dependent upon skin thickness and the frequencies recorded. It should not eliminate any of the frequencies within the range of recording and the choice lies between upper (high-cut or low pass) and lower (low-cut or high pass).

In addition, the quality of data production is dependent on the recording and display devices, the sophistication of the computer programs to reliably identify the initiation of muscle activity, the type of ground used, and obviating the use of long leads which contribute to noise and, therefore, contamination of the data. Several different types of ground have been utilized in human studies such as forehead, wrist, elbow, ear lobe and lip clips. For the current study, the ear lobe was selected due to ease of placement and distance from the experiment site.

Minimizing artifacts is essential for clear data recording and minimizing movements from adjacent muscles is very helpful. In the present study, the customized nose rest and the head halo provided stabilization during the chewing and static phases of the experiments (Fig. 4.1).

Cross-talk is a phenomenon commonly experienced in EMG recordings where adjacent muscle activity leads to electrical volume added to the data. Cross-talk can be minimized by standardizing the experimental conditions. Moreover, double deferential techniques can also assist in eliminating cross-talk[52, 53, 56, 58, 61, 66, 100]. This is the prime cause of criticism in telemetry EMG recording where cross-talk and noise can compromise the results[101].

Normalization is a standardization process for data acquisition which allows comparison between different subjects and with the same subject data on different occasions. It means normalizing SEMG levels to the percentage of an individual subject's MVC (maximum voluntary contraction) for each muscle group, hence reducing the variability between records.

Finally, correct processing of the data is essential and custom-made software programs are routinely used. IZZY® [102] provides an array of systematic offline analysis which allows the data from SEMG to be full wave rectified and further processed as CUSUM (Cumulative Sum), if required (Chapter 4 for details).

The purpose of this project was to study modulation in the muscles of mastication and the role played, particularly, by masseter muscle spindles during mastication. Furthermore, we aimed to highlight any relationship of the above to different facial heights as characterised by lateral cephalometric norms.

2 IMAGING

2.1 INTRODUCTION

Currently there is a wide array of imaging technology with innovative developments occurring at a phenomenal pace. Nearly every time one searches the world wide web something new is presented. At the time of writing, 3D video softwares are considered to be the next big thing [103]. The imaging industry finds wide application in defence, architecture, resource industries and general entertainment. In the medical field, it has immense application for diagnosis and potential to generate understanding not previously possible with 2D investigations. The classic examples are MRI and CT scans[27, 104-107].

In orthodontics, the focus is more based on the external features and soft tissues and 3D reproductions are invaluable not only for diagnosis in general medical scenarios, but more so for treatment evaluation and long-term growth changes. However, the majority of published work tends to come from Japan followed by European and USA researchers[108-110].

In this review, a brief historical background of the different imaging techniques used in the current study are presented, along with important findings from recent studies involving such technology.

Lateral Cephalometrics

Lateral cephalometric techniques have dominated in mainstream orthodontic, orthognathic and dentofacial growth studies for over seven decades. It is considered a versatile tool and has served the profession well since it was first introduced in 1931 by Broadbent[111]. Cephalograms have improved our understanding of growth through seminal research[112, 113]. It is an important diagnostic tool utilized extensively in orthodontics. One Google search brought out 28,200 items related to “lateral cephalometrics in orthodontics” while a similar search on PubMed found 808 documents on related topics. This highlights the volume of work done in the field.

There are two major limitations with cephalometrics; firstly, the additive radiation dosage of progressive films and, secondly, it is a two dimensional representation of the three dimensional craniofacial region. Moreover, the validity of cephalometric analyses has been questioned[114]and there have been concerns about the application of such simple analyses in diagnosis and treatment planning. Errors generally are attributed to orientation, geometry and association. A meta-analysis was conducted on six studies for random and systematic error along with repeatability and reproducibility[115]. Good repeatability was found for landmarks Me, PNS, ANS, S, Ptm, A and B whereas there was error for Ba and Ar on the x-coordinates. Hence, the choice of the previous landmarks was considered appropriate for the current study.

A number of computer software sites such as the Dolphin®, Mona Lisa® etc are available to analyse the lateral cephalogram with multiple analyses at a click or a mouse scroll. However, the computer analyses have also been criticized for error through loss of contrast, related to pixel size and poor calibration. Overall, the trained eye can effectively trace and resolve finer details up to 0.1mm or smaller[116] as compared to 2.48mm accuracy recorded from Active Appearance Models (AAM) which is a computer program designed to locate landmarks[117]. Such technology needs refinement with mathematical model in order to provide a higher level of accuracy and reproducibility.

Furthermore, the lateral cephalogram has been supplemented with other radiographic conventional images such as panoramic radiography, periapicals, and hand wrist films. The later is now being replaced by the cervical maturation index (CMI) which can be derived from standard lateral cephalograms reinforcing the trend towards minimizing radiation exposure in growing individuals.

There is a galaxy of analyses to choose from. One needs to be mindful of the analyses being a composite of several unique dimensions often with a signature eponym. So it is imperative to choose specific dimensions of relevance to the study at hand. For the current research, variables were selected from different analyses that provided published information for the vertical facial proportions; both for anterior and posterior regions (Details Chapter 4 Methods).

Lower face height is highly dependent upon muscle function, morphology and environmental factors[118]. Hence, analyses were selected to provide facial height information along with some new measurements to represent the length of the superficial and intermediate masseter muscle[31, 119, 120].

2.2 ULTRASONOGRAPHY

Ultrasonography (US), as the name implies, utilizes sound waves within the frequency of 2-18MHz that bounce off the tissue to provide the depth and density of the image conventionally captured on the screen as a real time image. It was developed for medical use in the 1940's with parallel developments across the Atlantic in the USA and Sweden. It has since been widely accepted as the safest investigating tool and is applied widely in obstetrics. However, it has applications across several medical disciplines including physiotherapy therapeutic outcomes for muscular-skeletal conditions.

US is advancing from 2D to 3D imaging whereby a series of 2D images are collated and rendered into 3D. The main limitation is cost as it needs a specialized probe and the image acquisition speed is relatively slow, especially in moving tissues such as contracting muscles. Further advances with colour Doppler technology are of value for imaging tortuous vessels. Generally speaking, conventional US serves well and is good for imaging muscles and soft tissues, but has poor acoustic impedance for bone.

Muscles of mastication have been studied extensively with US which has been considered to be a valuable, precise technique for analysing muscle shapes[8, 16, 17, 121].

Furthermore, US is superior to radiographs for soft tissue evaluation and definitely overcomes the radiation hazards. The surface topography for the masseter muscle is excellent due to its superficial anatomical position; however, the volume and cross-section assessments have been difficult [122]. Hence, the present study sought to evaluate these dimensions with a simplified approach and using arithmetical formulas.

A comprehensive meta analysis was recently presented [122] which reported masseter muscle thickness in contraction to range from 5-14.1mm[123, 124]. One could argue that this wide variation would be due to racial or ethnic diversity.

US has been extended to determine association between several variables such as the masticatory muscle thickness, TMD and bite forces [125]. The study found a positive correlation between masseter muscle thickness and posterior facial dimensions (Table 2.1). The study concluded that muscle thickness is related to vertical facial dimensions and bite force. Previous studies have had similar findings in large samples where not only negative relations between muscle thickness and anterior facial heights and mandibular lengths were noted but positive relationships existed with intergonial width and bizygomatic facial width according to anthropometric measurements[126, 127]. Likewise, a Swedish study consistently found a relationship between thin masseter and longer faces in females[128]. This study found US to be a reliable and accurate method for muscle assessment.

US has recorded thickness variations in masseter muscle contraction and relaxation[8] during function which would otherwise have been impossible with snap exposure type investigations.

Hatch and associates have clearly indicated the importance of multivariate analyses of the masticatory system for comprehensive diagnosis in dentistry[129]. They went so far as to include blood glucose levels along with bite force and cross-sectional area of the masseter muscle.

Overall, US has been documented as a reproducible and reliable investigating tool for masseter muscle assessment[18, 130] and could produce useful, non-radiological information to enhance orthodontic practice.

Author	Subjects	Masseter Muscle (US)	Lateral Cephalometric Variables	Facial assessment	Additional Variables	Findings	Deductions
Kiliaridis & Kalebo 1991	40 (40 ♀)	Thickness	- -	Facial Photographs	-	Significant	Thin masseter in longer faces in females only
Bakke *et al.* 1992	13 ♀	Thickness	Vertical	-	Bite force	Significant	Masseter thickness was correlated to anterior face height, vertical jaw relations, mandibular inclinations and bite force
Raadsheer *et al.* 1996	329(169♀)	Thickness	- -	Anthropometric (Farkas, 1994)	Body weight & height	Significant	Negative correlations muscle thickness & anterior facial height also with mandibular length, but positive relationship with intergonial width and bizygomatic facial width
Benington et al 1999	10 (6 ♀)	Volume, Thickness	7 Angular 2 Proportional variables LAFH LPFH	-	-	Highly significant	Muscle volume with posterior face height & thickness inversely with anterior face heights
Raadsheer *et al.* 1999	121 (63 ♀)	Thickness	Angular -	Anthropometric	Bite Force	Highly significant	Bite force was positively related to thickness of masseter and vertical & transverse facial dimensions, but negatively to mandibular plane and occlusal plane inclination
Satiroglu *et al* 2005	47 (23 ♀)	Thickness (Clenching)	Vertical patterns Angular measurements	-	BMI	significant	Thickness *inversely* related to vertical facial proportions
Kubo *et al.* 2006	5 ♂	Thickness	Masseter outline -	-	-	Highly significant	Contracted Masseter has better reproducibility than relaxed

Table 2.1Chronological review of the correlation studies for masseter muscle with US, anthropometry and lateral cephalograms with significant findings.

2.3 3D IMAGING

3D imaging has come a long way in the last 20 years, particularly since its introduction six decades ago [Thalmann-Degen 1944][131]. Primarily developed for application in industry[132] as an effective and non-invasive method, it quickly found its way into clinical applications, particularly in orthodontics[133] and oral surgery. 3D applications in restorative dentistry with the Cerec technique and CAD-CAM are already in main stream clinical application and boast high accuracy at the level of 70 microns [134]. Scanning of comparably smaller dimensions of hard surfaces with accurate reproduction has evolved over the past few years[108]. A similar level of precision is desired for facial soft tissue imaging. It's only a matter of time and refinement of technology until 3D imaging becomes a routine part of private orthodontic practice[135].

In orthodontics, 3D imaging was tested for the first time in 1981 with optical surface scans[136-138]. A major change came with the use of a hand-held scanner making the scan portable (McCallum *et al.;* 1996 in Moss[139]) followed by a probe to record 3D coordinates[126]. Imaging is sensitive to the surface acquisition and sufficient data are required to appreciate the subtle changes that occur in soft tissues over a period of time.

There are various types of imaging techniques ranging from stereo photogrammetry, to 3D laser scanning, vision-based scans like Moiré tomography along with the latest, safest and most cost- effective structured light 3D imaging[110]. Innovative approaches are under way to integrate the conventional lateral cephalogram with stereo photogrammetry and digital fusion. It is claimed that such techniques will obliterate errors through image sensors and 3D orientation [140].

Structured light creates a superficial shell-like reproduction of the face enabling the digitized topology of the face to be displayed in 3D without any ionizing radiation[140]. It is a simple and cost effective method of generating three-dimensional images with minimal time required for exposure, usually within one second.

The structured light (SL) technique provides reasonable accuracy when following certain protocols such as the exposure should be frontal with deviations of up to +/-15° only because with increasing profile view there is proportionally reduced accuracy. Linear measurements can be erroneous up to 1mm and if that is acceptable to the operator and duly accounted for it should not be of much concern. Likewise, the smaller inter-distance between the two cameras creates a limited field of view leading to diminished accuracy in z-coordinate measurements[141].

Overall, SL is considered a simple, cost effective and readily applied 3D imaging system[142]. It is based on stereoimaging and triangulation to produce a 3D image. From a light grid or pattern which is usually horizontal (Chapter 8). The mean absolute error for linear measurements with SL 3D imaging in the current study was 0.53mm[143].

Several reviews have evaluated these systems[144]. 3D imaging is valuable in assessing the growth changes in soft tissues over time, because previous investigating methods were not able to adequately assess soft tissue changes related to growth. A large scale growth study with 3D optical surface scanning[145] found significant changes in the vertical facial dimension with increase in the cross sectional cohort age range 5-10 years old. Moreover, they found a significant increase in dimension in the masseter muscle mass across the age range. Hence, age-related changes can be appreciated and quantified with 3D imaging. Furthermore, a longitudinal study analysing facial morphology changes also found similar results with an increase in the vertical dimension being gender dependent with greater significance in males[109].

Similarly, growth changes have been evaluated with 3D facial morphometry and Fourier analyses depicting changes in profile[146]. Another area where 3D imaging is used extensively concerns pre- and post-orthodontic treatment effects on soft tissues. The very controversial and anecdotal references often made regarding functional orthopaedic appliances has been backed with evidence[147-149]. Yet another highly contentious issue in orthodontics has been the extraction and non-extraction debate, where the treatment decision pendulum keeps swinging by the decade. A study in London[150] used 3D optical surface scans for patients with fixed appliances; one group with extractions and another without. The results were conclusive and laid the age-long controversy to rest with no significant facial soft tissue changes discernable in either study group.

Work is underway to fabricate user-friendly analysis methods especially as 3D imaging is relatively contemporary and lacking well-established quantification and comparison techniques. 3D studies conducted with Procrustes analysis have determined the translation of soft tissues in monozygotic and dizygotic twins [139]. Another study has used the Procrustes registration method in conjunction with 3D CT scan and Virtual Reality Modelling Language (VRML) to assess the registration error associated with such techniques and found it to be within +/- 1.5mm in most parts[151]. A South Korean study[152] found negligible error of 0.37mm and less than 0.66% magnification with laser scanners. They claim that the soft tissue rendering was highly reliable and reproducible. However, one would argue about the safety of such imaging as the study was not conducted on humans but mannequins.

2.3.1 ANTHROPOMETRY

Leslie G Farkas has been instrumental in providing guidelines for soft tissue measurements and tools for assessment with accuracy[153]. The work was conducted when 3D imaging was not in the mainstream. However, the landmarks and analyses have been easily translated into 3D studies and provide valuable information (Methods Chapter 4). Defining aesthetics can be challenging and quantifying the minor details for overall understanding of the aesthetically pleasing perception has been aptly described in Anthropometrics and Art in the Aesthetics of Woman’s Faces[154].

3 D ANALYSES

Unique to anthropometry and 3D imaging is the ability to measure and evaluate the transverse dimensions of the face. Although a large list of measurements are presented from the frontal aspect in the Farkas textbook[126] only the ones used in the current study will be considered.

Two horizontal planes commonly used for facial widths are the bizygomatic diameter, inter- zygonion (zy-zy), and mandibular or lower face width, inter-gonion (go-go). Generally, such evaluations assist in picking up asymmetry greater than 2mm[155]. These dimensions form indices for facial proportions. Thus, the Facial Index was calculated with horizontal (zy-zy) as a percentage of vertical from nasion and gnathion (n-gn). Similarly, the (go-go) width is used in the Jaw Index with stomion to gnathion as the lower face proportion (Fig 8.3). Details of indices used in this study are given in Fig 8.3 in Chapter 8.

The midline landmarks were used for vertical proportions which were Index of lower jaw to facial height and Index of Jaw to facial height. These proportions are related to the lateral cephalogram and the correlations can be beneficial substitutes in progressive evaluation over the course of the treatment.

2.3.2 OTHER IMAGING TECHNIQUES

MRI and CT scans have been utilized in orthodontic practices along with the latest Cone Beam CT Scans. However, the use of such imaging needs to be justified and avoided when possible.

Previous work[104] has affirmed that lower face height and masseter muscle thickness have inverse correlation. Generally, the increased lower face height individuals will present with classic cephalometric characteristics such as large gonial angles, steep mandibular planes and reduced posterior face height. The masseter muscle cross-section has been measured with MRI's and found to be significantly smaller by nearly 30% in the dolichofacial types[104]. CT scans have been used to find correlations between facial length/ width and masticatory muscle cross sections[107]; however, the routine clinical use of such investigation would be questionable.

A recent study has compared volume of masseter muscle derived from MRI with vertical facial dimensions and found volume to be more relevant than cross-sectional area[156]. Moreover, the posterior face height was significantly correlated to masseter muscle volume. Although, these methods are highly sophisticated, they have major issues such as cost and higher radiation dosage with CTscans.

2.4 PREDICTIVE EQUATIONS

In orthodontics, in particular and dentistry, in general, it is beneficial to predict changes in growth, development and tooth eruption for diagnosis, treatment planning and patient education. Generally, tooth size predictive tables have been widely utilized[157, 158] .Several variations are present and different models of predictive equations are available (Chapter 7,8). Some common examples of use are predictive variables for upper canine impactions[159], soft tissue variations following orthodontic and orthognathic surgical procedures[160] and space analyses[161].

However, there is limited literature regarding the use of predictive equations for surrogate values from different investigatory modalities. Such information could not only obviate the need for multiple tests for the individual but may reduce radiation based investigations when frequent observation intervals are required. A safe and cost-effective alternative to diagnosis and treatment planning would be desirable.

The aim of the present study was to analyse and correlate facial data from three different imaging sources: lateral cephalometrics, ultrasonography and 3D facial scanning and to identify relationships that might be translated into future mathematical models for diagnosis and predictions.

3 AIMS & OBJECTIVES

3.1 STATEMENT OF THE PROBLEM

Most of the contemporary work in orthodontics is geared towards the hard and soft tissues surrounding the oro-dental region, with little emphasis on the active muscle forces in immediate proximity. The aim of the current study was to find functional relationships of the masseter muscle with craniofacial variables. Due to the dynamic nature of the masseter muscle it needs to be investigated from physiological and orthodontic perspectives utilizing multiple modes of investigation. Hence, for this study EMG, lateral cephalograms, ultrasonography (US) and 3D imaging were used in a sample set of young adults. Special attention was given to non-invasive characteristics and cost-effectiveness of the selected investigating tools.

3.2 HYPOTHESIS

- Muscle spindle responses from masseter muscles will differ between various vertical facial proportions.
- US variables will be closely related to facial skeletal proportions, particularly the mandible
- 3D facial imaging variables will be correlated to the underlying skeletal and masseter muscle dimensions as acquired from lateral cephalometric and US sources respectively.

3.3 POSSIBLE CLINICAL APPLICATIONS

The central purpose of this study was to evaluate masseter muscle spindle responses in varying facial height individuals and correlate them with other investigations. Strong correlations could be translated into meaningful *predictive equations* as an alternative to multiple invasive investigations. However, the mathematical model needs to be simple and user-friendly. Better understanding of the facial musculature may benefit clinical outcomes involving manipulation of face heights; in particular, the dento-facial orthopaedics.

4 MATERIALS AND METHODS

4.1 EMG STUDY

4.1.1 SUBJECT SELECTION

A total of 31, young, healthy subjects with no past TMD symptoms were selected from the University of Adelaide student pool for the first stage of EMG experiments. Sixteen females of mean age 23 years (range 19-28 years) and 15 males with mean age 23 years (range 21-38 years) gave written informed consent to the experimental procedures which included local infiltration anaesthesia and one lateral cephalometric radiograph. Six subjects were repeated to verify experimental reproducibility. However, data from 28 subjects were analysed due to unsatisfactory quality and technical problems in 3 subjects.

This cohort also participated in ultrasonography (US) and 3D facial imaging studies. The protocol of experiments was approved by the Human Ethics Committees of the University of Adelaide and the University of South Australia which, in turn, conformed to the Declaration of Helsinki. Written and verbal explanation was given for the experiments and risks, albeit minimal, were highlighted. Ethics documents are attached in Appendix.

4.1.2 SKIN PREPARATION AND ELECTRODE PLACEMENT

The skin over the right and left masseter muscle and digastric muscle belly was prepared with a 70% alcohol swab and surface bipolar electrodes (200x Duo-Trode disposable; Duotrode®, Myo Tronics Inc, WA) were attached. The cut off point for impedance was kept below 5 Ω. If there was high resistance, further preparation with abrasive was done to lessen thick skin surface layers. Due care was given not to create abrasive injury to the skin while male participants were requested to shave beards.

4.1.3 IMPRESSIONS

Upper dental arch impressions were taken on the bite platform with both upper and lower plates in juxtaposition. This created less fatigue for the subject while the impression material took 3-5min to set. High quality, reproducible and dimensionally stable impression material Express STD Firmer Set (3M Express™, Michigan) was utilized. The head of the subject was guided so that the upper anterior teeth were placed perpendicular to the bite plate. After the upper impression had set, the left central incisor (21) was cleared from the impression to allow the perpex orthogonal probe to rest on the labial surface of 21. Every effort was made to clear impression material from mesial, distal, palatal and labial surfaces so that stimulation would be unimpeded. On the lower biting bar a simple 2-3mm thick impression was made as a flat platform. This provided cushioning during dynamic opening and closing cycles, preventing tear of the impression material in the lower incisal region (Fig. 4.1).

4.1.4 ORTHOGONAL PROBE

The height and position of the stimulator was adjusted individually with a jig. The probe was in contact with the labial surface at the middle third of 21. The preload was adjusted, especially during the random stimulations, to prevent slack between the stimulations.

4.1.5 EXTERNAL MOVEMENT MINIMIZATION TO REDUCE LEVEL OF NOISE

The bite platforms were separated by 13mm. Alligator clips were attached to the surface electrodes and earth was provided by an ear clip. The subject was then assisted into the impression on the bite platforms and their head was stabilized and immobilized by a specially built head halo (Fig.4.1). An adjustable nose piece contacted comfortably on the nasion region.

Artefacts arising from head movement were minimized or eliminated by the rigid head halo and nose bridge rest, particularly during mastication. Therefore, the confidence in reflex activity occurring purely from the muscle under observation was enhanced. The Engineering Department (Mr I Linke and associates, The University of Adelaide) helped develop the test apparatus which was an innovation for the current research thesis.

Subject feedback was also programmed with variables on the screen derived and tailored to each individual. The feedback monitor provided the subject with visual display of their effort levels and cues which were easy to follow.

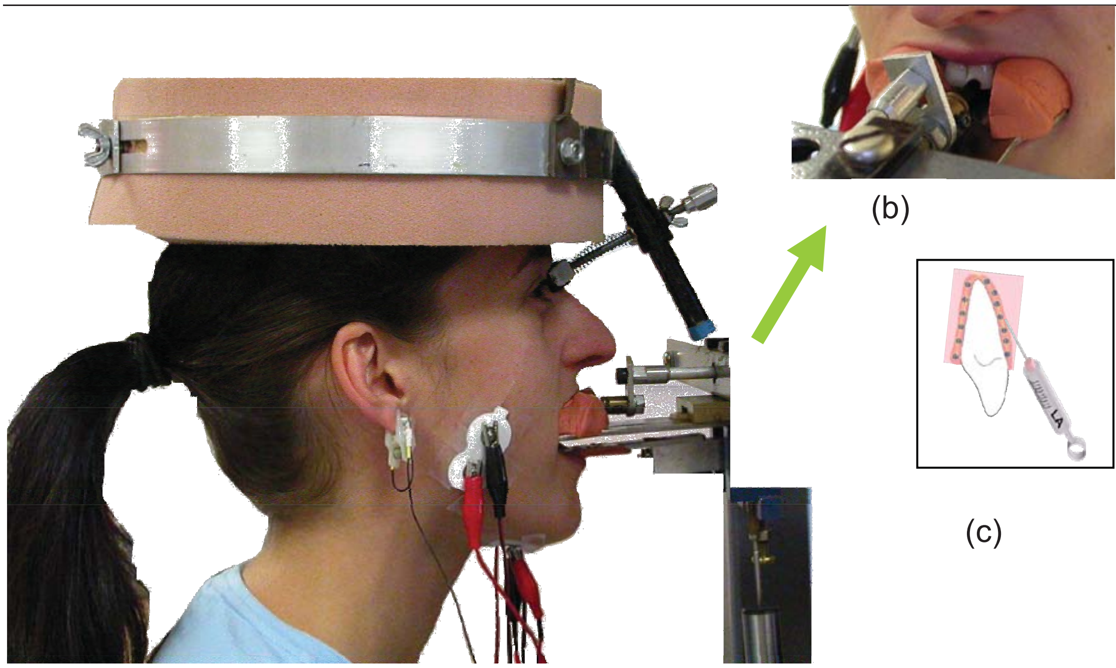
4.1.6 LOCAL ANESTHESIA

The above measurements were then repeated immediately following local infiltration anaesthesia (2-2 ½ mls of Lignocaine HCl with adrenaline 1:80,000) which was administered to the upper and lower central peri-incisal periodontium and the incisive papilla. Local anaesthetic was considered effective when subjects reported the loss of sensation on the lips and tongue. After anaesthesia, the tooth stimulations were not perceived by the majority of the subjects; while some reported vibrations on the back of their skull.

4.2 EQUIPMENT DETAILS

A complex, custom-made device was developed by Türker *et al* [7] to investigate the variable demands of human masticatory neuromuscular control. The apparatus was designed to record EMG activity from muscles of mastication, particularly in dynamic mode. This provided an opportunity to record reflexes, modulations in jaw muscle activity, velocities and chewing cycles. Moreover, it allowed simulation of natural rhythmic chewing which provided information during normal movements as opposed to data obtained from purely experimental conditions.

The equipment hardware comprised a DC motor with an amplifying system, bite plates, computer monitors and compensators (Fig.4.2). The DC motor translated vertical jaw movements into horizontal vectors that could be measured. The stimulus could also be delivered at a predetermined intensity and frequency. The upper bite plate was 3mm thick while the lower was approximately 5mm thick with the impression *in situ*. A 2mm freeway space was provided between the plates to simulate the natural resting distance. Standard desktop computers were fitted with a 12-bit DAQ card (National Instruments, NI PCI-6040E). The compensator was controlled by a microprocessor (Mitsubishi Electric MSA 0654) with a PID control (Proportional-Integral-Derivative) with a loop rate of 10kHz. This provided control of



(b)

(c)

(a)

Fig. 4.1. Subject seated comfortably throughout the experiment session. EMG recorded bilaterally from the masseter and the digastric muscles: (a) biting on a custom made impression; (b) left central incisor mechanically stimulated by an orthogonal probe;(c) local anaesthetic was infiltrated to eliminate periodontal mechanoreceptor input during the spindle trials. An ear clip provided the electrical ground. (Permission obtained from subject for publication, see appendix)

the position and force of the lower bite plate. A large range of options with regard to experimental positions, force delivery and frequency could be fed into the system.

The software comprised LabView® 6.02 (National Instruments) along with a further modification of the IZZY® program for off-line analyses. Surface EMG recordings were rectified and low pass filtered (100 Hz) followed by normalizing and final analyses. The Lab View® computer program translated required specifications to the compensator which, in turn, provided a stimulus profile, jaw positions and degree of resistance with a high degree of precision.

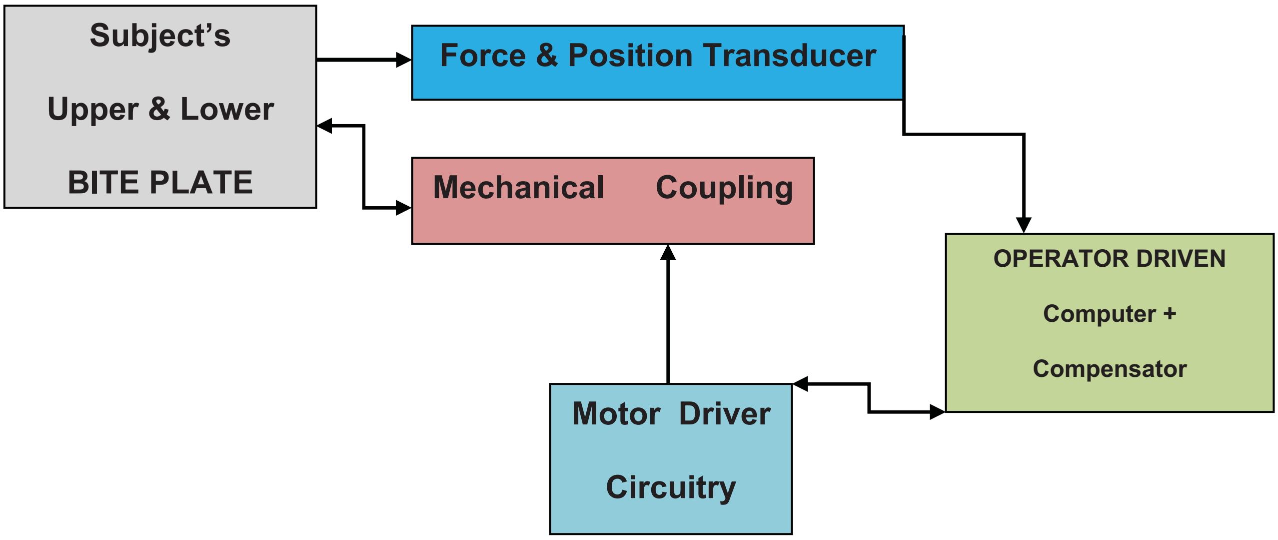


Fig 4.2. Line diagram of the EMG recording device used to simulate the masticatory system. The hardware and software are linked together to generate precise force, stimuli variables, and to adjust to individual biting rhythms.

4.2.1 EQUIPMENT SAFETY

This device fulfilled the safety regulations for use in healthy adults, whereby a maximum force of +/-35N is generated and is within the normal physiological opening and closing separations. Earth for the participating subject was provided by an ear clip. In the event of power loss, the lower bite plate returned to its resting position without sudden jolting, thus preventing any potential damage to the oro-facial region.

4.2.2 VISUAL FEEDBACK

Unlike the majority of EMG studies where maximum voluntary contractions (MVC) are provided as visual feedback, the current study requires special feedback due to the dynamic nature of the recording of data. This was measured from the rhythmic chewing data as the mean opening and closing for each subject from online analysis. Once this was established, data were fed into the visual display screen (Fig.4.3) and the subject was instructed to bite to the level of an overlapping white bar over a centrally positioned red bar displayed on the screen.

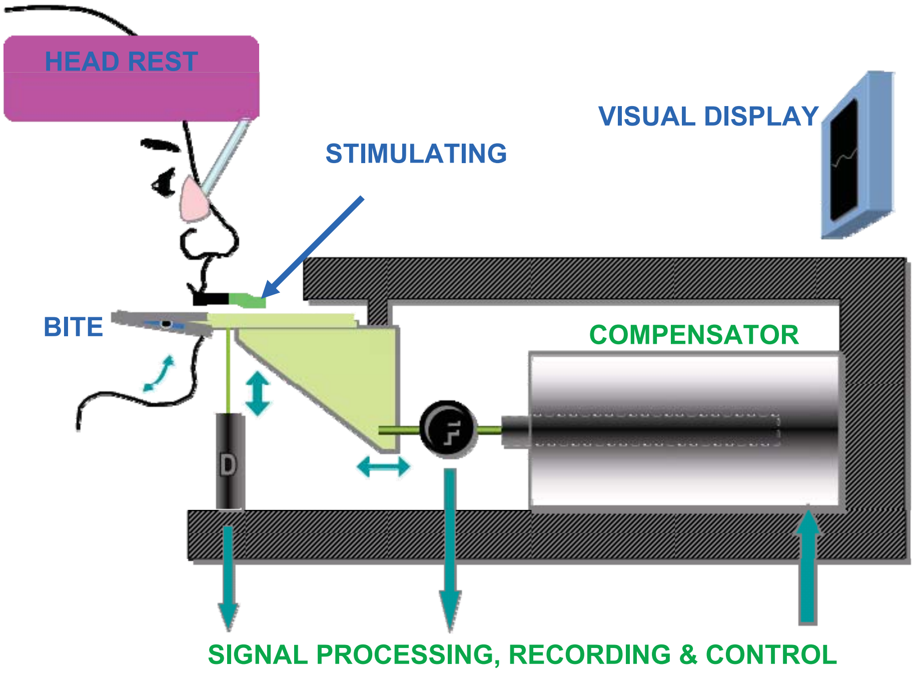


Fig. 4.3. A fixed reference area for the upper and lower jaws with bite plates was programmed for static and dynamic phases of rhythmic chewing. A sensitive compensator transferred pre-determined resistance to the bite plates. The visual display provided the feedback for required level of biting force during the static phase of each experiment.

4.2.3 STIMULUS PROFILE

Three different levels of force were delivered randomly during each phase of the experiments. The control, or pre-load, was 0.2 N and low stimuli and high stimuli were around 0.8 and 1.6N respectively (Fig.4.4). The stimulus profile was highly reproducible and was monitored on the visual display during the experiment.

The orthogonal stimulus was applied to the labial centre of the left central incisor. Axial stimulation was used previously[162] but had its limitations in the dynamic phase as it would not be in juxtaposition during mastication. This meant that there was lack of contact in certain cyclical phases with no stimulus rendered. Hence, in order to make the system reliable and reproducible orthogonal stimulus was chosen. The probe was applied directly onto the mid-labial surface of the upper left central incisor (21).

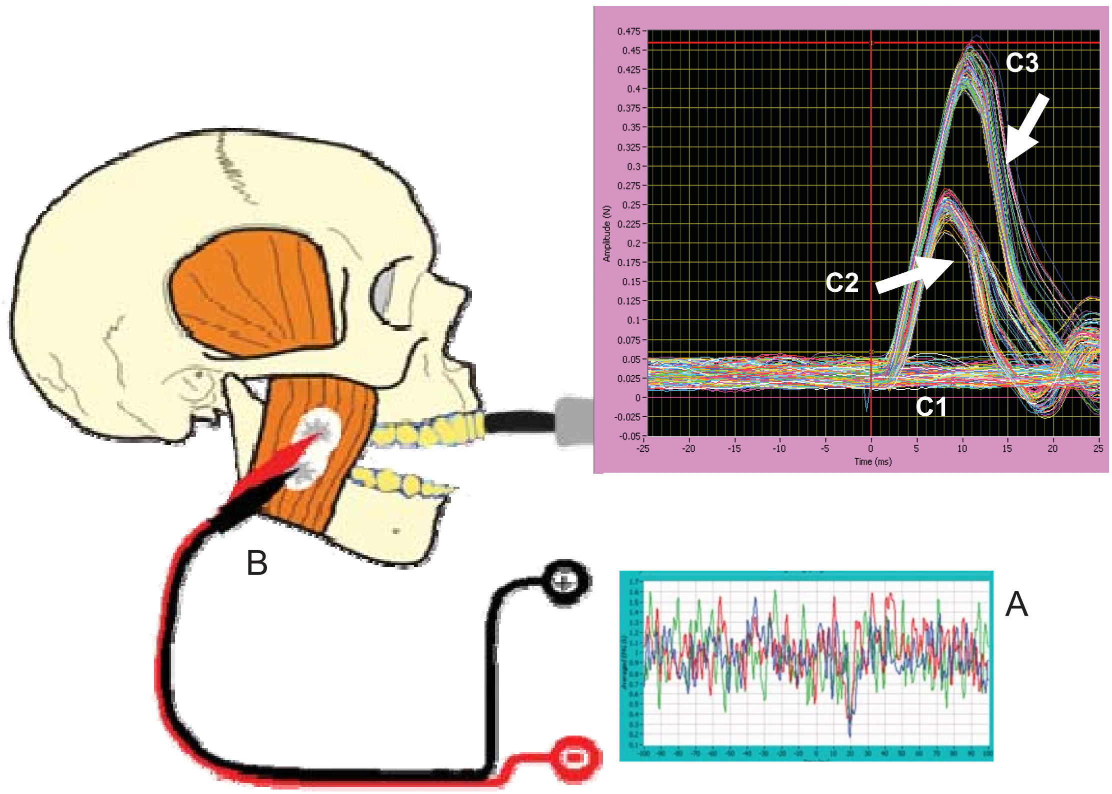


Fig. 4.4. EMG (A) was recorded from the masseter (B) during both Non-LA and LA trials. Examples of control (continuous 0.2N: C1), low (0.8N: C2) and high (1.6N: C3) force stimulations are shown.

Randomization is essential to counteract expectation and prediction by the subject. Inter-stimulus interval was individualized for each subject and was triggered at set points of open mouth and close mouth during the dynamic movements. Similarly, for static recordings the midpoints of closed and open mouth were utilized. The number of stimuli were adequate to help counterbalance the level of noise.

4.2.4 EMG RECORDINGS

The EMG signal was amplified at x5000 and band pass filtered at cut off frequencies of 20-500 Hz. All recordings were made with the Lab View® program (National Instruments) with seven channels (Fig.4.5).

Initially, rest position was recorded. This provided a baseline and assisted in evaluating noise during later analysis. A brief cycle of dynamic movement was recorded to determine the opening level which was to be used for the maximum static recording that followed thereafter. The *“dat result”* was converted into *“txt”* file and mid-open and mid-close distance was recorded. Unlike earlier work where the MVC (Maximum Voluntary Contraction) at various percentages was utilized for the subject feedback, customized open and closed mouth cycles were analysed with the IZZY® program. Values were then entered in for the High and Low static recordings. Hence, each subject had its own dynamic variable for feedback. Thus, the initial recordings were made on Dynamic approximately 300 triggers (mixed = low, high & control) and Static with High (100 triggers) and Low (100 triggers), details in Chapter 5.

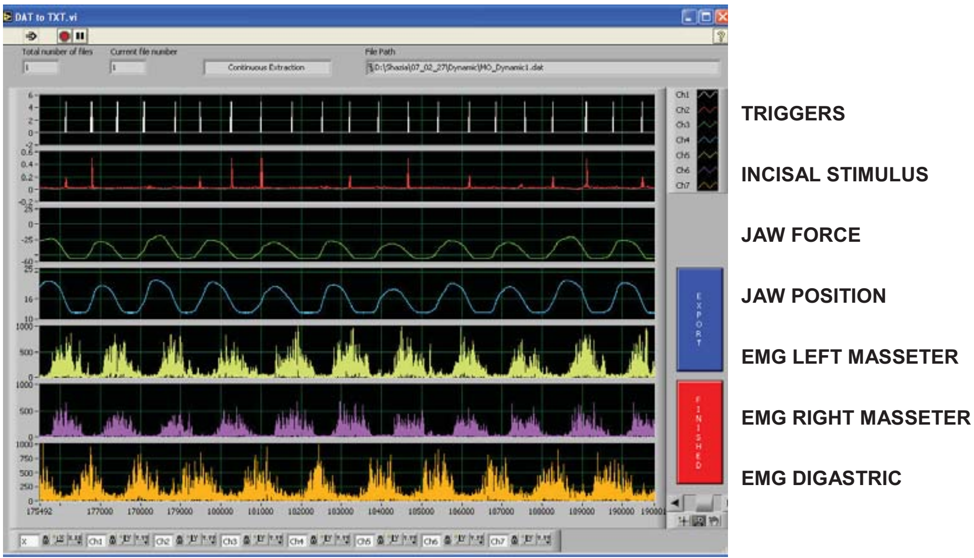


Fig 4.5. Screen shot of the computer generated triggers (white), profile of stimulus (red), Jaw force (green), Jaw position in opening and closing (blue) and raw EMG recorded from left masseter (yellow), right masseter (pink) and anterior digastric (orange). Total of seven channels were operating at the time of the experiments.

4.2.5 OFF LINE DATA ANALYSES

The data were analysed off-line by generating CUSUMS and the conventional normalization of reflexes as detailed below.

4.2.6 CUSUMS

The muscle spindle reflexes can be analysed with cumulative sums which are regarded as a more accurate representation of overall muscle activity[163].

In this study, the EMG was full-wave rectified and extracted around the trigger time for all conditions from -25 to +75ms. Cumulative sums (CUSUMS) of all reflexes were constructed (Fig.4.6) for all conditions using the method described by Brinkworth and Türker[102], which defines precise points of latencies, duration and strengths of the masseter muscle spindle reflexes.

CUSUM is calculated by

CUSUM (t) = $\sum_{tp-}^{t} bw \{ EMG(T) - \overline{EMG(To)} \}$

Where bw is the bin width in ms (inverse of sampling frequency in kHz), $\overline{EMG(To)}$ is mean pre-stimulus EMG while tp- is the pre-stimulus analysis time used. EMG is normalized to 1 and its units are k. Thus, the units of CUSUM are k.ms.[102]

Latency of the reflex is reflected in the number of synapses in the circuit. Percentage of the reflex change and duration help estimate strength of the connection between stimulated afferent nerves and the muscle. It also helps eliminate noise from the trace. Population CUSUM reflexes were calculated for all conditions across subjects (Fig.4.6). All reflexes analysed in this study are from surface EMGs obtained following effective local anaesthesia to evoke the spindle reflex.

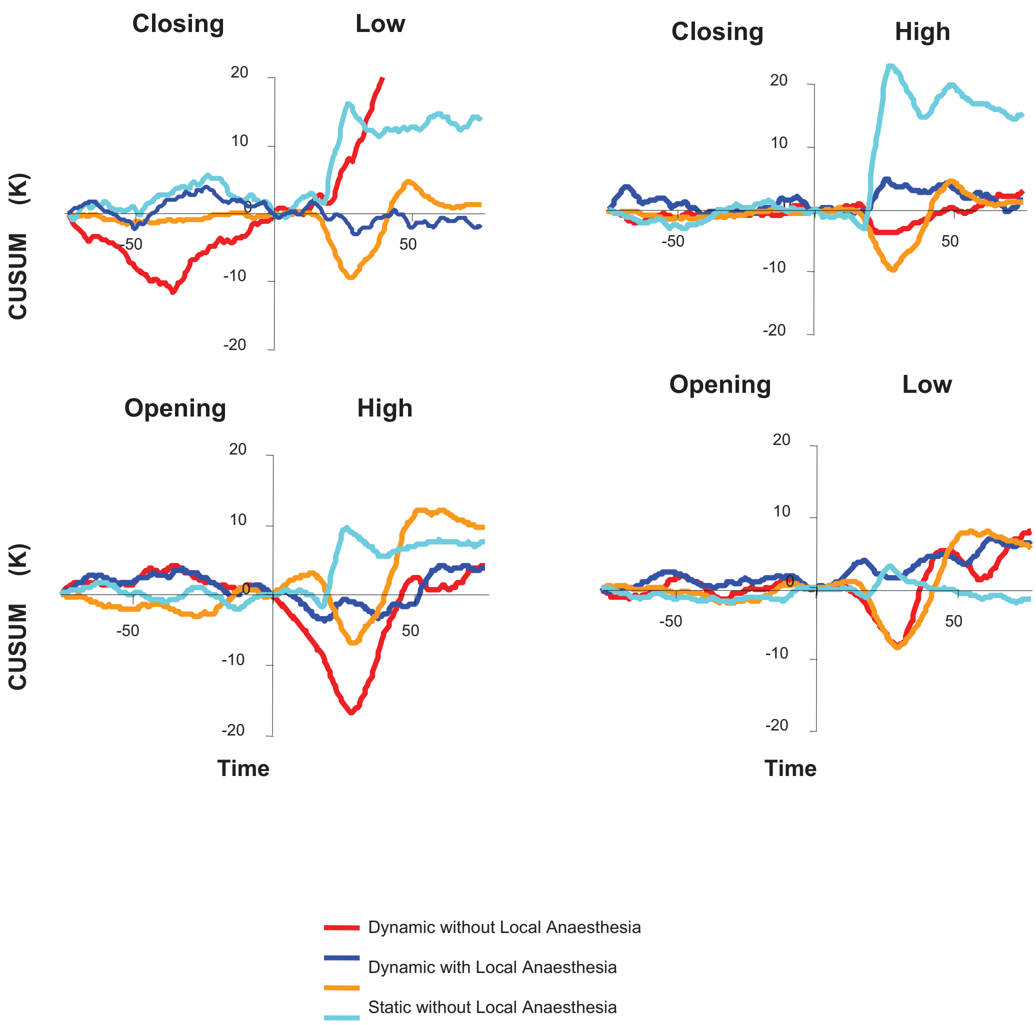


Fig. 4.6. CUSUM (Cumulative Sum) of left masseter reflex response to mechanical stimulation of left central incisor for all conditions are traced. The muscle spindle reflex (teal) is uninhibited following local anaesthesia and shows excitation in all conditions (results from one subject).

4.3 REFLEX ANALYSES

Rectified EMG was extracted around the trigger time (-50 to +75 ms).The extracted EMGs were sorted with regard to stimulus intensity and phase of movement. The dynamic with no stimulus trials were used to detrend the dynamic trials using a method similar to that reported by Hück *et al*., (2005)[83]. Briefly, the averaged rectified dynamic with stimulus trial was divided point-by-point by its respective non-stimulus representation. A 20 ms window starting 15 ms post-stimulus time was constructed and the reflex area (the integral above or below the pre-stimulus mean) was calculated. The strength of the reflex was recorded by expressing it as a percentage of the pre-stimulus area[164] between -5 and -25 ms.

2 repeated measures ANOVAs (SPSS 15.0®) were performed for:

1. Reflexes obtained prior to local anaesthesia (periodontal mechanoreceptor + muscle spindle response).
2. Reflexes obtained during local anaesthesia (muscle spindle response only).

When interactions between main factors were present, post hoc testing of means was conducted. The level of significance was set at 5% and, for post hoc tests, p-values were corrected using the Bonferroni method.

4.4 LATERAL CEPHALOMETRICS

Standard lateral cephalograms were taken with a magnification factor of 1.083 with the Siemens Nador 2 SR system (Bensheim, Germany) with specifications of 220V, 50 Hz and 10amp (Dr Jones & Partners Medical Imaging centre, Adelaide). The focus-film distance was 150cm and mid-sagittal plane- film distance was 11.5cm. The exposure time was 0.64sec with 75kv and 20mA.

Cephalometric tracings were performed on standard acetate paper (3M Unitek, Orthodontic Products, Monrovia, CA 91016, USA) with a 0.3mm graphite mechanical pencil in a darkened room for masseter muscle length evaluation only. All other measurements and digitization were done by a single investigator (SND) with computerized cephalometric software programs.

Dolphin® (Los Angles, California, USA) and Mona Lisa® (Tidbinbilla, Canberra, Australia) (Fig. 7.3A) cephalometric analysis software systems were used to evaluate selected linear, angular and proportional facial skeletal variables from scanned images of the lateral cephalograms.

4.4.1 ANALYSES ON DOLPHIN

The Dolphin® 10.5 version (Los Angles, California, USA) program was used for generating various analyses. The analyses selected specifically measured linear, proportional or angular anterior and posterior vertical face heights. Three analyses were chosen from the software: Roth-Jarabak, McLaughlin and Eastman.

The Roth-Jarabak analysis provided

1) Gonial angle (Ar-Go-Me)
2) Upper Gonial angle (Ar-Go-Na)
3) Lower Gonial angle (Na-Go-Me)
4) Ramus Height (Ar-Go)
5) Corpus Length (Go-Me)
6) P-A face height (S-Go/N-Me)
7) Jarabak Anterior ratio
8) Anterior Face Height (AFH)
9) Posterior Face Height (PFH)

The MacLaughlin analysis provided FMA (MP-FH) and the Palatal-Mandibular Angle. Finally, the Eastman analysis complemented with LAFH/TAFH percentage, LPFH (Go-MxP) and UPFH (S-MxP).

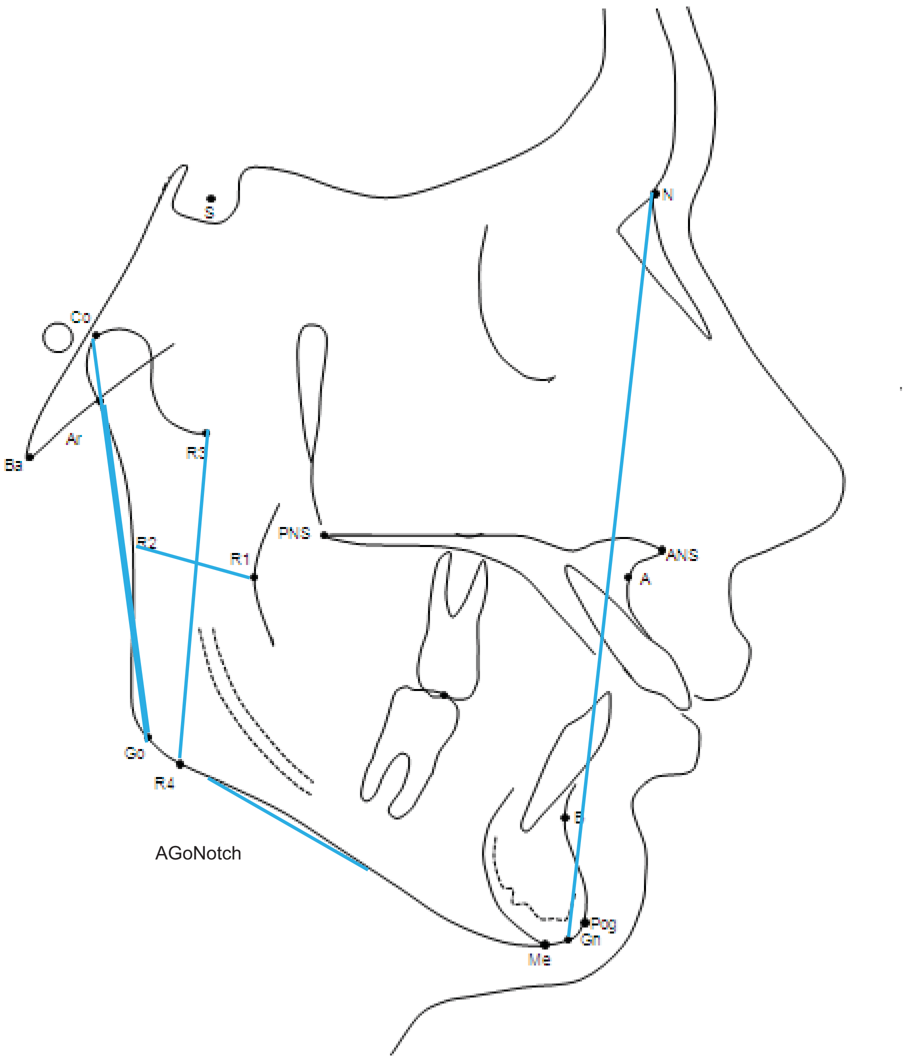


Fig 4.7. Linear cephalometric measurements, Co-Go, Ar-Go, R1-R2, R3-R4, N-Gn, AGoNotch.

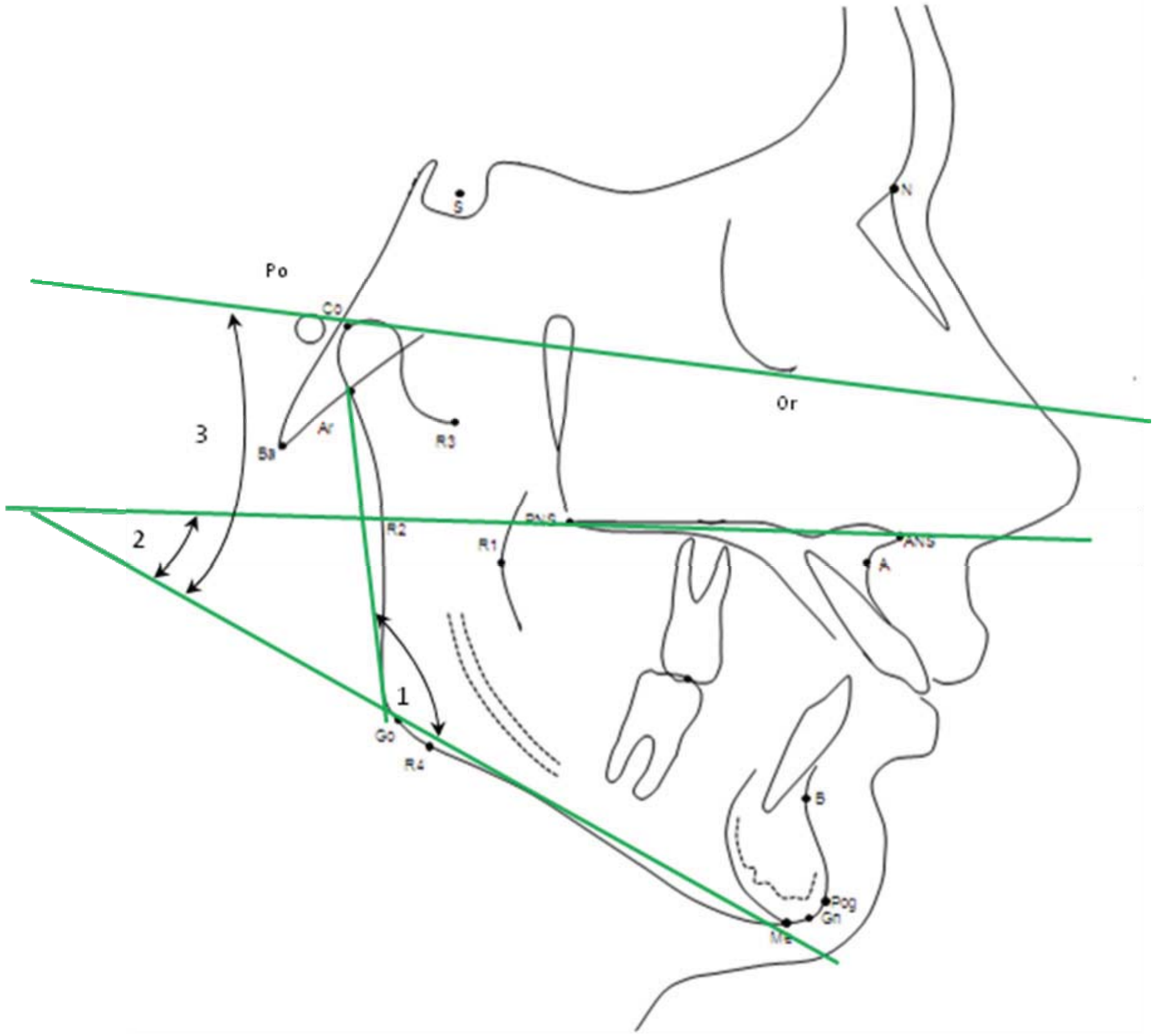


Fig 4.8. Angular measurements from lateral cephalogram 1.GoAngle, 2.Max-Man, 3.FH-Man

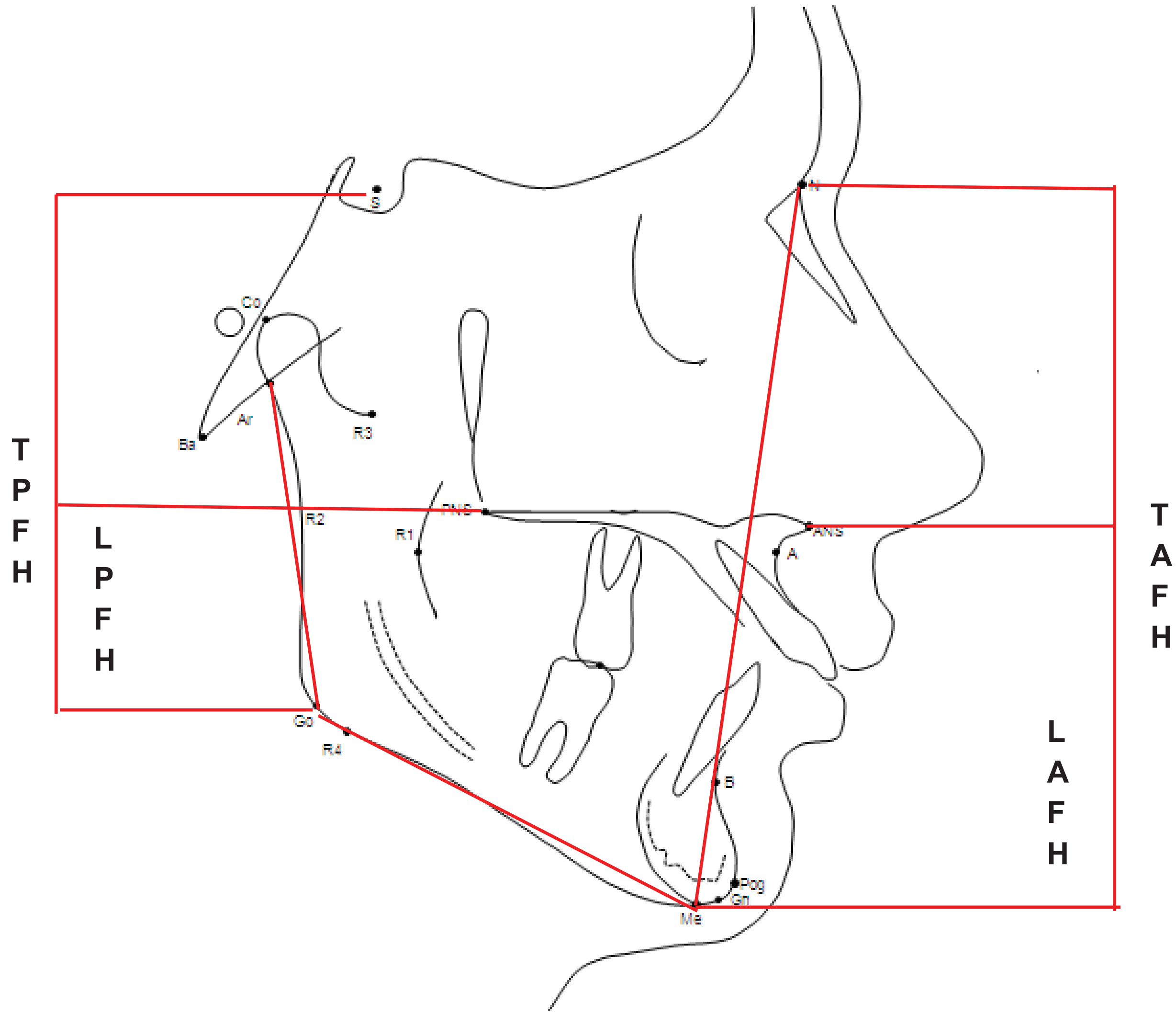
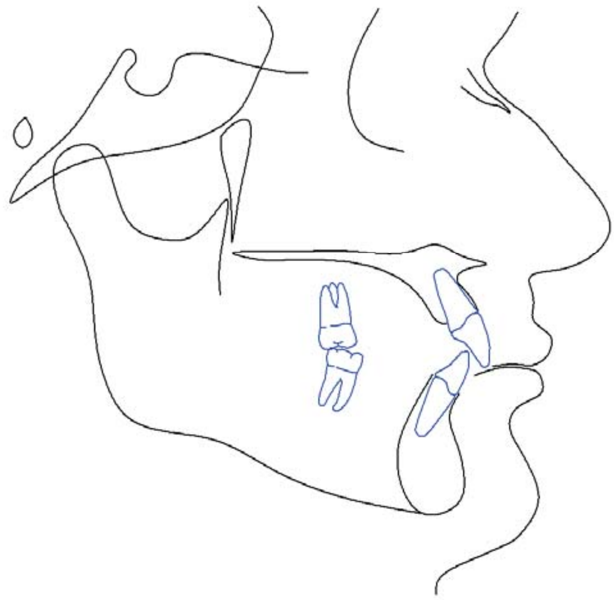


Fig 4.9. Proportional variables selected were LAFH/TAFH,LPFH/TPFH, ArGo-GoMe and Jarabak Ratio (NMe/GoAr)

22/11/2007 Initial Analysis: Jarabak Norm:Other

	Value	Norm	Std Dev	Dev Norm
CRANIAL BASE				
Saddle/Sella Angle (SN-Ar) (°)	124.8	124.0	5.0	0.2
Anterior Cranial Base (SN) (mm)	77.1	77.3	3.0	-0.1
Posterior Cranial Base (S-Ar) (mm)	38.6	37.0	4.0	0.4
MANDIBLE				
Gonial/Jaw Angle (Ar-Go-Me) (°)	123.5	120.8	6.7	0.4
Mandibular Body Length (Go-Gn) (mm)	88.8	80.0	4.4	2.0 **
Upper Gonial Angle (Ar-Go-Na) (°)	61.0	49.0	7.0	1.7 *
Lower Gonial Angle (Na-Go-Me) (°)	62.5	72.0	6.0	-1.6 *
Ramus Height (Ar-Go) (mm)	49.8	53.0	4.5	-0.7
A-P RELATIONSHIP				
SNA (°)	85.5	82.0	3.5	1.0 *
SNB (°)	85.4	80.9	3.4	1.3 *
ANB (°)	0.0	1.6	1.5	-1.0 *
Convexity (NA-APo) (°)	-4.9	2.5	3.0	-2.5 **
CRANIAL BASE / MANDIBLE				
Articular Angle (°)	131.8	138.0	6.0	-1.0 *
Sum of Angles (Jarabak) (°)	380.0	380.0	6.0	0.0
Jarabak Anterior Ratio (x100)	86.9	90.0	4.0	-0.8
MP - SN (°)	20.0	33.0	6.0	-2.2 **
Nasion-Gonion Length (mm)	117.5	134.4	4.0	-4.2 ****
Y-Axis Length (mm)	132.5	140.0	6.0	-1.3 *
Facial Plane to SN (SN-NPog) (°)	87.8	82.0	4.0	1.5 *
Posterior Face Height (SGo) (mm)	80.8	90.0	5.0	-1.8 *
Anterior Face Height (NaMe) (mm)	107.9	139.0	5.0	-6.2 ******
P-A Face Height (S-Go/N-Me) (%)	74.9	65.0	4.0	2.5 **
Y-Axis (SGn-SN) (°)	56.6	67.0	5.5	-1.9 *
SKELETAL / DENTAL				
IMPA (L1-MP) (°)	101.9	95.0	7.0	1.0 *
FMIA (L1-FH) (°)	64.8	65.7	8.5	-0.1
L1 - Facial Plane (L1-NPo) (mm)	0.6	1.0	2.0	-0.2
U1 - NPo (mm)	5.1	5.0	2.0	0.1
U1 - SN (°)	114.4	103.1	5.5	2.0 **
Mand Plane to Occ Plane (°)	14.1	18.6	5.0	-0.9
DENTAL				
Interincisal Angle (U1-L1) (°)	123.7	130.0	6.0	-1.1 *
SOFT TISSUE				
Lower Lip to E-Plane (mm)	-5.7	-2.0	2.0	-1.9 *
Upper Lip to E-Plane (mm)	-6.8	-8.0	2.0	0.6



22/11/2007 Initial Analysis: Jarabak Norm:Other

	Value	Norm	Std Dev	Dev Norm
CRANIAL BASE				
Saddle/Sella Angle (SN-Ar) (°)	112.5	124.0	5.0	-2.3 **
Anterior Cranial Base (SN) (mm)	67.4	75.3	3.0	-2.7 **
Posterior Cranial Base (S-Ar) (mm)	33.5	35.0	4.0	-0.4
MANDIBLE				
Gonial/Jaw Angle (Ar-Go-Me) (°)	120.9	122.9	6.7	-0.3
Mandibular Body Length (Go-Gn) (mm)	74.2	75.2	4.4	-0.2
Upper Gonial Angle (Ar-Go-Na) (°)	47.1	52.0	7.0	-0.7
Lower Gonial Angle (Na-Go-Me) (°)	73.8	71.2	6.0	0.4
Ramus Height (Ar-Go) (mm)	43.3	48.5	4.5	-1.2 *
A-P RELATIONSHIP				
SNA (°)	83.1	82.0	3.5	0.3
SNB (°)	81.7	80.9	3.4	0.2
ANB (°)	1.4	1.6	1.5	-0.2
Convexity (NA-APo) (°)	3.2	4.9	3.0	-0.6
CRANIAL BASE / MANDIBLE				
Articular Angle (°)	157.6	140.3	6.0	2.9 **
Sum of Angles (Jarabak) (°)	391.1	386.6	6.0	0.7
Jarabak Anterior Ratio (x100)	90.8	93.0	4.0	-0.5
MP - SN (°)	31.1	33.0	6.0	-0.3
Nasion-Gonion Length (mm)	107.4	127.8	4.0	-5.1 *****
Y-Axis Length (mm)	123.3	131.0	6.0	-1.3 *
Facial Plane to SN (SN-NPog) (°)	81.5	80.5	4.0	0.3
Posterior Face Height (SGo) (mm)	75.3	82.5	5.0	-1.4 *
Anterior Face Height (NaMe) (mm)	111.3	128.5	5.0	-3.4 ***
P-A Face Height (S-Go/N-Me) (%)	67.7	65.0	4.0	0.7
Y-Axis (SGn-SN) (°)	65.8	67.0	5.5	-0.2
SKELETAL / DENTAL				
IMPA (L1-MP) (°)	99.1	95.0	7.0	0.6
FMIA (L1-FH) (°)	52.1	64.8	8.5	-1.5 *
L1 - Facial Plane (L1-NPo) (mm)	8.1	1.5	2.0	3.3 ***
U1 - NPo (mm)	11.2	5.0	2.0	3.1 ***
U1 - SN (°)	108.1	102.8	5.5	1.0 *
Mand Plane to Occ Plane (°)	18.2	17.4	5.0	0.2
DENTAL				
Interincisal Angle (U1-L1) (°)	121.7	130.0	6.0	-1.4 *
SOFT TISSUE				
Lower Lip to E-Plane (mm)	-0.1	-2.0	2.0	1.0 *
Upper Lip to E-Plane (mm)	-0.9	-6.0	2.0	2.6 **

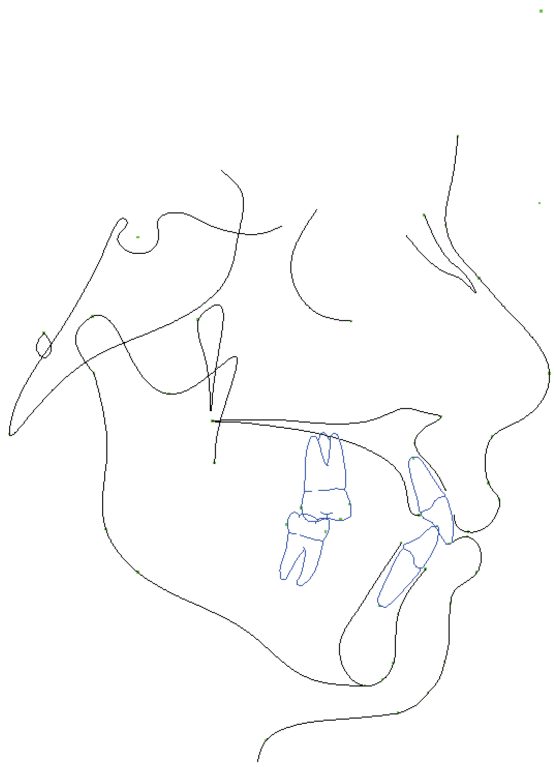


Fig 4.10 Examples of Dolphin® images for reduced (above) and average face height (below) subjects

4.4.2 MANUAL TRACING

Conventional manual tracing was performed to estimate masseter muscle length by the origins and insertions derived from the bony landmarks[31, 120]. Cephalometric tracings were performed on standard acetate paper (3M Unitek, Orthodontic Products, Monrovia, CA 91016, USA) with a 0.3mm graphite mechanical pencil in a darkened room. All tracings and digitizations were done by a single investigator (SND) in order to overcome inter-examiner variability.

The architecture of the masseter muscle is complex with the multi-pennate and layered internal organization of superficial, intermediate and deep attachments[30]. The decision had to be made to measure the superficial and intermediate masseter muscle attachments as the deep component would be impossible to estimate from the lateral cephalogram alone. Detailed anatomical attachment levels [Ebert, 1939; Schumacher, 1961][30] and "Sehnenspiegel" –internal aponeuroses- are parasagittally aligned from the zygomatic arch tubercle anteriorly to the most posterior portion of the arch almost to the level of the coronoid process as the masseter progresses from superficial to deep fibres.

4.4.2.1 Superficial Masseter Muscle

The superficial masseter muscle length estimate was made from the zygomatic tubercle to the gonial angle with a digital caliper, to two decimal places.

4.4.2.2 Intermediate Masseter Muscle

For landmark clarity and enhanced reproducibility, the posterior extreme of the zygomatic process was taken as the point of origin and gonion as the area of insertion. These estimates provided the relative measure for the intermediate masseter. It is apparent that the measurements for the intermediate should be less than superficial by 5-35%[30].

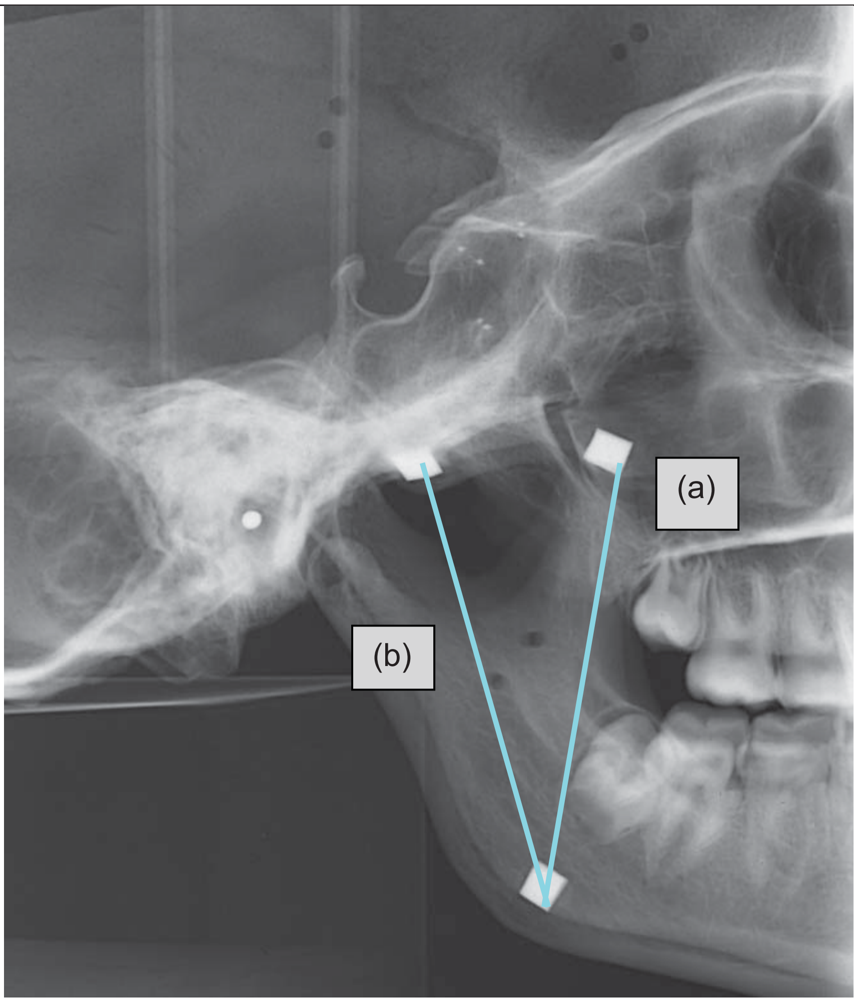
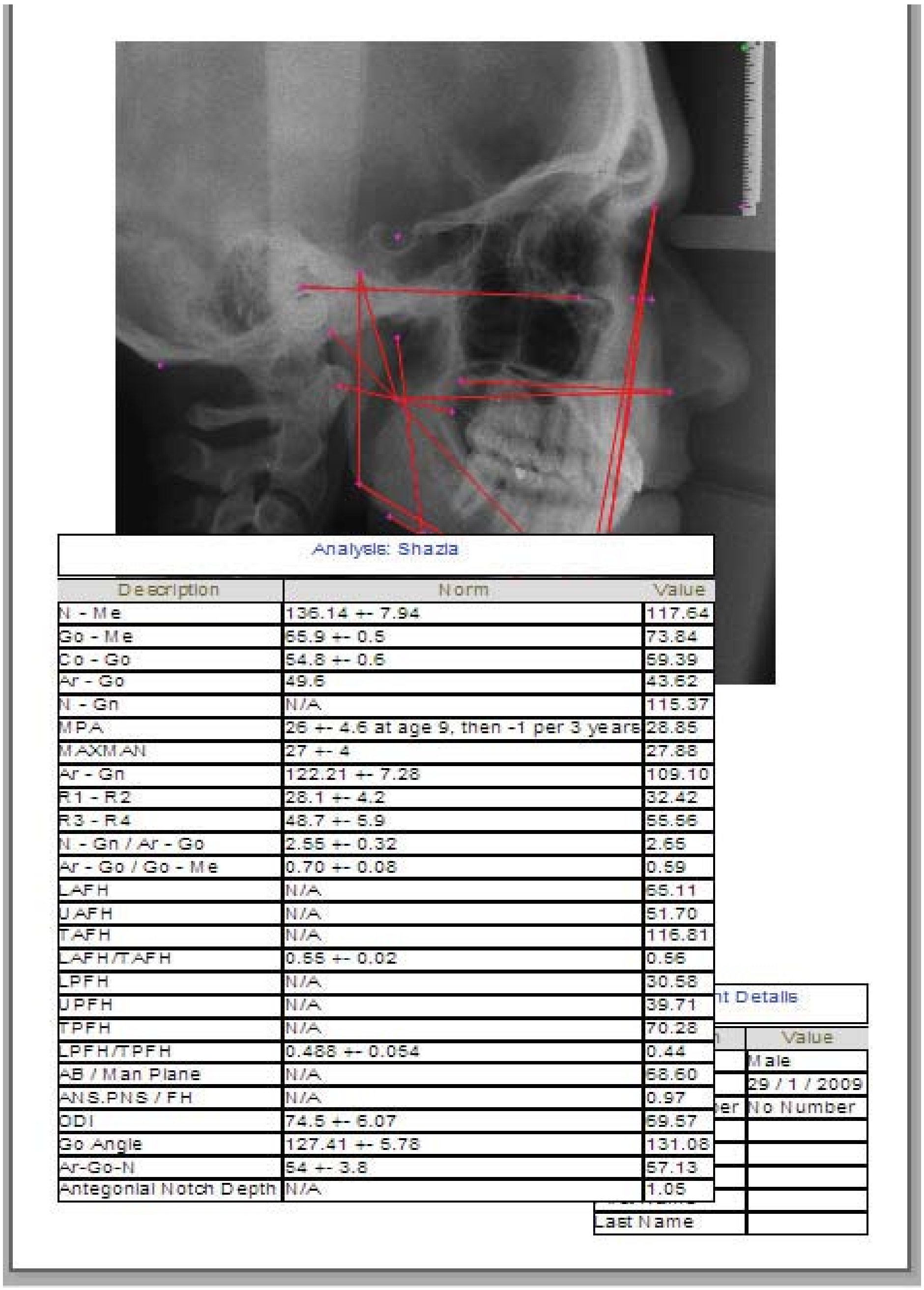


Fig. 4.11. Estimation of masseter muscle lengths from the pilot study (Chapter7) with dried skulls and lead markers, (a) represents the superficial masseter muscle while (b) estimates the intermediate masseter muscle.

The measurements were then corrected to the 8% magnification factor of the lateral cephalogram. Ten percent of the sample was randomly selected and retraced for repeatability. Dahlberg's statistic was calculated.



Analysis: Shazia

Description	Norm	Value
N - Me	136.14 +- 7.94	117.64
Go - Me	65.9 +- 0.5	73.84
Co - Go	54.8 +- 0.6	59.39
Ar - Go	49.6	43.62
N - Gn	N/A	115.37
MPA	26 +- 4.6 at age 9, then -1 per 3 years	28.85
MAXMAN	27 +- 4	27.88
Ar - Gn	122.21 +- 7.28	109.10
R1 - R2	28.1 +- 4.2	32.42
R3 - R4	48.7 +- 5.9	55.56
N - Gn / Ar - Go	2.55 +- 0.32	2.65
Ar - Go / Go - Me	0.70 +- 0.08	0.59
LAFH	N/A	65.11
UAFH	N/A	51.70
TAFH	N/A	116.81
LAFH/TAFH	0.55 +- 0.02	0.56
LPFH	N/A	30.58
UPFH	N/A	39.71
TPFH	N/A	70.28
LPFH/TPFH	0.488 +- 0.054	0.44
AB / Man Plane	N/A	68.60
ANS.PNS / FH	N/A	0.97
ODI	74.5 +- 6.07	69.57
Go Angle	127.41 +- 5.78	131.08
Ar-Go-N	54 +- 3.8	57.13
Antegonial Notch Depth	N/A	1.05

Fig 4.12. Screen shot of Mona Lisa® program with custom made variables specific for the analyses used in the current study.

The specific variables required for further analysis were custom defined in the Mona Lisa program which provided:

1) Ricketts Analysis with R1-R2 and R3-R4
2) Overbite Depth Indicator (ODI) from Kim[165]
3) Ante-gonial Notch Depth

All variables from Dolphin® were grouped together into a composite analysis creating an extensive list (Fig.4.12).

4.5 ULTRASONOGRAPHY

Eleven volunteers from the original EMG cohort (8 females; age range 22-30 years) participated in the US experiments. Each subject was provided with a detailed information sheet and written consent was obtained. All participants had natural and healthy dentitions, were free of dental symptoms at the time of the experiments and displayed no discernable cranio-facial dysmorphic traits. Approval was obtained from both the University of Adelaide and University of South Australia Human Ethics Committees.

All scans were conducted in a semi-darkened room. Each subject was comfortably seated with the left side of the face towards the examining sonographer (Dr K Thoirs). Images were obtained in a plane between the zygomatic tubercle to the gonial angle following the protocol of the pilot study (Chapter 7). Subjects were instructed to bite down into intercuspal position with maximum clench. This position could be held between 10-15sec comfortably.

After image acquisition, three measurements of the masseter muscle were made with the inherent electronic calipers i.e the length, thickness and cross-sectional area (Details in Chapter 7). The length of the masseter muscle was measured as the distance between the zygomatic tubercule and the gonial angle. The thickness was measured as the maximum distance between the superficial and deep aspects of the masseter muscle (Fig. 7.2, Chapter 7).

Cross-sectional area was measured using a trace tool on the machine which allowed the sonographer to manually define the boundaries of the muscle. The volume of the masseter muscle was calculated for each subject with the formula

$V=1/3\pi\ r.h$, where V is cone volume, r is radius and h is height.

Extended Field Of View (EFOV) Ultrasonography acquired one image from which all measurements were made. EFOV was chosen because the field of view of the transducer did not cover the entire length of the masseter muscle due to the limited size of the transducer face. EFOV allows an image to be generated beyond the size of the transducer[166].

4.6 3 D IMAGING

Finally, for the 3D imaging ten volunteers from same original cohort (8 females; age range 22-30 years) were recruited with ethics approval from the University of Adelaide. Each subject was requested to sit comfortably on a back supported chair in natural head posture in a well lit room. Instructions were given to keep the eyes open if possible and to have the hair tucked so that the face could be imaged clearly.

The structured light projector was placed 1.5 metres away from the subject and the horizontal light grid was flashed for 20 seconds (Details in Chapter 8). Two frontal images were captured along with one profile image of the left side.

The data were then computer processed to perform the triangulation. The raw scans of the subjects produced approximately 200,000 3D points. The raw scans were further processed in Vr Mesh (http://www.vrmesh.com/) to remove unwanted background data and noise. The area of interest was manually extracted from the raw scan and then uniformly re-sampled which removed points from a scan to produce constant inter-point distance. The re-sampled scans were de-noised with a filter and then converted into 3D mesh-triangles for measurement.

4.6.1 3D IMAGING ANALYSES

The images were then analysed on VrMesh (http://www.vrmesh.com/) software (details in Chapter 8). The variables provided information of vertical and transverse and facial measurements.

4.6.2 3D INDICES

The landmarks and indices selected for 3D imaging were located by enhancing the image by scan magnification or rotations.
The landmarks were described with Greek or Latin terminology similar to that in lateral cephalometrics.
Overall six indices were used:

1. Facial Index	n-gn x 100/zy-zy
2. Jaw Index	sto-gn x 100/ go-go
3. Index of jaw /facial width	go-go x 100/ zy-zy
4. Index of jaw width / facial height	go-go x 100/ n-gn
5. Index of lower jaw / facial height	sn-gn x 100/ n-gn
6. Index of jaw / face height	sto-gn x 100/ n-sto

These represented the vertical and transverse proportions in the mid-face and lower jaw regions, derived from Farkas[126].

4.6.3 TRANSVERSE DIMENSIONS

The 3D scan was rotated right and left to precisely position the zygonion as the most lateral point on the zygomatic arch. Even though palpation would be preferred, the scans produced distinct shadows that assisted recognition of the bizygion diameter (zy-zy) which represented the transverse dimension of the upper face. The bigonial diameter (go-go) represented the width of the lower face. Palpation is generally advantageous for precise positioning of gonion (most lateral point on the mandibular angle) landmark.

4.6.4 PERPENDICULAR MEASUREMENTS

Midline landmarks and inter-distances generated perpendicular measurements.
Nasion (n) point is the same for bony and soft tissue and is relatively easy to identify. It presents as a slight ridge in the midline at the nasal root. Subnasale (sn) is the midpoint between the columella base and the lower border of the nasal septum intersecting with the surface of the upper lip. It too was relatively easily identified on the 3D images when, for better viewing, the image was rotated by 30°. Stomion (sto) is the centre point in the imaginary crossing of vertical facial midline and the lip fissures and is easily identified when lips are competent with a prominent philtrum. Again, stomion is a very easy and reproducible landmark in 3D scans. Gnathion (gn) is defined as the lowest point on the median lower border of the mandible. However, it proved somewhat difficult in the 3D scans with noise and lack of smooth reproduction of the chin region. Face height was measured from nasion to gnathion (n-gn) and mandibular height from stomion to gnathion (sto-gn).

4.6.5 TANGENTIAL / CURVILINEAR MEASUREMENTS

The curvilinear measurements were included to supplement the data; particularly, as 3D images have the unique advantage of measuring surface contours without rendering them to simple straight lines.

The mandibular curvilinear arc, extending from left tragus to subnasale to right tragus (t-sn-t) was measured. The tangential mandibular depth was measured from tragus to gnathion (t-gn) from the left aspect only. A second angular measurement was generated from tragus to gonion (t-go) and gonion to gnathion (go-gn) and defined as the mandibular inclination.

4.7 STATISTICAL ANALYSES

There was an adequate sample size for the EMG study (n=28) but there was a subsequent attrition of the cohort over the period of time with a relatively small sample available for the 3D imaging (n=10). Overall, the results were normally distributed. Descriptive statistics were presented for the different modalities ie EMG, lateral cephalogram, US and 3D imaging (Chapters 5,6,7,8). For the smaller sample size Spearman's rank order correlations were considered a better method of statistical analyses, but as predictive equations were required, Pearson correlations were finally utilized. Pearson correlations (**r**) were generated due to the normal distribution of the data. The correlations gave us an idea of the significant relationships between and among the modalities studied. Moreover, these could be compared with previous studies.

Subsequently, it was deemed necessary to categorize large data sets with different variables into factors. Hence, Factor Analyses was used and generated with the statistical package SAS 9.1 (SAS Institute, Inc. Cary, NC. USA). Eigenvalues assisted in factor scores and solutions. This allows the data to be visually assessed from scree plots along the elbow effect hyper association. The general guidelines suggested for Eigenvalues are to have values less than 1 as these assist in determining the number of factors to retain for the final analyses. ANOVA were generated to assess the model outcome for a typical linear regression which was the case for the data and hence the applicability of predictive variance to generate predictive equations. More specific details are presented in Chapters 5-8 in the Results section.

4.7.1 RELIABILITY

The reliability of the imaging techniques were subject to two different types of test. Firstly, Dahlberg's statistic was utilized for repeatability for lateral cephalometrics, US and 3D imaging where intra-examiner reliability was evaluated.

Secondly, in order to find the degree of reproducibility between different methods, the Bland and Altman method[167] was utilized. Hence, our work showed a high reproducibility for US and caliper measurements. Likewise, with US with 3D imaging and lateral cephalogram with US showed high agreement. Limits of measurements were set at 95% Confidence Intervals (CI).

With 3D imaging, the landmarks were located on 3 different occasions. We were able to identify the landmarks that had higher reproducibility as compared to others purely by the fact that their position was easy to identify on repeated trials.

4.7.2 DAHLBERG'S STATISTIC

This test evaluates the method error. Overall, the digitization and cephalometric analysis from the Dolphin and Mona Lisa programs showed good reproducibility. For masseter lengths the Dahlberg statistic was 1.08 (good reproducibility) for manual tracings and caliper readings. It is not possible to repeat surface EMG due to its inherent issues with exact and precise placement of electrodes and biological changes[98, 168]. Details in Chapters 5-8.

4.7.3 BLAND AND ALTMAN

Good agreement which was set at 95% of the confidence level was found with the pilot study with skulls where direct caliper measurements were taken and compared with US e-tool measurements for simulated masseter muscle lengths (Chapter 7). Therefore, we were confident with the methodology when assessing the masseter in live volunteers.

4.7.4 PREDICTIVE EQUATIONS

One of the aims of the study was to produce meaningful predictive equations hence obviating the need to repeat ionizing radiation investigations. We duly acknowledge the relatively small sample size; however, it provides a platform for future investigations.

Predictive equations can be generated by several different statistical methods. However, a simple cost-effective method would use typical linear regression models whereby a sufficient sample would usually be in the order of 15-20 and the expected strong relationships would provide significant equations. This is generally the viable option with one to one relationships and where short equations are most likely to be used but, if three or more predictors are used with overall weak relationships, then one would require larger sample sizes and more sophisticated statistics to generate meaningful predictive equations (Chapter 8).

The predictive equations were generated with SAS 9.1 (SAS Institute, Inc. Cary, NC. USA) and the significance was suggested by p-value ≤ 0.05 at 95% confidence. Predictive equations were formulated for lateral cephalometric, US and 3D imaging to provide potential alternatives for information normally gained through lateral cephalograms. Some variables and landmarks showed higher significance than others providing stronger relationships and more significant predictive equations (Chapters 7, 8).

The results and their discussions are presented in the form of four papers prepared for publication.

Chapter 5	Modulation of Masseteric Reflexes by Simulated Mastication (**accepted Journal of Dental Research**)
Chapter 6	Masseter length determines Muscle Spindle Reflex excitability during jaw closing movements (submitted American Journal of Orthodontics and Dentofacial Orthopedics)
Chapter 7	Study of function and form for human masseter muscle with ultrasonography and lateral cephalometrics (to be submitted Investigative Radiology)
Chapter 8	Analysis and correlations of human masseter muscle with 3D imaging, ultrasonography and lateral cephalometrics (to be submitted American Journal of Orthodontics and Dentofacial Orthopedics)

In addition components of the research have been presented at professional and scientific meetings in oral and poster formats (Details in Appendix).

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5 MODULATION OF MASSETERIC REFLEXES BY SIMULATED MASTICATION

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5.1 ABSTRACT

It is well known that limb muscle reflexes are modulated during human movements. However, little is known about the existence of equivalent masticatory muscle reflex modulation. We hypothesised that masticatory reflexes would be modulated during chewing so that smooth masticatory movements occur. To examine this hypothesis, we studied the modulation of inhibitory reflexes evoked by periodontal mechanoreceptor activation and of excitatory reflexes evoked by muscle spindle activation during simulated mastication. In 28 participants, 1 and 2 N mechanical taps were delivered to the incisor. Responses to these taps were examined using spike-triggered averages of the masseteric electromyogram. In order to differentiate between periodontal mechanoreceptor and muscle spindle mediated reflex components, experiments were performed prior to, and in the presence of, periodontal anaesthesia. Both periodontal mechanoreceptor and muscle spindle reflexes were reduced during simulated masticatory movements.

KEY WORDS

Periodontal Mechanoreceptors, Masseter, Reflexes, Muscle Spindles, Electromyography

5.2 INTRODUCTION

Afferent feedback from peripheral and muscle receptors is modulated in human limbs so that movement is both effectively controlled and allowed to occur smoothly[79]. However, it is not known whether similar modulation of input to jaw muscles occurs during masticatory movements. Studies on functional connectivity between human orofacial afferents and jaw muscle motoneurons during movement pose significant technical challenges hence, other than two recent studies: Hück et al. [83] and van der Bilt *et al*.[169]; all such investigations to date have used static jaw conditions [reviewed in Türker[3]]. The study of van der Bilt *et al.* found no modulation of the muscle spindle reflex during various phases of jaw closing. However, in that study, no comparison was made to EMG-matched static reflexes. Therefore, the degree of muscle spindle reflex modulation by masticatory movement has yet to be investigated. No studies have been conducted that measure periodontal mechanoreceptor reflex modulation during jaw movement.

In the present study we sought to test the hypothesis that periodontal mechanoreceptor and muscle spindle reflexes are modulated during mastication. Specifically, we hypothesised that, periodontal mechanoreceptor reflexes which are protective in nature, would be modulated downward only when small stimulus intensities were applied to a tooth. In regard to muscle spindle reflexes, we hypothesised that the extent of reflex modulation would depend on movement phase, i.e. there would be a diminution of the reflex strength during the opening phase compared to the closing phase.

5.3 METHODS

5.3.1 PARTICIPANTS

Experiments were approved by the University of Adelaide human ethics committee and conformed to the Declaration of Helsinki. A total of 28 participants (16 females; age range 19-38 years) signed informed consent. All had natural and healthy dentitions.

Experimental set-up

Participants sat in a dental chair adjusted for height such that the horizontal plane of their upper dental arch was aligned with the upper bite plate of a custom-built mastication apparatus[7]. Bite plates were coated with a semi-rigid dental impression material (3M Express™, Michigan) moulded to each of the participants' teeth. The upper impression had a rectangular region cut away to expose the upper left central incisor so it could be contacted by a tooth stimulator.

Electromyography (EMG) recording

Following skin preparation, adhesive EMG electrodes were affixed to the skin over the left masseter. Participants were grounded via an ear-clip electrode. EMG was amplified (1000 – 10000x), filtered (20-1000Hz) and sampled at 2000Hz. EMG presented to participants as online feedback for the STATIC segments of the experiment was full-wave rectified and low-pass filtered (cut-off frequency 1Hz).

5.3.2 PROTOCOL

Experiments were divided into two main portions: DYNAMIC and STATIC. During the DYNAMIC phase, participants "chewed" against a spring-like resistance provided by a servo-controlled motor. Resistance to jaw closing was inversely proportional to jaw gape, i.e. minimal at maximal opening and maximal (~60N) at occlusion. Participants were instructed to "chew" at a natural pace. Three predetermined stimulus waveforms were sent to a dedicated proportional-integral-derivative loop which controlled the tooth stimulator. These were superimposed upon a constant tooth preload force (0.1N) and consisted of a half sinusoidal 2N pulse (HIGH STIMULUS), a half sinusoidal 1N pulse (LOW STIMULUS) and pulse of zero amplitude (NO STIMULUS) randomly sorted and delivered in response to a trigger that occurred each time the jaw passed through the mid-gape point (~14mm measured interincisally) (Fig. 5.1). 300 randomly-ordered, stimuli were delivered (100 LOW STIMULUS, 100 HIGH STIMULUS, and 100 NO STIMULUS).

Rectified EMG recorded around the trigger during opening and closing (NO STIMULUS) was averaged off-line and used as EMG feedback for the STATIC portions of the experiment. STATIC tasks were performed with the dynamic lower bite bar set in position-hold mode at each participant's mid-gape jaw position. Participants contracted isometrically to two levels i.e. either "HIGH EMG" (corresponding to the EMG evoked during jaw-closing around mid-gape) or "LOW EMG" (corresponding to the EMG evoked during jaw-opening) whilst the tooth was stimulated at random intervals.

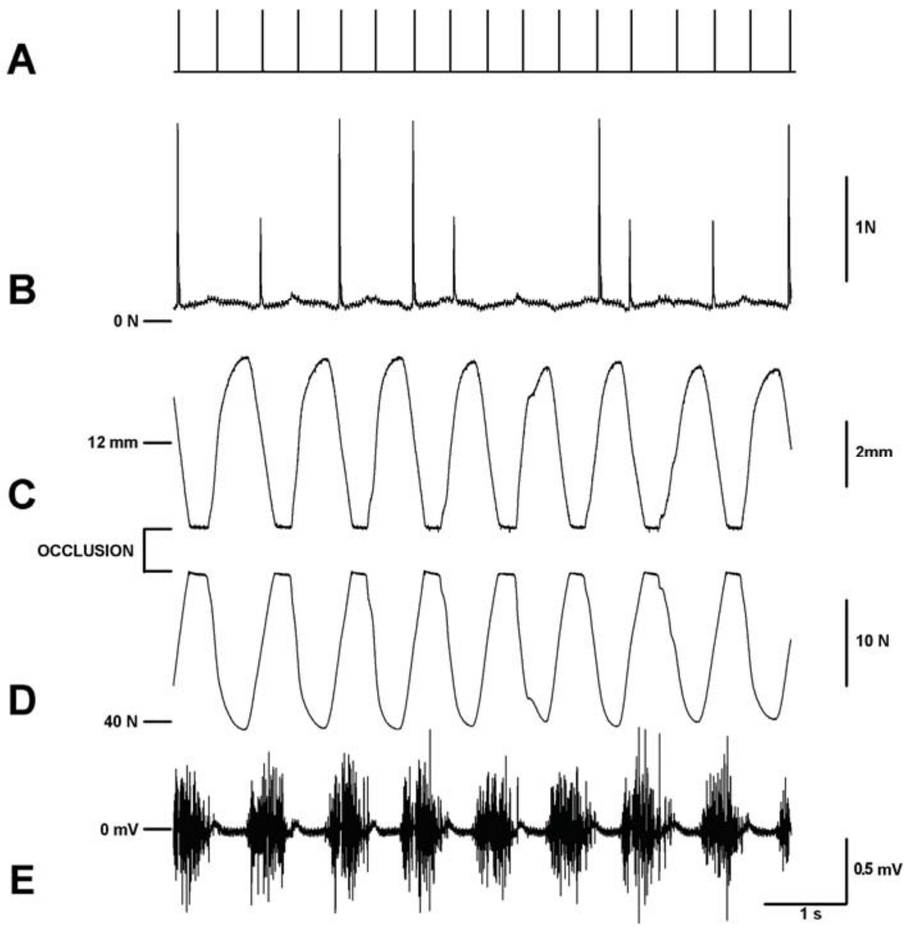

Fig. 5 1. Raw data recorded during a DYNAMIC experiment (n=1). A. Triggers generated each time jaw crossed mid-gape (12 mm on this subject); B. Forces applied to the upper left central incisor tooth at each trigger randomly (0, 1 and 2N). C. Jaw gape measured as interincisal distance. D. Mandibular resistance force. E. Masseteric EMG.

Once all procedures had been performed, approximately 4ml of local anaesthetic (LA) solution (Xylocaine™, Dentsply) was administered to the upper and lower central peri-incisal periodontium and the incisive papilla. Once anaesthesia had been achieved, the experimental procedure was repeated.

5.3.3 DATA ANALYSIS

Rectified EMG was extracted around the trigger time (-50 to +75 ms). The extracted EMGs were sorted with regard to stimulus intensity and phase of movement. The DYNAMIC with NO STIMULUS trials were used to detrend the DYNAMIC trials using a method similar to that reported by Hück et al.[83] Briefly, the averaged rectified DYNAMIC with STIMULUS trial was divided point-by-point by its respective NO STIMULUS representation. A 20 ms window starting 15 ms post-stimulus time was constructed and the reflex area (the integral above or below the prestimulus mean) was calculated. The strength of the reflex was recorded by expressing it as a percentage of the pre-stimulus area [170] between -25 and -5 ms (Fig. 5.2).

Three-way repeated measures ANOVAs (SPSS 15.0®) were performed for the data set obtained prior to local anaesthesia (periodontal mechanoreceptor + muscle spindle response) and the data set obtained during local anaesthesia (muscle spindle response only). The main factors included in the analysis were JAW CONDITION (static or dynamic), LEVEL OF BACKGROUND EMG (static: high or low; dynamic: close or open) and STIMULUS INTENSITY (1 N or 2 N).

When interactions between main factors were present, post hoc testing of means was conducted. The level of significance was set at 5% and, for post hoc tests, p-values were corrected using the Bonferroni method.

5.4 RESULTS

Prior to the administration of local anaesthetic, the reflex response elicited by mechanical stimulation of a tooth was inhibitory. During local anaesthesia the same stimulus evoked an excitatory reflex (Fig.5 2).

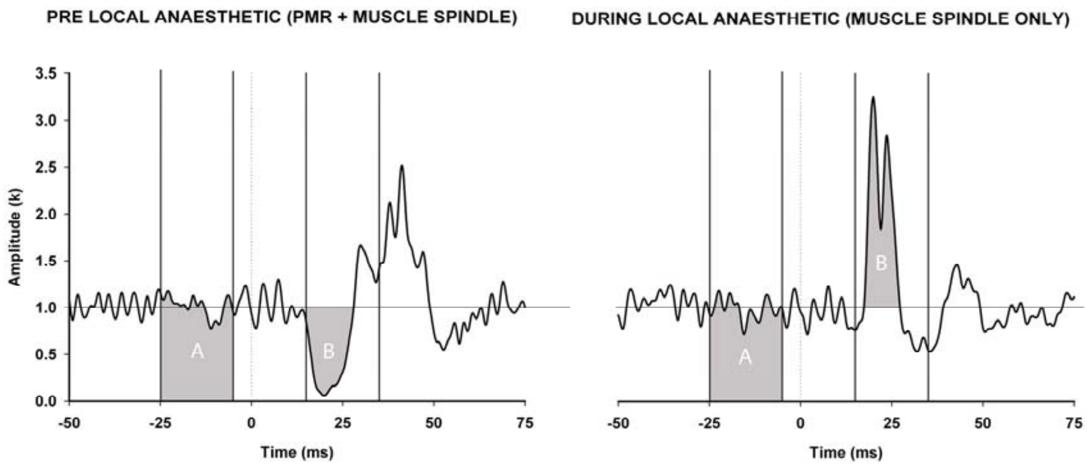

Fig. 5.2. Inhibitory and excitatory reflexes elicited by mechanical stimulation of a central incisor. Left panel shows the average reflex response (n=50) recorded from the masseter prior to local anaesthetic application. The reflex is composed of an inhibitory response that begins ~15 ms after the stimulus was delivered (t = 0 ms). Right hand panel shows the response elicited by the same stimulus during the application of local anaesthetic. The reflex is composed of an excitatory response that begins ~20 ms. Reflexes are recorded from the same subject under the same stimulus conditions (static jaw, 2N stimulus intensity). Reflexes have been amplitude-normalised by dividing each bin by the pre-stimulus mean (k). Amplitude units for the reflexes are expressed in "k" an arbitrary unit of amplitude that is a function of the average amplitude of the pre-stimulus mean. The calculation of reflex strength was calculated for each reflex by expressing the area B as a percentage of the area A.

5.4.1 MODULATION OF REFLEXES PRE-LOCAL ANAESTHETIC

Prior to local anaesthetic there was a significant interaction ($p < 0.001$) between JAW CONDITION (static or dynamic) and the LEVEL OF BACKGROUND EMG (matched for opening and closing). During jaw closing reflex inhibition was not significantly modulated when compared to its EMG matched static condition. In contrast, during opening there was a significant ($p < 0.001$), 49% reduction in reflex inhibition. Furthermore, under static conditions there was a significantly ($p < 0.001$) smaller inhibition (40% smaller), with the level of EMG that corresponded to closing i.e. higher EMG levels corresponded with smaller reflex inhibitions (Fig. 5.3). There was no interaction between STIMULUS INTENSITY and any of the other factors, suggesting that the reflex was equally modulated regardless of the stimulus intensity. However, the main effect of STIMULUS INTENSITY was statistically significant ($p < 0.001$); the 2N stimulus giving an inhibition that was on average 59% larger than the 1N stimulus.

5.4.2 MODULATION OF REFLEXES DURING LOCAL ANAESTHETIC

During local anaesthesia there was a significant interaction between JAW CONDITION (static or dynamic) and the LEVEL OF BACKGROUND EMG (matched for opening and closing). During jaw closing reflex excitation was significantly ($p < 0.05$) downwardly-modulated (45% smaller) compared to static. In contrast, during opening there was no difference in reflex excitation (Fig.5.3). There was no interaction between STIMULUS INTENSITY and any of the other factors. However, the main effect of STIMULUS INTENSITY was statistically significant ($p < 0.001$); the 2N stimulus giving an excitation that was 55% larger than the 1N stimulus.

5.5 DISCUSSION

In this study we have examined the extent and direction of reflex modulation that occurs during masticatory movements. The compound response of the masseter to a sharp mechanical stimulation of a tooth is a strong, short-latency inhibition which acts to halt jaw-closing, thereby minimizing damage to the teeth. This response is not solely inhibitory; also containing a superimposed excitatory response arising from concomitant vibration-induced excitation of muscle spindles. Therefore, when a tooth is stimulated, the inhibition strength is sufficient to not only inhibit muscle activity, but also to overcome the effect of concurrent muscle spindle-derived excitation. We have demonstrated that the compound inhibitory reflex response is significantly reduced during jaw movement. Interestingly, this modulation was only apparent when the jaw was opening. This seems a counterintuitive finding given that the tooth would seldom encounter mechanical stimulation during jaw opening. One explanation for this might

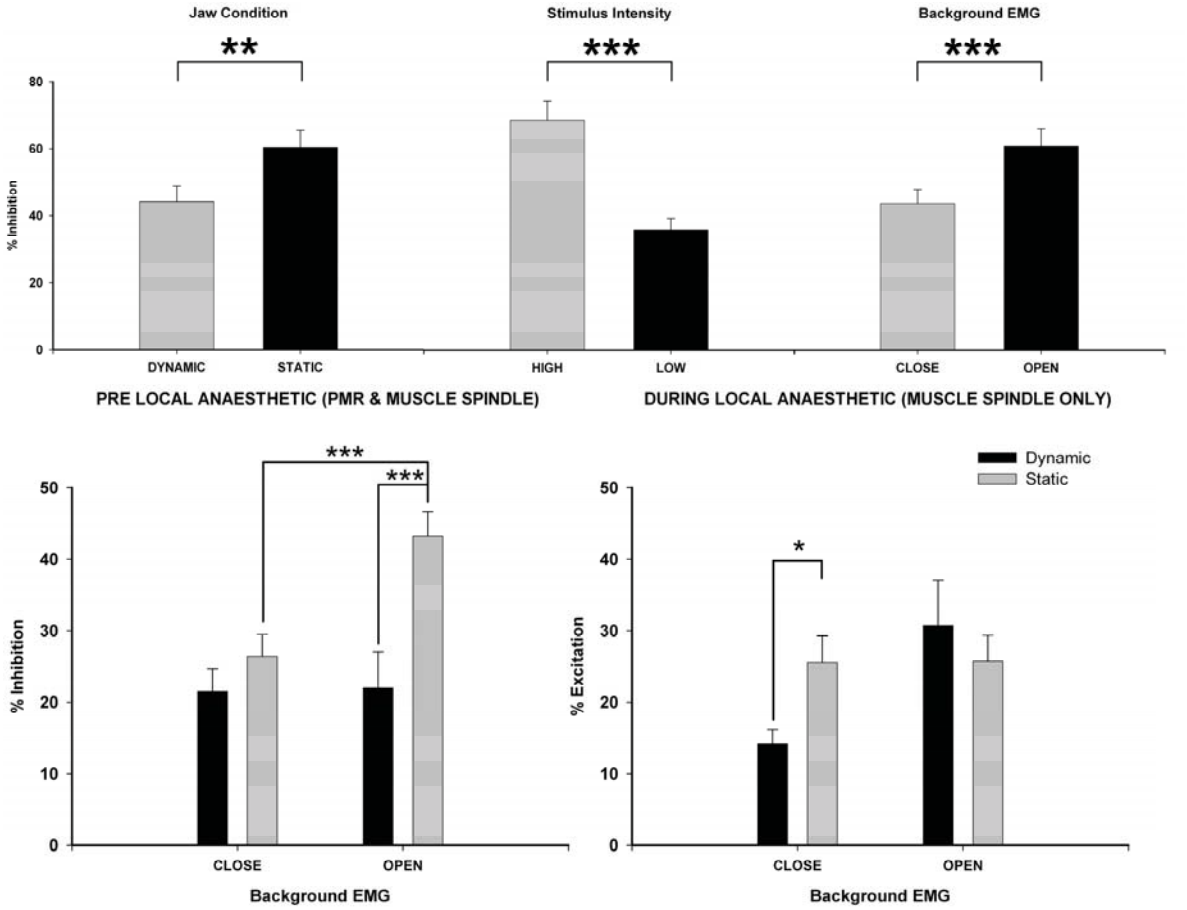

Fig 5.3 The effect of jaw movement condition and background EMG on the strength of reflex inhibitions and excitations of the masseter. Left panel shows the average + SEM (standard error of the mean) reflex response (n=28) expressed as percent inhibition recorded from the masseter prior to local anaesthetic application. The significant interaction between jaw movement condition and background EMG is evident in the difference between static opening and dynamic opening and also the difference between static and dynamic opening. Right panel shows the average + SEM reflex response (n=28) expressed as percent inhibition recorded from the masseter during periodontal anaesthesia. The significant interaction between jaw movement condition and background EMG is evident in the difference between static closing and dynamic closing.

be that, during opening, the muscle spindles are more excitable and hence, the evoked reflex responses contain a larger excitatory component. Evidence to support this is available from our muscle spindle-only reflex results. Therefore, we suggest that the reflex sensitivity of periodontal mechanoreceptors is not modulated by movement per se; the modulation that occurs independently of the muscle spindle modulation is attributable to the level of background excitation. When the background EMG was high, i.e. the jaw was closing or the EMG feedback was matched to this, the level of inhibition was smaller. This effect is well recognised (Türker[171] and may represent a form of automatic gain control that works in an opposite way to that seen for excitatory responses. As this is the very first study where PMR input to jaw muscle motoneurons are studied during jaw movements, it is difficult to compare our results with previous work. Similar experiments on rabbits show the jaw-opening reflex elicited by weak electrical stimuli is dramatically reduced during mastication, whilst that elicited by strong stimuli increased in size (Lund and Olsson, 1983). Similar results are observed with noxious electrical stimulation in humans[91]. We hypothesised that reflexes elicited by low intensity (1N) innocuous mechanical stimuli would be 'gated' during mastication whereas those reflexes elicited by stronger (2N), potentially damaging stimuli, would not decrease. With the stimulus intensities we used we were unable to show this hypothesised dichotomous response. There was no interaction between the factors of stimulus intensity and jaw condition, indicating that the reflex inhibition was reduced equally for both stimulus intensities tested. In order to test this hypothesis properly, much stronger stimuli may need to be used. This is not, of course, possible in a human study as a large number (n=300) of very strong mechanical stimuli may induce permanent damage on the stimulated tooth.

This is the first time the modulation of jaw muscle spindle reflexes during movement has been studied using a direct comparison with an EMG-matched static control condition. Furthermore, it is the first time jaw muscle spindle modulation has been studied using a stimulus that only activates the jaw muscle spindles. Our hypothesis, that the jaw muscle-spindle evoking reflex would be facilitated during jaw-closing and inhibited during jaw-opening, has to be rejected on the strength of the current data. We found no significant reduction of the reflex during jaw opening and, in opposition to our hypothesis, a weakening of the reflex during closing. Our finding, that the reflex was significantly depressed during the closing phase, is not without precedent however. Multiple studies on the lower limb have found a generalised reflex depression relative to respective control values obtained during static conditions at similar background EMG levels[172-175]. Furthermore, the assumption that feedback from muscle spindles should be most significantly diminished during jaw opening so that counterproductive forces of the jaw elevator muscles can be avoided[169], does not necessarily have analogy in limb studies. Neither modulation of the quadriceps H-reflex[176], nor the biceps femoris stretch reflex[172] follow the classical pattern of reciprocal inhibition between antagonistic muscles during walking. The gain of reflexes in both of these muscles increases during the times when the muscles are lengthening rapidly and is then modulated-downward during shortening. While it would seem a pattern of reciprocal inhibition would be appropriate for maximisation of efficient movement in the jaw, the current evidence suggests this is not a significant feature of the motor control strategy in the human masticatory system. Significantly, experimental evidence suggests that the jaw depressors do not impose a reciprocal inhibition onto the elevators[177]. The fact that gape is generally minimal during the rhythmic activity of chewing and that actual physiological stretching of the masseter only occurs physically, i.e. when opening to bite, may suggest that a system of presynaptic inhibition of jaw muscle spindles is unnecessary in the jaw system. While the simulated mastication performed by the subjects in this study was rhythmic, the necessity for an intraoral appliance to bite on meant that the chewing gape was in a range that imposed significantly more stretch, and hence excitatory input, onto the masseter than would occur during natural mastication.

A significant difference between the findings of our study and that of van der Bilt *et al.*[169] is that, in their study, reflexes during opening were negligible. In order to overcome the limitations imposed by having no background EMG during the opening phase, we opted to load the jaw in both the opening and closing directions. This tactic ensured a background level of EMG, and reflex size large enough to enable reliable reflex analyses. However, it also introduced a significant limitation to our results. During the opening phase in our study, the jaw would have been depressed passively and therefore, any reciprocal inhibitory process might not have been engaged. Furthermore, the eccentric activation of the masseter that occurred during the opening phase restricts the applicability of our results, in terms of analogy to natural rhythmic movement in the masticatory apparatus, to the closing phase only. We, therefore, conclude that masseter muscle spindle sensitivity is depressed significantly during the jaw-closing phase of simulated mastication compared to EMG-matched static jaw conditions. The extent to which masseteric muscle spindle reflex modulation occurs during jaw opening may only be ascertained using a method such as the H-reflex, where a reliable reflex can be obtained with little background EMG. In conclusion, we have shown that the compound inhibitory response of the masseter to tooth stimulation is modulated downward during jaw opening movements. This is probably the result of a concurrent enhancement of muscle spindle excitability during opening. Additionally, we have shown that the excitatory muscle spindle response is downwardly modulated during the closing phase of movement rather than the opening phase. While this result went against our hypothesis, it suggests that the jaw reflex system exhibits similar properties to limb reflex systems.

The current findings add to our understanding of how mastication is automatically controlled in humans. A broader understanding of this control system will allow the further development of scientifically sound methods to treat masticatory related dysfunctions.

5.5.1 ACKNOWLEDGEMENTS

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