5. LITERATURE REVIEW

There is a vast expanse of literature regarding the structure and function of the masticatory system. However, the majority of knowledge regarding the jaw has been derived from experimental animal preparations. Where human studies have been performed they have been limited to stimulation of the labial surface of the incisor teeth under static conditions, or stimulation of the entire lower jaw during limited, minimally controlled chewing movements.

From previous experiments the major reflex generating receptors in the jaw have been identified. The two main receptor groups are periodontal mechanoreceptors (PMRs) and muscle spindles. However, other receptor systems, such as auditory, skin and mucosal receptors, can not only generate reflexes but also modulate the reflex activity generated by PMRs.

PMRs are located around the tooth root and respond when put under tension. There are two main classes of PMRs. One set responds with excitation to the jaw closing muscles when excited by a slowly rising stimuli, the other set responds with a strong inhibition when excited by a rapidly rising stimuli. Muscle spindles respond rapidly to a stretch or vibration of the jaw and/or teeth by causing the jaw closing muscles to contract. However there is also evidence that they may also be responsible for a longer latency excitatory response.

5.1. JAW PHYSIOLOGY

Motoneurons are efferent neurons that innervate skeletal muscle fibres (Schmidt, 1985). The group of motoneurons that control an entire muscle is referred to as the motoneuron pool for that muscle. Together the motoneuron and the muscle fibres that it innervates are known as a motor unit (Liddell & Sherrington, 1925). Hence, the motor unit includes the entire cell body, the dendrites and the axon of the motoneuron, in addition to the muscle fibres innervated by the axon (Calancie & Bawa, 1990).

Studies on limb muscles have shown that all muscle fibres of a motor unit lie within a single muscle (Stuart & Enoka, 1983) and that there is a one-to-one relationship between the discharge of a motoneuron and all the muscle fibres it controls (Bigland-Ritchie *et al.*, 1979). In addition, it is known that each muscle fibre is innervated by only one motoneuron (Burke, 1981) and each fibre within a motor unit has similar metabolic properties (Burke *et al.*, 1971; Nemeth *et al.*, 1986), although the muscle fibres of a given motor unit are widely scattered over a cross-section of the muscle (Nemeth *et al.*, 1981).

5.1.1. JAW MUSCLES

The jaw muscles differ from each other both structurally and functionally (Møller, 1966; McDevitt, 1989). The jaw-closing muscles, such as the masseter and temporalis, have architectural features that suit them for force production. Conversely, the jaw-opening muscles, such as the digastric, are better designed to produce velocity and displacement (van Eijden *et al.*, 1997).

The masseter is divided into a superficial and a deep portion. The superficial portion is more active in force production whereas the deep portion, with its vertically orientated muscle fibres and a high density of large and complex muscle spindles (Eriksson & Thornell, 1987; Eriksson *et al.*, 1994), is believed to be more involved in the postural control of the mandible (Eriksson *et al.*, 1984; Hannam & McMillan, 1994). Both portions of the masseter are active only 40-70% during incisal biting compared to intercuspal clenches (Blanksma & van Eijden, 1995). This may be due to active inhibition of the masseter during incisal biting because of the increased loading on the temporomandibular joint.

The digastric muscle is formed by an anterior and posterior belly linked by an intermediate tendon and is active in depression and horizontal positioning of the mandible. The temporalis also has two components. The

anterior portion is reportedly involved in the positioning of the jaw and provides tonic activity for the rest position, while the posterior component is involved during chewing by moving the jaw up and down towards the working side (Blanksma & van Eijden, 1990).

Not only are there functional differences between the jaw muscles, there are reports the components of the muscles perform differently. It has been claimed that the masseter has a recruitment pattern that differs from that of the anterior temporalis muscle (Hagberg *et al.*, 1985). Another study found that during static clenches no difference was found between the regions of the temporalis however the anterior and posterior regions differed during chewing, while the superficial and deep masseter functioned differently during both clenching and chewing (Blanksma & van Eijden, 1995). Furthermore, while one study found differences between the stretch reflex gains in temporalis and masseter muscles, it was concluded that the likely cause was the larger distance of the reflexly-activated muscle fibres in the masseter with respect to the electrodes, rather than an actual difference in reflex sensitivity (Lobbezoo *et al.*, 1993). There is even evidence that the digastric muscle of the rabbit (which has a single belly, does not contain connective tissue partitions and all fibres arise from the same tendon and insert into a single broad site) contains two, possible three functional sub-regions (Tsuruyama *et al.*, 2002). Indicating that even a muscle that appears to have only one region may have divisions capable of acting independently. The difference between muscle properties may be partially explained by the presence of different fibre compositions.

5.1.2. MUSCLE FIBRE TYPES

As with all skeletal muscles, the masticatory muscles contain three distinct types of motor units. The slow-twitch (S or type I) fibres (Taylor, 1976) are oxygen dependant and as such are generally found in the deep parts of muscles where the blood supply is greatest. S fibres are innervated by small motoneurons and hence are the first to be recruited during voluntary contraction due to the large input resistance in the motoneuron (Henneman, 1985). They produce relatively small contractile forces but are resistant to fatigue being able to maintain a constant contractile force for very long periods. The fast, fatigue-resistant (FR or type IIA) fibres can use both oxygen and glycogen for fuel. FR fibres are innervated by intermediately sized motoneurons and as such are recruited during moderately intensive tasks. They produce relatively fast and strong twitches and while they are fatigue resistant the contractile force does decrease over time. The fast, fatigable (FF or type IIB) fibres are glycolitic and are generally found on the

superficial layer of skeletal muscles. FF fibres are innervated by the largest motoneurons and are only recruited during powerful voluntary contractions; they produce the fastest and strongest twitches but fatigue quickly (Burke, 1968; Brooke & Kaiser, 1970; Burke *et al.*, 1973). Human jaw muscles also contain a relatively large proportion of fast-contracting units of intermediate fatigability (F (int.) or type IIC), which are rare or non-existent in normal adult limb muscles (Dubowitz & Brooke, 1973).

The human masseter of young adults contains mostly S fibres and FR fibres are rare or lacking (Eriksson, 1982), while the digastric contains S, FR and FF fibres in approximately equal proportions and size. The masseter of elderly humans contains significantly lower proportions of type S fibres when compared to young adults; in contrast it is the FF fibres that decrease with age in the digastric (Monemi *et al.*, 1999). This difference shows that age-related alterations in fibre type composition are muscle and region specific, probably reflecting muscular differences in genetic programs and epigenetic influences.

5.1.3. PERIODONTAL MECHANORECEPTORS

Within the tooth-supporting tissues, there are receptors with very similar histological and physical characteristics to the cutaneous mechanoreceptors (Linden *et al.*, 1994). These receptors are the periodontal mechanoreceptors (PMRs), and their activity corresponds directly to the amount, rate and direction of force applied to the teeth (Trulsson *et al.*, 1992; Hannam & McMillan, 1994; Trulsson & Johansson, 1994; Türker *et al.*, 1997).

5.1.3.1. ANIMAL STUDIES

In animals four types of specialized nerve endings have been described to exist in the periodontal space: Ruffini-like, spindle and expanded nerve endings found near the root apex and coiled nerve endings found near the mid-range of the tooth (Maeda *et al.*, 1990). Despite the difficulty in stimulating the PMRs in isolation, a number of findings have been made in regards to the neuronal wiring that may exist in the jaw. Animal studies (Pfaffmann, 1939a & b; Ness, 1954; Linden & Millar, 1992) have shown that touch, pressure, and displacement sensitivity of teeth are periodontal sensations, not pulpal; the majority of PMRs are located close to the apex of the tooth root; they respond to tension, but not compression (Cash & Linden, 1982), they have low activation thresholds, and they adapt slowly to changes in force. The second, less numerous, class of receptor are located

between the gingiva and the apex, they also respond only to tension but have a high activation threshold, and are rapidly adapting (reviewed in Linden, 1990).

There are conflicting reports regarding the location of PMR cell bodies within the brain depending on the type of analysis used. Histochemical studies that have injected radioactive dyes directly into the trigeminal ganglion or mesencephalic trigeminal nucleus in animal brains (rats, cats and monkeys) and traced all the receptors coming from individual teeth (Byers *et al.*, 1986; Byers & Dong, 1989). These studies have shown that the receptors closest to the apex of the tooth root have cell bodies in the mesencephalic trigeminal nucleus while those located in the middle of the root have cell bodies in the trigeminal ganglion (Byers, 1985). However a physiological study (Linden & Scott, 1989), and a histochemical study on the molar teeth of rats (Byers & Holland, 1977), have shown the opposite, receptors in the apex of the tooth root have connections to the trigeminal ganglion while those in the middle of the root have cell bodies in the mesencephalic trigeminal nucleus. Although it has been shown that the distribution of receptors in cat canine and monkey incisor teeth is similar, it is claimed that the receptors around rat continuously erupting teeth are different (Byers & Dong, 1989).

5.1.3.2. HUMAN STUDIES

While some researchers have claimed that there may only be one type of receptor in humans and that the rates of adaptation and threshold properties are dependant on the location of the receptor within the periodontal ligament (Cash & Linden, 1982; Linden & Millar, 1988b) this seems unlikely as two distinct types of receptors have been categorised. By recording from the inferior alveolar nerve using tungsten electrodes, two different receptor types have been identified (Trulsson *et al.*, 1992; Trulsson, 1993; Trulsson & Johansson, 1994). The 'saturating group' that had a pronounced saturation tendency with the sensitivity to changes in force diminishing above 1N; and the 'non-saturating' group which display a gradually increasing discharge rate (linear response) to forces up to, and above, 5N. It was also found that the non-saturating receptors do not lose their dynamic sensitivity, even when they are loaded with high static forces. Hence the saturating and non-saturating receptors are likely analogous to the slow and fast adapting receptors as seen in animal studies. Additional studies have suggested that the slow-rate-sensitive receptors may have an excitatory connection, while the fast-rate-sensitive receptors appear to induce powerful inhibitory synaptic potentials on the jaw-closers (Brodin *et al.*, 1993b; Türker *et al.*, 1994).

If the properties of human PMRs around the incisor teeth follow the same pattern as seen in animal studies (i.e., slow-rate-sensitive receptors close to the root and fast-rate-sensitive receptors closer to the gingiva) then axial stimulation will stimulate few of the slow-rate-sensitive receptors, as most will undergo compression. Hence if the axial stimulus profile contains only slowly rising force components then it is likely not to activate PMRs and little or no reflex response due to PMRs will be present. In contrast, slow orthogonal stimulation will stimulate the slow-rate-sensitive PMRs; and in such studies an excitatory response has been found (Brodin *et al.*, 1993b; Yang, 1999). On the other hand, when the stimulus profile has faster components the fast-rate-sensitive PMRs will be stimulated regardless of the stimulus direction and a similar reflex response will be elicited. Static orthogonal experiments have established this response as inhibitory (Sessle & Schmitt, 1972; Matthews, 1975; van der Glas *et al.*, 1984; Türker *et al.*, 1997; Yang & Türker, 1999).

5.1.4. MUSCLE SPINDLES

The cell bodies of the masseter muscle spindle afferents are located in the mesencephalic trigeminal nucleus (Szentagothai, 1948) with processes from the cell bodies making monosynaptic contact with the jaw-closer motoneurons (Taylor *et al.*, 1995). In contrast to the findings in most agonist/antagonist muscle groups in humans, the jaw closing muscles contain a large numbers of muscle spindles while the jaw-opening muscles do not (Taylor *et al.*, 1976).

While it is established that the deep masseter has the majority of the muscle fibres in animals (Karlsen, 1965; Maier, 1979; Lennartson, 1980) there are conflicting reports as to whether it is the deep (reviewed in Rowlerson, 1990) or superficial (Kubota & Masegi, 1977) masseter that contains the majority of muscle spindles in humans.

It has been suggested that unlike muscle spindles in the limb muscles, the muscle spindles in the jaw are more important for the development of large, fast forces, rather than for the maintenance of static posture (Scutter & Türker, 2001) although this is not the view held by all researchers (Eriksson *et al.*, 1984; Hannam & McMillan, 1994).

Most low threshold mechanoreceptors are phasically stimulated during the closing phase of mastication, the notable exceptions are spindle afferents, which although active during closing, fire during jaw opening (reviewed in Lund, 1991).

5.1.5. OTHER ORAL RECEPTORS

Although it has been shown that the PMRs and muscle spindles are the most important sources of peripheral feedback for the production of reflex activity during mastication (Morimoto *et al.*, 1989; Morimoto & Nagashima, 1989) there are many other receptors in the intra- and peri-oral area including: temporomandibular joint, auditory, Golgi tendon as well as cutaneous and mucosal receptors.

Little is known about the temporomandibular joint afferents except that some encode displacement, others velocity and they have a preferred direction (reviewed in Lund, 1991).

Auditory receptors contained in the ears can, if stimulated, produce a reflex decrease in the level of jaw muscle activity similar to that found in response to stimulation of PMRs (Meier-Ewert *et al.*, 1974; Sato *et al.*, 1994).

Golgi tendon organs have been identified in the jaw muscles of kittens (Lund *et al.*, 1978) and possibly in the jaw of rabbits (Lund & Matthews, 1979) but there is little or no data on what they do, or even if the exist in the jaws of other animals.

Recordings have been made from the receptors contained in the skin, hair and mucosa of anesthetised rabbits (Appenteng *et al.*, 1982b). The results showed that while over half of the hair afferents were excited during mastication most of the skin afferents were not. However an experiment on awake humans undergoing mechanical stimulation of the incisor has shown that the reflex response was modulated by tactile stimulation (Tucker & Türker, 2001).

5.2. ELECTROMYOGRAPHY AND FORCE

5.2.1. BITE FORCE

There is evidence that the masticatory forces are controlled very precisely and that these forces change from bite to bite, depending on the consistency of the bolus (reviewed in Türker, 2002). For this reason it is important to study both the bite force and EMG activity of the jaw muscles in order to ascertain the cause of these changes so the process of mastication can be better understood.

One group has reported the average bite force between the first molars was 396N for women (Hagberg *et al.*, 1985). While an earlier study showed that at maximum voluntary contraction (MVC) the bite force was approximately 900N on the first molar (Anderson, 1956b). In this case the mean maximum whole tooth load during chewing was found to be between 110-150N (12-17% MVC) compared with an average maximum of 60-80N (7-9% MVC) and the average loads for most foods was found to be 3-18N (0.33-2% MVC). The loads tended to be larger towards the end of the chewing sequence than at the beginning. With the last one or two loads frequently being larger, more prolonged or separated from the rest by a longer time interval (Anderson, 1956a). Hence normal chewing tended to be between 1 and 10% MVC (depending on the substance) with values approaching 20% MVC towards the end of the chewing cycle. However, while the study by Anderson (1956) used 'raw' carrot for mastication it was cubed and placed in 37^0 water for a number of minutes before the experiments. This would likely have softened the carrot leading to lower EMG levels. Hence, a more recent study utilising an artificial food to emulate hard foodstuffs, such as carrot and peanuts, found masticatory EMG levels of 41-66% MVC (Slagter *et al.*, 1992).

5.2.2. EMG / FORCE CORRELATION

5.2.2.1. STATIC CONDITIONS

Theoretical predictions suggest that EMG activity should level off as tension rises (Bernstein, 1967), though this is generally not observed. The first description of relationship between integrated SEMG and force during voluntary isometric contraction in human concluded that the relationship was linear (Lippold, 1952). However, subsequent studies have found that the association is more complex. Investigations have shown that the EMG/force relationship depends on the muscle under investigation. The physiological

reasons for the various relationships most likely reflect differences in muscle fibre type composition and their contractile properties. A non-linear EMG/force relationship in the first dorsal interosseous (the EMG levelled off at high force levels) underlines different activation strategies during slow voluntary isometric and isotonic contractions at a whole muscle level (Madeleine *et al.*, 2001).

Experiments performed over the full dynamic range (10-90% MVC) have shown a linear response for adductor pollicis, soleus, first dorsal interosseous and vastus lateralis. A non-linear ($y=bx^a$ $a>1$, below 30% then linear above) response has been shown in biceps brachii, brachioradialis and from both the lateral and long heads of the tricepts brachii (Woods & Bigland-Ritchie, 1983). An earlier study also showed significant non-linearity between integrated EMG and muscle tension for m. biceps brachii (Komi & Buskirk, 1970). These results suggest a linear EMG/force relationship for muscles of near uniform fibre composition and non-linear relationships for muscles of mixed fibre composition.

In back muscles different relationships have been identified depending on the activity. A linear increase in flexion-rotation and extension-rotation torque resulted in an exponential increase in the total EMG output (Kumar & Narayan, 2001). This is in contrast to results from axial rotation not involving flexion of the spine where a linear increase in the magnitude of the axial torque produced a near linear increase in EMG (Pope *et al.*, 1986) for an isometric sub-maximal contraction.

Previous studies on jaw muscles have identified both linear (Ralston, 1961; Yemm, 1977; Blanksma *et al.*, 1992; Tortopidis *et al.*, 1998) and curvilinear (Pruim *et al.*, 1978; Watsell & Devlin, 1987) relationships between increasing bite-force magnitude and the integrated EMG of jaw-closing muscles. While one study indicated that linearity or non-linearity of the EMG/force relationship in the jaw is a determinant, among other variables, of the direction of the resultant force (Mao *et al.*, 1996). Another study found that the relationship between EMG and force was linear for the anterior temporalis, but not for the masseter or posterior temporalis muscles (Haraldson *et al.*, 1985). A linear relationship was described between the bite force and surface EMG of masticatory muscles in a further study (van Eijden *et al.*, 1990), however only forces above 50N were tested. In studies performed by Blankma et. al. a linear relationship was found between intra-muscular EMG and bite force in both masseter (Blanksma *et al.*, 1992) and temporalis muscles (Blanksma & van Eijden, 1990), although there was a difference in activity between the components of the muscles depending on the direction of bite force. While another study has shown a linear

relationship between the SEMG of both masseter and anterior temporalis with bite force (Ferrario *et al.*, 2004).

It has been suggested that the two main situations when curvilinear EMG/force relations may exist are when a muscle is activated close to maximum (Kuroda *et al.*, 1970) or when synergistic muscles contract simultaneously to produce a resultant force (Dul *et al.*, 1984). These results indicate that studies that use both EMG and total jaw force should note the jaw separation, ensure that large levels of muscle contraction are not used and attempt to create experiments were synergistic muscle contractions are avoided or reduced.

In a test to determine endurance, subjects were asked to exert maximum bite force on their right side for as long as possible. It was found that the EMG of the right masseter muscle was closely related to bite force but the activity of the other muscles (left, right anterior, posterior temporalis and left masseter) was not (Haraldson *et al.*, 1985).

5.2.2.2. DYNAMIC CONDITIONS

Unlike static experiments conducted on the cat soleus muscles during isometric contractions utilising electroneuromuscular stimulation, which showed a linear relationship between EMG and force under normal contraction levels (Guimaraes *et al.*, 1994), a highly non-linear relationship has been found to exist during dynamic contractions (Guimaraes *et al.*, 1995). Thus indicating that movement itself causes changes in the EMG/force relationship, or that activation of muscles other than the soleus are confounding the results. Although the shape of the EMG/force relationship in this study was found to be non-linear it was similar between cats and at different velocities of movement. This finding was confirmed in the cat plantaris muscles where the EMG was used to accurately predict the force in the cat hindlimb during locomotion, despite the non-linear relationship (Herzog *et al.*, 1998). Further research has also found that through the use of artificial neural networks the EMG from the soleus muscle could be used to predict muscle forces given sufficient training (Liu *et al.*, 1999).

Research on the EMG/force relationship in the masticatory system during dynamic conditions is limited. However there is a suggestion that the tissue deformation caused by the contraction of the jaw closing muscles may influence the EMG/force relationship at high activity levels (Liu *et al.*, 2004).

5.2.3. FATIGUE

Fatigue can be regarded as a decrement in the output force from a motor unit, usually as the result of repeated activation (Burke, 1981) or, any reduction in the maximal capacity to generate force or power output (Vøllestad, 1997). Fatigue can be the result of the failure of: neuromuscular transmission, the muscle fibre action potential, excitation-contraction coupling or the contractile machinery itself.

The two types of fatigue are 'peripheral' and 'central'. Central fatigue is a progressive reduction in the voluntary activation of a muscle during exercise and is caused by a reduction in the frequency of motoneuron firing, or a reduction in the number of motor units utilised. Peripheral fatigue is produced by changes at, or distal to, the neuromuscular junction (Gandevia, 2001) and follows changes in the local pH, an increase in lactic acid concentration and t-tubule failure.

Central fatigue can be, at least in part, overcome by asking the subject to perform a maximum contraction for a short time then, just before they stop, asking them to make a 'super' effort (Bigland-Ritchie, 1981); experiments such as this show that there is almost always a 'reserve' of muscle activity during maximum voluntary contractions. While this method can work for some muscles others such as plantarflexors (Belanger & McComas, 1981; Bigland-Ritchie *et al.*, 1986), abdominal muscles (Gandevia *et al.*, 1990) and jaw-closers (Lyons *et al.*, 1996) cannot be voluntarily driven to maximum contractions.

One way to assess the level of maximum voluntary contraction and fatigue is to use twitch interpolation (Merton, 1954; Hales & Gandevia, 1988). This method involves interpolation of a supramaximal electric shock to the motor nerve during an attempted MVC. Any motor units not recruited by volition or not driven to maximum frequency add extra force directly following the stimulus. If this experiment is continued then a reduction in the voluntary, as well as the electrically evoked contraction level is observed. By following this method the relative contributions of peripheral and central fatigue can be extrapolated. Peripheral fatigue corresponds to the reduction in electrically evoked contraction levels while central fatigue is the difference between the voluntary contraction level and that evoked by the stimulus.

Fatigue can be altered by the administration of drugs. One study (Kalmar & Cafarelli, 1999) has shown that giving subjects caffeine prior to trials can increase both the voluntary activation at MVC and the time to fatigue.

Jaw-closing muscles differ from limb muscles in that maximum force does not decline following sustained isometric contractions (Junge & Clark, 1993) and there is no change in brief maximal contraction or force levels during or after various fatigue-inducing isometric tasks suggesting a lack of contractile failure in the jaw-closing muscles (Clark & Carter, 1985). Also the EMG/force ratio increases in fatiguing limb muscles but not jaw-muscles. For these reasons neuromuscular fatigue does not appear to accompany sustained contractions of jaw muscles and pain intolerance, rather than neuromuscular fatigue, may be the limiting factor of a sustained submaximal, or even maximal, clenching effort. Hence findings of reflex alterations during a sustained contraction of the first dorsal interosseous muscle (Harrison *et al.*, 2000) may not apply to the jaw.

5.2.4. CROSS-TALK

There is strong evidence that the reflexes observed in small muscles, such as the digastric, could be caused by cross-talk from adjacent, more active muscles, such as the masseter. Cross-talk results when potentials from adjacent muscles reach the recording site through volume conduction, thus contributing to the EMG signal (van Vugt & van Dijk, 2000). Experiments on cross-talk have shown that an SEMG signal detected on the skin above a leg muscle, and having a peak-to-peak amplitude of up to 17% of a signal detected above a neighbouring muscle, may be due to cross-talk rather than to activation of the muscle below the electrode (De Luca & Merletti, 1988).

5.2.5. ELECTROMYOGRAPHIC RECORDING METHODS

While there is no doubt that intra-muscular EMG (IM-EMG) has superior recording characteristics (Türker, 1993) almost all studies into human jaw reflexes use surface EMG (SEMG) to quantify the reflex response of the muscle (Ottenhoff *et al.*, 1992a; Takada *et al.*, 1995; Türker *et al.*, 1997; Louca *et al.*, 1998). Reasons for this may include the fact IM-EMG recordings give a more localised view, which is not desirable if the whole muscle response is required, intra-muscular electrodes are more expensive and much more invasive than surface electrodes and IM-EMG recordings require faster and more expensive equipment due to higher frequency characteristics. The main benefit of IM-EMG is that it is not subject to as much artefact (cross-talk, stimulus and movement) as the SEMG and the motor-unit action potentials are not as filtered (Türker *et al.*, 1999).

To determine if there was a difference between the EMG activity of the superficial and the deep portions of the masseter muscle surface and intra-muscular electrodes were recorded during specific static and dynamic tasks with no difference observed (Belser & Hannam, 1986). Furthermore, no difference has been found in the identification, latency or duration of reflexes when comparing IM-EMG and SEMG (Wittek *et al.*, 2001). However differences in the relative strengths of the reflexes are expected as the muscle recording sites are not the same, and the amount of noise in surface EMG records is greater.

One study has found that the IM-EMG/force relationship in human vastus lateralis (knee extensor) muscle averaged across subjects detected from eight electrode sites showed almost the same linear correlation in spite of different electrode locations. This suggests that if all muscle fibres participate in the same action at the same time, the averaged normalised integrated IM-EMG recorded from any place in the muscle using wire electrodes could reflect the total activities of that muscle, even if the muscle is large (Onishi *et al.*, 2000). Thus indicating that the localized recording made using IM-EMG may correspond just as well to the total muscle activity as the overall recording made using SEMG electrodes.

The relationship between the muscle and the skin always changes during motion (Vigureux *et al.*, 1979) hence SEMG cannot be used due to the changing electrode/muscle relationship; in contrast, IM-EMG recordings are suitable for detecting the EMG of single muscles selectively, even during motion.

One of the problems in EMG investigations is the repeatability of measurements. Duplicate observations are not normally made, or if made not adequately reported. While one group reports that reproducibility is better with SEMG than IM-EMG (Komi & Buskirk, 1970), the reason for this may be due to the problem in ensuring the exact position for the second recording session when using IM-EMG recordings. This problem is reduced in the SEMG as a whole muscle response is normally achieved, and markers can be used to ensure the exact location of the electrode.

5.3. CHEWING

The underlying rhythm of mastication is produced by a group of cells located in the brainstem that are collectively known as the central pattern generator or CPG (reviewed in De Laat, 1987a). Experiments have shown that the isolated brainstem had the ability to generate the basic features of mastication and that the pattern was not due to signals generated by the vascular or respiratory systems (Dellow & Lund, 1971). The actions of the CPG can be divided into three processes: production of the masticatory rhythm, generation of jaw, tongue and facial muscles activity patterns and the coordination of the masticatory muscles (reviewed in Nakamura & Katakura, 1995).

Although the CPG sets the rhythm for mastication and alternately activates the openers and closers, control of this process is largely dependent on sensory feedback (reviewed in Lund, 1991). Either sensory inputs from the mouth or descending commands from the forebrain can activate the CPG (Lund & Olsson, 1983). However far more muscle activity is generated if resistance counteracts the closing movement (Goldberg & Tal, 1978; Ottenhoff *et al.*, 1992a), and the reflex effects of sensory afferents, and their actions on the CPG, can change dramatically from one phase of movement to another (Rossignol *et al.*, 1988). Furthermore, CPG-induced depolarising potentials in masseter motoneurons are often too small to cause a discharge in fictive mastication where peripheral feedback is not possible (Kubo *et al.*, 1981). For this reason it is necessary to discover what the jaw reflexes contribute to the final pattern of rhythmical jaw movements and which receptors are responsible for them.

5.3.1. CHEWING RATES

The rate of chewing in various animals appears to be governed not only by the type of food being eaten (Peyron *et al.*, 2002; Inoue *et al.*, 2004) and the relative age of the animal (Weijs *et al.*, 1989) but also by the size and weight of the jaw; smaller animals tend to have faster mastication rates than larger animals. The chewing rate for mice is reported to be 5-7Hz (Kobayashi *et al.*, 2002) while it is slower in the larger rabbit, 3.3-4Hz (Morimoto *et al.*, 1985). One study found that the rate of mastication in cats varied between 2.5Hz when chewing hard liver up to 4Hz when lapping milk (Thexton *et al.*, 1980). Another study found that at around 2.7-2.9Hz the rate of mastication in cats (Hiraba *et al.*, 2000) was similar to the 3Hz found in the possum (Hiiemae, 1975). Rates below 3Hz have been reported for humans (Morimoto *et al.*,

1984) as well as rates around as 1-1.7Hz (Hiiemae *et al.*, 1996), while a rate of just over 1Hz has been reported for the cow (Schleisner *et al.*, 1999).

5.3.2. RECEPTORS INVOLVED IN CHEWING

The significance of studying the responsiveness to physiological loading relates not only to the understanding of the tooth support mechanism but also clinically to an appreciation of the loss of support occurring with periodontal pathology and to the effects of orthodontic loading (Moxham & Berkovitz, 1995). During mastication, the forces that are applied to the teeth displace them in their sockets. This stimulates PMRs. The nature of this connection is unclear due to the difficulty in stimulating the PMRs without at the same time activating other receptor in the peri-oral region (Sato *et al.*, 1994). Forces applied to a tooth can stimulate receptors in the periodontal ligament, gingival mucosa, dentine, pulp, alveolar bone, periosteum, jaw muscles, temporomandibular joints and ears (reviewed in Türker *et al.*, 1999). Of these the two main forms of jaw reflex feedback during mastication are PMRs and muscle spindles (Morimoto *et al.*, 1989).

Regardless of how carefully teeth are mechanically stimulated three types of receptors will be activated: PMRs, muscle spindles and acoustic (vibration) receptors (reviewed in Türker, 2002). Sounds can produce reflex changes in jaw muscles (Meier-Ewert *et al.*, 1974). While the sound of a tap alone is sufficient to evoke an inhibitory reflex in the masseter (Sato *et al.*, 1994), which is similar to, albeit smaller than, that resulting from periodontal receptors (Cadden *et al.*, 1996), their main action appears to be facilitation of PMR activity (van Steenberghe *et al.*, 1981; van der Glas *et al.*, 1988). To overcome this effect experiments generally require human subjects to wear headphones through which white noise is played in order to mask any acoustic vibrations, hence the response of the PMRs and muscle spindles can be found without interference from vibration receptors.

5.3.3. CHEWING IN ANIMALS

Most of the current knowledge about the structure and function of the jaw muscles comes from animal species with which investigators have used widely varying reduction techniques. While it has been claimed that the muscle spindles are the main contributors to reflex generation of additional muscle activity during mastication (Vallbo *et al.*, 1979), a number of experiments have been undertaken to determine their relative importance in

the reflex control of mastication, with varying results. The main problem with animal experiments is that they are rarely conducted under 'normal' conditions with many of the experiments conducted on anaesthetised, decerebrate animals (Morimoto *et al.*, 1982; Westberg *et al.*, 2000) or brainstem preparations (Tanaka *et al.*, 1999). Despite this limitation, the relative contributions of PMRs and muscle spindles has been inferred in animal experiments by first taking measurements of chewing forces then removing the input to the brain from the muscle spindle afferents, usually by placing lesions in the brain stem destroying the tract to the mesencephalic trigeminal nucleus, and repeating the experiment. One such study on anaesthetized rabbits has shown that muscle spindles are only responsible for approximately 20% of the developed muscle activity, with PMRs responsible for the other 80% (Morimoto *et al.*, 1989; Morimoto & Nagashima, 1989). Experiments performed on monkeys have shown that after lesion of the mesencephalic trigeminal nucleus on one side there was a preference to chew on the contralateral side, however if both sides were destroyed there was no side preference or change in the pattern of mastication (Goodwin & Luschei, 1974). However care must be taken when interpreting these findings as the mesencephalic trigeminal nucleus also contains a number of PMR cell bodies (Byers *et al.*, 1986; Byers & Dong, 1989) that would also have been destroyed.

Electrical stimulation of the cerebral cortical masticatory area in rabbits induces rhythmic jaw movement that resembles natural mastication (Bremer, 1923), and when objects are inserted between the teeth during induced movement the EMG of the masseter increases (Lavigne *et al.*, 1987). It has also been found that the force and EMG increased with increasing hardness of the chewing strip used (Hidaka *et al.*, 1997). The increased masseteric response has been found to precede the onset of tooth contact from the second masticatory cycle after application of the strip, but never the first cycle (Komuro *et al.*, 2001). Furthermore, the early increase in EMG disappeared after lesion of the muscle spindle afferents to the brain. In contrast, no such change occurred after blocking periodontal afferents by transection of both the maxillary and the inferior alveolar nerves. The feed forward control of the increase in masseter EMG is therefore dependent mainly on sensory inputs from the muscle spindles, but little on those from the periodontal receptors. However, it should be noted that these experiments were performed on anaesthetized rabbits and as such the contribution of higher centres on the chewing cycle was removed, and unlike the above studies by Morimoto et. al. (1989) only the additional EMG activity before tooth contact was used. The combination of these two findings show that muscle spindles are important for the extra muscle activity associated with anticipation of resistance (in a feed forward manner) where as PMRs are

more important for the direct sensory feedback of food (in a feedback manner).

Reflex studies on awake cats has found that jaw-opening (inhibitory) reflexes are facilitated during jaw-opening and inhibited during closing (Chase & McGinty, 1970). However, it has been found that when rabbits chew hard food the reflex changes in the masticatory muscles are similar to those found in an unloading reflex or in a reflex after tooth tap. The unloading reflex is considered to be elicited as an off response from the sudden shortening of the muscle spindles while the loading response to hard food is attributed to the PMRs. Both are characterized by inhibition of the jaw closing muscles and excitation of the opening muscles (Yamada & Haraguchi, 1995). Although other studies have shown that the jaw-opening reflex in anesthetised rabbits is suppressed after the first time resistance is encountered during mastication (Lavigne *et al.*, 1987) and a large jaw-closing reflex (excitation) becomes prevalent (Morimoto *et al.*, 1989).

5.3.4. CHEWING IN HUMANS

While there are papers with detailed descriptions on mastication in a number of animals there is no comparable descriptions of human mastication that include the sequential changes in the form of the masticatory cycle and in the patterns of muscle activity (reviewed in Lund, 1991). The main limitation in human experiments is that all experimental changes and recordings are indirect (Macaluso *et al.*, 1998). This introduces extra complexity not only in the recording and interpretation of data but also in the experimental equipment used. Due to this problem there have been relatively few studies conducted on humans during simulated mastication. In one of the only sets of experiments to investigate simulated mastication under controlled conditions Ottenhoff and colleagues used a device that produced a force that countered jaw closing while subjects opened and closed their mouths at a set rate. The force was then suddenly removed allowing the jaw to move freely. One of the main findings from this work was that 80% of the muscle activity generated when counteracting the applied force disappeared in the first cycle that the force was removed (Ottenhoff *et al.*, 1992b). In similar experiments it has been shown that the activity of the masseter increases within about 25ms when loading is not expected, though the strength of the response is substantially less than the reduction due to unexpected loss of resistance.

Anticipatory activity commences 100ms before jaw loading when it is expected (Abbink *et al.*, 1999b), however this anticipatory activity is

relatively small and the jaw-closer muscles respond mainly through reflex loops, even when loading is predicted. It has also been shown that the speed of the chewing cycle does not influence the evoked jaw reflexes, faster jaw movements resulted in a larger anticipatory and background EMG level but the reflex responses are unchanged (Abbink *et al.*, 1999a). From these results it can be concluded that receptors adapt very quickly, and make a large contribution to jaw muscle activity. However, due to methodological limitations it was not certain which receptors were responsible for these responses, or their relative contributions. More recent testing has shown that the application of local anaesthetic did not affect the response of the masseter or temporalis muscles to loading during simulated mastication (Abbink *et al.*, 2002). However, the stimulus force was delivered to the entire lower jaw rather than one or two teeth as would be found during normal mastication.

Additional studies on human during stimulated mastication have shown that only very weak stretch reflexes are induced during jaw opening; where as strong reflexes can be elicited during jaw closing (van der Bilt *et al.*, 1997). Indicating that reflexes are modulated during jaw movement. The source of this modulation may lie in the CPG (Lund, 1991). During the closing and opening phases of mastication the CPG sends alternating excitatory and inhibitory signals to the jaw-closing muscles. Throughout closing the excitation sent by the CPG may make the jaw-closers more susceptible to peripheral originating input, while the inhibition during opening would have the opposite affect. Efferent fibres from the CPG are also known to influence the motoneurons of the digastric in both a pre-and post-synaptic manner (Lund & Olsson, 1983).

In a study into human mastication subjects were given model foods with varying hardness (Peyron *et al.*, 2002). It was found that almost all EMG and jaw movement parameters measured were affected by increasing the hardness of the model foods; and the food hardness modifications were found to be the strongest during the first five stokes, beginning as early as the first stroke and lasting for the entire sequence. This early onset of task adoption indicates a reflex origin, while the persistence of the changes may indicate the use of higher brain centres, or the contribution of muscle spindles in a feed forward manner as found in rabbits (Komuro *et al.*, 2001). An early study investigating receptors during mastication in humans involved injecting anaesthetic to block intraoral and temporomandibular joint receptors (Schaerer *et al.*, 1966). Mastication was not prevented but subjects reported great difficulty manipulating food and keeping it between their teeth. However no analysis was performed to quantify the changes.

Some of the best indications of the importance of PMRs in humans is that mastication in edentulous subjects is less efficient, and the masticatory forces in these individuals falls to around 20-40% of the dentate value (Haraldson *et al.*, 1979; Slagter *et al.*, 1993). Experiments that have investigated this affect have involved the determination of maximum bite force both before and after the application of local aesthetic (Lund & Lamarre, 1973; Orchardson & MacFarland, 1980). There is also evidence that dentate and edentate people employ different chewing strategies (Heath *et al.*, 2002). Furthermore, in complete denture wearers the maximum bite force is considerably lower than in dentate people. One study has suggested that this may partially be due to ill-fitting dentures and reported an immediate improvement in the maximum force generation upon insertion of new or realigned dentures (Leyka *et al.*, 2000). However, another study found that, particularly for elderly patients with severe bone resorption, a delayed improvement should be expected (Müller *et al.*, 2002) suggesting that the quality of the fit is not the only issue.

5.4. JAW REFLEXES UNDER STATIC CONDITIONS

Since simulated mastication in humans is quite difficult, and the results are not easily interpreted, many researches have confined their studies to static jaw experiments where the conditions can be better controlled. However the main problem with static experiments is that it is not possible to assume that the reflexes will operate in the same way during mastication (Bawa & Stinkjaer, 1999; Yang & Türker, 1999).

During such experiments it is important to control the response from the two main receptor classes: proprioceptive, which detect the motion, position or activity of a muscle or limb by responding to stimuli arising within the organism; and exteroceptive, which respond to stimuli from outside the body.

5.4.1. PROPRIOCEPTIVE RECEPTORS

It is important to control the activity of proprioceptive receptors as their output can have a direct influence on the level of motoneuron excitation (and visa versa), this is important as different levels of activity have the potential to cause different reflex responses (Türker, 1988; Miles *et al.*, 1989a; Yamamura & Shimada, 1992; Yamamura *et al.*, 1993). Controlling muscle activity is generally performed by providing the subject with feedback from rectified and smoothed EMG derived from a muscle under investigation (van der Glas *et al.*, 1984; Miles & Türker, 1987; Draper, 1990). However despite the fact that the general tendency of the jaw is to counter-balance the 'active' side with an appropriate level of force to reduce the threat of strain to the temporomandibular joint (Dessem & Taylor, 1989) there is no guarantee that muscles, other than the one for which feedback is provided, will be contracting at a level similar to that which is required. There is also evidence that if the frequency of single motor unit firing is provided as feedback other motor units within the same muscle will significantly vary their firing rate over time even though the rate of the unit under control does not change (Nordstrom & Miles, 1991). Hence the level of motoneuron excitation can never be ensured unless it is under direct control by the subject.

5.4.2. EXTEROCEPTIVE RECEPTORS

In humans, a stimulus applied to the midline of the jaw elicits reflexes in the jaw-closing muscles on both sides symmetrically and simultaneously.

Reflexes are always elicited bilaterally and no overall significant differences have been observed between sides for occurrence, latency, duration or amplitude of the jaw-jerk, or for the latency or duration of the silent period (Kossioni & Karkazis, 1999), or for electrical or mechanical stimulation of PMRs (van der Glas & van Steenberghe, 1988).

Unlike in animal studies, many different experiments have shown that while a reflex response in the masseter can be easily identified, the human digastric has little or no reflex activity (Goldberg, 1976). Near painful electrical stimuli to skin and mucosal receptors do not initiate activation of the digastric in humans (Yemm, 1972a), and while the response to sudden downwards movement of the mandible is a contraction of the masseter and temporal muscles, it does not induce a reflex response in the digastric (Lund & Olsson, 1983; De Laat, 1987b). However trains of large electrical stimuli applied to the lip have been shown to elicit mid to long latency excitations in the digastric (62ms) albeit a little unreliably and only for a brief period before habituation (Cadden *et al.*, 1997).

In contrast to the above studies Ostry et al. found that unloading of jaw-opener muscles in humans elicited a short-latency decrease in EMG activity (20ms) followed by a short-duration silent period in these muscles while similar behaviour in response to unloading was observed for spindle-rich jaw-closer muscles (Ostry *et al.*, 1997). However, as previously discussed there is strong evidence that the small EMG changes seen in the digastric record could be caused by cross-talk from the masseter (or changes in the electrode/muscle interaction during unloading) rather than altered activity of the motoneurons controlling the digastric. A further study, this time conducted under dynamic conditions, has shown a small reflex response in the digastric with a latency the same as that seen in the masseter, but with a maximum occurring significantly later (Abbink *et al.*, 1998a). Thus indicating that while cross-talk may explain some of the activity digastric reflexes might only be present in humans during movement of the jaw.

One source of difference between the jaw muscles is that, unlike the jaw-closers, the jaw-opening muscles do not contain muscle spindles (Taylor *et al.*, 1976; Kubota & Masegi, 1977; Rowlerson, 1990). This anatomical finding illustrates that there are fundamental differences in the wiring of the sensory feedback between the jaw closing and jaw opening muscles. However since this finding is common across species it does not explain the differences between animals (where jaw-opening reflexes are easily elicited) and humans (where the jaw-opening reflexes are difficult to elicit). Rather the difference may have more to do with the mass of the jaw. Heavy jaws do not need highly developed jaw-opening reflexes as the weight of the jaw is normally sufficient to cause passive opening. In addition smaller animals tend to chew

at higher rates than larger animals (Hiiemae *et al.*, 1996; Kobayashi *et al.*, 2002), thus higher velocities of jaw movement, and hence more activity in the jaw-opening muscles, is required. It is also possible that the type of preparation used, for example using decerebrate animals (Collier & Lund, 1987), may change the relative excitability of reflexes.

5.4.3. DIFFERENCES BETWEEN SUBJECTS

Although the basic movements during mastication are said to be the same, there is a large degree of variability between individuals, food types and even between chewing cycles (reviewed in Lund, 1991) indicating that the sensory feedback, reflexes and/or conscious reactions to stimuli are different. Most studies on jaw reflexes do not split the results of males and females (van der Glas *et al.*, 1985; Brodin *et al.*, 1993b; Türker & Jenkins, 2000). However, one study has shown females had a shorter latency and larger amplitude jaw-jerk response than males, while the duration was the same (Kossioni & Karkazis, 1994); and another study has shown gender differences in the response to electrical stimulation of the lip (Lyons *et al.*, 2002). It has also been reported that the duration and shape of the masseteric motoneuronal discharge is altered by age (O'Connor & Türker, 2001). However, age and sex have been shown not to have a strong effect on human masticatory performance, rather the number of functional tooth units and the bite force have been confirmed as the key determinants (Hatch *et al.*, 2001). Despite this finding the possibility of differences between the sexes and ages illustrates the necessity of ensuring studies that compare subject groups have similar gender and age ratios.

It is known that large and highly significant inter-subject differences exist in almost all reflexes. Recent consumption of drugs, such as caffeine, can influence experimental results (Kalmar & Cafarelli, 1999), the difference between the chewing patterns of different people is greater than the variations found between different foods (Heath *et al.*, 2002), and that age can alter motor unit action potential shape (O'Connor & Türker, 2001). Further subject factors may include: periodontal health, tooth crowding, recent usage, tooth angle, periodontal history and even the amount of gamma activity present. While every effort can be made to ensure subjects have healthy teeth and gums with no history of orthodontic treatment or periodontal disease, some subject differences such as tooth separation and angle are unavoidable. While some hypotheses can be made with regards to the affect that these parameters may have on the jaw reflexes, they have yet to be tested.

It has been reported that respiration alters the stretch reflex observed in the jaw-closers (Otani-Saito *et al.*, 2001). The jaw-jerk reflexes of the masseter and anterior temporalis was found to be higher during nasal compared to oral respiration, while there was no change in the reflex observed in the posterior temporalis. This may also contribute to experimentally-derived inter-subject differences, as some people are more likely to be nasal breathers than others.

5.4.4. ORTHOGONAL STIMULATION OF TEETH

The easiest way to mechanically stimulate teeth is to tap the labial surface of incisor teeth with a probe. This approach has been tried by a number of researchers (Hannam *et al.*, 1970; Matthews, 1975; Bonte *et al.*, 1993; Sato *et al.*, 1994). Most studies have shown that tooth tapping in humans produces jaw reflexes that are multi-phasic and comprise a complex sequence of short- and long-latency inhibition and excitation (Louca *et al.*, 1998), although many of the secondary and later reflexes may be partly artefactual (Brodin *et al.*, 1993b; Türker & Powers, 1999). It has also been found that the reflex response to mechanical stimulation of the incisor is reproducible over the course of a year (van der Glas *et al.*, 1984) and is influenced by the level of muscle activity (Bessette *et al.*, 1973). In one study, brisk orthogonal taps elicited inhibition with a latency of 13ms and duration of 37ms, followed by an excitation at 71ms lasting for 29ms (Türker *et al.*, 1994), while in another study the inhibition latency was found to be 20ms (Yang & Türker, 1999). One group has shown that the reflex response of both humans and rats to orthogonal stimulation of the incisor teeth was similar, and that it varied with respect to the background muscle activity; low activity showed excitation while high activity showed inhibition (Yamamura & Shimada, 1992; Yamamura *et al.*, 1993). It has also been shown that in response to mechanical stimulation of the teeth small human motor units in the masseter show excitation while larger units show inhibition (Yamamura & Shimada, 1993). Although doubt has been cast over these findings due to the extremely long post-stimulus analysis time (>1s) meaning factors other than reflex responses, such as reaction time events, may have been analysed.

Contrary to most findings, one group found that the main inhibitory response was not abolished after the application of local anaesthetic and hence concluded that PMRs were not responsible for this reflex (Hannam *et al.*, 1970). The problem with this study was that only a very small amount of anaesthetic was used (0.5mL). This amount has been found to be enough to remove pain perception from the tooth but not pressure. Thus, more recent

studies in which larger quantities of anaesthetic were used and the reflexes disappeared, or were drastically reduced (van der Glas *et al.*, 1985; Türker & Jenkins, 2000), are more likely to be correct. In this study the subjects could not feel the stimulation on their teeth, rather they reported small vibrations coming from the back of their skulls.

To further investigate the role of PMRs in the production of masseteric inhibition following mechanical tooth stimulation a study has been performed on subjects with dentures (Brodin *et al.*, 1991). The results of which showed that while the threshold was increased the reflex inhibition was not completely abolished despite the lack of natural teeth and hence PMRs. The cause of this persistent activity was attributed to mechanoreceptors situated in the mucosa, which took over the functional role of the absent PMRs. However the size and type of stimulation used strongly suggests that receptors remote to the peri-oral area were stimulated.

One of the problems with the above form of stimulation is that the taps contain very high frequency components, much higher than is found during normal mastication (Türker *et al.*, 1997) unless extremely brittle food is used. It is known from both animal (Linden & Millar, 1988a) and human (Linden *et al.*, 1995) studies that PMRs are sensitive to both total force as well as rate of force application, to overcome this problem the concept of pre-load was introduced (Brodin *et al.*, 1993b). Pre-load involves holding the probe against the tooth at a low force level (0.5-1N) in between stimulation, this removes the high frequency components that could be found at the beginning and end of the stimuli when the probe took up the slack and makes the taps more like pushes. Only by using preload can the exact stimulus profile be applied to the tooth. Following this method it was found that slow, smooth forces applied on teeth evoked short (10-15ms) and long-latency (35-70ms) excitations in the EMG rather than inhibitions (Türker *et al.*, 1994) and local anaesthetic removed the response (Brodin *et al.*, 1993b).

While rapidly rising forces generate powerful shot latency inhibitory reflexes in the jaw-closers and reduction in the bite force, slowly rising forces usually induce an excitatory reflex response in the masseter and a reduction followed by an increase in the bite force (Yang & Türker, 1999). As discussed previously, there exist two distinct anatomical pathways linking periodontal mechanoreceptors and the jaw muscle motoneurons that may account for the two distinct reflex responses (Türker *et al.*, 1994; Türker, 2002). Namely some receptors respond to slowly rising forces, while others are activated by rapidly changing components of the force stimulus (Trulsson & Johansson, 1994).

Orthogonal tooth unloading experiments have shown that the unloading reflex is comprised of a short latency excitation (13ms), inhibition (20ms) and long latency excitation (40ms), hence the response to rapid unloading is similar to rapid loading (Türker & Jenkins, 2000). The short latency excitation is likely to be the result of spindles, and since the inhibition disappeared after the application of local anaesthetic it is likely that it originates from the periodontal region, in particular PMRs. The nature of the late excitation however is still unknown it may be a long-latency component of the stretch reflex, it may be caused by PMRs with excitatory connections to the jaw-closers or it may be artefactual.

Studies in animals clearly show the existence of the neural structures for an early excitatory reflex seen in both loading and unloading experiments. In decerebrate cats (Dessem, 1995) it has been shown that tooth displacement induces short-latency depolarisations in spindle cell bodies in the mesencephalic nucleus of the trigeminal nerve and in the motoneurons of the jaw elevator muscles. Similar short-latency excitatory reflexes in the masseter have been observed following intra-oral stimulation in rats (Funakoshi & Amano, 1974).

5.4.5. AXIAL STIMULATION OF TEETH

Unlike during static orthogonal experiments using rapid mechanical stimuli, there is positive feedback to the jaw-closing muscles during mastication (Ottenhoff *et al.*, 1992a & b; van der Bilt *et al.*, 1995; Abbink *et al.*, 1998a). Three possible reasons for this are: the forces encountered during chewing are more like slow pushes than fast taps; the movement of the jaw modulates the reflexes; and/or the direction of the stimulus on the tooth is important. Since it is known that PMRs code the direction and magnitude of force applied to the teeth (Trulsson *et al.*, 1992; Dessem, 1995) it is likely that the direction of force application is a contributing factor. Hence experiments that study human jaw reflexes due to axial simulation will provide a more accurate picture of what happens during normal activity than those utilising orthogonal stimulation. However, to date, little or no information exists as to the response of human incisor teeth to axial stimulation.

6. METHOD FOR QUANTIFYING RESPONSES FROM INTRA-MUSCULAR AND SURFACE ELECTROMYOGRAPHY

Measuring human reflex responses from EMG traces in an accurate, repeatable and reliable way with a high degree of specificity has traditionally been a difficult task. This chapter describes a new method that can be used to quantify reflex responses from both surface and intra-muscular electromyogram (EMG). This technique extends the classical cumulative sum (CUSUM) calculations by defining precise points for the calculation of latencies, durations and strengths to facilitate automatic reflex detection and permit the strength of a reflex to be defined in absolute units. The effect of varying the pre-stimulus time, the number of trials averaged and the amount of filtering used on the identification and classification of reflex parameters are also investigated. Furthermore, the effect of noise on these values, and how to remove it, is discussed.

The new method, which is an expansion of the CUSUM analysis, is compared and contrasted with the more common threshold-crossing method in two different muscles: masseter and first dorsal interosseous, in experiments utilising both mechanical and electrical stimulation. There are a number of advantages to using the new method; not only does the modified CUSUM method detect reflexes earlier than threshold-crossing methods but also the strength and duration are less susceptible to averaging and filtering parameters while giving a better indication of the reflex size.

The data suggests that a pre-stimulus analysis period of at least 100ms be used to correctly identify the variability inherent in EMG traces. It is also concluded that for subtle reflexes 50 stimuli should be the minimum number used when spike trigger averaging is employed, as lower numbers are associated with much greater pre-stimulus variability. Zero-phase filtering the rectified averaged EMG traces is recommended as this makes it easier to identify significant changes in the electrical activity of the muscle in question. In addition, noise estimation and removal from averaged rectified EMG recordings yields results that are a more accurate representation of the synaptic activity of the motor units in question.

This chapter is an edited version of the manuscript “A method for quantifying reflex responses from intra-muscular and surface electromyogram” by R.S.A. Brinkworth and K.S. Türker, which has been published in the Journal of Neuroscience Methods 122(2): 179-193 (2003).

6.1. INTRODUCTION

Human reflex studies can be used as tools to investigate the synaptic connections between afferent systems and motoneurons. However, measuring reflex responses in a repeatable way with a high degree of specificity and reliability has traditionally been a difficult task. It has been shown that single electromyogram (EMG) traces are not reliable for reflex measurements (Lavigne *et al.*, 1983). Instead, several manipulations have been suggested to improve and convert EMG records into quantifiable forms. The most common technique is full-wave rectification and averaging of the EMG around the time of stimulation (Hannam *et al.*, 1969; Jenner & Stephens, 1982). However the number of stimuli required to yield a reliable result, and the pre-stimulus time used to ascertain the base level of activity, varies markedly from study to study (Evans *et al.*, 1989; van Boxtel *et al.*, 1993; Hodges & Bui, 1996; Harrison *et al.*, 2000).

It is difficult to ascertain the exact latency and duration of a reflex from the EMG record, even with rectification and averaging, due to the variability inherent in such recordings (Garnett & Stephens, 1980). To overcome this problem cumulative sum (CUSUM) calculations have been utilised. Even though the original CUSUM calculations were constructed to illustrate subtle changes in the peri-stimulus time histogram of single motor unit records (Ellaway, 1978) the same calculations have been successfully applied on rectified averaged EMG recordings (Brodin *et al.*, 1993a). Although changes in the EMG are made clearer by using the CUSUM confusion still exists as to the exact point of reflex onset, how large a deflection must be to be considered significant and how to overcome noise found in EMG records. It has also been common practice to rely on observer judgement for the determination of reflex parameters (Brodin & Türker, 1994) rather than constructing computer algorithms that are less subjective and yield data that can be easily, and accurately, reproduced (Hodges & Bui, 1996). Moreover, to date, CUSUM calculations have not been used to indicate the magnitude of the reflex response relative to the baseline EMG.

In the CUSUM, increases from the mean are shown as positive slopes, while decreases are recognized as negative slopes (Garnett & Stephens, 1980). When the CUSUM starts to turn (turning point) it corresponds to the EMG crossing the pre-stimulus mean. By using this value, reflex latencies corresponding to the start of a trend can be found rather than waiting for the EMG to cross a predefined threshold. Determining how large a CUSUM deviation (EMG area) must be before it is classified as a significant event is difficult. To overcome this problem Türker and colleagues proposed the concept of the error box. If the CUSUM deviation is greater than the

maximum pre-stimulus deviation (in either direction) then a significant event has occurred (Türker *et al.*, 1997). An improved version of this error box approach has been adopted, and a computer program written for automatic detection.

Numerous automatic EMG analysis methods have been recommended (van der Glas *et al.*, 1995; Abbink *et al.*, 1998b; Leader *et al.*, 1998) and a number of reviews published (van Boxtel *et al.*, 1993; Hodges & Bui, 1996). However, almost all of the current methods involve determining the time that the EMG signal crosses a predefined amplitude threshold. This occurs despite the fact the amplitude of the EMG at a particular point is not sufficient for determining either the start, nor end, of a burst of EMG activity (Marple-Horvat & Gilbey, 1992). To overcome this problem many researchers utilise time windows of various widths. By passing the signal through a window a number of data points around the time in question are used to determine if a significant event has occurred. Windows are also used to decide if an event has sufficient duration to be considered significant. The problem with using windows is that there is no set optimal width. If the window is too short then brief spikes in the EMG may be classified as significant events even when they are not. If the window is too long then time distortion will occur, thus causing errors in the temporal calculations. In addition, it will be difficult for the signal to cross the amplitude threshold as more and more large values are required to increase the value in the window, hence significant events may be missed. This problem is then compounded since there is no clear choice for the amplitude threshold value (Winter, 1984).

By using the area of an EMG signal rather than isolated points, or groups of points, a more accurate indication of overall muscle activity over time can be achieved (Loeb & Gans, 1986; Marple-Horvat & Gilbey, 1992). In addition, studies have indicated that by analysing the area subtle reflexes that may not have large amplitudes are revealed (Türker & Jenkins, 2000).

The aims of this study were four fold. Firstly, to show that EMG analysis based on area (i.e. CUSUM) yields a better representation of the activity of motor units than those based on amplitude as the strength of a reflex is indicative of electrical activity over time rather than at one point in time. Secondly, to demonstrate that the time of a turning point in the CUSUM (EMG crossing the pre-stimulus mean) is a better indication of reflex latency than the time that the EMG crosses a defined amplitude threshold level. Thirdly, to illustrate the effect different pre-stimulus analysis times have on reflex detection. Finally, to show that noise estimation and removal should be used in EMG reflex studies so that the strength of reflexes can be accurately determined.

For the purposes of completeness two different reflex complexes from two different muscles: masseter and first dorsal interosseous (FDI), and two different stimulation methods: mechanical and electrical, were used to show the accuracy and versatility of the new method against the classical threshold-crossing method.

6.2. METHODS

6.2.1. SUBJECTS

The experiments were approved by the Human Ethics Committee of the University of Adelaide and conformed to the Declaration of Helsinki. Three subjects were used in the masseter experiments while one subject was used for the FDI experiment. All subjects were neurologically normal adult volunteers who gave written informed consent (age 18-25 years). The general detail of the masseter experiment has been given elsewhere (Brodin *et al.*, 1993b), and the FDI experiment was adapted from the literature (Evans *et al.*, 1989; Harrison *et al.*, 2000) hence they are summarized only briefly below.

6.2.2. EMG RECORDING

In all cases the intra muscular (IM-EMG) and surface EMG (SEMG) activity was amplified between 300 and 3000 times, high-pass filtered at 5Hz and then sampled at 5kHz using a 12-bit A/D; in addition IM-EMG was fed into a filter box where it was rectified and low-pass filtered (0.1Hz) to serve as visual feedback to the subject.

EMG recordings were performed as follows. Standard silver/silver chloride gel surface electrodes were placed over the belly of the muscle of interest to record the SEMG. To record intra-muscular activity two Teflon® insulated silver wires were inserted into a depth of approximately 1cm into the target muscle using a 25G needle. The end 3mm of the wires were striped of the insulation and offset from each other by 1cm to ensure recording from a suitable area.

6.2.3. NOISE ESTIMATION

In all experiments, and for each subject, three trials with no muscle activity were randomly placed within the experimental protocol and used to determine the noise level in the system. Each trial contained 50 stimuli. The noise trials were rectified then averaged together (n=150); the minimum value in the pre-stimulus period was defined as the noise level and subsequently subtracted from all rectified averaged EMG traces. Post-stimulus values were not used in the noise trials.

6.2.4. REACTION TIME

To determine if the observed changes in the EMG records were indeed reflexes and not conscious reactions, the minimum reaction time for each muscle to the stimulus used was calculated. This involved increasing the time between stimuli to between 8 and 10 seconds, and asking the subject to contract the muscle when a stimulus was felt. Due to the large and consistent nature of muscle electrical activity generated by conscious contractions, the reaction times were easily identifiable from the normal reflex responses and the background level.

6.2.5. MEASUREMENTS FROM THE MASSETER

The subjects were asked to bite into impression material mounted on two fixed bite bars with their upper left incisor positioned on a tooth rest. A tooth stimulator was used to stimulate the upper left incisor of the subjects (Brodin *et al.*, 1993b) with stimuli delivered randomly between 1 and 3 seconds.

The rising edge of the stimulus force was a half-sinusoid with a maximum of approximately 3N (plus 1N preload) reached in 20ms. This level was then held for a further 80ms to ensure that the unloading phase did not induce a stimulus that interfered with the one elicited from the rising phase. Subjects bit at 10% maximum voluntary contraction (MVC) of the left masseter throughout the experiments.

6.2.6. MEASUREMENTS FROM THE FIRST DORSAL INTEROSSEOUS

A single electrical pulse, width 0.1ms, was delivered at random intervals between 2 and 4 seconds via ring electrodes attached on either side of the proximal interphalangeal joint of the right index finger. Stimulus strength of 7 times the threshold of perception (7T) was used. A constant muscle contraction of 20% MVC was achieved with the right hand placed flat on a table and an isometric abduction of the index finger performed against resistance. The SEMG electrode was placed over the FDI muscle in the direction of the muscle fibres, the IM-EMG electrode was placed between the SEMG recording sites to a depth of approximately 1cm. The electrical stimulus artefact was suppressed by using a custom-built stimulus artefact suppressor.

6.2.7. OFFLINE ANALYSIS

The formula used for the calculation of the CUSUM was:

$$CUSUM(t) = \sum_{t_{p-}}^{t} bw(EMG(T) - \overline{EMG(T_0)})$$

where $\overline{EMG(T_0)}$ = mean pre-stimulus EMG (normalised to 1), t_{p-} = is the pre-stimulus analysis time used and bw is the bin width in ms (inverse of sampling frequency in kHz). The bin width value is included as a correction for the sampling rate, it provides normalisation and ensures that a comparison can be made between studies that use different sampling rates. Using 'k' as the units for normalised EMG (where mean pre-stimulus value equals 'k') then the units for CUSUM become 'k.ms'. 1k.ms represents the level where the product of change from the mean (in percentage) and reflex time (in ms) is 1. For example, a reflex that is on average 20% above the pre-stimulus base line for 10ms will have a reflex size of 2k.ms while a reflex that is 10% below the base line for 20ms will have a reflex size of 2k.ms.

Analysis involved zero-phase band-pass filtering the EMG (surface 5 to 500Hz, intra-muscular 50 to 1500Hz), rectifying the signals, extracting a defined time period from around the stimuli, averaging the signals followed by applying a zero-phase 5-bin moving average filtering (approximately 500Hz low pass) for IM-EMG and 11-bin filtering (approximately 200Hz low pass) for SEMG (Basmanjian & De Luca, 1985). Zero-phase filtering was used as other filters produce phase shifts that result in erroneous temporal calculations (van Boxtel *et al.*, 1993). Low-pass filtering was used to reduce the occurrence of non-genuine zero-crossings (caused by the inherent 'spikes' in EMG traces due to the synchronous activity of the motor units) and ensure that reflexes with low amplitudes but large durations could be identified.

The number of trials (n) averaged together for the masseter experiments (10, 25, 50, 100, 150, 200, 250 and 400) and the pre-stimulus time used in both the masseter and FDI experiments (25, 50, 75, 100, 125, 150, 200, 300, 400 and 500) were varied to illustrate the effect these parameters have on the CUSUM and the associated error box (i.e. pre-stimulus variability).

EMG normalisation was achieved through dividing the averaged trace by the average pre-stimulus value; this had the effect of making the pre-stimulus average level (k) equal to one. CUSUMs of the normalised averaged EMG data were then constructed.

The resulting data were analysed to compare two different methods of reflex/burst detection: the threshold detection method (Marple-Horvat & Gilbey, 1992; Abbink *et al.*, 1998b; Harrison *et al.*, 2000) adapted from recommendations in a review of the literature (van Boxtel *et al.*, 1993; Hodges & Bui, 1996), and the new modified CUSUM method proposed in this paper. Calculations from both methods were performed both with and without noise removal. The reflex parameters measured are described below and illustrated in Figure 6.1. A computer program was written to follow these guidelines and ensure the results were both objective and repeatable.

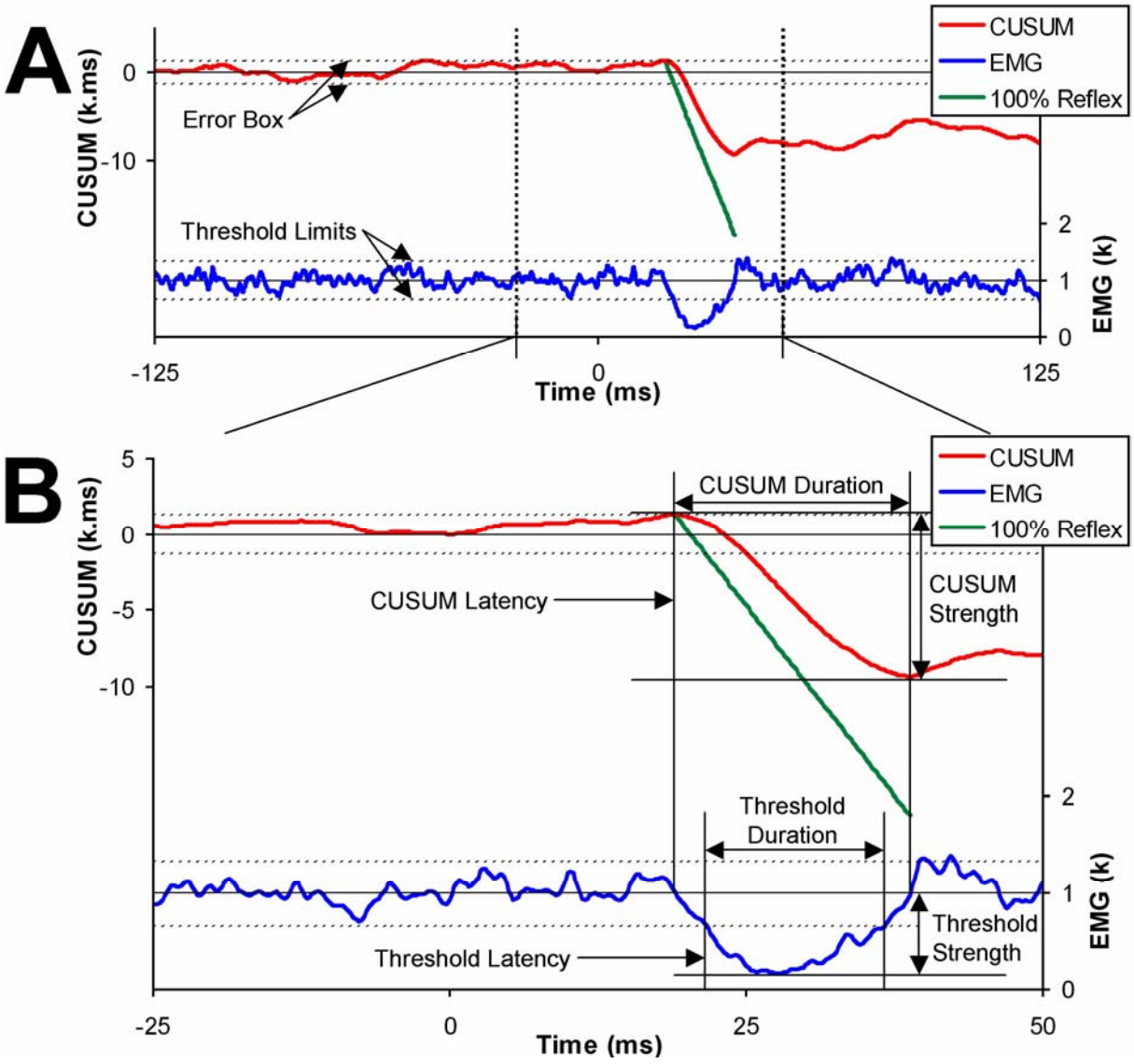
6.2.7.1. REFLEX OCCURRENCE: THRESHOLD-CROSSING METHOD

A significant event was registered if the average value of a six-bin window of the EMG trace, and the following five windows, was more than three standard deviations from the mean (as defined by the pre-stimulus period). In this way both the amplitude of the signal, and the duration above the significance level, were used to determine if a significant event had occurred. A window rather than a single data point was used in this analysis. This approach was adapted since measuring the amplitude of a signal at a point for comparison with a predetermined threshold level is not strictly sufficient for defining the start of a burst of activity in an EMG signal. By definition, a burst must last a number of time periods rather than a single data point (Marple-Horvat & Gilbey, 1992), however the exact number of data points is still a matter of conjecture. Various previous studies have used differing times (Neafsey *et al.*, 1978; Di Fabio, 1987; Lee *et al.*, 1987; Thompson & McKinley, 1995) although using the 95th percentile value of the pre-stimulus durations exceeding the limit has been suggested (van der Glas & van Steenberghe, 1981). The main problem with using the 95th percentile of the pre-stimulus durations exceeding the limit is that if the post-stimulus period was identical to the pre-stimulus period then a number of events would be classified as significant even though they were the same as happened pre-stimulus. The definition of reflex occurrence used in this chapter has been adapted from the literature (Hodges & Bui, 1996).

6.2.7.2. REFLEX OCCURRENCE: MODIFIED CUSUM METHOD

A significant event was classified if the vertical distance between two consecutive CUSUM turning points (EMG crossing the pre-stimulus mean) was more than 100% of the maximum pre-stimulus variation (error box). This method is different to the Threshold-crossing method as it uses area over time rather than amplitude over time.

Figure 6.1: Reflex Characteristics



EMG and CUSUM characteristics of a masseteric inhibitory reflex (n=150) elicited from axial stimulation of the upper left incisor and recorded from the left masseter using intra-muscular electrodes from a subject contracting at 10% MVC and sampled at 5kHz; A) entire record, B) close-up of inhibition showing reflex characteristics. The CUSUM latency was determined from the turning point of the CUSUM (corresponds to the EMG crossing the pre-stimulus mean); the threshold latency corresponded to the time the EMG crossed the pre-stimulus amplitude threshold. The CUSUM strength is the ratio of the CUSUM reflex deviation to the maximum possible reflex (corresponds to EMG area) while the threshold strength is the maximum EMG deviation from the pre-stimulus level. The CUSUM duration is defined as the time to the next CUSUM turning point (also the time the EMG again crosses the pre-stimulus mean) and the threshold duration is the time that the EMG re-crosses the amplitude threshold.

6.2.7.3. REFLEX LATENCY: THRESHOLD-CROSSING METHOD

The threshold latency was defined as the first point in the first window when a significant event was identified. This definition of latency is different from that used by Hodges and Bui. Rather than selecting the mid-point of the window as the reflex latency, the first point is used as it more closely corresponds to the start of the change in the EMG. While this does cause a phase advance (+0.5ms or plus two and a half bin widths) in the detected latencies, the latency is still later than that found using the modified CUSUM method. This is because the signal takes time to move from the mean to the level of significance.

6.2.7.4. REFLEX LATENCY: MODIFIED CUSUM METHOD

The CUSUM latency was defined as the time of the turning point that initiated the reflex (significant event). This corresponds to the time that the EMG crosses the pre-stimulus mean; it is not the time that the CUSUM crosses the error box. In this way the start of a significant event can be found rather than waiting for a level to be reached.

6.2.7.5. REFLEX STRENGTH: THRESHOLD-CROSSING METHOD

The maximum (for excitation) or minimum (for inhibition) in the averaged, rectified, normalised value of a reflex was defined as the threshold strength. The units were percentage difference from pre-stimulus mean (%PSM), for example an excitation maximum that was twice the value of the pre-stimulus mean would be 100%PSM (i.e. 100% increase) while an inhibition minimum that was half the pre-stimulus mean would be –50%PSM (i.e. 50% decrease).

6.2.7.6. REFLEX STRENGTH: MODIFIED CUSUM METHOD

The vertical distance between two CUSUM turning points of a reflex (that is the area between the EMG trace and the pre-stimulus mean) was defined as the reflex strength. As the CUSUM has been normalised this value had little meaning, so to make it easier to understand, and to compare it to the threshold strength, it was converted into a percentage. Due to normalisation of the EMG, the CUSUM and the bin width, the largest possible inhibitory reflex corresponds to a CUSUM slope of –1 per ms (i.e. 0 EMG level). Hence, the strength of the largest possible inhibitory reflex response is given as the duration (in ms) of the reflex. Thus CUSUM reflex strength is the deviation of the reflex (EMG area) divided by the duration of the reflex event, and

reported as a percentage. It is possible for an excitatory reflex to have strength in excess of 100%; this corresponds to an average EMG activity of more than twice the base level, or a more than 100% increase in the area above the pre-stimulus mean. This definition of strength is superior to the Threshold-crossing method as it indicates the total amount of change in the EMG activity rather than just the maximum (or minimum) value.

6.2.7.7. REFLEX DURATION: THRESHOLD-CROSSING METHOD

The threshold duration was found by subtracting the latency of the reflex from the time of the end of the reflex. The end of the reflex was calculated in a similar way to the threshold latency (Hodges & Bui, 1996) except that it was taken as the last value in the last window, not the first value in the first window. As with the latency this introduced a temporal discrepancy, this time a phase lag (-0.5ms or minus two and a half bin widths).

6.2.7.8. REFLEX DURATION: MODIFIED CUSUM METHOD

The CUSUM reflex duration was the horizontal distance between the start of a reflex, as defined by the CUSUM latency, and the next turning point. This is equivalent to the time period between the EMG crossing the pre-stimulus mean twice (Figure 6.1B).

6.3. RESULTS

The modified CUSUM method has been developed in the laboratory over a number of years and by observing reflex responses in many subjects. Four subjects participated in experiments to clearly illustrate the principles involved in the new methodology, three in the masseter experiments and one in the FDI.

6.3.1. REACTION TIME

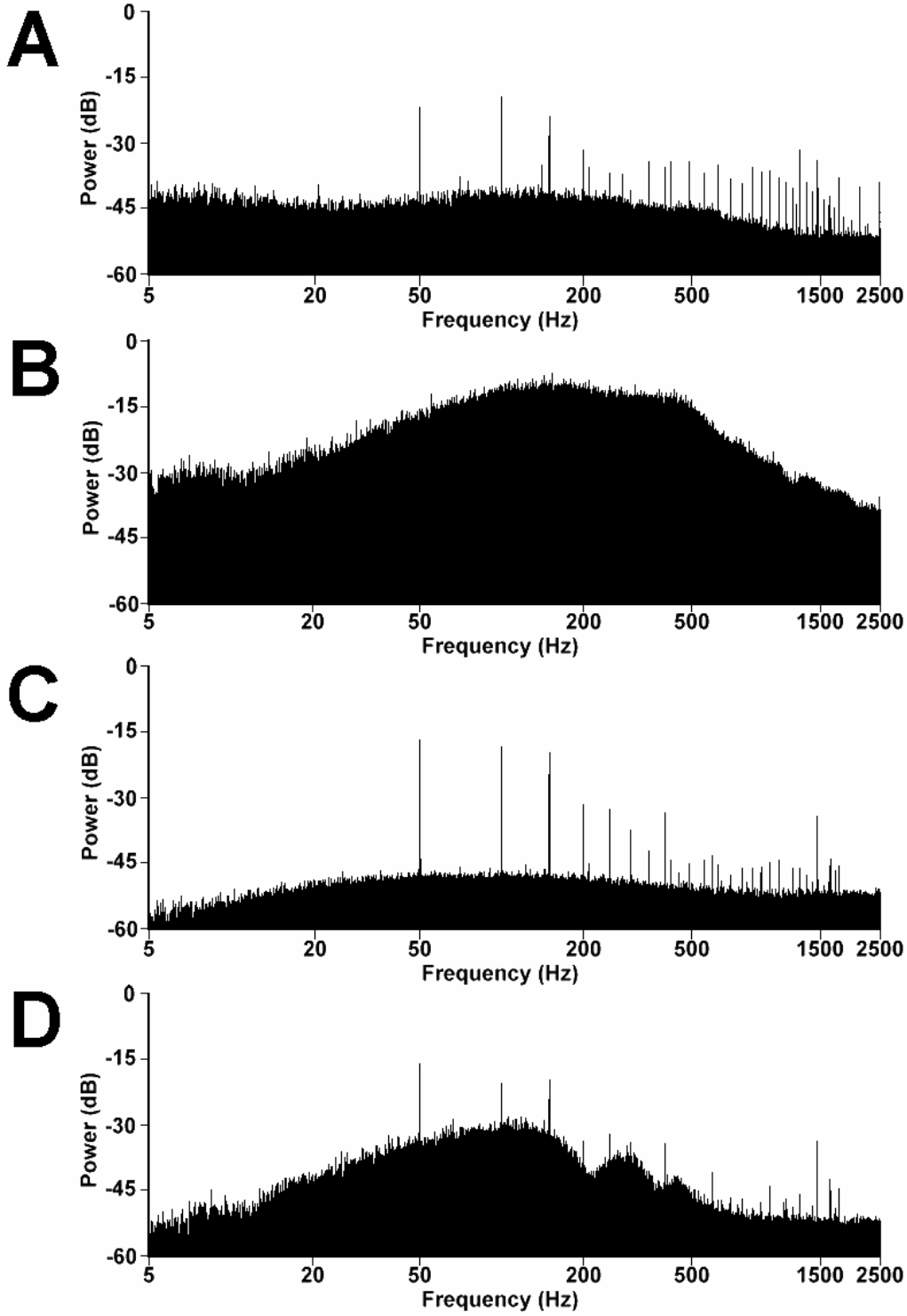
The reaction time for the non-painful mechanical stimulation of the teeth as recorded in the masseter was found to be 140ms. The reaction time for strong (7T) electrical stimulation of the hand was found to be 125ms.

6.3.2. NOISE

The noise removal procedure was applied as described in the methods. This procedure ensured that the DC offset introduced by the inherent noise was removed from the averaged rectified EMG. To determine if this noise removal procedure was valid a spectral analysis of the data was performed. The results shown in Figure 6.2 are from the FDI experiment but are indicative of those found in all experiments. As can be seen from the non-contracting trials (0% MVC), the largest noise contribution (-20 to –35dB) is from spikes at specific frequencies most likely coming from the mains power supply and the resulting harmonics (50Hz, 100Hz, 150Hz, etc.) with white noise at a significantly lower power (approximately –45dB).

While it is true that the noise in the intra-muscular recording was larger than that found in the surface (as seen in the non-contracting trials), the mains power noise spikes that can be found in the SEMG during active muscle contraction are not identifiable in the IM-EMG due to the increased signal amplitude (>15dB). Hence the noise had less of an impact on the results derived from the IM-EMG traces. The low frequency component in the IM-EMG signal (<50Hz) and the high frequency component in the SEMG (>500Hz) both correspond to noise (Basmanjian & De Luca, 1985) and were filtered out before further analysis was performed.

Figure 6.2: Power Spectral Analysis Of FDI Experiment



A) 0% MVC intra-muscular EMG, B) 20% MVC intra-muscular EMG, C) 0% MVC surface EMG and D) 20% MVC surface EMG. Only 5Hz high pass filtering was performed on the data before spectral analysis. While a 50Hz notch filter could have been used to remove the spikes in the signals associated with electrical interference and the harmonics, its use may cause phase distortions and remove some of the EMG signal. Noise removal, as discussed in the text, drastically reduces the noise while not inducing any phase changes, and retaining the entire EMG signal.

6.3.3. Effect Of Varying Pre-stimulus Period And Stimulus Number

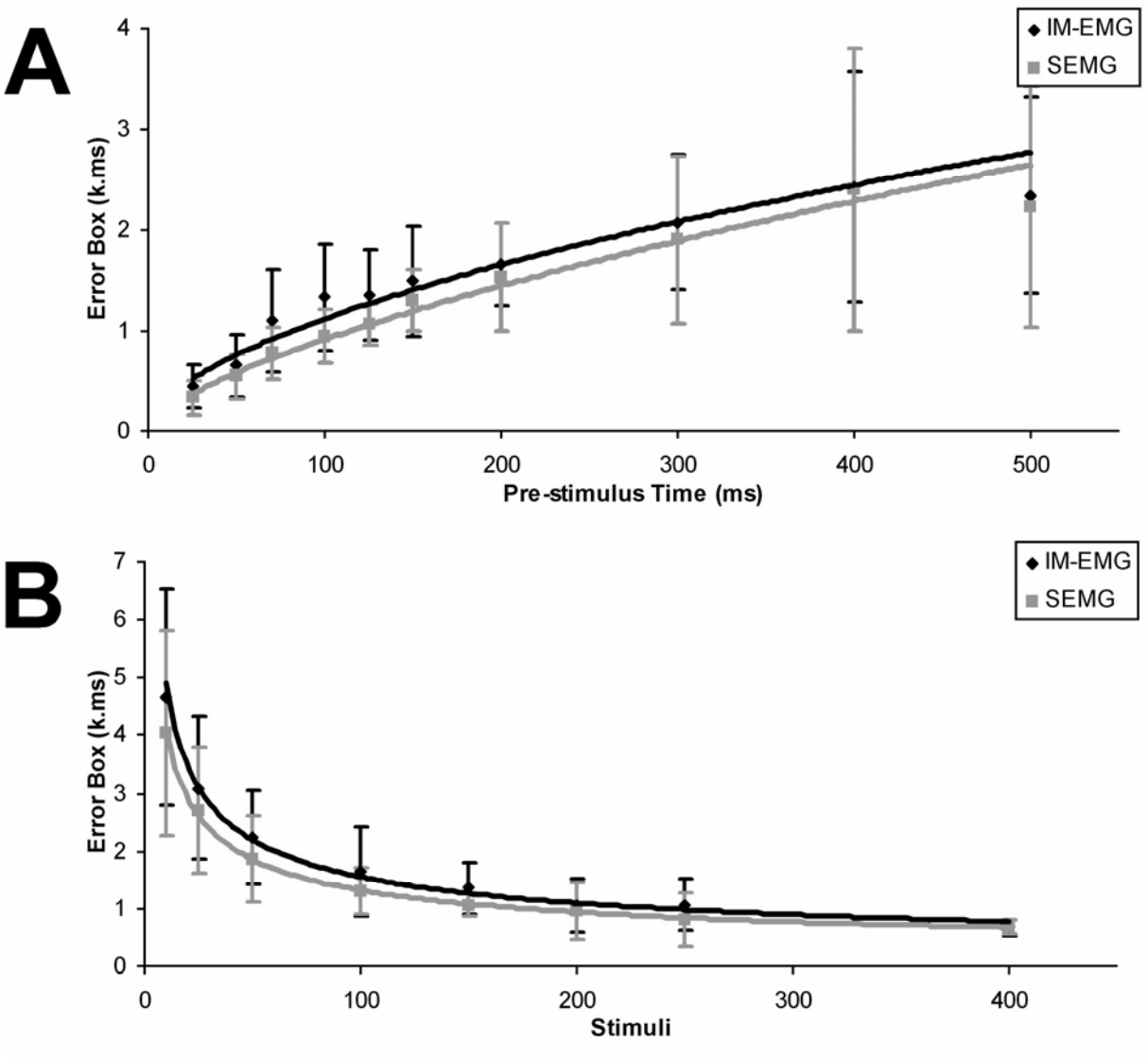
Figure 6.3A shows the effect on the CUSUM error box of averaging different numbers of stimuli while Figure 6.3B shows the effect on the CUSUM of using various pre-stimulus times. Univariate analysis on the data showed that there was no significant difference between the sizes of the error boxes in IM-EMG compared to SEMG recordings ($p>0.05$) under either of the two conditions (changing pre-stimulus time or changing the number of stimuli). The results indicate that by increasing the pre-stimulus time the error box increases. The use of a short pre-stimulus time (<100ms) causes the pre-stimulus variation to be underestimated and hence occurrences that were not reflexes may be identified as significant events. Conversely pre-stimulus times that are too long (>250ms) overestimate the EMG activity close to the stimulus and thus subtle reflexes may be more difficult to detect. While low stimulus numbers are associated with large pre-stimulus variability there is little change in the error box once 150 stimuli are reached (Figure 6.3B).

In the present experiments, the number of stimuli chosen for each experiment was 150 while the pre-stimulus times were set to the same as the post-stimulus times required to ensure the complete capture of all reflex activity. For the masseter this was 125ms, while for the FDI it was 200ms.

6.3.4. Effect Of Varying Filtering Parameters

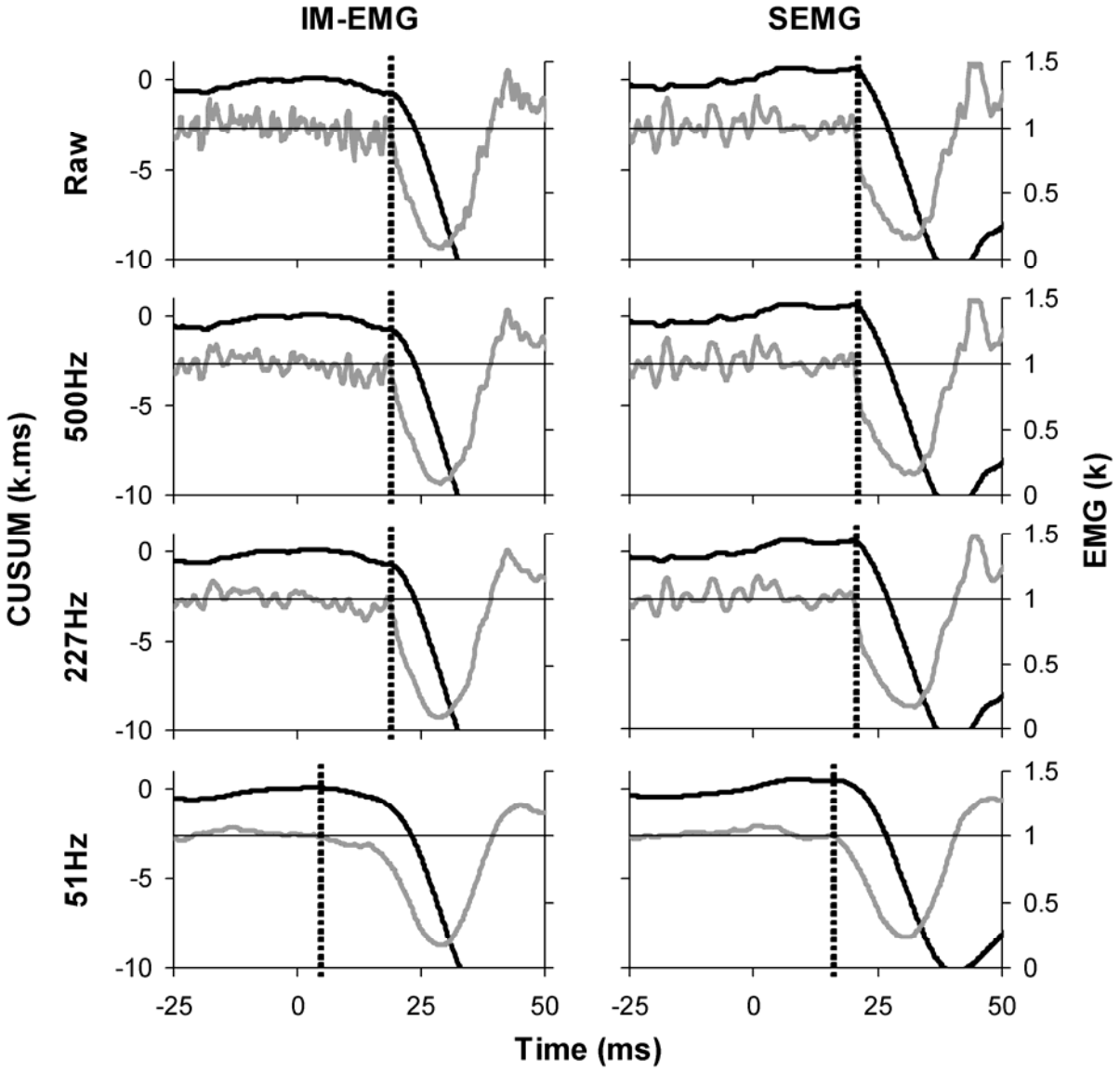
Figure 6.4 indicates the effect of varying the low-pass cut-off frequency applied to the averaged rectified EMG trace for both IM-EMG and SEMG. The SEMG has less high frequency components than the IM-EMG, hence is affected less by the filtering, while the shape of the CUSUM is almost unchanged with a low cut-off frequency above 227Hz. The latency of the inhibitory reflex was found to be approximately 19-20ms in both IM-EMG and SEMG recordings. However, when high levels of filtering are employed (i.e. 51Hz low-pass) then the EMG no longer crosses the pre-stimulus average and the latency calculations become erroneous, 4ms for IM-EMG and 16ms for SEMG. This is despite the fact that, even with DC removed, approximately 90% of the power in the rectified and averaged IM-EMG and SEMG was present below 50Hz.

Figure 6.3: Altering Pre-Stimulus Analysis Time And Number Of Stimuli



The effect of changing the A) pre-stimulus analysis time and B) number of stimuli averaged on the size of the error box. The muscle contraction level was 10% MVC. IM-EMG and SEMG represent intra-muscular and surface EMG recordings respectively. The number of averages used for A) was 150 while the pre-stimulus time used for B) was 125ms. Values are an average from all three masseter experiments and the error bars represent one standard deviation. The trend lines are all of the form ax^b and all R^2 values are greater than 0.94. Increasing the pre-stimulus time increased the error box size while increasing the number of stimuli had the opposite effect.

Figure 6.4: Effect Of Zero-Phase Low-Pass Filtering



How the averaged rectified EMG (grey) and CUSUM (black) from the masseter of one subject (n=150) in response to a 3N stimulus delivered at time 0 changes with different levels of low-pass filtering. Filter cut-off frequencies on the left with raw indicating no filtering; IM-EMG and SEMG represent intra-muscular and surface EMG respectively. The x-axis is time (in milliseconds), the left y-axis is CUSUM (k.ms) and the right y-axis is normalised EMG (k). In each case the broken line denotes the latency of the inhibitory reflex. Filtering reduces the number of times the EMG crosses the pre-stimulus mean, therefore the number of CUSUM turning points making reflex identification easier. However, if too much filtering is performed (i.e. 51Hz) then changes in the latency calculations can result as turning points not caused by noise will be removed.

6.3.5. MASSETER

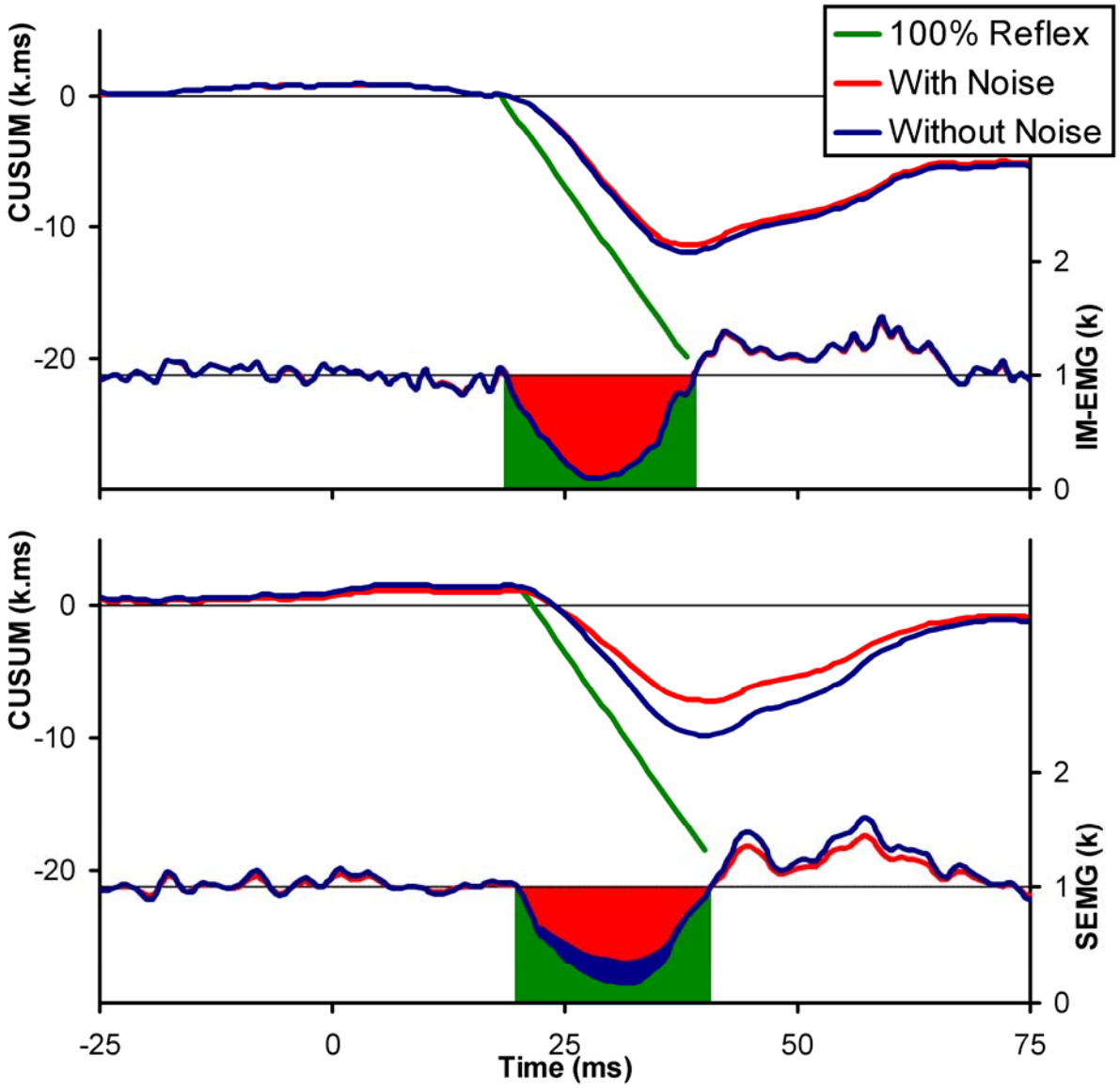
In all three subjects a mechanical stimulus of 3N with an average preload of 1N generated an inhibitory reflex response. The rectified averaged (n=150) EMG of the masseter muscle and its CUSUM during an inhibitory reflex was studied to illustrate the new methodology. Figure 6.5 illustrates the reflex elicited in the masseter by mechanical stimulation of the tooth for both SEMG and IM-EMG with and without noise removal, also shown is the maximum possible reflex levels. It is noted that the removal of noise from the intra-muscular trace had almost no effect on the strength of the reflex; in contrast, there is a significant difference between the strength of the SEMG reflex before noise removal compared to after. In fact the strength (both area and maximum) of the reflex determined from the SEMG after noise removal is similar to that found from the intra-muscular trace.

The stimulus caused an inhibitory reflex response in the masseter that was detectable in both the intra-muscular and surface EMG using both the CUSUM and threshold methods. All results are shown in Table 6.1.

The CUSUM of the EMG displayed a consistent pattern of a downward followed by an upward phase at all stimulus conditions. The downward phase illustrated the consecutive bins in the EMG that had less activity than the pre-stimulus level. On the other hand, the upward phase illustrated the consecutive bins in the EMG record that had more activity than the baseline level. In no trial did the upward phase of the CUSUM cross the zero level before the reaction time set in. Therefore, the upward phase was simply caused by delayed spikes (Türker & Cheng, 1994) and there was no reflex response after the inhibitory reflex was completed.

The average latency of the inhibitory reflex using the CUSUM method was 20ms for intra-muscular and 19ms for surface recordings. Using the threshold method, the latency was 22ms for both recording methods. The latencies determined by the threshold method were always longer than those found using the CUSUM method. The average duration of the reflex was 19ms in intra-muscular and 20ms in surface recordings using the CUSUM method while using the threshold method it was 17ms and 16ms in the IM-EMG and SEMG recordings respectively. The durations determined by the threshold method were always shorter than those found using the CUSUM method. Noise removal did not have an effect on the calculated latency or duration of the reflex.

Figure 6.5: Effect Of Noise Removal - Jaw



How noise removal changes both the normalised A) intra-muscular (IM-) and B) surface (S) EMG recordings of an inhibitory reflex in the masseter for one subject (n=150), the entire peri-stimulus time period (125ms pre-stimulus and 125ms post-stimulus) is not shown. Variables were a masseter contraction level of 10% MVC, an average preload of 1N and a 3N mechanical stimulus delivered at time 0. Light grey boxes around the EMG and light grey lines on the CUSUM show the 100% levels for the reflexes. There was little change in the area of the reflex after noise removal in the IM-EMG, -51% before compared to –56% after noise removal; however the SEMG reflex area increased substantially from –36% to –47%.

Table 6.1: Results Of Masseter Experiments

	Subject					
	1		2		3	
	noise	-noise	noise	-noise	noise	-noise
Latency						
IM-EMG C (ms)	19	19	19	19	23	23
SEMG C (ms)	16	16	20	20	22	22
IM-EMG Th (ms)	21	21	19	19	24	24
SEMG Th (ms)	21	21	21	21	23	23
Duration						
IM-EMG C (ms)	20	20	20	20	18	18
SEMG C (ms)	23	23	21	21	18	18
IM-EMG Th (ms)	16	16	18	18	16	16
SEMG Th (ms)	16	16	19	19	15	15
Strength						
IM-EMG C (%)	-50	-53	-56	-59	-46	-56
SEMG C (%)	-38	-42	-40	-55	-29	-44
IM-EMG Th (%PSM)	-79	-84	-85	-89	-69	-84
SEMG Th (%PSM)	-71	-78	-57	-78	-48	-73

IM-EMG, SEMG, C and Th represent intra-muscular and surface EMG recordings and CUSUM and threshold-crossing analysis methods respectively. Latency and duration measurements were unaffected by the removal of noise.

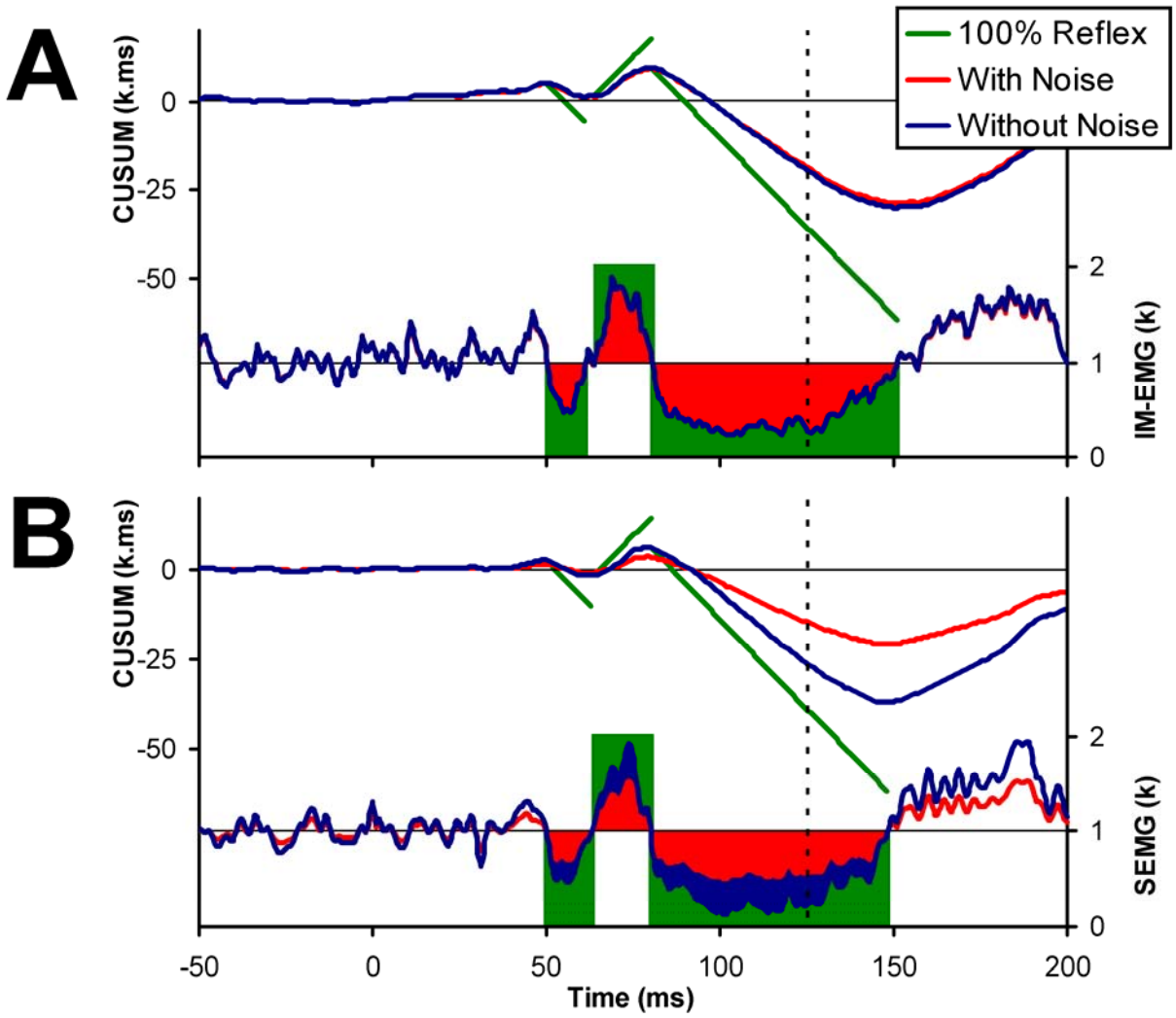
Before the noise removal, the average strength of the reflex as derived from the CUSUM method was -51% from intra-muscular and -36% from the surface recording. After the removal of noise the intra-muscular value increased slightly to -56%, while the surface strength increased to -47%. A similar result was found when the maximum reflex value was studied using the threshold-crossing method. The intra-muscular reflex maximum was -78%PSM before and -86%PSM after noise removal; the surface reflex maximum was -59%PSM with noise and -76%PSM without noise.

6.3.6. First Dorsal Interosseous

The rectified averaged (n=150) EMG of the FDI muscle and its CUSUM during a complex reflex response was studied to illustrate this new methodology. Figure 6.6 illustrates the reflex response elicited in the FDI by electrical stimulation of the right index finger for both SEMG and IM-EMG with and without noise removal, also depicted is the theoretical maximum reflex levels for each reflex. Note that, as with the masseter recordings, the removal of noise from the intra-muscular trace had almost no effect on the strength of the reflex, while there is a sizable difference between the strength of the SEMG reflex before noise removal compared to after.

The stimulus caused a complex reflex response in the FDI that was detectable in the intra-muscular and surface EMG using both detection methods. Visible reflexes in the EMG traces were early inhibition, excitation and late inhibition. The latency, duration and strength of each reflex are shown in Table 6.2. Unlike the masseter trials the later reflexes were larger than the preceding reflexes indicating that secondary reflexes were real.

Figure 6.6: Effect Of Noise Removal – FDI



How noise removal changes both the A) intra-muscular (IM-) and B) surface (S) EMG recordings of a complex reflex response in the first dorsal interosseous (FDI) of one subject (n=150) at a contraction level of 20% MVC. The stimulus (7T) was delivered at time 0. The reaction time (125ms) is shown by a vertical dotted line. Light grey boxes around the EMG and light grey lines on the CUSUM show the 100% levels for each reflex. As with the masseter results there was little change in the IM-EMG recordings after noise removal but a large change was found in the SEMG.

Table 6.2: Results Of FDI Experiment

	Reflex					
	IN1		E		IN2	
	noise	- noise	noise	- noise	noise	- noise
Latency						
IM-EMG C (ms)	51		64		80	
SEMG C (ms)	50		64		80	
IM-EMG Th (ms)	54		67		84	
SEMG Th (ms)	55		67		83	
Duration						
IM-EMG C (ms)	11		16		71	
SEMG C (ms)	13		16		69	
IM-EMG Th (ms)	4		10		52	
SEMG Th (ms)	5		11		61	
Strength						
IM-EMG C (%)	-34	-35	49	51	-54	-56
SEMG C (%)	-18	-33	27	48	-36	-63
IM-EMG Th (%PSM)	-53	-56	92	96	-75	-78
SEMG Th (%PSM)	-31	-55	53	95	-50	-89

IM-EMG, SEMG, C and Th represent intra-muscular and surface EMG recording methods as well a CUSUM and Threshold-crossing analysis methods respectively. IN1, E and IN2 indicate early inhibition, excitation and late inhibition respectively. Note, as with the masseter experiments, latency and duration measurements in the first dorsal interosseous (FDI) experiment were the same with and without noise.

6.4. DISCUSSION

A new method (modified CUSUM) that allows automated determination of the existence, latency, duration and strength of reflexes from EMG traces has been discussed. The results show that the modified CUSUM method yields latency results that are earlier, and durations that are consistently longer, than those found using the threshold method. The results are free from time (phase) delays caused by traditional low-pass filtering (Abbink *et al.*, 1999b) or curve fitting (Takada *et al.*, 1995) the data, and are significantly easier to perform than complex calculations such as wavelets (Panagiotacopulos *et al.*, 1998).

6.4.1. COMPARISON OF RESULTS WITH PREVIOUS STUDIES

Although it is not new to define the latency of a reflex from the time the EMG crosses the pre-stimulus mean (Evans *et al.*, 1989) most previous reflex studies have used the amplitude of the EMG (Thompson & McKinley, 1995; Abbink *et al.*, 1998b; Harrison *et al.*, 2000) as opposed to area in order to determine if, and when, a significant event has occurred. Calculations based on the area of the EMG are superior to measuring amplitude, as the CUSUM will detect subtle but consistent changes in the EMG not attainable through amplitude methods. Area is also the best indicator of the level of effort represented by the EMG signal. There is also empirical evidence that such integrals are closely correlated with the frequency modulation of individual motoneurons and consequently the net synaptic depolarisation that impinges on the motoneuron pools (Loeb & Gans, 1986).

Results from the masseter muscle inhibitory reflex response correspond well with other findings of 20ms latency for both horizontal loading (Yang, 1999) and unloading (Türker & Jenkins, 2000) however, the reflex duration as determined by the CUSUM method was found to be longer than reported elsewhere (Reynolds & Thexton, 1978). The most likely cause of this discrepancy is the method used to calculate the duration in this paper, which takes into account the entire reflex, not just the portion above the threshold level. Using the portion of the signal that is above the threshold for calculations of duration will drastically underestimate reflexes, especially subtle ones.

Although the shape of the reflexes elicited from the FDI were similar to that found in the literature the large late inhibitory reflex in the FDI was not studied and an early excitation was identified before the early inhibition (Evans *et al.*, 1989). While it is true that the late inhibition did last longer

than the reaction time, and hence may not be totally a reflex, there were a number of reasons why it was included in this paper but not in the literature. Firstly, the onset of the inhibition occurs before the reaction time and hence is, at least initially, a reflex. The rate of stimulation used in previous studies (up to 9Hz) (Evans *et al.*, 1989; Harrison *et al.*, 2000) was too fast to permit the peri-stimulus time required to fully define the reflex. Previous studies also used much lower levels of stimulation (about 2T), thus lower threshold fibres may have been stimulated. Moreover, while it is proposed that secondary and later EMG responses are likely to be, at least in part, artefactual (Moore *et al.*, 1970; Türker & Cheng, 1994), the inhibition phase analysed here is much larger than any of the preceding responses and therefore does represent a different reflex pathway. Finally, the subject was asked to maintain the EMG level at 20% MVC hence, if anything, this inhibitory reflex was reduced by maintaining a high level of motoneuron excitation (Türker, 1988).

6.4.2. DETECTION OF A SIGNIFICANT EVENT

Little consensus exists in literature regarding methods for determining a significant event from the EMG (Hodges & Bui, 1996). For a comparison to be drawn between different muscles, experimental conditions, subjects' and/or subject groups, it is critical that there is accuracy and constancy in the determination of a significant event. Computer-based automatic analysis of EMG records enables minimization of variation between measurements of different individuals by incorporating common decision criteria among multiple operators. "In addition, digital signal processing rejects errors associated with scaling of time and amplitude which are inherent in visual inspection of an analogue signal" (Takada *et al.*, 1995). When analysing EMG records it is essential to define and maintain a set of clear, accurate and repeatable criteria for the detection of significant events. If the methods for determining a reflex from EMG traces is purely subjective (i.e. based solely on observer judgement) then the results will also be subjective. If, on the other hand, the analysis method is completely definable then the results will not be subjective and researches that wish to validate the work may do so with confidence that the same methods are being used.

In the new method, a significant change is no longer defined as one that causes the CUSUM to cross the error box, as was the case in an earlier publication (Türker *et al.*, 1997). A significant event is now described as a vertical displacement of the CUSUM that is greater than the amplitude of the error box without a turning point (EMG equal to 1) in between (Figure 6.1).

We suggest that this new approach minimizes erroneous recognition of significant events from CUSUM traces.

6.4.3. NOISE

EMG recordings can be contaminated by many different sources of electrical noise (Basmanjian & De Luca, 1985; Loeb & Gans, 1986) and despite good experimental design, and averaging the EMG around the stimulus to increase the signal to noise ratio (Heckman *et al.*, 1995), some noise will still remain. Assuming good averaging parameters and interference that is not time-locked to the stimulus, then the noise is approximately a DC value added to the rectified averaged EMG. This will have a number of detrimental effects. Firstly it will increase the pre-stimulus average value making it appear that the level of muscle activation was higher. Secondly it will act as the lowest limit for the EMG signal making inhibition appear smaller (this is especially apparent at low muscle contraction levels). Finally, due to the increased pre-stimulus mean, any excitations will be underestimated. The identification and removal of this constant offset is necessary if the strengths of reflexes are to be accurately reported.

Approximating and removing this constant DC value can solve this problem. By performing readings while there is no active muscle contraction, the minimum DC value can be approximated and then subtracted from the rectified averaged traces to give a reading almost entirely free of white noise and mains power interference. However, caution is advised when finding the noise level, as incorrect values will have a large influence on the calculated strength of an SEMG reflex. On the other hand the IM-EMG strength should not be affected as much due to the increased signal-to-noise ratio in IM-EMG recordings. The best way to determine the noise level for a particular experiment is to perform a number of trials in which there is no muscle activity. These trials should then be averaged together and the minimum value used as the noise level. Due to variations in electrode quality and placement as well as small changes in the electronics (such as amplifier noise which is dependant on temperature) the noise level found for one person on one occasion should not be used for a second experiment. It is also important to note that the noise removal procedure identified above will not remove cross talk from the EMG recording. An SEMG signal detected above a muscle and having a peak-to-peak amplitude of up to 16.8% of a signal detected above a neighbouring muscle may be due to cross talk rather than to activity of the muscle below the electrode (De Luca & Merletti, 1988). Thus, in cases where cross talk may be a significant problem, IM-EMG

recordings are recommended above SEMG recordings as the amount of cross talk is reduced (Türker, 1993).

It has been claimed that even during rest most skeletal muscles are still partially active (Yemm, 1976) therefore removing the noise value as defined in this text may also remove some of the EMG. This was found not to be the case under the conditions tested for two main reasons. Firstly, the minimum pre-stimulus value found in the noise trials was used hence reducing the possibility of removing part of the EMG signal. Secondly, spectral analysis of the noise trials (Figure 6.2) showed a large number of spikes at specific frequencies (i.e. multiples of 50Hz) that did not correspond with an EMG signal (likely interference from mains power), and a uniform frequency distribution at very low power levels (-45dB) indicative of white noise. This indicated that there was no significant EMG contribution to the defined noise values, and thus no detectable EMG activity during rest in the muscles used.

The method of noise removal described in this paper will not alter the points on the EMG relative to each other; rather it will move them equally relative to the zero level. Hence calculations such as reflex latency and duration that rely on signal characteristics relative to the signal itself (i.e. the pre-stimulus mean or standard deviation) will not be affected. On the other hand, calculations such as strength that rely on the signal's absolute value will be affected. In such cases the values determined once noise removal has been performed will be a more accurate representation of the true value.

6.4.4. IM-EMG VERSUS SEMG

While there is no doubt that IM-EMG has superior recording characteristics (Türker, 1993) most reflex studies use SEMG (Ottenhoff *et al.*, 1992a; Takada *et al.*, 1995; Louca *et al.*, 1998). Reasons for this may include the fact that IM-EMG recordings give a more localised view, which is not desirable if the whole muscle response is required, intra-muscular electrodes are both more expensive and invasive, and IM-EMG recordings require faster and more expensive equipment due to the higher frequency characteristics inherent in the signal. The main benefit of IM-EMG is that it is not subject to as much artefact (cross-talk, stimulus and movement) and has a greater signal-to-noise ratio than the SEMG (Türker, 1993).

Despite these differences, the occurrence rate, latency and duration of the reflexes evoked in these experiments were similar (or the same) in both IM-EMG and SEMG recordings; this is consistent with other findings (Wittek *et*

al., 2001). Although there was a large discrepancy in the strengths of the reflexes using the two recording methods, the differences were reduced when noise removal was employed. This suggests that, provided an appropriate noise correction is used, results from SEMG recordings may be as accurate as those found using IM-EMG.

6.4.5. REFLEX STRENGTH

On average, the percentage change in the area of the EMG represents the effective strength of the synapses activated by the stimulus. The term 'effective' should be stressed to indicate that predictions of the synaptic connection on the motoneurons are indirect and that the responses observed in the EMG depend not only upon the strength of the synaptic connection but the pre-stimulus firing rate of the active motor units underlying various activity levels in the EMG (Türker & Miles, 1989). A strong stimulus will probably affect all the active motor units and change their firing rates (Türker & Cheng, 1994). However, the level of change in the firing rate of the motor units will depend upon their pre-stimulus firing frequency (Miles & Türker, 1986). Consequently it is expected from the frequency principle that the duration (and therefore CUSUM strength) of the inhibitory reflex will be reduced with an increase in the background level of firing (Türker, 1988). This stresses the importance not only of noting the percentage reflex change in the CUSUM of the rectified averaged EMG but also the total duration of the reflex in order to envisage its impact on external forces.

6.4.6. EFFECT OF STIMULUS NUMBER AND PRE-STIMULUS TIME

Extended pre-stimulus times have the problem that, if the time between stimuli is short, a response to the previous stimulus may not have finished before the next pre-stimulus period commences, thus yielding erroneous data. Large pre-stimulus times may also overestimate the EMG variability and hence identifying subtle reflexes will be difficult. On the other hand, pre-stimulus times that are too short underestimate the EMG variability and can classify normal fluctuations in the EMG as reflexes. It is recommended that the pre-stimulus time used be the same as the post-stimulus time needed to ensure all reflex events are included in the record. It is also not recommended to use pre-stimulus times less than 100ms, as the low frequency components of the rectified averaged EMG may not be accurately estimated.

It is known that increasing the number of stimuli averaged together increases the signal-to-noise ratio (Heckman *et al.*, 1995). A low number of averaged traces can lead to noisy EMG records and make the identification of subtle reflexes difficult. It is recommended that a minimum of 50 stimuli be used while 100 or more is suggested if such a number is feasible. However large reflexes such as H-reflexes or motor evoked potentials produce changes in the EMG that are clearly identifiable at much lower stimulus numbers.

6.4.7. EFFECT OF FILTERING

While some filtering is desirable to reduce the variation and non-genuine zero-crossings produced by noise and/or synchronous discharges of motor units, excessive filtering can cause large errors in the temporal calculations as depicted in Figure 6.4. To diminish the possibility of errors produced by excessive filtering, the averaged rectified EMG traces should not be filtered with cut-off frequencies less than 200Hz. The cut-off frequency used for the low-pass filtering of the averaged rectified IM-EMG signal (500Hz) was higher than that selected for SEMG (227Hz) due to the higher frequency characteristics in the signal. While using a reduced cut-off frequency for IM-EMG traces resulted in a smoother signal (Figure 6.4) there was little, if any, change in the CUSUM or the resulting reflex characteristics indicating that a range of filtering levels on the averaged rectified trace (500-200Hz) is acceptable.

6.4.8. EXPERIMENTAL APPLICATIONS

The modified CUSUM method described in the text can be used for any EMG reflex analysis where spike-triggered averaging is employed. Used in conjunction with the recommended filtering characteristics, pre-stimulus times and number of stimuli, as well as noise correction, it will provide reliable, reproducible and accurate results.

7. Response Of Human Jaw Muscles To Axial Stimulation Of An Incisor Tooth

The role of periodontal mechanoreceptors (PMRs) in the reflex control of jaw muscles has thus far been mainly derived from animal studies. To date the work that has been done on humans has been limited and confined mostly to orthogonal stimulation of the labial surface of the incisor teeth. The purpose of this study was to investigate the response of the masseter and digastric muscles in humans to controlled axial stimulation of the upper left central incisor, both before and during a local anaesthetic block of the PMRs.

Ten neurologically normal young adult females were tested, each on two separate occasions to confirm the reproducibility of the results. It was found that the reflex response in the masseter was modulated by the rate of rise of the stimulus used and, to a lesser degree, the level of background muscle activity. There was little detectable change in the activity of the digastric muscle under the conditions tested and what was found could be attributed to cross talk from the masseter. The reflex responses obtained were significantly different between subjects, however retesting the same subject on a different occasion yielded similar results. The results indicate that the most common response of the masseter muscle to brisk axial stimulation of the incisor is a reflex inhibition at 20ms, followed by a late excitation at 44ms. However, it is possible that this late excitation could be due to delayed action potentials and hence be artefactual. As the application of a local anaesthetic block removed or significantly reduced both of these responses, it was concluded that they originated from the PMRs. Unlike during orthogonal stimulation, slowly rising stimuli did not produce any excitatory reflex activity. This indicated a difference in jaw reflexes to forces applied in different directions, possibly due to the activation of different receptor types when stimulating the tooth in either the orthogonal or axial directions.

This chapter is an edited version of the manuscript “Response Of Human Jaw Muscles To Axial Stimulation Of The Incisor” by R.S.A. Brinkworth, K.S. Türker and A.W. Savundra, which has been published in the Journal of Physiology 547(1): 233-245 (2003).

7.1. INTRODUCTION

Experiments in animals indicate that a large amount of feedback for jaw closing muscles comes from periodontal mechanoreceptors (PMRs) (Morimoto *et al.*, 1989; Morimoto & Nagashima, 1989). However, due primarily to limitations in methods, this is yet to be confirmed in humans. Previous studies on humans have involved orthogonal stimulation of teeth, i.e. mechanically stimulating the labial surface of the incisor (van der Glas *et al.*, 1985; Brodin *et al.*, 1993b). The current study differs from previous studies as it involves a novel stimulation technique, axial stimulation. The main aim of the present study was to investigate the contribution of PMRs to human masseter and digastric muscles using axial stimulation. To this end, changes in the surface electromyogram (SEMG) of masseter and digastric muscles to a number of different mechanical stimulus profiles were investigated. An additional aim was to test the reproducibility of the results by retesting the subjects on a second occasion.

During mastication the forces that are applied to the teeth displace them in their sockets, thus stimulating PMRs. The nature of the resulting feedback is unclear due to the difficulty in stimulating the PMRs without activating other receptors in the peri-oral region at the same time (Sato *et al.*, 1994). Mechanical stimuli activate a number of receptors that may have different synaptic connections to the motoneuronal pool. Forces applied to a tooth can stimulate receptors in the area of application, i.e. the mechanoreceptors in the gingiva and the periodontal space (the PMRs). However, tooth stimulation can also activate vibration, stretch and position sensitive receptors in and around the jaws (reviewed in Lund *et al.*, 1983), and the vibration sensitive receptors in the inner ear (van Steenberghe *et al.*, 1981).

The easiest way to mechanically stimulate teeth is by means of tapping the labial surface of an incisor with a probe (orthogonal stimulation). This approach has been tried by a number of researches with most studies showing tooth tapping in humans produces jaw reflexes that are multi-phasic and comprise a complex sequence of short- and long-latency inhibitory and excitatory reflexes (van der Glas *et al.*, 1985; Louca *et al.*, 1996). Although many of the secondary and later reflexes may be partially, or fully, artefactual (Moore *et al.*, 1970; Türker & Cheng, 1994; Türker & Powers, 1999).

One of the problems with many of the experimental mechanical stimuli used is that the taps contain very high frequency components. It is known from both animal (Linden & Millar, 1988a) and human (Trulsson & Johansson, 1994) studies that PMRs are sensitive to the total force as well as the rate of force application. To overcome the problem of high frequency components in

the stimuli the concept of preload was introduced (Brodin *et al.*, 1993b). This involves holding the probe against the tooth at a low force level (0.5-1N) in between stimuli. By using a preload, the high frequency components that were contained in the beginning of the stimuli, when the probe took up the slack in the stimulus delivery system, can be reduced. It was therefore put forward that an exact stimulus profile could only be applied to a tooth if a preload was used (Türker *et al.*, 1997).

To identify the reflex responses that originate from PMRs, a number of studies have utilised a local anaesthetic (LA) block (Sessle & Schmitt, 1972; Türker & Jenkins, 2000). These studies illustrated that the PMRs are responsible for the majority of the reflex response of the human jaw muscles to stimulation of the incisor teeth.

Since it is known that PMRs code the direction of force applied to the teeth (Trulsson *et al.*, 1992; Dessem, 1995) it is likely that the direction of force application is a contributing factor in the reflex response of the human jaw muscles. Hence experiments that study human jaw reflexes due to axial stimulation are likely to provide a more accurate picture of what happens during normal incising activity than those utilising orthogonal stimulation. However, this experiment was not intended to directly compare axial and orthogonal stimulation of teeth, but rather provide the next logical step in experimental design, ensuring the direction of the mechanical stimulus is similar to that encountered during mastication.

7.2. METHODS

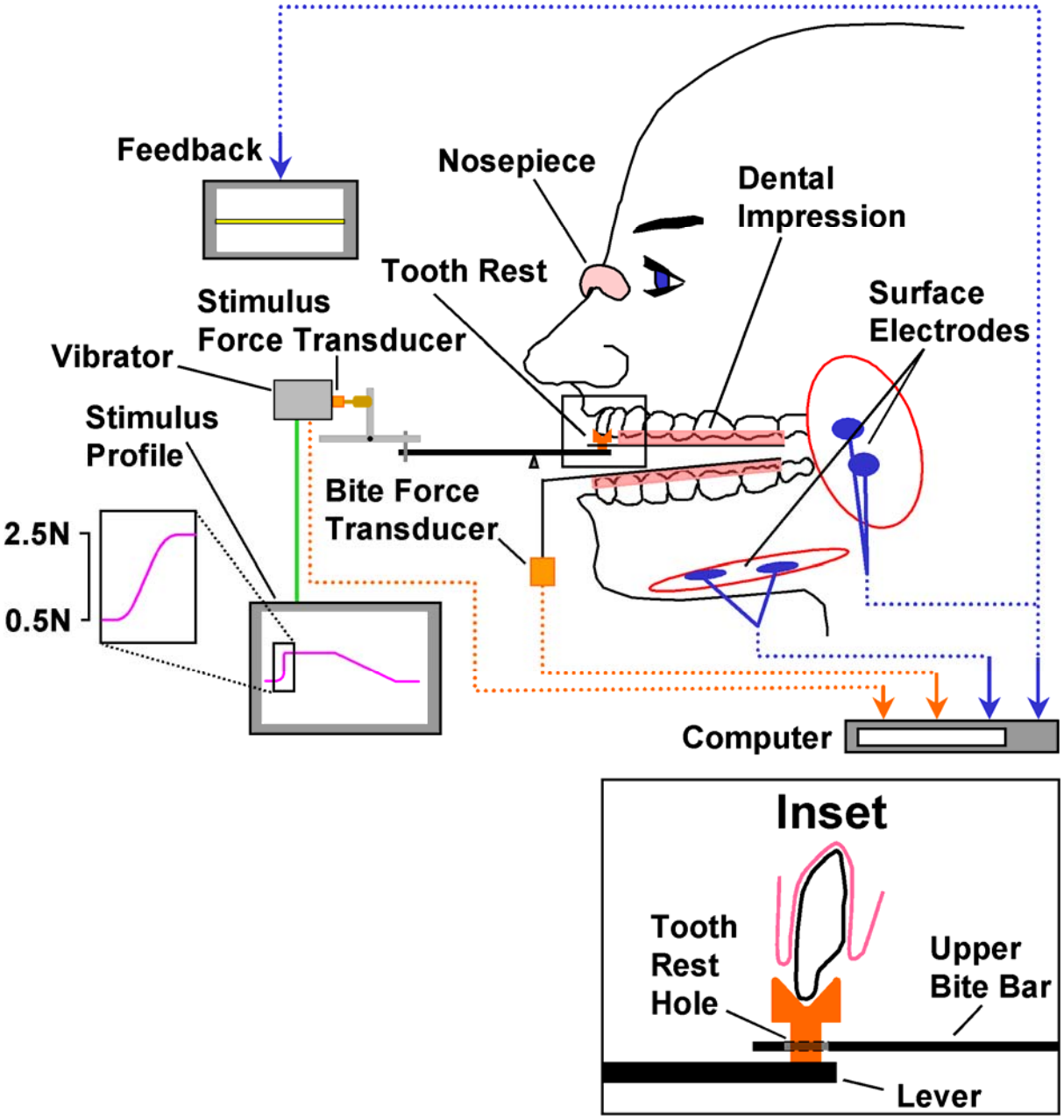
Written informed consent was obtained from ten adult female volunteers with healthy teeth and gums, and no history of orthodontic treatment or dysfunction. The age range of the subjects was 18 to 25 years. Each subject was tested twice, no less than 1 week or more than 8 weeks apart. The experiments were approved by the Human Ethics Committee of The University of Adelaide and all procedures used conformed with the Declaration of Helsinki.

7.2.1. PROTOCOL

Subjects were instructed to bite into impression material (Formasil II, Heraeus Kulzer-Wehrheim) mounted on two bite bars with their upper left central incisor sitting on a tooth rest; the mean separation distance of the bars was set to 13mm. A tooth stimulator, shown in Figure 7.1, was used to stimulate the upper left central incisor. Movement of the subject's head was minimized by the use of a fixed nosepiece, which also counteracted against the axial forces applied to the tooth. The dental impression was cut away from around the incisor so it could be contacted and stimulated by a small brass tooth rest (Figure 7.1 inset). The impression of the subjects' upper teeth was taken in such a way to direct the force of stimulation along the long axis of the tooth. This involved slightly tilting the head forward while taking the impression of the upper teeth. During the experiment the subject was required to lean into the nosepiece, this reduced this possibility of the head tilting backwards. In addition, the tooth rest was not fixed to the lever or upper bite plate allowing for small amounts of subject movement while ensuring stimulation along the long axis of the tooth.

A computer generated force profile was used as the input signal to a small mechanical vibrator connected to the tooth via a lever system and tooth rest. Since the sound of a tap is sufficient to alter the reflex response of masticatory muscles (van der Glas *et al.*, 1988; Sato *et al.*, 1994), the subjects wore headphones through which white noise was played during the experiment at approximately 80dB in order to mask acoustic vibrations. This level of noise was sufficient to mask speech at normal volume.

Figure 7.1: Experimental Set Up – Incisal Stimulation



The subject bite into impression material mounted on two bite bars with their upper left central incisor resting on a tooth rest that fit into a hole in the upper bite bar (inset). Movement of the subject was further minimized by the use of a fixed nosepiece. The nosepiece also counteracted against the axial forces applied to the tooth. A computer then produced the desired force profile randomly between one and a half and three seconds. Two force transducers, one located in the motor arm and the other below the lower bite bar, picked up the stimulus force applied to the tooth and the total force generated by the jaw respectively. Both the masseter and digastric EMG activity were recorded as well as the outputs of both force transducers; in addition, the SEMG of the masseter was fed into a filter box and used for online feedback.

Surface electrodes were placed on the skin overlying the left masseter and digastric muscles in order to detect the SEMG activity. The EMG was amplified (3000x) and band-pass filtered (cut off frequencies of 5 and 1000Hz) before recording. The subject was given online feedback showing their current level of muscle activity so it could be maintained at a specified level throughout the trial. For this purpose the masseter SEMG was full wave rectified and low pass filtered (cut off frequency 0.1Hz) then presented to the subject on an oscilloscope screen. Grounding of the subject was achieved by the use of a lip-clip electrode (Türker *et al.*, 1988). To ensure correct force application, the stimulus force was recorded via a transducer located on the arm of the vibrator. A bite force transducer was connected to the lower bite bar (see Figure 7.1) to measure the forces generated by the subject's jaw musculature. This was done to find the overall response of the masticatory muscles to the stimulus as it has been shown that the EMG of one or two jaw muscles may not indicative of the overall bite force, which is the net response of all jaw muscles (Yang & Türker, 1999). All data channels were sampled at 12-bits and 2kHz using a specially designed (LabVIEW® National Instruments) computer program detailed in Appendix 1.

It is necessary to apply the stimuli at random intervals so the subject cannot predict when they will occur. Hence there will be little, if any, EMG change due to anticipation of the stimulus (Ottenhoff *et al.*, 1992b). An inter-stimulus interval between 1.5 and 3 seconds was used. Throughout the experiment three bite levels were used: 5%, 10% and 20% of maximum voluntary contraction (MVC). These contraction levels were selected as most people can sustain them for the expected duration of the trials, and they represented the lower limits of muscle contraction levels likely to be used during mastication (Anderson, 1956a & b; Slagter *et al.*, 1992). The stimulus profile used was a slow sinusoidal as described in the literature (Türker *et al.*, 1997). This, along with a preload of approximately 0.5N, ensured that high frequency components in the stimuli were minimized. These parameters maximized the possibility of obtaining excitatory reflexes while still containing the required parameters for inhibitory reflexes as established for orthogonal stimulation (Türker *et al.*, 1997). Three different stimulus rise times were used: fast (12ms), medium (20ms) and slow (90ms). A pre-load of 0.5N and a maximum delivered force of 2N were selected as the non-varying stimulus parameters.

Once all conditions had been performed, approximately 4mL of LA (Xylocaine™ - lignocanine hydrochloride with adrenaline 1:80,000) was administered to the gingival and palatal region in order to block the PMRs from all upper front teeth (canine to canine). Once the LA block was in place, the experimental procedure was repeated. The success of the LA was insured

when the subject could no longer feel the stimulus around the teeth; this took approximately 15 minutes to achieve. Some subjects reported feeling the fast stimuli as a faint vibration at the base of the skull. In total, nine different mechanical conditions were performed twice on a given day, once before LA and then once during LA block. The subjects then came back on a separate occasion to undertake the experiment a second time.

It is known that the teeth are displaced in their sockets following mechanical stimulation and successive taps do not permit them to return to their original position. In fact, the first few taps displace the tooth by different amounts only reaching a relatively stable position after a number of stimuli (Moxham & Berkovitz, 1995). In order to overcome this change, the first 10 taps of each trial were not included in the analysis so a steady state response could be found. Each trial lasted approximately two and a half minutes, and subjects were asked to stay on the bite bars for three consecutive trials before coming off to rest.

7.2.2. ANALYSIS

During the off-line analyses, each stimulus was analysed to ensure that it fell within defined parameters. If the preload or tap level deviated more than 0.4N from the desired value, it constituted a significant change from the desired stimulus profile and was removed from further analysis.

The digitised EMG signals were zero-phase band-pass filtered (5-500Hz) and full wave rectified. A defined time period from around each stimulus was then extracted (pre- and post-trigger time of 125ms) then averaged together ($n \approx 50$) and finally zero-phase low-pass filtered (200Hz). EMG normalisation was achieved by dividing the averaged trace by the mean pre-stimulus value; this had the effect of making the pre-stimulus average level (k) equal to one. Following this, cumulative sums (CUSUMs) of the normalised averaged EMG data were constructed (Ellaway, 1978) from which a dedicated Excel® (Microsoft) macro extracted various reflex characteristics such as latency, duration, strength and occurrence. Details of which are given in Chapter 6 and briefly summarised below.

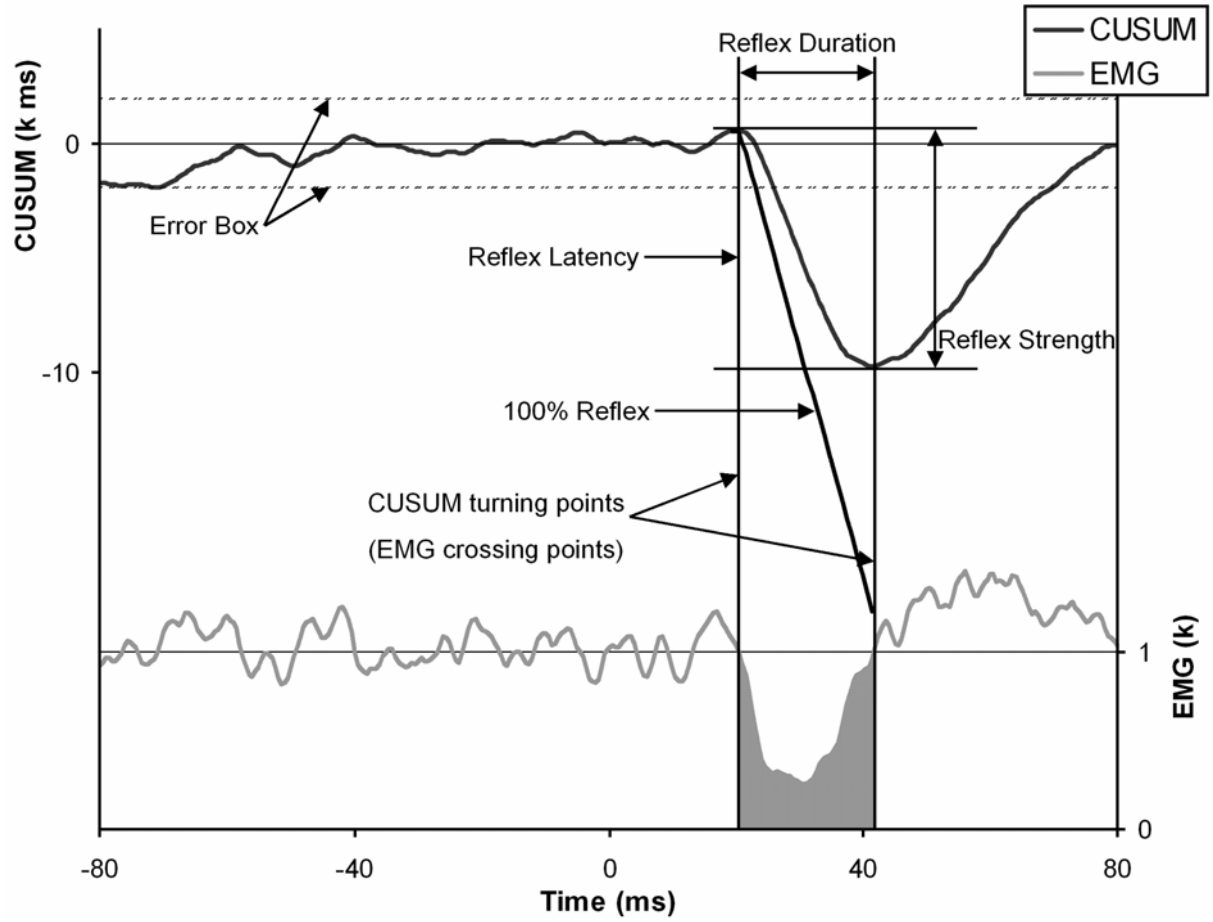
Figure 7.2 illustrates the measured reflex characteristics. A symmetrical error box was constructed that took into account the largest pre-stimulus CUSUM deviation in either direction (Türker *et al.*, 1997). If the post-stimulus CUSUM deviated by an amount greater than the error box, without a turning point (i.e. EMG crossing the pre-stimulus mean) in between, then a reflex was recorded. The latency and end of a reflex were defined as the

turning points at the start and end of a reflex respectively. The duration of a reflex was defined as the difference between the latency and the end. Since the EMG was normalised, the largest possible inhibition corresponded to a CUSUM slope of -1 (EMG=0), hence all reflex strengths were calculated as a percentage of this theoretical maximum. Excitations were given a positive percentage while inhibitions were defined as negative.

Averaged bite force records were assembled in the same way as EMG records, but normalisation comprised of subtracting the average pre-stimulus bite level from the trace to observe the change in bite level, hence making it possible to compare the changes between trials with different pre-stimulus averages. The bite force was averaged then passed through a 9-bin moving average filter centred on the current value (equivalent to zero-phase low-pass filtering at 111Hz). Figure 7.3 illustrates the measured reflex characteristics from the bite force record.

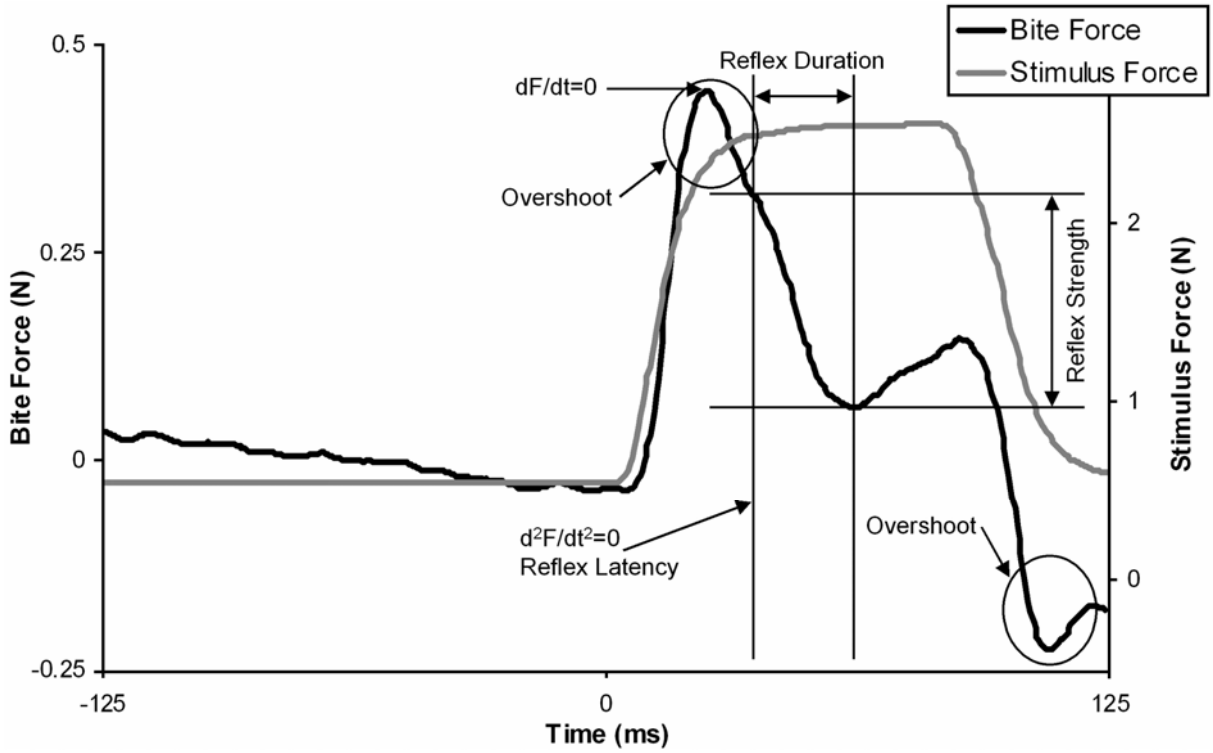
A change in the bite force was only classified as a reflex if its strength was greater than twice the maximum pre-stimulus variation (error box). As with the EMG latency, the bite force latency was defined as the point where the bite force started to turn (derivative equals zero). The end of the reflex was defined as the next turning point and the duration as the difference between the latency and the end. The strength of the reflex was the vertical distance (amplitude) between the latency and the end. The main problem encountered in the bite force analysis was the overshoot that was present in a number of the records. When the stimulus was applied to the tooth, a small movement of the head was induced causing the bite force record to follow the shape of the stimulus superimposed upon it. However, in many of the fast stimulus conditions an overshoot was produced at the end of the rising and falling phases of the stimulus. Therefore, the first turning point in the bite force following the stimulus corresponded to the peak of the overshoot, not the start of a reflex. Hence, if calculations showed that the latency of the reflex was the first turning point after the rising edge of the stimulus then the next time the slope changed (second derivative equals zero) was taken as the latency of the reflex. Only by using the second derivative could the reflex latency be established when it occurred close to the end of the rising edge of the stimulus. To ensure that this analysis method yielded accurate results the following precaution was taken: if the latency of a reflex in the bite force recording did not occur between 7 and 25ms after an associated inhibition in the EMG, then an exception was generated and the records closely scrutinized. Although this was rare, the most common reason for this was excessive variability in the bite force recordings. Under such circumstances the bite force reflex was removed from further analysis.

Figure 7.2: EMG Reflex Parameters



Characteristics of the masseteric inhibitory reflex (n=50) elicited from axial stimulation of the upper left central incisor and recorded from the left masseter of a subject biting at 5% maximum voluntary contraction before the application of local anaesthetic; the stimulus was delivered at time 0. The reflex latency was determined from the CUSUM turning point (equivalent to the point the EMG crossed the pre-stimulus mean). The strength was the ratio of the CUSUM reflex deviation to the maximum possible reflex (corresponds to the EMG area). The reflex duration was defined as the time until the next CUSUM turning point. Excitatory reflexes were measured in the same way as inhibitions (time the EMG crosses the pre-stimulus mean and the area between the EMG and the pre-stimulus mean), the only difference being that excitations occurred above the pre-stimulus mean while inhibitions were below.

Figure 7.3: Bite Force Reflex Parameters



Characteristics of the reflex seen in the bite force record elicited from axial stimulation of the upper left central incisor and recorded from the lower jaw of a subject contracting at 20% MVC before the application of local anaesthetic (n=50). The average pre-stimulus bite force was subtracted to clearly illustrate the change. Both the rising and falling edges of the stimulus can be seen in the bite force as well as the overshoots after both the rising and falling edges of the stimulus. The first turning point (first derivative, $dF/dt=0$) after the stimulus corresponded to the peak of an overshoot, not the start of a reflex, since it occurred before, or directly after, the corresponding EMG change. The reflex latency was determined from the inflection after the first turning point (second derivative, $d^2F/dt^2=0$) this is likely to correspond to the genuine latency of the bite force reflex since it occurred approximately 15ms after the reflex inhibition in the EMG. The reflex duration was defined as the time between the latency and the next turning point. The reflex strength was the force change between the start and end of the reflex.

7.2.2.1. STATISTICS

To determine if the values found from the day 1 and day 2 recordings were different, paired t-tests were used. If the difference was not significant then the data were grouped together before further analyses were performed. In this way only data whose populations were not significantly different were pooled.

To determine if the stimulus parameters had any effect on the recorded values, a univariate analysis of variance was performed. The model searched for direct as well as 2-way and 3-way interactions between the three stimulus parameters (bite level, stimulus rate and local anaesthetic). In addition, a factor that indicated the subject was included to see if there were significant differences between subjects. If an interaction was significant then lower level interactions and direct influences were ignored.

Binary logistic regression was performed to ascertain if any of the stimulus parameters affected the number of reflex responses observed. Chi squared tests were used to discover if there was any difference between the number of reflex occurrences observed on day 1 compared to day 2.

For all tests, the level of significance was set to 5%. Results are given in the form mean ± one standard deviation; time calculations are given to the nearest 0.5ms, force values are given to 0.01N accuracy, while strength and frequency calculations are given to the closest percent.

7.3. RESULTS

The data were separated into the three reflex conditions as observed from the EMG records, early excitation (E1), inhibition (IN) and late excitation (E2); and one from the bite force record, change in bite force due to reflex (BR). The observed changes in the bite force record were only ever inhibitory.

Although the rate of occurrence was low, E1 was elicited at least once in 5 of the 10 subjects before LA and in all 10 subjects during LA. IN and E2 were found in all 10 subjects both before and during the application of LA, however the occurrence rate during LA was drastically reduced. BR was found in all subjects before LA and 7 out of the 10 during LA, however as with IN and E2, the occurrence rate during LA was reduced.

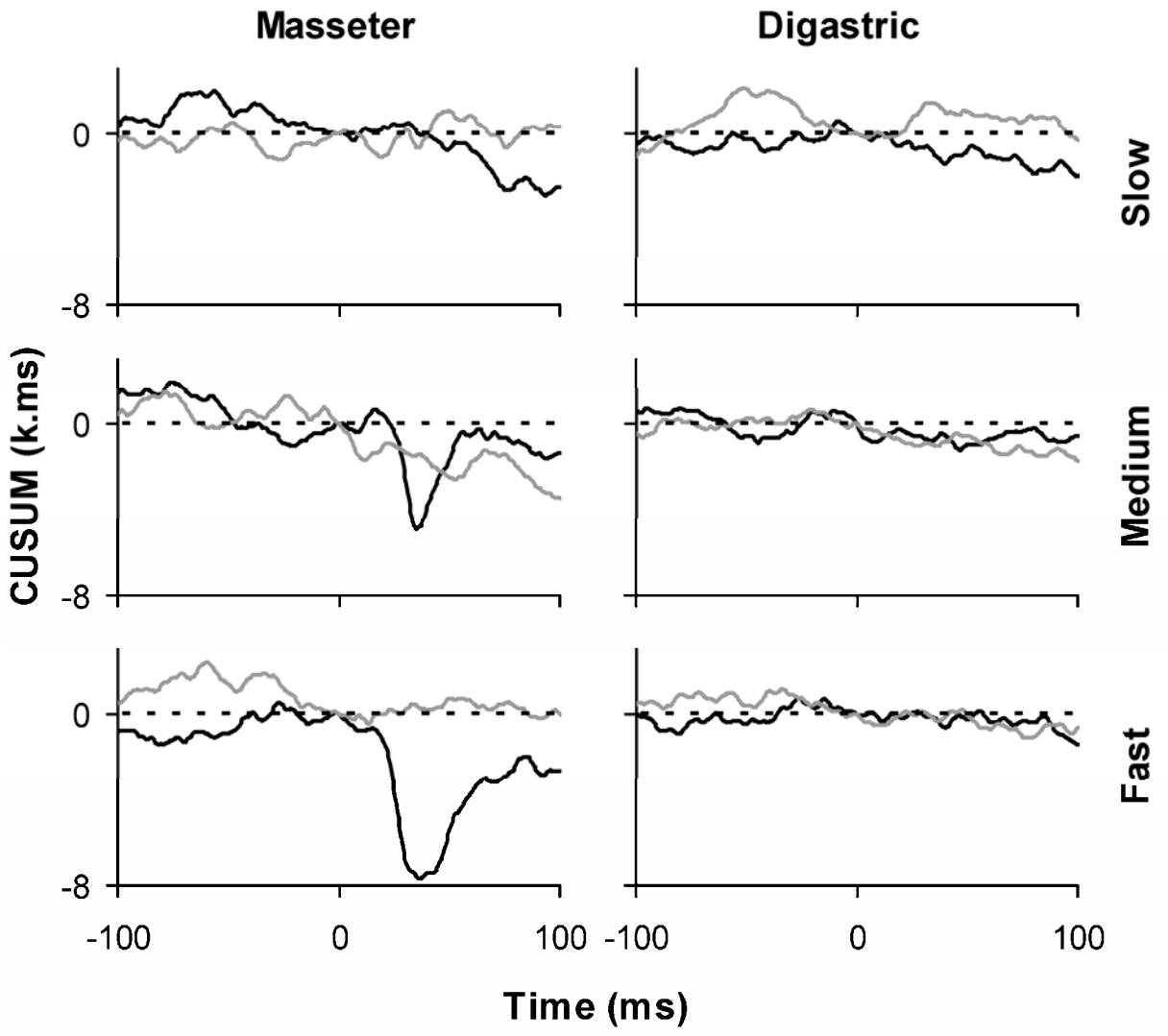
Figure 7.4 is an example of the results from one subject during trials conducted at 20% MVC. It illustrates the CUSUM of the masseter and digastric muscles to slow, medium and fast stimuli both before and during a LA block. The only time a masseteric response was elicited was during a medium or fast push before the LA block. There were no significant deflections in the CUSUMs associated with the digastric under any of the stimulus conditions.

Figure 7.5 shows the effect of LA on both the CUSUM of the masseter and the bite force recorded from one subject during a 10% MVC contraction of the masseter. As described above, the start of the stimulus profile was evident in the bite force records under both conditions. The large negative deflections in both the force and CUSUM traces are drastically diminished during LA indicating a reduction in the inhibitory reflex.

7.3.1. MASSETER

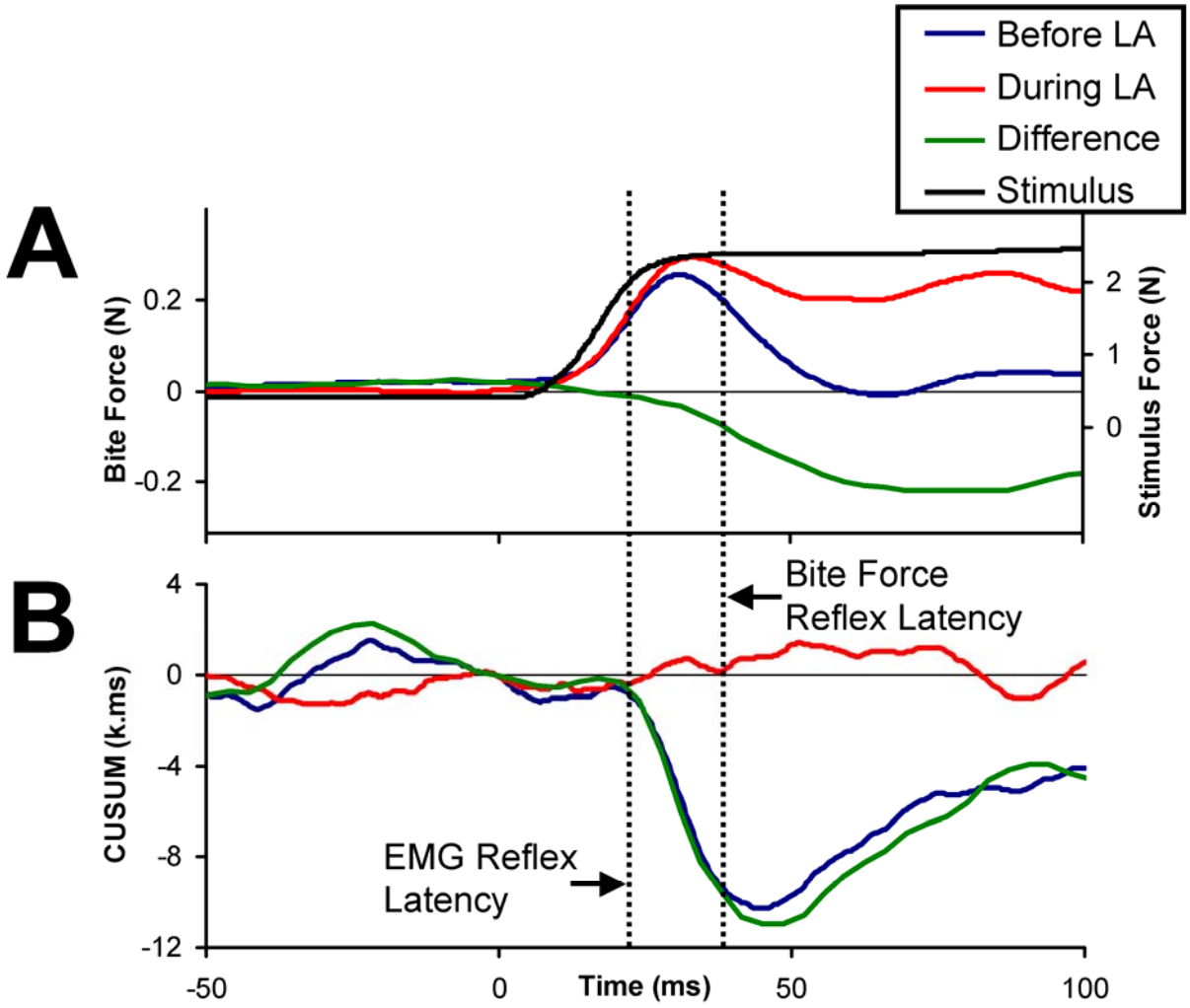
The effect that both stimulus rate and LA block had on the latency, strength and duration of the three reflex events seen in the masseter (E1, IN and E2) is illustrated in Figure 7.6 and described in detail in the following sections.

Figure 7.4: Jaw Muscles Response To Various Stimuli



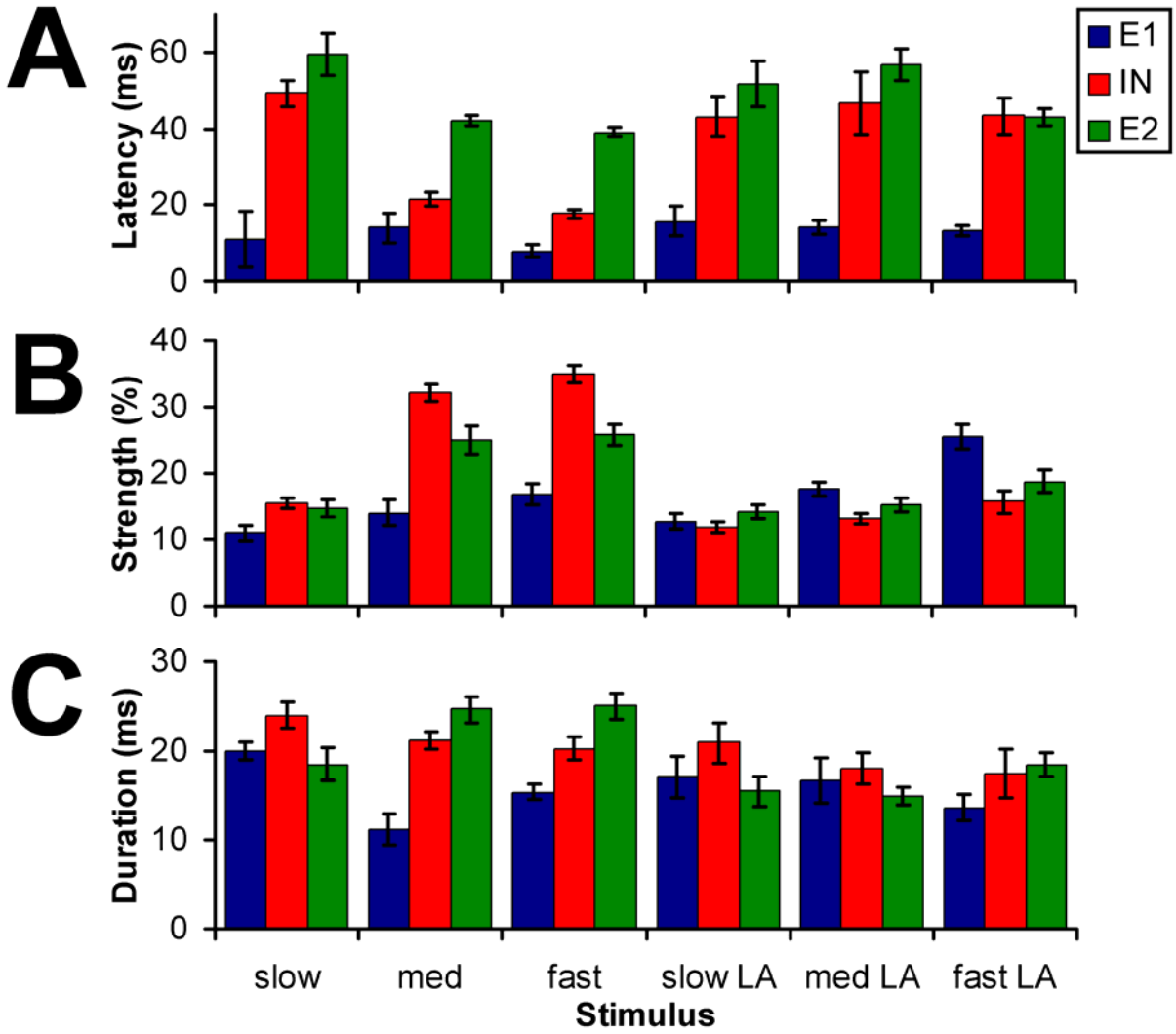
Cumulative sums (CUSUMs) of the rectified averaged (n=50) SEMG response of the masseter and digastric muscles from one subject to various rise time stimuli (fast: 12ms, medium: 20ms and slow: 90ms). All stimuli commenced at time 0. The EMG activity of the masseter was set at 20% MVC. Results from both before (dark trace) and during (light trace) the application of local anaesthetic are shown. The reflex activity seen in these records is an inhibition (downward movement of the CUSUM) followed by a late excitation (upwards movement of the CUSUM) before local anaesthetic in the masseter in response to a medium or fast push stimulus, no sizable digastric activity was present. This was indicative of most results.

Figure 7.5: Effect Of Anaesthetic – Incisal Stimulation



Affect of a local anaesthetic (LA) block on the bite force and CUSUM of the masseter muscle from one subject biting at 10% MVC. The stimulus force (medium stimulus rate) is included to illustrate its effect on the bite force record. The average pre-stimulus bite force was subtracted from the two bite force recordings so the change due to the activation of PMRs could be clearly seen. The difference traces (contribution due to activation of PMRs) were calculated by subtracting the result obtained during local anaesthetic from that found before local anaesthetic. All reflex activity in both the CUSUM and the bite force records were removed by the application of LA. The delay between the start of the inhibitory reflex and the reflex reduction in the bite force (18ms) is included for comparative purposes.

Figure 7.6: Reflex Characteristics In The Masseter Verses Stimulus



The effect of altering stimulus rise time on the (A) latency, (B) strength and (C) duration of reflexes seen in the masseter both before and during the application of a local anaesthetic block. E1, IN E2 and LA stand for early excitation, inhibition, late excitation and local anaesthetic respectively. Columns indicate the average value while the error bars show the standard error of the mean. Strength is given as an absolute value so inhibition can be easily compared to the excitations. The effects that the stimulus conditions had on the measured reflex parameters are discussed in the relevant sections in the text and shown in Table 7.1.

7.3.1.1. EARLY EXCITATION

A reflex was classified as E1 if it was excitatory and the latency was less than 25ms. Due to the low number of occurrences of this reflex (19 occurrences each for day 1 and day 2 from 180 records) it was not possible to perform meaningful paired t-tests or univariate analysis on the data. Hence none of the reflex statistics were combined, and no information on what stimulus conditions altered the reflex latency, strength or duration was available.

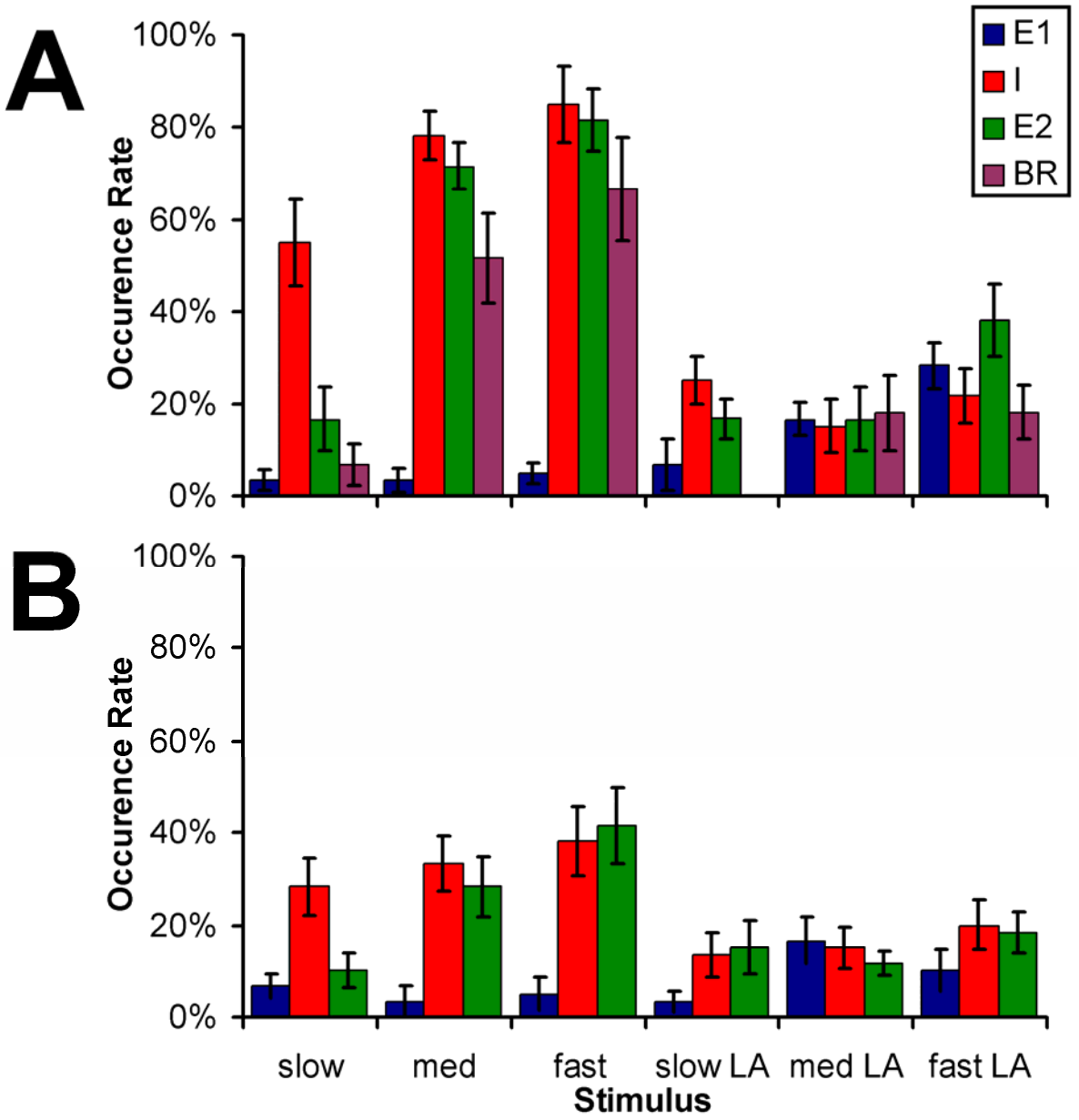
E1 had an average latency of 13 ± 6ms, an average strength of 22 ± 21% and an average duration of 16 ± 6.5ms as observed on day 1. The latency, strength and duration of E1 on day 2 were 13.5 ± 5.5ms, 18 ± 6% and 14 ± 5.5ms respectively. It should be noted however that the mean and standard deviations of the E1 strength of day 1 are skewed by the presence of two unusually large excitations (>90%) from one subject that were not observed either on day 2 or in any other subject.

There was no significant difference between day 1 and day 2 in the number of occurrences of E1. Both the application of LA ($p<0.001$) and the rate of stimulus application ($p<0.005$) affected the occurrence frequency of E1. No inter-subject differences were observed, however this might be due to the low number of E1 occurrences (n=38 from 360 trials). As illustrated in Figure 7.7A while the rate of E1 incidence was low before LA (regardless of the stimulus rate) during the application of LA increasing the stimulus rate increased the number of E1 reflexes observed. The best stimulus condition to use in order to elicit E1 was a fast stimulus during the application of a LA block, although even then it occurred in only 28% of the trials.

7.3.1.2. INHIBITION

Paired sample t-tests on the results of IN showed that there were no differences between day 1 and day 2 recordings for latency or strength of IN, but there was a significant difference between the durations of the reflexes evoked on the different days ($p<0.005$). Hence the data from the two days was combined for further latency and strength analysis while separate reflex duration analysis was performed for the day 1 and day 2 recordings. Table 7.1 shows that in addition to significant inter-subject differences for each of the measured statistics, all stimulus conditions had significant effects on at least one of the measured values.

Figure 7.7: Reflex Occurrence Rates – Incisal Stimulation



The effect of altering stimulus rise time on the occurrence of reflexes seen in the A) masseter and B) digastric both before and during a local anaesthetic block. E1, IN, E2, BR and LA stand for early excitation, inhibition, late excitation, reflex in bite force and local anaesthetic respectively. Columns indicate the average occurrence rate over the ten subjects while the error bars show the standard error of the population. Bite level is not shown as it had no affect on the detection rate of the reflexes. The inhibition was the most often evoked reflex in the masseter before LA and increasing the stimulus rate increased the detection rate for all three reflexes. Early excitation was the only masseteric reflex to increase in frequency during the local anaesthetic block. Many of the reflexes detected in the digastric, particularly before the application of LA, were simultaneously recorded in the masseter, indicating they were the result of cross talk.

Table 7.1: Results Of Analysis On Reflex Data – Incisal Stimulation

Reflex	Statistic	Effected By	Significance
Inhibition	Latency	Push*LA	†††
		Subject	††
	Strength	Push*LA	†††
		Subject	†††
	Duration day1	Bite	†
		Subject	†
	Duration day 2	Bite	††
		LA	††
		Subject	†††
Late Excitation	Latency day 1	Push*LA	†
	Latency day 2	Bite*Push*LA	†
		Subject	††
	Strength	LA	†††
		Push	†
		Subject	†††
	Duration	LA	††
		Subject	†
Bite Reflex	Latency	Push	†††
	Strength	LA	†
		Subject	†††
	Duration	Push*LA	†
		Subject	†††

Early excitation is not included in the table due to the low sample number. * indicates an interaction between two conditions, † represents $p<0.05$, †† represents $p<0.01$ and ††† represents $p<0.001$. Inhibition duration and late excitation latency have the day 1 and day 2 results separated as paired sample t-tests showed that they were significantly different. There were highly significant inter-subject differences for each statistic (except late excitation latency for day 1 and bite reflex latency) indicating that the choice of subject is a critical factor in the determination of each average value.

The number of observed inhibitions was not significantly different between day 1 and day 2. As with E1, the stimulus rise time ($p<0.05$) and LA ($p<0.001$) had significant affects on the number of occurrences of IN. Figure 7.7A shows the increase in occurrence of IN with the increase in the speed of the stimulus before LA and a large reduction in the number of reflexes observed during LA. Hence, the best stimulus condition to elicit an inhibitory reflex was a fast stimulus before LA. The bite level had no significant influence on the number of IN reflexes. Furthermore, there was a significant difference in the number of occurrences between subjects ($p<0.001$).

While the combination of stimulus rate and LA had a significant impact on the IN latency ($p<0.001$) there were two main groups. The longest latencies (and largest variability) of IN occurred during the application of LA or when a slow stimulus was used, in which case the average latency was 46.5 ± 20ms (reflex observed 29% of the time); on the other hand when a medium or fast stimulus was used before the application of LA the latency was 19.5 ± 11ms (incidence of reflex was 82%). Similar to the latency, the strength of IN was weakest during LA or when a slow stimulus was used, -14 ± 5%, compared to a medium or fast stimulus, -34 ± 10% ($p<0.001$). Although the bite level had a statistically significant effect on the duration of IN on day 1 ($p<0.05$), the difference between the three levels was only 1.5ms (19 ± 6.5ms for 5%, 18.5 ± 7.5ms for 10% and 21 ± 9.5ms for 20% MVC). However, for the data collected on the second experimental day, a bite level of 20% MVC resulted in a significantly reduced duration when compared to the other levels ($p<0.01$), 20 ± 8ms for 20% MVC versus 23.5 ± 8.5ms for 5 and 10% MVC. The application of LA also decreased the duration ($p<0.005$) with an average before LA of 24 ± 8.5ms (74% occurrence rate) and the average during LA being 16.5 ± 5.5ms (21% occurrence rate).

7.3.1.3. LATE EXCITATION

A reflex was classified as E2 if it was excitatory and the latency was longer than 25ms. There was no difference between day 1 and day 2 in the strength and duration of E2. However, a paired samples t-test showed there was a significant difference for the latency ($p<0.005$) measurements between day 1 (43.5 ± 14ms) and day 2 (44.5 ± 11ms).

There was no significant difference between the number of occurrences of E2 in day 1 compared to day 2. As with E1 and IN, the rate of stimulus delivery ($p<0.001$) and the application of LA ($p<0.001$) but not the bite level ($p>0.05$) affected the number of occurrences of the reflex. Figure 7.7A shows the increase in the number of occurrences of E2 with increasing speed of the

stimulus. Just like IN, the best stimulus condition to elicit an E2 response was a fast stimulus before LA.

As shown in Table 7.1 the statistical analysis on the latency of E2 shows a complex interaction between all three stimulus parameters ($p<0.05$). In general, the slower the stimulus rise time the longer the latency of the reflex while no real trend with LA and bite level was evident. The largest E2 reflexes were observed before LA ($p<0.001$), with an average strength of 24 ± 12% (57% incidence rate) or when the fast or medium stimulus was used ($p<0.05$), strength of 23 ± 12% (69% incidence rate). The smallest average reflex strength occurred during LA, 17 ± 7% (24% occurrence rate) and as a result of a slow stimulus, 14 ± 4% (observed 17% of the time). The E2 duration was longer before LA, 24.5 ± 9.5ms compared to during, 17 ± 5.5ms ($p<0.005$).

As well as to the standard statistical analysis, an additional test was performed to determine if there was any overlap between IN and E2. A univariate analysis of variance on the cessation time of IN showed that there was no difference regardless of whether there was a detectable E2 ($n=99$) following IN or not ($n=46$, $p>0.2$).

7.3.1.4. Reflex Change In Bite Force

BR was only ever inhibitory, the latency of which was significantly affected by the rate of stimulus application ($p<0.001$). The average latency of BR to a slow stimulus was 86.5 ± 6ms while the latencies evoked by the medium and fast stimuli were 37.5 ± 7.5ms and 32.5 ± 4.5ms respectively. The duration of BR was affected by a combination of stimulus rate and LA ($p<0.05$). The average duration due to the medium and fast stimuli before LA was 17.5 ± 6.5ms while the duration under LA, or to a slow stimulus was 21.5 ± 6.5ms. The average strength of BR underwent a significant decrease from 0.27 ± 0.20N before LA to 0.09 ± 0.05N during LA ($p<0.05$). As shown in Figure 7.7A the application of LA had a significant effect on the occurrence rate of BR ($p<0.001$). Prior to the LA, BR was rarely elicited by the slow stimulus (7%) but was observed 52% of the time in response to a medium stimulus and 67% to a fast stimulus. During LA, BR was not found in response to a slow stimulus and was elicited 18% of the time during the medium and fast stimuli.

7.3.2. DIGASTRIC

Figure 7.7B shows the reflex occurrence for each of the stimulation rates both before and during a LA block. No large reflexes or significant trends were seen in the analysis of the EMG recordings from the digastric muscle. The recordings made from the digastric muscle were of low amplitude and contained few recognisable reflexes. Only 5% of records from both day 1 and day 2 had a detectable E1 in the digastric before LA indicating little, if any, early excitation of the muscle. It was found that 78% of the IN reflexes recorded before the application of LA were also detected in the masseter with no significant difference between the paired latencies (digastric 26.5 ± 19ms, masseter 31 ± 21ms, $p>0.05$). A similar result was obtained for E2 with 83% of the detectable reflexes observed in the digastric EMG before LA also observed in the masseter. The application of LA increased the incidence of E1 ($p<0.005$), and decreased the incidence of IN ($p<0.001$) and E2 ($p<0.01$), however similar results were also seen in the masseter. Additionally, the reflex occurrence rates for all reflexes elicited during LA were 16%.

7.4. DISCUSSION

This is the first study to investigate the reflex response of jaw muscles to mechanical stimulation of the human incisor in the axial direction under controlled conditions. The results of the current study show that the main form of feedback to the masseter during fast and medium axial stimulation of the incisor is inhibition arising from the PMRs, while slow stimuli produce weak, or no, reflex activity. The results also suggest that there is no reflex response in the digastric muscle to any of the applied stimuli, and the changes seen in the SEMG are likely to be generated by cross talk from the masseter. These results further highlight the importance of PMRs for reflex control of the masticatory system.

The timing of the reflexes found in this study may be longer than the true latencies in the masticatory system, as there is an inherent delay in the method of stimulation. The time for the vibrator to move to 5% of the maximum stimulus value after receiving the start of the stimulus profile and the movement be picked-up by the strain gauges was on average 1ms slower than the theoretical time. This indicated a mechanical delay in the system. In addition, the stimulus profiles did not have sharp edges, hence not all receptors would have been stimulated at the same time, and the integration of multiple receptor inputs before a threshold could be reached would take time.

7.4.1. REFLEX ACTIVITY IN THE MASSETER MUSCLE

7.4.1.1. EARLY EXCITATION

The E1 response observed during axial stimulation has the same latency (13ms) as found during orthogonal tooth unloading experiments (Türker & Jenkins, 2000). While the latency was longer than the 7ms reported to exist during strong orthogonal tapping (Goldberg, 1971), the discrepancy is likely to be due to the higher rate of stimulation and lack of mechanical control in the previous study. Due to its latency, duration, excitatory nature, and the increased incidence during the LA block, this reflex is most likely to be due to the activation of muscle spindles in the jaw-closers that respond to stretch and vibration (Fukuyama *et al.*, 2000).

In the current experiment, conditions that were likely to activate the muscle spindles were deliberately avoided. If a stimulus stretches (Matthews, 1975) or vibrates (Orchardson & Sime, 1981) jaw muscles then the muscle

spindles will be activated and hence an E1 is likely to be generated. Fixing the head with tooth moulds and using a nosepiece to counter the axial force reduced both jaw movement and stretch in the current experiments. In addition, by using a stimulus with preload to reduce high frequency components in the stimulus, the possibility of stimulating the muscle spindles was further decreased. This was confirmed by the findings showing the occurrence of E1 was minimal and only increased when fast force rates (which have larger high frequency components) were used. The fact that E1 occurrences increased during LA application indicates that axial stimulation induces simultaneous activity in spindles and PMRs and that the latter part of the spindle response is obliterated by the simultaneously occurring stronger inhibitory response from the PMRs. During the LA block, PMR related inhibition was dramatically reduced hence exposing the early excitation. Therefore, during mastication these two pathways may be competing to regulate the activity in the jaw-closers.

7.4.1.2. INHIBITION

The most common, and strongest, reflex response seen in the masseter before LA was inhibition; the latency of which corresponded well with other findings of 20ms for both orthogonal loading (Yang & Türker, 1999) and unloading (Türker & Jenkins, 2000) but not with the 13ms latency found during orthogonal tapping (Türker *et al.*, 1994). The reason for the discrepancy between tapping and pushing is likely to be due to the mechanical delay, tapping is a much faster stimulus. The discrepancy between the duration of the reflex elicited in this study (21ms) and the one found during horizontal tapping (37ms) indicates that rate and/or direction of force application is an important factor in the inhibitory reflex duration. As with orthogonal experiments, the application of local anaesthetic drastically reduced both the frequency and strength of this reflex indicating that it is mainly generated by the activation of the PMRs (Brodin *et al.*, 1993b; Louca *et al.*, 1998).

By recording from the inferior alveolar nerve using tungsten electrodes, two different receptor types have been identified in humans (Trulsson *et al.*, 1992; Trulsson & Johansson, 1994). The 'saturating group' are more active in response to static forces less than 1N and loose their dynamic sensitivity above this value; and the 'non-saturating' group which displayed a linear response to forces up to 5N and keep their dynamic sensitivity even during the application of static forces above this level. These 'saturating' and 'non-saturating' receptors are likely to correspond to the slow and fast adapting receptors as seen in animal studies (reviewed in Linden, 1990).

If the properties of human PMRs follow the same pattern as seen in the animal studies, slow-rate-sensitive receptors close to the apex of the tooth root and fast-rate-sensitive receptors closer to the middle of the root, then axial stimulation will stimulate only a small number of the slow-rate-sensitive receptors, as most will undergo compression. Hence, if the axial stimulus profile contains only slowly rising force components then it is likely to activate only a small number of PMRs, as the slow-rate sensitive receptors will undergo compression and the fast-rate sensitive receptors will only be stimulated weakly, thus little or no reflex response will be seen. In contrast, when the stimulus profile has faster components, the fast-rate-sensitive PMRs will be stimulated and a reflex response is expected. Studies using orthogonal stimuli have shown that the slow-rate-sensitive receptors have an excitatory connection, while the fast-rate-sensitive receptors induce powerful inhibitory synaptic potentials (Brodin & Türker, 1994; Türker *et al.*, 1994; Türker *et al.*, 1997).

Although the application of LA reduced the occurrence of IN, some inhibitory activity was still observed (approximately 20% of the time). While a small amount of this activity may be due to the LA block wearing off, this effect would be minimal as any subjects who reported sensation to the tooth stimulation during the experiment had additional LA applied and the trial repeated. During the LA block, subjects reported no feeling in their teeth, only a vibration in the base of the skull in response to the fast stimulus. Therefore, the expected cause of any persistent reflex activity may not be contained within the periodontal space. Although an attempt was made to remove the contribution of the receptors in the ears by playing white noise to the subject these receptors, which are known to be inhibitory to jaw-closers (Sato *et al.*, 1994), may still have been partially activated by the vibration conducted through the jawbone. However it has been claimed that this influence should be much less than 20% (van der Glas *et al.*, 1988). During normal mastication, vibrations will only be significant during chewing of brittle food as soft foods will absorb much of the kinetic energy of the jaw. Therefore, this reflex may act as a constant 'break' on jaw-closer muscle activity during the chewing of brittle foods. Thus possibly facilitating other factors such as the elasticity (Yemm, 1976) and the length-tension (Mackenna & Türker, 1978) or velocity-tension relationship (van Willigen *et al.*, 1987) of the jaw muscles in stopping the jaws from forcefully coming together if an object yields suddenly (reviewed in Türker, 2002). It is also possible that PMRs remote to the area effected by the LA block were stimulated due to the mechanical coupling known to exist between teeth (Linden *et al.*, 1995; Trulsson & Johansson, 1996a; Johnsen & Trulsson, 2003).

7.4.1.3. LATE EXCITATION

Late excitation was the third, and final, reflex response elicited by the stimulus. Previous reports of late excitation latency fall in two groups, 40ms (van der Glas *et al.*, 1984; Türker & Jenkins, 2000) or 70-80ms (van der Glas *et al.*, 1985; Türker *et al.*, 1994) depending on the type of stimulation and analysis methods used. Although there is no consensus as to the nature of late excitation (Türker & Jenkins, 2000), the results of the present study suggest that it is altered by the same parameters that affect the latency, duration and occurrence of inhibition, and the incidence is reduced by the use of LA block. This indicates that the PMR's may be responsible for at least part of the E2 reflex. Alternatively, since the latency of E2 is closely related to the end of IN, it may be the result of delayed action potentials rather than a true reflex (Türker & Cheng, 1994; Türker & Powers, 1999).

If it is a true reflex, then the E2's not removed by the application of local anaesthetic may originate from remote locations such as skin, mucosal, long-loop spindle or temporomandibular joint receptors that are known, or are suspected, to have an excitatory effect on jaw-closing muscles (Schwaluk, 1971; Tucker & Türker, 2001). During normal mastication the function of these receptors may be to facilitate the jaw closing force so as to increase the ability to chew food, but since IN is stronger and occurs first, this increase in jaw muscle activity will only be present when conditions such as large and/or fast forces, which cause the reflex inhibition, are not present. In this way, larger forces are only developed when there is no danger of damage to the masticatory system (Ottenhoff *et al.*, 1992a & b).

7.4.1.4. REFLEX CHANGE IN BITE FORCE

The results indicate that the inhibition seen in the masseter EMG is, at least in part, responsible for BR. BR was always negative, was more prevalent when inhibition was stronger and the duration was comparable to that of IN. BR followed IN in all cases before LA, and the average EMG/force delay of 15.5 ± 5.5ms was similar to the delay of 15ms reported previously (Yang & Türker, 1999).

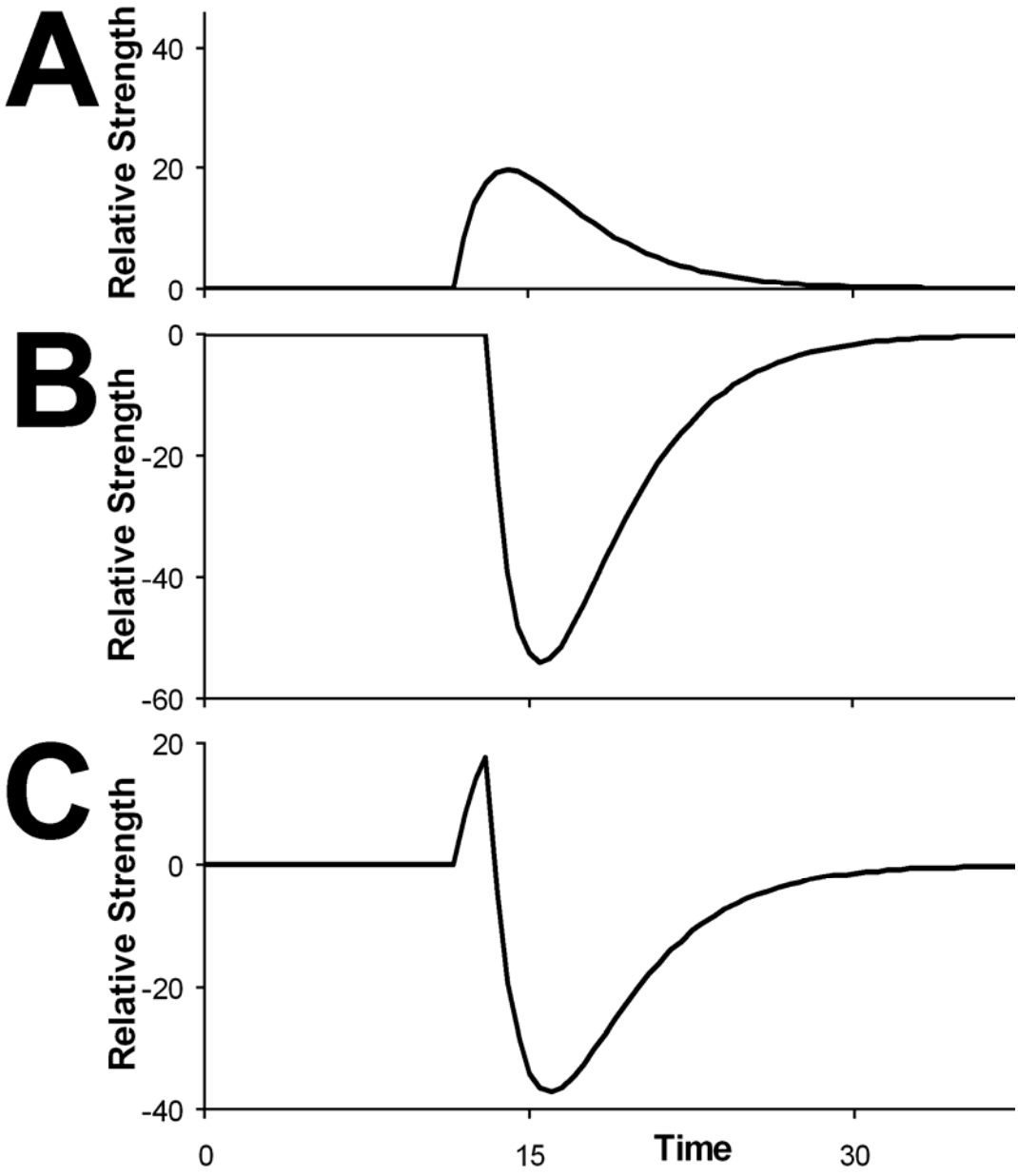
The most likely explanation for the stimulus profile appearing in the bite force record was that the stimulus force on the incisor caused a slight upward movement of the head. Since the bite force is measured from the lower bite bar this upward movement of the jaw would cause a small increase in the observed force and since this increase is time locked with the stimulus it would not be averaged out to zero. This would also explain the

existence of early excitation; spindles would be activated (due to jaw muscle stretch) as the upper jaw moved up while the lower jaw was stationary on the bite bar.

7.4.1.5. INTERACTIONS

With the analysis of surface EMG signals there is always the chance that secondary and tertiary responses are 'count' or 'synchronization' type errors of the primary response (reviewed in Türker, 2002). While this may be the case for the E2 response, it was not the case for IN as it was more prevalent and stronger than E1. This led to the hypothesis that the E1 and IN reflexes may be superimposed and early excitation may be stronger and more prevalent than indicated. By using LA, which is known to remove or significantly reduce IN, this theory has been tested in the literature (Türker & Jenkins, 2000) and in the current study. Under such circumstances, the occurrence of E1 increased substantially indicating that it does not originate from the PMR's and that it is often masked by the presence of the almost simultaneously occurring larger inhibitory reflex. Therefore, we hypothesise that after tooth stimulation masseter motoneurons receive an excitatory postsynaptic potential (EPSP) from the muscle spindles and a short time later a stronger inhibitory postsynaptic potential (IPSP) from the PMRs. The result is a small increase in the electrical activity sent to the masseter followed by a sharp decrease. This situation is illustrated in Figure 7.8.

Figure 7.8 Proposed Electrical Activity At Motoneuron



Diagrammatic representation of the electrical activity seen at a masseter motoneuron in response to mechanical stimulation of the incisor. A) Excitatory postsynaptic potential received as an input to motoneuron caused by activation of the muscle spindles. B) The inhibitory postsynaptic potential received as an input to the motoneuron caused by activation of the periodontal mechanoreceptors. C) The extra electrical activity sent to the masseter due to the superposition of the inputs. If the periodontal component is removed by application of local anaesthetic then the extra electrical activity sent to the masseter will only contain the spindle component and hence an excitation is much more likely to be observed in the EMG. Late excitation is not depicted as it is likely to be delayed action potentials caused by the inhibition rather than a second excitatory postsynaptic potential.

7.4.2. REFLEX ACTIVITY IN THE DIGASTRIC MUSCLE

In humans it has been shown that while a reflex response in the masseter, and other jaw-closing muscles, can be easily identified, the digastric, and other jaw-opening muscles, have little or no reflex activity (Goldberg, 1976). Even painful electrical stimuli to skin and mucosal receptors, which are known to generate a jaw-opening reflex in animals, do not initiate activation of the digastric in humans (Yemm, 1972a). While sudden downward movement of the mandible causes a strong contraction of the masseter and temporal muscles, it does not induce a reflex response in the digastric muscle (Lund & Olsson, 1983; De Laat, 1987b). It has also been reported that there is no response in the digastric muscle to the unloading of incisors (Türker & Jenkins, 2000). In contrast, multiple, rapid trains of high intensity electrical stimuli applied to the lip have been shown to elicit mid to long latency excitations in the digastric (62ms) albeit a little unreliably and only briefly before habituation occurs (Cadden *et al.*, 1997). Additionally, small reflex response in the digastric, with a latency the same as that seen in the masseter but with a maximum occurring significantly later, (Abbink *et al.*, 1998a) has been found to exist during jaw movement.

One source of difference between the jaw muscles is that, unlike the jaw-closers, the jaw-opening muscles do not contain muscle spindles (Taylor *et al.*, 1976). This anatomical finding illustrates that there are fundamental differences in the wiring of the sensory feedback between the jaw closing and jaw opening muscles. However since this finding is common across species it does not explain the differences between animals (where jaw-opening reflexes are easily elicited) and humans (where the jaw-opening reflexes are difficult to elicit). Rather the difference may have more to do with the mass of the jaw. Heavy jaws do not need highly developed jaw-opening reflexes as the weight of the jaw is normally sufficient to cause passive opening. In addition smaller animals tend to chew at higher rates than larger animals, thus higher velocities of jaw movement, and hence more activity in the jaw-opening muscles, is required. It is also possible that the type of preparation used, for example using decerebrate animals (Collier & Lund, 1987), may change the relative excitability of reflexes making it easier to elicit a response than would normally be the case.

The current study indicates that, when observed, 'reflexes' in the digastric are more likely the result of cross talk from the masseter rather than altered activity of the motoneurons controlling the digastric. Cross-talk results when potentials from adjacent muscles reach the recording site through volume conduction, thus contributing to the EMG signal (van Vugt & van Dijk, 2000). Experiments on cross talk have shown that when using surface electrodes up to 16.8% of a signal detected above a muscle may be due to

cross-talk rather than to activation of the muscle below the electrode (De Luca & Merletti, 1988).

The results of the current study show that the detection rates of reflexes in the digastric were low, and when they were detected they were almost always detected in the masseter at the same latency. Hence, the combination of low EMG and 'reflex' amplitudes as well as different wiring and conjoint reflex responses with the masseter muscle leads to the conclusion that there may be no genuine reflex activity in the digastric muscle in response to axial stimulation of the incisor. One way to test this hypothesis would be to use intra-muscular rather than surface electrodes to record the digastric EMG activity, as intra-muscular recordings are less susceptible to cross talk (Türker, 1993).

7.4.3. EXPERIMENTAL CONSIDERATIONS

The differences found between the two testing days were minimal, and while both the inhibition duration and the late excitation latency were statistically different between the two testing days the difference in the means (3ms and 1ms respectively) was small.

It is known that the frequency of PMR discharge is effected by the rate at which the forces are applied to the teeth (Linden *et al.*, 1995); and that under static orthogonal stimulation conditions, slowly rising stimuli are more likely to elicit an excitatory response in comparison to rapidly rising ones (Brodin *et al.*, 1993b; Türker *et al.*, 1994; Türker *et al.*, 1997). However, this was found not to be the case with axial stimulation, as slowly rising stimuli were likely to produce a small inhibitory reflex if anything, and an increase in the EMG over time was not observed. The reason for this is likely to be the position of the two PMR types within the periodontal ligament (reviewed in Türker *et al.*, 1999).

In line with the 'frequency principle' of inhibition established for motor units (Miles & Türker, 1986), the present study indicates that increasing bite force slightly decreases the duration of the inhibition in the range of bite levels used. The range of bite levels (5 – 20% MVC) were chosen as higher levels have been associated with an increase in muscle fatigue (Kroon *et al.*, 1986). It has also been shown, in previous research where the stimuli were delivered orthogonally, that the inhibition was greatest at low levels of contraction (van der Glas *et al.*, 1984). Additionally, while one study has shown that when chewing hard artificial foods the peak level of muscle activity was larger than that used (Slagter *et al.*, 1992) another study showed

that 5 – 20% MVC was close to that encountered during mastication of normal foods (Anderson, 1956a & b). Thus, during regularly encountered muscle contraction levels there is only a small decrease in the inhibitory reflex duration due to an increase in muscle activity; assuming that the reflexes operate in the same way under dynamic conditions.

There were large and highly significant inter-subject differences found in almost all reflex statistics measured. While gender and age were tightly controlled (only young females were tested) other subject factors may have contributed to this controversy. These may include, but are not limited to: periodontal health, tooth crowding, recent tooth usage, tooth angle, periodontal history and the amount of gamma-motoneuron activity present. While every effort was made to ensure subjects had healthy teeth and gums with no history of orthodontic treatment or periodontal disease, some subject differences were unavoidable.

While the results of the current study are definitive, the practical uses of the inhibitory reflex during axial loading are not yet known. If the same reflexes hold during normal mastication as during static axial experiments, then inhibitory reflexes in the jaw-closer muscles will be produced at every tooth contact, making chewing an inefficient process. There is evidence that these findings may not be directly applicable to dynamic conditions. Previous findings on human mastication (Ottenhoff *et al.*, 1992a; Abbink *et al.*, 1999b) have shown that additional muscle activity increases when resistance is encountered between the teeth. Furthermore, investigations on chewing animals (Morimoto *et al.*, 1989) have shown that extra muscle activity is generated when the molar teeth encountered resistance, and that this activity is drastically reduced following the destruction of the PMR input to the central nervous system. Hence the reflex contribution of the PMRs may be altered depending on the tooth that is stimulated (incisor or molar), the movement or position of the jaw, and the stimulus parameters used such as rate of force application.

8. Response Of Human Jaw Muscles To Axial Stimulation Of A Molar Tooth

The reflexes of the main jaw-closer muscles (masseter and anterior temporalis) on both sides of the jaw were investigated using surface electromyography to observe reflex activity following mechanical stimulation of the 1st right upper molar tooth at various forces under a number of levels of jaw muscle activity.

As with analogous studies performed on the incisor, three distinct reflex events were identified in the EMG before the earliest conscious subject reaction: early excitation, inhibition and late excitation. However, contrary to observations found during studies on the incisor, excitation, not inhibition was the primary reflex response. The application of a local anaesthetic block around the stimulated molar showed that the primary agents in eliciting the observed reflexes were not contained within the periodontium of the stimulated tooth. A diminished representation of periodontal mechanoreceptors around the molar teeth and more elaborate root structures, hence a more solid connection to the jaw and consequently less tooth movement, were deemed the likely reason for the distinction between the reflex responses of the incisal and molar regions.

In addition to the reflex studies, the minimum reaction time of a number of subjects was determined to permit the distinction of a reflex event and an event that could be a conscious subject reaction. It was found that the reaction time of the temporalis muscles was significantly shorter than those of the masseter, while no significant difference was found between the left and right sides.

Overall, the data showed that the presence or absence of background muscle activity and subject variability were the main causes of changes in the reflex response, provided the level of the stimulus was greater than 3N. The application of local anaesthetic had no impact on the reflexes evoked.

This chapter is an edited version of the manuscript “Response Of Human Jaw Muscles To Axial Stimulation Of A Molar Tooth” by R.S.A. Brinkworth, C. Male and K.S. Türker, which has been published in Experimental Brain Research 159(2): 214-224 (2004).

8.1. INTRODUCTION

Reflex activity in jaw-closer muscles has been investigated previously with regard to afferent feedback arising from the orthogonal (Brodin *et al.*, 1993b; Louca *et al.*, 1996; Yang & Türker, 1999; Türker & Jenkins, 2000) and more recently axial (Chapter 7) stimulation of human incisors. However, comparatively less effort has been made to describe the reflex activity elicited from stimulation of molar teeth (Yamamura & Shimada, 1992; Yamamura *et al.*, 1993), likely because of the technical difficulty in delivering precise mechanical stimuli to the area. As the incisors are neither structurally nor functionally the same as the molars, it cannot be assumed that reflex activity observed during incisor investigations will act as a legitimate representation of reflex activity found during stimulation at any other point along the dental arch. This study is therefore a pilot investigation into the reflex characteristics observed in jaw-closer muscles following precise mechanical stimulation of a molar tooth.

Periodontal Mechanoreceptors (PMRs) are stretch receptors located in the periodontal region. There are several different types of PMRs, and they can be defined categorically via the location of their cell bodies, by their morphology, by the stimulus properties through which they are activated and by their response properties (Linden & Millar, 1988b; Linden & Scott, 1988). PMRs are described as being one of the major contributors to the extra muscle activity developed to overcome resistance between the teeth during induced chewing in anaesthetised animals (Morimoto *et al.*, 1989); and in humans these receptors can provide both positive and negative feedback to the jaw-closer muscles (Lavigne *et al.*, 1987; Türker, 2002).

Human studies on PMR-mediated reflexes have indicated the presence of two largely autonomous pathways innervating the jaw-closers, one excitatory and the other inhibitory (Türker *et al.*, 1994; Türker *et al.*, 1997). The excitatory pathway has been suggested to perform a number of functions: it may contribute essential activity to food-holding behaviour (Trulsson & Johansson, 1996b), or it may behave as a compensation reflex to control chewing force (Brodin *et al.*, 1993a). The inhibitory pathway reduces muscle activity when normal biting encounters an unexpectedly hard object (van der Glas *et al.*, 1984; Bjornland *et al.*, 1991; Bonte *et al.*, 1993) or when an object between the teeth unexpectedly fractures (Miles & Madigan, 1983; Türker & Jenkins, 2000). In all cases the inhibitory response specifically deals with an unexpected event and in a protective fashion.

Previously, research on reflexes involved orthogonal stimulation (i.e. stimulating the labial surface) of incisor teeth (reviewed in Türker, 2002). Depending on the rate of stimulation, orthogonal stimuli activate

populations of receptors at the apex and/or around the fulcrum, and therefore induce either excitation, if the stimulus is slow, or inhibition if a more rapid stimulus is applied (Brodin *et al.*, 1993b; Türker *et al.*, 1994; Türker *et al.*, 1997). A recent experiment involving axial stimulation of the incisor (Chapter 7), where the receptors around the apex were compressed, showed that rapid stimuli produced the same inhibition as seen in orthogonal stimulation, however slow stimuli did not produce the previously described excitation. In both cases (axial or orthogonal stimulation) the application of a local anaesthetic block removed, or significantly reduced, the observed inhibitory reflex activity, indicating PMRs were involved in the generation of the reflex.

Investigations into the contribution of various jaw muscles to bite force have identified that, in humans, the masseter and temporalis muscles are more active during full occlusal clenches (100%) than during incisal biting, where the masseter is active at 60% of maximum and the temporalis 35% (Blanksma & van Eijden, 1995). This may be due to active inhibition of the muscles during incisal biting because of the increased loading on the temporomandibular joint or because the position of the jaw is not ideal for temporalis contraction during incisal biting where the jaw is protruded. There is a significant increase in the maximum bite force as the bite point moves posterior from the incisors, with maximum force obtained at the first molar tooth (Spencer, 1998). Previous experiments (Blanksma & van Eijden, 1995; Yang & Türker, 1999) indicate greater bite force used and a larger utilisation of the temporalis during bite tasks involving posterior teeth. There is also evidence that the masseter and temporalis muscles have different compositions (van Eijden *et al.*, 1997) and operate optimally at different jaw angles (van Eijden *et al.*, 1988), indicating both physical and functional differences. Consequently, investigations of posterior teeth should involve a recording from both the temporalis and masseter muscles, as it cannot be assumed that they will operate in the same way.

Unlike during incisal stimulation, or tapping the lower jaw with a reflex hammer, where the stimulus is applied to a point approximating the midline of the jaw, selectively stimulating a single molar tooth, like natural mastication, sets-up distinct activity in the ipsi- and contralateral sides of the jaw (Møller, 1976). It has been suggested that there are distinctions between the activity of these two sides to minimise the strain on the temporomandibular joint during normal function (Hylander, 1978). Whereas these data referred to changes in bite force production, it is reasonable to suspect that differences may also exist in the reflex activity between the ipsi- and contralateral paired muscles, as the stimulus is only applied to one side

of the jaw. This study will attempt to elucidate those differences by recording from the masseter and temporalis muscles on both sides of the jaw.

The aim of this study was to determine the reflex responses of masseter and temporalis muscles on both sides of the jaw, as well as the reflex change in the bite force to axial stimulation of the 1st upper right molar tooth. To this end both the stimulus intensity and the level of background activity were altered both before and during the application of a local anaesthetic block to determine the contribution of each. Furthermore, to facilitate the distinction between a reflex and a conscious subject reaction, the reaction time of each muscle and the bite force were determined.

8.2. METHODS

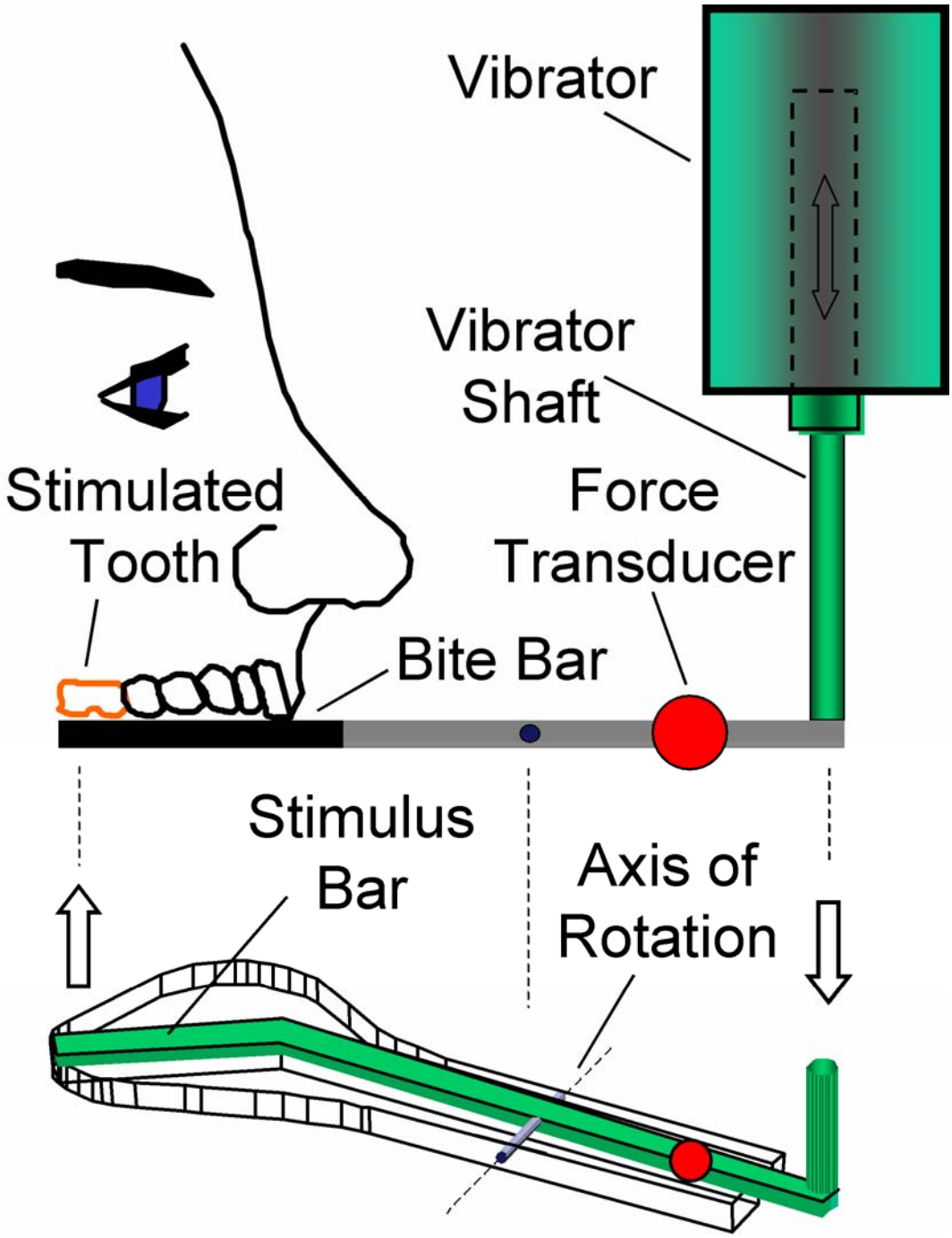
8.2.1. SUBJECTS

Experiments were carried out on nine healthy subjects aged from 18 to 26 years (five males and four females) from whom written informed consent was obtained. All subjects had normal dentition with no sign of open bite, and individuals with a history of orthodontic treatment at the 1st molar site were excluded from participation. The experiments were approved by the Human Ethics Committee of The University of Adelaide and conformed to the Declaration of Helsinki.

8.2.2. STIMULATION PROCEDURE

The apparatus used to stimulate the molar teeth is shown in Figure 8.1 and comprised of a small linear motor (LDS model V201) in addition to a hinged lever installed within the upper bite bar. The motor pushed down on the lever, which subsequently provided an upward force on the tooth. The forces were configured (in a force shape program) and delivered randomly using a computer and amplifier. A microprocessor controlled compensator box (custom built microprocessor based programmable controller with loop rate of 1kHz) was used to maintain the force on the tooth within suitable parameters and to ensure the reproducibility of stimulus profiles. A strain gauge on the lever measured the force delivered to the tooth. To maintain head position, impressions of the subject's teeth were taken (Express STD, 3M) which covered the teeth to the gum line. Once the subject placed his/her teeth into this rigid mould, it was not necessary to bite to keep the jaws from moving since the mould kept the teeth and hence jaws in place. The position of the head was further secured by a nosepiece, which reduced movement of the head in the vertical plane. The mean separation distance of the bite bars was set to 12mm (Türker, 1988). The dental impression material was cut away from around the upper incisors so that the probe-shaft would be allowed unobstructed movement. The impression material was trimmed at the contact site, although the impression of the first molar remained in position, fixed with a dental adhesive (VPS Tray Adhesive, 3M) providing a perfect mould of the crown of the target tooth to serve as the 'probe-cap'. A transducer connected to the lower bite-bar picked up the force generated by the lower jaw.

Figure 8.1: Experimental Set Up – Molar Stimulation



The subject bit into impression material (not shown) mounted on two bite-bars (only the upper bar is depicted). Once set, the impression material was removed from the upper incisors and around the upper right first molar to permit unfettered movement of the stimulus bar. The impression of the first molar was retained to act as a tooth rest and was attached to the stimulus bar with adhesive. The motor applied a vertical force to the stimulus bar that in turn applied the force on the molar. The applied force was monitored by a digital control system to ensure an accurate force profile and that the probe remained in contact with the tooth at all times, regardless of the level of muscle activity.

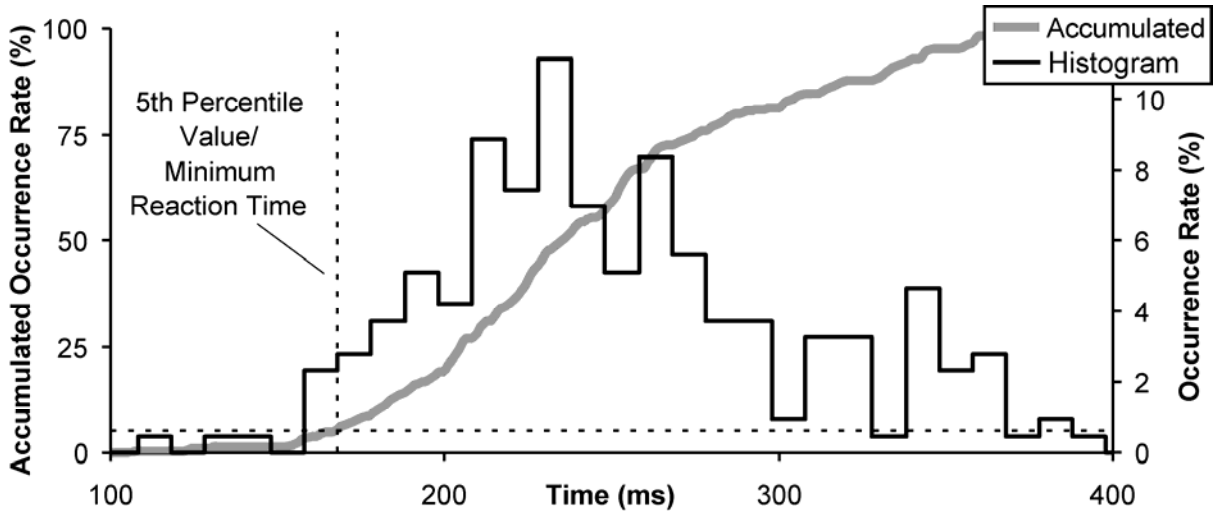
Bipolar surface electrodes were placed over the ipsi- and contralateral masseter as well as both anterior temporalis muscles to record their electromyogram (EMG). The subjects were grounded using a lip-clip electrode (Türker *et al.*, 1988). The EMG was amplified (3000x) and band-pass filtered (5-1000Hz) before being digitised (12-bit) and recorded (2kHz). The first right upper molar was stimulated with a constant rate of force (120N/s) and the maximum level was held for over 100ms. The preload (constant force on the molar between stimuli) used in the experiment was 0.5N. Subjects were given on-line feedback of the activity of their right temporalis muscle relative to their maximum voluntary contraction (MVC). The total bite force was recorded in addition to surface EMG (SEMG) recordings from the right and left temporalis and the right and left masseter.

8.2.3. REACTION

In order to facilitate the discrimination between a reflex event and a conscious subject reaction, preliminary experiments were undertaken to determine the reaction time to molar stimulation. Six subjects (three male, three female), who also participated in the reflex trials, were recruited. Between 20 and 25, 6N stimuli were delivered randomly to the first molar of each subject with a time between stimuli of 8 to 10s. Subjects were instructed to maintain a constant 1% MVC of the right temporalis muscle between stimuli and “bite hard” in response to a stimulus.

The minimum reaction time was defined as the value that is at the lower 5% of the distribution histogram built from individual measurements of each record for each subject and for each muscle. This process, illustrated for the results from the masseter in Figure 8.2, involved the construction of histograms and the determination of the 5^{th} percentile point, i.e. the value that is less than 95% of the data.

Figure 8.2: Reaction Time – Molar Stimulation



The histogram and accumulated occurrence rate (percentile distribution) values for the reaction time in the masseter muscles. The bin width of the histogram is 10ms, the bin width for the accumulated distribution is 2ms, while the actual measurement of the reaction time was done using 0.5ms bins (2kHz sampling rate). The 5th percentile value, or the minimum reaction time, is the time when the accumulated occurrence rate crosses 5% and was found to be 168ms in the masseter muscles.

8.2.4. REFLEX

All subjects participated in the reflex study. Each trial consisted of 50 stimuli delivered at one of three stimulus forces: 3, 6 or 8N. The stimuli were delivered axially to the crown of the tooth at random intervals between 2 and 4s. Trials were conducted using three levels of muscle activity: 0, 1 and 5% MVC of the right anterior temporalis. These levels were selected to represent the absence of voluntary activity, the presence of activity at a very low level and the average (Anderson, 1956b) or lower limit (Slagter *et al.*, 1992) of bite force observed during normal mastication respectively. Feedback of muscle activity was provided through the use of a low-pass filter (cut-off of 1Hz) and oscilloscope. Feedback was not required for the 0% MVC trials as the subject was asked to remain as relaxed as possible, although a small amount of non-voluntary activity during these conditions could not be ruled out. Stimulus conditions were randomised.

Upon delivery of the nine different stimulus combinations (three stimulus strengths at three levels of background muscle activity), local anaesthetic (2ml Xylocaine 2% with adrenaline 1:80,000, ASTRA) was administered to the mucosa directly above the molar tooth and on the palatal side. After a period of 10-15 minutes, when the local anaesthetic block had taken effect (evidenced by no reported sensation to application of stimuli), the trials were repeated.

8.2.5. ANALYSIS

Offline analysis involved zero-phase band-pass filtering the EMG (5-500 Hz), rectifying the signals, extracting a defined time period from around the stimuli (for reflex trials pre- and post-stimulus times were set at 125 ms, for reaction trials the pre-stimulus time was 125 ms and post-stimulus 400 ms), averaging them for each trial (n=50 for reflex and n=1 for reaction) and finally zero-phase low-pass filtering (333 Hz). Zero-phase filtering is required to ensure that no temporal (phase) distortions are introduced into the signals, hence ensuring the accuracy of timing measurements. Zero-phase band-pass filtering was achieved by passing the data through a 2^{nd} order equal ripple filter with a ripple of 0.05dB, then time reversing the signal, passing it back through the same filter, and then time reversing the signal again. Zero-phase low-pass filtering was achieved by using a moving average filter centred on the time point of interest.

Due to the need to overlook reflexes in the reaction time experiments the error box (maximum pre-stimulus variations in the CUSUM of the EMG; for

detail see Chapter 6) was increased from the 100% of pre-stimulus variation used in the reflex trials to 300%. This precaution ensured that only the largest variations were used in reaction time experiment.

Each stimulus was analysed to ensure that it fell within defined parameters. If the force on the molar deviated by more than 0.2N it was removed. In addition, if the level of pre-stimulus EMG activity varied by more than 20% from the desired (set) level then that stimulus was also removed. These two steps ensured that the data corresponded as closely as possible to those specified by the trial condition.

Cumulative sums (CUSUMs) of the normalised averaged EMG data were constructed (Ellaway, 1978), from which a specially written computer program extracted various reflex characteristics such as latency, duration and strength (area). The EMG and force analysis procedures are detailed in Chapters 6 and 7 respectively, hence are only covered briefly below.

When averaged over a sufficiently large number of trials, the noise in a rectified EMG signal that is not time locked to the stimulus becomes approximately D.C. This noise was estimated for each subject and each recording method by finding the minimum DC value in the average of the no contraction trials. This value was then subtracted from all other EMG levels to give an almost noise free signal (Chapter 6).

EMG traces were full wave rectified and normalised to the pre-stimulus average. All EMG reflex strengths were calculated as a percentage of the theoretical maximum inhibition with excitations as positive and inhibitions as negative, i.e. –100% corresponded to a complete cessation of all EMG activity, and 100% corresponded to the average EMG level during the reflex being twice that of the background level. The units for CUSUM graphs are k.ms representing the average pre-stimulus EMG level (k) integrated over time (ms).

Averaged bite force records were assembled in the same way as EMG records but normalisation comprised of subtracting the average pre-stimulus bite level from the trace to get a change in bite force that could be compared between trials that had different pre-stimulus averages. The bite force was averaged then zero-phase low-pass filtered at 111Hz. A change in bite force was classified as a reflex only if the strength was greater than twice the maximum pre-stimulus variation (similar to the error box approach for the EMG records).

8.2.5.1. STATISTICS

To establish what, if any, effect the stimulus parameters had on the recorded values, an analysis of variance (ANOVA) was performed. A natural logarithmic transform was used on a measured parameter (reflex latency, strength or duration) if there was sufficient departure from normality (Brinkworth *et al.*, 2004). If a parameter was determined to be significant then a Bonferroni post-hoc test was performed, to determine where the differences were. The model used looked for direct as well as 2-way interactions between the experimental parameters (muscle, bite level, stimulus level and local anaesthetic). In addition, a subject parameter unique to each participant was included in the statistical model to ascertain if there were significant differences between subjects. If an interaction was significant, then direct influences were ignored. If the ANOVA determined a parameter to be significant but the post-hoc test could not identify where the difference was, that parameter was ignored. Only if no significant differences were found to exist between data sets were the results pooled.

Binary logistic regression was performed to ascertain if any of the experimental parameters affected the number of reflex occurrences. Chi-squared tests were used to discover where any differences were.

For all tests the level of significance was 5%. Results are given in the form of mean (upper limit, lower limit) where the upper and lower limits are equal to the 95% confidence limits (1.96 times the standard error of the mean) and represent the range in which the actual mean is 95% likely to be contained. It is important to note that the confidence intervals for data that were transformed are not symmetrical about the mean. If the lower confidence limit was less than zero for any parameter, other than inhibition strength, then the lower limit was taken as zero. Reflex time calculations are given to the nearest 0.5 ms, reaction time calculations are given to the nearest millisecond, strength and occurrence rate calculations are given to 0.1% accuracy.

8.3. RESULTS

8.3.1. REACTION

The pooled results indicated no statistical difference between the reaction times for the two masseter muscles, and the right and left temporalis were not different. While the two muscles were statistically different from each other ($p<0.001$), no difference was found between the masseter reaction time and that identified in the bite force. In all cases, one or more EMG signals increased before a change in bite force was detected; however, in some cases the masseter reaction times were later than the bite force reaction time. The 5% values were found to be 103ms for the temporalis, 168ms for the masseter and 165ms for the bite force record.

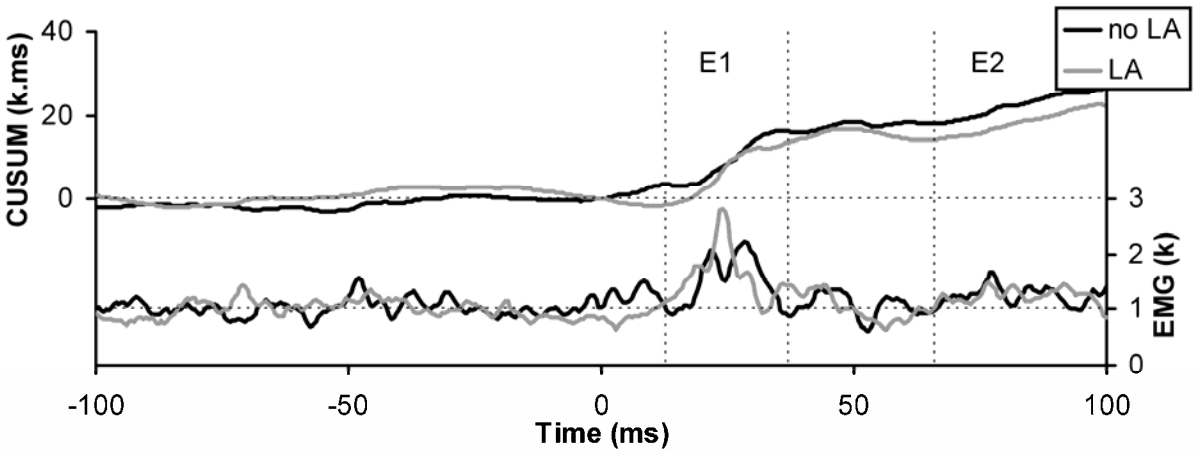
8.3.2. REFLEX

The main findings in the reflex analysis were a lack of large inhibitory reflexes, which are seen during incisor stimulation, and the observation that the application of local anaesthetic did not significantly alter the reflex response elicited. An example of these findings is depicted for one subject in one muscle in Figure 8.3.

Three groups of significant deviations in the integrated EMG record (CUSUM) were found before the reaction time. Hence, three EMG reflex conditions were identified: early excitation (E1), inhibition (IN) and late excitation (E2). The EMG reflexes were classified according to the conditions set out in the relevant sections. Only one mechanical reflex condition was observed: change in bite force due to reflex, which was always excitatory. Detail is provided in the relevant sections, and an example of the results from one subject in one condition is provided in Figure 8.4. The excitations are visible in each muscle as positive slopes in the CUSUMs, while the reflex increase in the bite force is only discernible after the stimulus artefact and the associated overshoot (for details see Chapter 7). Any change in the recorded traces after the reaction time was discounted.

No significant differences were observed between the two sides of the jaw for any of the reflex parameters measured; however, the duration of late excitation tended to be longer in the temporalis muscle than in the masseter.

Figure 8.3: Effect Of Anaesthetic – Molar Stimulation



The averaged (n=50) EMG, and associated CUSUMs recorded from the left temporalis of a subject in response to an 8N stimulus delivered axially to the 1st upper right molar commencing at time zero, during a sustained 5% maximum voluntary contraction of the right temporalis muscle, both before and during a local anaesthetic (LA) block of the region surrounding the stimulation area. The EMG pre-stimulus mean (k) has been normalised to one. A large early excitation (E1) is visible in both traces as is a small, but consistent, late excitation (E2) with latency of approximately 66 ms in both records. Both excitations are seen as positive slopes in the CUSUMs, which is a measurement of the area above the pre-stimulus mean. No inhibitory reflex is evident in this record. As with findings in other jaw muscles utilising different stimulus sizes and background EMG levels, the application of LA had no significant or consistent effect on the evoked reflex response.

Table 8.1 shows the reflexes found in the EMG traces and the stimulus parameters that had a significant effect on the measured values. The interactions are described in the sections below. Where there was no significant difference between conditions or muscles, the data were pooled.

Figure 8.5 shows the reflex latencies of the EMG reflexes and the associated mechanical reflex. From the latencies it appears that the reflex increase seen in the bite force was caused by the late excitation; however, any reflex change as a result of early excitation or inhibition may have been masked by the stimulus artefact and overshoot respectively.

Figure 8.6 shows the reflex occurrence rates as a factor of both bite level and stimulus force. There was no significant difference found between the occurrence rates of the reflexes in the various recorded muscles or in response to the application of local anaesthetic; hence the data were pooled in this figure to illustrate the significant factors in reflex occurrence, i.e. bite level and stimulus force.

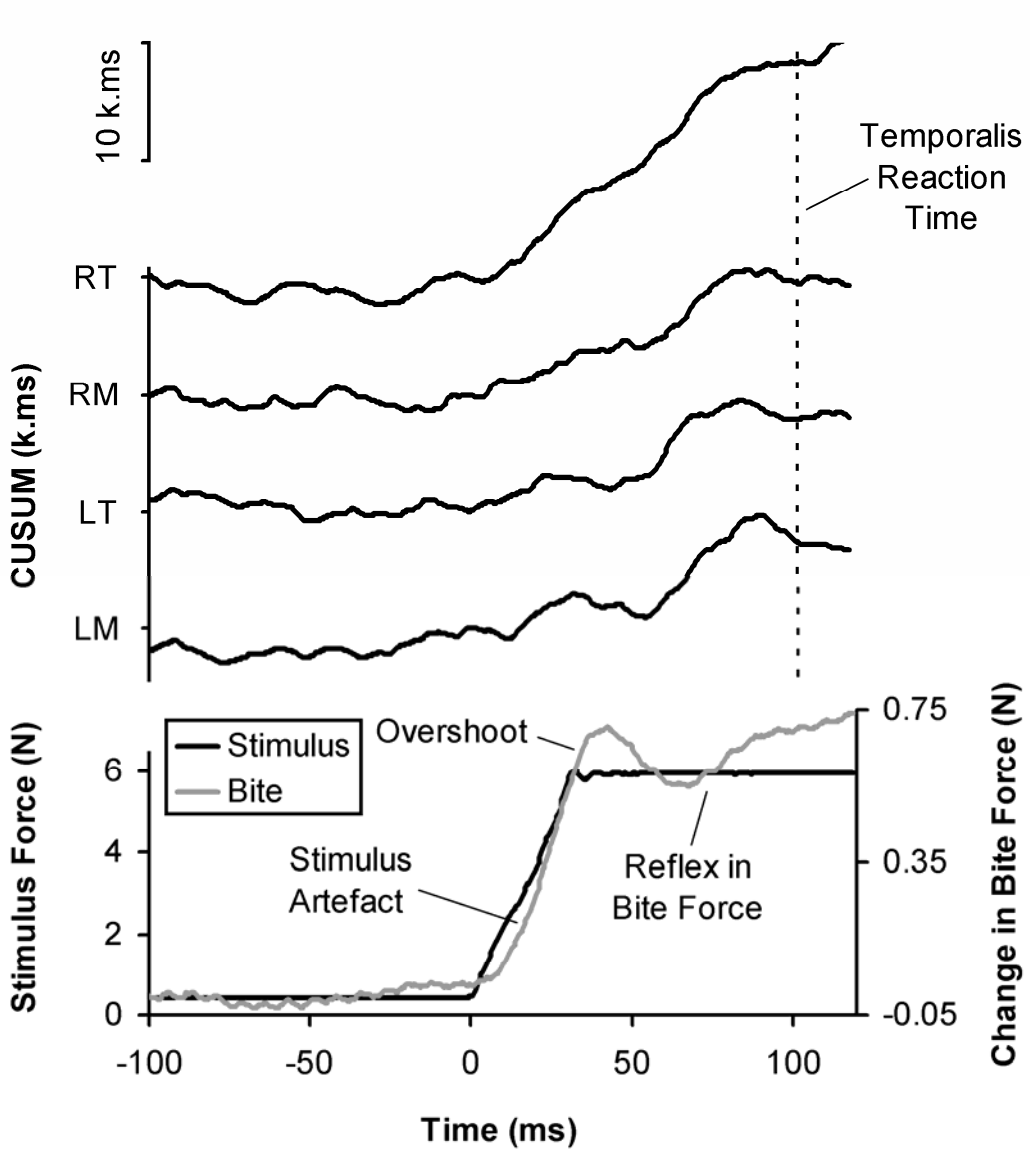
Figure 8.7 shows the averaged EMG traces recorded from one subject in response to all bite and stimulus levels before the application of a local anaesthetic block. There was little or no response at 0% MVC; however, excitations, and some inhibitions, became visible in the presence of conscious muscle activity and when larger stimuli were used. The results were similar after a local anaesthetic block was applied.

8.3.2.1. EARLY EXCITATION

If a reflex was excitatory and occurred within the first 25ms after the commencement of the stimulus force it was defined as early excitation (E1). The only factor that influenced the occurrence of E1 was the bite level ($p<0.01$); with the occurrence rate being significantly lower in the absence of voluntary muscle activity, 3.7% (confidence interval: 6.7%, 1.0%), compared to either 1% MVC, where the occurrence rate was 17.6% (22.8%, 12.5%), or 5% MVC, where the occurrence rate was 15.9% (20.8%, 10.9%).

At 9.5ms (10.5ms, 9ms) the average latency of E1 was not significantly altered by any of the experimental parameters, but was not normally distributed and hence required a logarithmic transform.

Figure 8.4: Reflex Response – Molar Reflexes



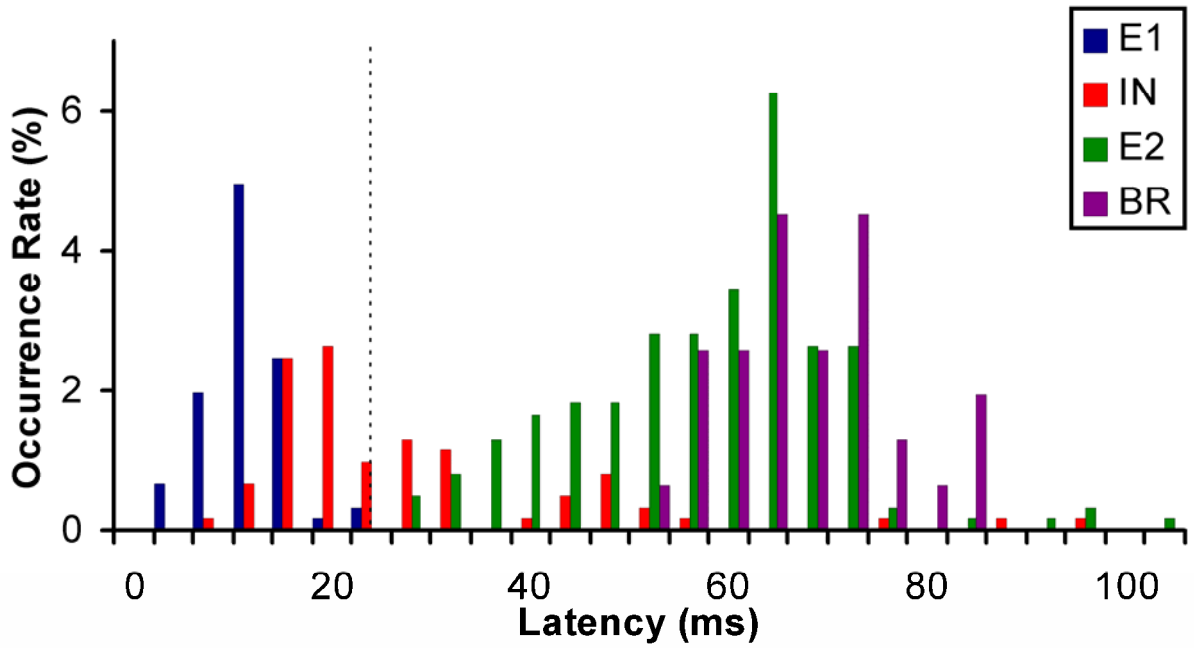
The CUSUMs of the responses from the right temporalis (RT), right masseter (RM), left temporalis (LT) and left masseter (LM) as well as the change in bite force (light trace) to a 6N stimulus (dark trace) applied at time 0 from one subject. The bite force is given as a change relative to the pre-stimulus average. Before integration to construct the CUSUM, the EMG traces were normalised so that the pre-stimulus average (k) was one. The tendency for the CUSUM graphs to increase after the stimulus is applied shows that the dominant reflex response was excitation. Any changes after the reaction time are discounted as they may indicate a conscious subject reaction rather than a true reflex. The dark continuous line in the bottom trace indicates the actual stimulus force delivered to the molar tooth as it is recorded in series. Note the precise control of the stimulus force by the compensator box, which insured that the preload and the applied force profile stayed constant throughout the experiment. The significant events in the bite force record, stimulus artefact, overshoot and reflex in bite force, are also show.

Table 8.1: Results Of Analysis On Reflex Data – Molar Stimulation

Reflex	Statistic	Transform	Effected By	Significance
Early Excitation (E1)	Occurrence		Bite Level	††
	Latency	Logarithm		
	Strength	Logarithm	Subject	†††
			Bite Level	†††
	Duration	Logarithm	Bite Level	††
Inhibition (IN)	Occurrence		Subject	††
			Bite Level	†
	Latency	Logarithm	Subject	†††
			Bite Level	†
	Strength		Subject	†††
			Bite Level	†††
			Stimulus Level	†
	Duration			
Excitation (E2)	Occurrence		Subject	††
			Bite Level	†††
			Stimulus Level	†††
	Latency		Subject	†††
			Bite Level*Stimulus Level	††
	Strength	Logarithm	Subject	††
			Bite Level	†††
	Duration	Logarithm	Muscle	†
Reflex in the Bite Force (BR)	Occurrence		Subject	††
			Bite Level*Stimulus Level	††
	Latency		Subject	†
			Stimulus Level	††
	Strength			
	Duration			

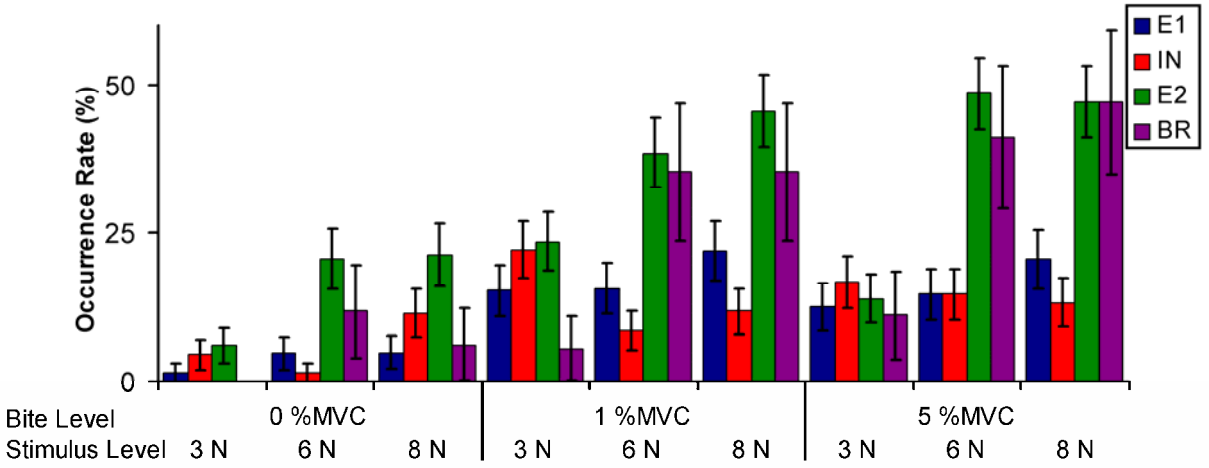
The symbol * indicates an interaction between two conditions. The sign † represents p<=0.05, †† and ††† represent p<=0.01 and p<=0.001 respectively. If the data were not normally distributed a natural logarithm transform was employed as indicated. Some reflex statistics were not affected by any of the stimulus parameters; these are indicated with blank rows. The subject and bite level had the most influence on the measured parameters; the stimulus level and muscle recording had small influences, while the application of local anaesthetic had no significant impact.

Figure 8.5: Reflex Latencies – Molar Stimulation



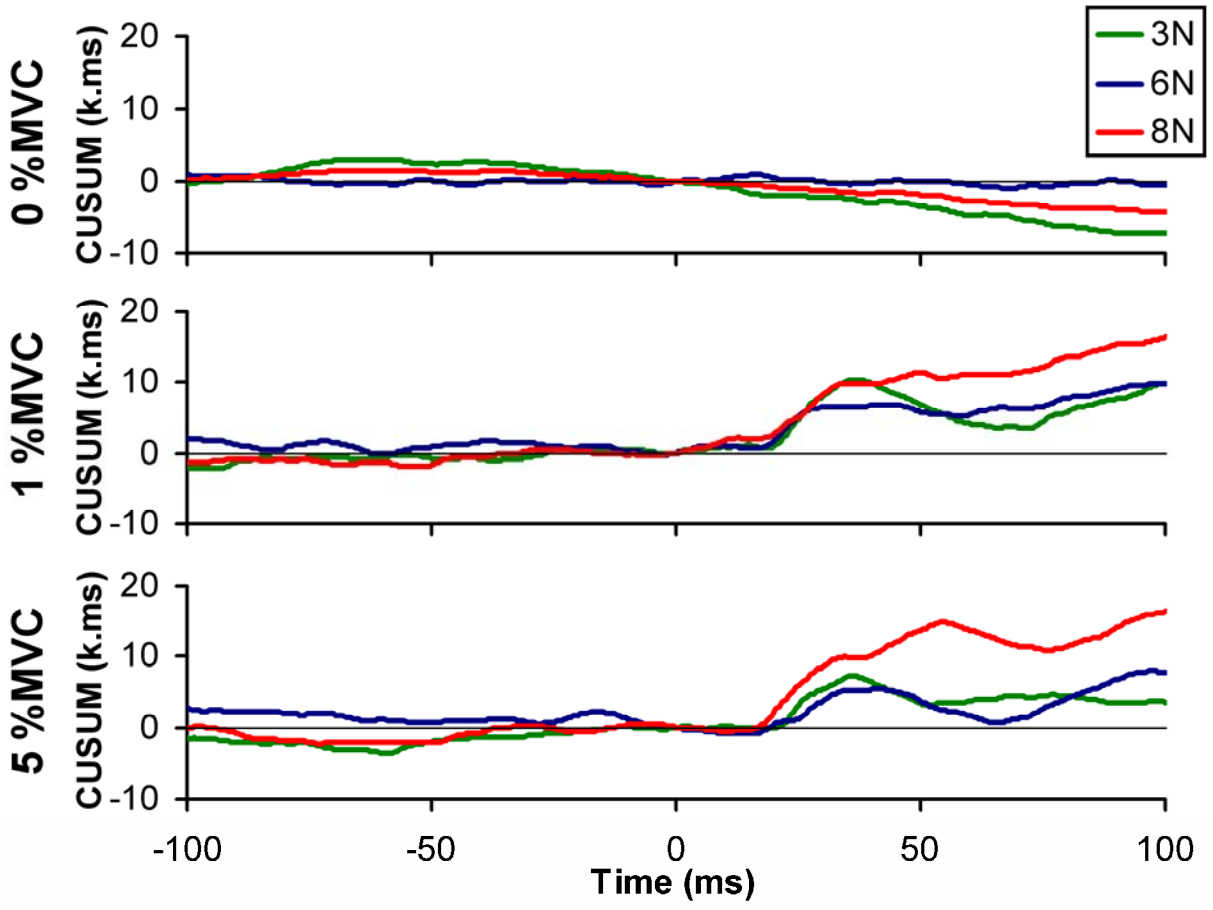
The distribution of the reflex latencies observed. E1, IN, E2 and BR represent early excitation, inhibition, late excitation and reflex change in bite force respectively. The dashed vertical line at 25ms indicates the end of E1 and start of E2 classification. The data represent all recorded muscles and conditions. The bin width used was 4ms and is indicated on the graph (i.e. latencies between the tick marks labelled 20ms occurred between 20 and 24 ms). As discussed in the text E2 is the most likely cause of BR due to the association between their directions (excitatory) and the average delay between their onsets, around 10 to 15 ms as derived from the raw data.

Figure 8.6: Reflex Occurrence Rates – Molar Stimulation



The occurrence rate of early excitation (E1), inhibition (IN), late excitation (E2) and reflex seen in the bite force (BR) as a factor of bite level and stimulus level. In the absence of voluntary muscle activity (0% MVC, maximum voluntary contraction) the occurrence rates of all reflexes were significantly reduced, although the presence of a small number of inhibitions showed that 0% MVC did not always correspond to a total absence muscle activity, i.e. there was a small amount of non-voluntary activity in some cases. In addition, the late excitation and reflex in the bite force were both increased when a 6N or 8N stimulus was used, compared to a 3N stimulus. Error bars represent one standard error of the population. The data represent results taken from all recorded muscles and subjects.

Figure 8.7: Reflexes Elicited at Different Bite and Stimulus Levels



The CUSUMs of the EMGs recorded from the left temporalis muscle of one subject in response to 3N (light trace), 6N (medium trace) and 8N (dark trace) stimuli, during 0% (upper box), 1% (middle box) and 5% (lower box) maximum voluntary contraction (MVC) before the application of local anaesthetic. There are no reflexes seen at 0% MVC, however once voluntary muscle activity is present there are clear excitatory reflexes (positive slopes without turning points) and the occasional inhibition (negative slopes without turning points).

The strength of E1 was affected by both the bite level ($p<0.001$) and subject ($p<0.001$) and required a logarithmic transform to ensure normality. The strength of the reflex was lower at 0% MVC, 15.8% (20.7%, 12.0%) than during a 1% MVC, 41.7% (48.1%, 36.2%) or 5% MVC, 44.8% (51.6%, 39.0%). No significant difference was found to exist between any of the recorded muscles.

As with both the occurrence and strength, the duration of E1 was found to be significantly different at 0% MVC, 47.5ms (75.5ms, 30ms) when compared to either 1% MVC, 18ms (19.5ms, 16.5ms) or 5% MVC, 20.5ms (23ms, 18ms) ($p<0.01$). However, the low occurrence rate and large variability may make the duration measurement at 0% MVC suspect. The duration measurements of E1 also required a logarithmic transform.

8.3.2.2. INHIBITION

The occurrence of the inhibitory reflex (IN) was affected by both the subject ($p<0.01$) and the presence of muscle activity ($p<0.05$). The occurrence of IN was significantly lower at 0% MVC, 5.8% (9.1%, 2.5%) than that at 1% MVC, 14.3% (19%, 9.6%) or 5% MVC, 14.9% (19.7%, 10.1%). The presence of any inhibitions at 0% MVC was confirmation that some latent muscle activity existed during the period of enforced relaxation.

The latency of IN was altered by the subject ($p<0.001$) and the bite level ($p<0.05$). During active muscle contraction the average latency of IN was found to be 22.5ms (27ms, 19ms) at 1% MVC and 19.5ms (23ms, 16.5ms) at 5% MVC; however, at 0% MVC the latency was significantly longer, and more variable at 34.5ms (52ms, 23ms). The IN latency data were transformed using a natural logarithm.

The subject ($p<0.001$), bite level ($p<0.001$) and stimulus level ($p<0.05$) all affected the strength of the IN. With an average strength of –12.5% (-16.4%, -8.5%) the IN in the absence of voluntary muscle activity was significantly lower than during a 1% MVC, -37.9% (-41.3%, -34.5%) or a 5% MVC, -36.1% (-39.9%, -32.4%). It was also found that a 6N stimulus elicited a stronger reflex than an 8N stimulus, -37.6% (-44.2%, -31.0%) compared to –30.3% (-36.0%, -24.5%), although this difference was only slight.

The duration of IN was not significantly altered by any of the experimental parameters and was found to be 19.5ms (20.5ms, 18.5ms).

8.3.2.3. LATE EXCITATION

If a reflex was excitatory and occurred later than 25ms following the commencement of the stimulus, but before the reaction time, it was defined as late excitation (E2). Subject ($p<0.01$), bite level ($p<0.001$) and stimulus level ($p<0.001$) were the parameters that affected E2 occurrence rate. The development of voluntary muscle activity increasing the occurrence rate from 15.8% (21.0%, 10.6%) at 0% MVC to 35.7% (42.2%, 29.2%) at 1% MVC and 36.1% (42.6%, 29.5%) at 5% MVC. It was also less likely that a reflex would be elicited in response to a 3N stimulus, 14.8% (19.6%, 10.0%) than a 6N, 36.3% (43.0%, 29.7%) or 8N, 38.6% (45.4%, 31.8%) stimulus.

In addition to the subject ($p<0.001$), the latency of E2 was affected by the interaction of bite force and stimulus force ($p<0.01$). During voluntary muscle activity the latency of the reflex elicited from a 3N stimulus was significantly shorter, 45.5ms (49.5ms, 41ms) than when a 6N or 8N stimulus was used, 57ms (59ms, 55ms). However, in the absence of voluntary muscle activity the latency was unchanged with stimulus force and was found to be 59ms (65.5ms, 53ms), significantly longer than in response to a 3N stimulus during voluntary muscle activity.

The strength of E2 required a logarithmic transform for normality and was affected by both the subject ($p<0.01$) and the bite level ($p<0.001$). As with many of the stimulus parameters it was the presence of voluntary muscle activity, not the level of activity, that influenced the strength of E2: 14.8% (17.6%, 12.5%) at 0% MVC, 37.9% (41.6%, 34.6%) at 1% MVC and 34.6% (38.3%, 31.2%) at 5% MVC.

The average duration of E2 was the only reflex statistic that was found to be different between the recorded muscles ($p<0.05$). While there was no difference between the left and right sides, the duration of E2 was significantly longer in the temporalis muscles, 26ms (28ms, 23.5ms) than in the masseter, 22ms (23.5ms, 20ms). A logarithmic transform was employed to ensure normality of the E2 duration measurements.

8.3.2.4. REFLEX IN BITE FORCE

If a reflex was seen in the bite force record following the stimulus rise phase and the associated overshoot (Chapter 7), it was defined as a reflex in the bite force (BR) and was found to only ever be excitatory. Different subjects had different occurrence rates of this reflex ($p<0.01$) while the interaction of bite level and stimulus level also influenced the occurrence rate of BR ($p<0.01$). BR was seen 5.9% (12.3%, 0%) of the time when a there was an absence of voluntary muscle activity, 8.3% (17.4%, 0%) if a 3N stimulus was

used and there was voluntary muscle activity and 39.7% (51.3%, 28.1%) of the time when there was voluntary muscle activity and a 6N or 8N stimulus was used.

The latency of BR was different for different subjects ($p<0.05$) and was later when a larger stimulus was used ($p<0.01$). In response to a 3N stimulus the latency was 56ms (58.5ms, 53ms) while a 6N and 8N stimulus elicited reflexes at 63ms (66ms, 60ms) and 71ms (75ms, 67ms) respectively.

None of the experimental parameters affected either the strength or duration of BR. The average strength observed was a 1.4% (1.6%, 1.2%) increase from the pre-stimulus average, while the average duration was found to be 40.5ms (45ms, 36.5ms).

8.4. DISCUSSION

The pattern of activity observed during this investigation displays characteristics similar to that found during static orthogonal (Louca *et al.*, 1998) and axial (Chapter 7) stimulation of the incisor. Ramped mechanical stimulation of the incisor produces short latency inhibitions and late excitation reflexes (Louca *et al.*, 1996). If the stimulus contains faster components, then it is also possible to elicit an early excitation due to the stimulation of muscle spindles (reviewed in Türker, 2002). Furthermore, if the stimuli are rapid taps rather than ramped stimuli, then a sequence of short- and long-latency inhibitions and excitations can be elicited (van Steenberghe *et al.*, 1981), not unlike the reflex response in the current study, but with inhibitions that are stronger and occur more frequently.

Unlike a number of studies on incisor teeth that have found PMRs to be responsible for the majority of the reflex activity observed (Sessle & Schmitt, 1972; Türker & Jenkins, 2000); it is clear from the results of this investigation, specifically due to the limited effect of local anaesthetic, that the PMRs are not the primary receptor responsible for the reflex activity observed during stimulation of the 1st molar tooth. It is also important to note that the relatively long duration of the experiment did not reduce the effectiveness of the local anaesthetic (van der Glas *et al.*, 1985). The length of this experimental paradigm was comparable to the study presented in Chapter 7 where the application of local anaesthetic had a large impact on incisal reflexes, and extra anaesthetic was applied if subjects reported a return of sensation to the target area.

The most likely source of the reflex activity seen in this study are receptors remote from the periodontal space such as the muscle spindles and/or those contained within the temporomandibular joint, which could be stimulated by the small jaw stretch following tooth stimulation.

8.4.1. RECEPTORS

Although reflexes caused by the stimulation of PMRs in incisor teeth are reported in response to stimuli as low as 0.25 N (Louca *et al.*, 1996), and a small number of receptors surrounding the molar teeth can be stimulated at the same force level (Johnsen & Trulsson, 2003), the current study has shown that at least 6N is required to elicit molar reflexes reliably in the jaw-closer muscles of humans. Experiments performed on rats (Tabata & Hayashi, 1994), monkeys (Byers & Dong, 1989) and humans (Trulsson & Johansson, 1996a; Johnsen & Trulsson, 2003) have shown that from the

incisors to the molars, the number of PMRs surrounding each tooth decreased along the dental arch, with the fewest receptors found around the molar teeth. It is possible that these reduced numbers of PMRs around the roots of the molar teeth are only present to contribute in a protective capacity, and as such they may only be a factor when a force occurs on a molar tooth very rapidly or at force much stronger than the 8N used in this study.

The average loads for denture wearers chewing most foodstuffs is close to the stimulation forces used in the current study, in the range of 3 to 18N (Yurkstas & Curby, 1953). However, the mean maximum load on a single healthy molar tooth during chewing of normal foodstuffs is reported to be more than 10 times the stimulation forces used (Anderson, 1956b), while the lower range of bite forces are close to 8N (Anderson, 1956a) or even higher (Slagter *et al.*, 1992). Hence, a future study involving a protocol utilising both faster and larger force applications is required to rule out PMR involvement in molar reflexes completely.

Few histochemical studies have been performed on nerve endings in the human periodontal space. One study on the nerve endings surrounding the first premolar classified three distinct types of endings: encapsulated corpuscles, bush-like endings and free-nerve endings (Fukuda & Tazaki, 1994), but no Ruffini endings, which are believed to be the primary mechanoreceptors in the periodontal ligament (reviewed in Wakisaka *et al.*, 2000), while another concluded that the role of a number of nerve endings in the human premolar might be the control of blood flow, not the encoding of force (Nakamura *et al.*, 1992). The above mentioned studies, along with the current finding of little to no PMR involvement in jaw muscle reflexes at low to medium force levels means that the presence of a small number of mechanoreceptors around the molar can not be ruled out, although the possibility is slight.

8.4.2. REFLEXES

While the possibility exists that many, if not all described reflexes observed following early excitation are artefactual (Türker & Powers, 1999), the increased size and occurrence rate of late excitation compared to early excitation are evidence to the contrary. As the muscle spindles are the likely agent behind the excitatory reflex activity, evidence of inhibitory events must be either artefactual, the result of a phase advancing action potentials (i.e. 'silent-periods'), or via some residual PMR activity occurring in a neighbouring location due to mechanical coupling of the teeth (Linden *et al.*,

1995; Trulsson & Johansson, 1996a; Johnsen & Trulsson, 2003). Although the inhibition was weaker and less frequent than the early excitation, it was not possible to determine if the inhibitory event was real or an artefact, as this requires single motor-unit recordings, and the construction of peri-stimulus frequencygrams (Türker & Cheng, 1994).

One possible source of error with the analysis method used is that the CUSUM is very sensitive to slight, but consistent changes in the EMG, especially at the low levels of activity used in this study. Hence it is likely that event though the occurrence rates were very low, a number of the reflexes found during the 0 activity level trials were the result of postural changes (i.e. a small change in jaw position with the stimulus) rather than true reflexes. This is particularly true when looking at the 'inhibitions' during a trial with no voluntary muscle activity, the presence of which highlights the difference between zero voluntary contraction and an absence of recordable muscle activity.

8.4.2.1. EARLY EXCITATION

The latency of early excitation found in this study was earlier than that found in incisor studies (Türker & Jenkins, 2000), and the strength and occurrence rates were higher (Chapter 7). Overall, the results suggest that the muscle spindles may play a greater role in the reflexes elicited in the molar region than in the incisor region. This is possibly due to the larger and more extensive root structure of the molar teeth, meaning that they are fixed more strongly to the jawbone; hence more vibrations are likely to be translated through the tooth to the muscle spindles, as there is less mechanical damping than in incisors. Furthermore, the bite force records from the lower jaw suggest that while the upper jaw moved up slightly, the lower jaw stayed in position. This slight upwards movement of the head would stretch the jaw muscles since the lower jaw cannot move with the rest of the head as it is attached to a solid lower bite bar. This slight stretch stimulus is sufficient to generate a muscle spindle based excitatory reflex in the jaw-closers (Türker & Jenkins, 2000).

8.4.2.2. INHIBITION

The latency of the inhibitory reflex seen in this study was comparable to that seen in incisal studies utilising horizontal loading (Yang & Türker, 1999), horizontal unloading (Türker & Jenkins, 2000) and axial stimulation (Chapter 7). However unlike previous results (Brodin *et al.*, 1993b) the application of local anaesthetic did not influence the occurrence rate and

strength of the reflex indicating it was not the result of PMR activity around the stimulated molar tooth. One possible source of this result is that the reflex is caused by the activation of PMRs located around the premolar and/or front teeth which were not anaesthetised and may have been activated as a result of the mechanical coupling known to exist between teeth (Johnsen & Trulsson, 2003). It is also possible that the movement of the head against the lower bite bar during the application of the stimulus generates extra pressure on all the lower teeth hence involving a PMR related reflex that would not be abolished by the application of local anaesthesia around the upper molar teeth. The other possibility, as discussed above, is that the inhibition is not real and merely the result of the phase advanced, and hence synchronised, action potentials by the preceding excitatory response inducing a silent period in the EMG.

8.4.2.3. LATE EXCITATION

The occurrence of late excitation has been previously described in the human masseter in response to both stretch (Miles *et al.*, 1995) and unloading (Miles & Poliakov, 1997) of the jaw. Although at approximately 45% (with muscle contraction and a stimulus of 6 or 8N), the reflex was less common with molar stimulation than the late excitation occurrence rate of 72% found during incisal stimulation (Chapter). However, it is possible that some or all of the late excitatory reflexes found during incisal stimulation are artefacts of the strong preceding inhibition, something that did not occur in molar stimulation. The receptors involved in this reflex, as in other molar induced reflexes as mentioned above, are most likely to be spindles, temporomandibular joint receptors and/or other non-anaesthetised PMRs.

8.4.2.4. REFLEX CHANGE IN BITE FORCE

The BR was only excitatory and occurred in close association with the late excitatory reflex response. This is similar to previous research (Poliakov & Miles, 1994; Miles *et al.*, 1995) where a change in bite force was associated not with the jaw jerk induced monosynaptic spindle reflex but with the slow stretch induced late excitatory reflex. They found that the interspike interval underlying the short latency reflex was not greatly altered, but the interspike intervals underlying the long latency reflex was overtly shortened which was the cause of the prominent increase observed in the bite force. Although applied on the maxillary molar tooth, the stimulus used in the current study is similar to the stretch stimulus used previously and hence is likely to induce similar short and long latency reflexes originating from the spindles,

non-anaesthetised PMRs and temporomandibular joint receptors and generating an explicit increase in the bite force immediately following the late excitatory reflex response.

8.4.3. EXPERIMENTAL PARAMETERS

All reflex events in this study showed that the reflex strength increased as a result of the presence of some voluntary muscle activity, regardless of its magnitude. This common response pattern during the presence of voluntary activity, and the decreased strength and occurrence of inhibitory reflexes compared to incisor studies, suggests a reduced degree of fine reflex control in the posterior region of the jaws when compared to the anterior. Reflex duration was largely unaffected by the experimental factors, and while slightly longer than those described previously (Chapter 7), they remain comparable.

The reflexes observed were common in all recorded muscles with only small variation in the reflexes between muscles, this was despite the fact that only the right temporalis muscle was used as feedback and consequently was the only muscle guaranteed to be contracting at the specified level. There may be concerns relating to the legitimacy of the data from the remaining three muscles due to the possibility the contraction level may have been different. However, the general tendency of the jaw is to counter-balance the 'active' side with an appropriate level of force to reduce the threat of strain to the temporomandibular joint (Dessem & Taylor, 1989), and previous experiments on masseter stretch receptors have shown a high degree of reflex symmetry (van der Glas & van Steenberghe, 1988; Miles & Poliakov, 1997). Furthermore, this experiment has shown that it is the presence of voluntary muscle activity, not the level, which is of primary importance.

8.4.4. METHODOLOGICAL CONSIDERATIONS

Molar stimulation in humans has been attempted in the past (Yamamura *et al.*, 1993); however, the study had numerous methodological issues that made the interpretation of the results difficult. Of paramount importance was the choice of a one second post-stimulus period for reflex analysis. Since it is known that the reaction time of masseter muscles to incisor stimulation is far below this value at 140ms (Brodin *et al.*, 1993a), and this experiment has established the reaction time for molar stimulation to be 103ms for

temporalis and 168ms for masseter muscles, the majority of the measured response in the above molar stimulation study was not reflex in origin.

It has been shown that stimulation of the skin can cause a net increase in the EMG level seen during stimulation of human teeth (Tucker & Türker, 2001). Hence the nosepiece used to secure the subject's head position and ensure that the vertical forces applied to the teeth were balanced may have caused a change in the evoked reflexes. Future experiments, utilising a topical local anaesthetic on the nose are required to rule out this possibility.

There are a number of methodological limitations when using surface EMG, not the least of which is cross talk between the muscles of interest and others near-by (Türker, 1993). It has been found that a surface EMG signal detected above a muscle in the leg, and having a peak-to-peak amplitude of up to 16.8% of a signal detected above a neighbouring muscle, may be due to cross talk rather than to activity of the muscle below the electrode (De Luca & Merletti, 1988). This is even more relevant when recordings are taken from the face, because of the close proximity of a number of muscles. Recordings from the masseter may also contain activity from muscles such as the zygomaticus major, platysma and risorius, while recordings from the temporalis may contain activity from the orbicularis oculi or other near-by muscles. To overcome this potential source of noise it would be necessary to perform intra-muscular recordings from the muscles of interest.

8.4.5. CONCLUSIONS AND IMPLICATIONS

Although maximum bite force is much higher in the molar region than in the incisors, and breakdown of the morsels occur in the molar region, this study has shown that PMRs around the molar teeth do not contribute significantly to the development of bite force when the stimuli are directed vertically and are less than 8N. However, the existence of a positive feedback loop that originates from receptors other than the molar PMRs is obvious. The divisions between the anterior and posterior regions of the dental arch are now somewhat clearer with decreased sensory feedback within the posterior region, at least under static conditions.

Before any regional generalisations are made a future investigation will be required to ascertain the activity present at 10 - 20% MVC, which is described in one study as the maximum force observed during natural chewing behaviour (Anderson, 1956b), while another claims that the upper limit is closer to 60% MVC (Slagter *et al.*, 1992). It will also be necessary to utilise both faster and stronger stimulus profiles. Most importantly, it is

crucial to determine if these results hold during rhythmic movement of the jaw since animal studies have shown that modulation of jaw reflexes during movement occurs not as an exception but as a rule (reviewed in Lund, 1991).

9. EMG, Force and Discharge Rate Analysis of Human Jaw Reflexes in Response to Axial Stimulation of An Incisor Tooth

Reflex studies utilising controlled stimulation along the long axis of human incisors are relatively new, and the effects that various stimulus parameters have on the elicited reflex are not fully understood.

Twelve subjects were recruited to determine the effect of varying contraction level, stimulus force and amount of constant force applied between stimuli on the reflex response of the masseter muscle. Multi-unit intra-muscular electromyogram (EMG) was recorded alongside surface EMG to determine whether any differences existed between the two. Furthermore, cumulative peri-stimulus dischargegrams were constructed to determine whether events seen in the EMG were real or artefactual.

Axial stimulation of the incisor induced a response in the EMG comprising of peak-trough-peak, with the trough the most dominant. The bite force record showed only a reduction (relaxation) in response to the stimulation. The most significant experimental factor affecting the reflex occurrence and strength was the stimulus force, while subject-to-subject variation influenced almost all recorded parameters. Although the latency, duration and occurrence rates were not significantly different, the strength of the responses was greater in intra-muscular recordings compared with the surface recordings.

Discharge rate analysis showed that approximately two-thirds of late excitations detected in the EMG did not correspond to an increase in the firing rate of the underlying units; hence they were due to the clustering of action potentials following the inhibition and not to a change in the membrane potential of the motoneuron. It was also found that the duration of the trough, as seen by the reduced cumulative discharge rates in the underlying units, was longer than indicated by the EMG.

This chapter is an edited version of the manuscript "EMG, Force and Discharge Rate Analysis of Human Jaw Reflexes in Response to Axial Stimulation of the Incisor" by R.S.A. Brinkworth, and K.S. Türker, which has been published in Experimental Brain Research *In Press* (2004).

9.1. INTRODUCTION

Mechanical stimulation of teeth evokes sensations of touch, pressure and pain (Anderson *et al.*, 1970), and produces reflexes (Hannam *et al.*, 1968; Hannam & Matthews, 1969; Sessle & Schmitt, 1972; Brodin *et al.*, 1993a) that are likely to be important in mastication (Ahlgren, 1969; Ottenhoff *et al.*, 1992b; Abbink *et al.*, 1999b). Human studies have shown that these reflexes are present mainly in the jaw closing muscles, not the jaw-openers (Goldberg, 1976; Lund & Olsson, 1983; Yamada *et al.*, 1985; De Laat, 1987b; Louca *et al.*, 1996; Türker & Jenkins, 2000).

Animal studies (Byers & Dong, 1989) have shown that the majority of periodontal mechanoreceptors (PMRs) are located close to the apex of the tooth root with a fewer number located closer to the middle of the tooth root. It is also known that PMRs respond to tension, but not compression (Cash & Linden, 1982). Orthogonal stimulation, which historically was the preferred method of experimental tooth stimulation, activates both groups of receptors, so it is not possible to determine the contribution of each. Applying stimuli along the long axis of the tooth thereby reduces the involvement of receptors at the root apex making it possible to determine the contributions of receptors at different positions around the tooth.

The human masseter is one of the main jaw-closer muscles and is divided into two parts: superficial and deep. The superficial portion is more active in force production whereas the deep portion, with its vertically orientated muscle fibers and a high density of large and complex muscle spindles (Eriksson & Thornell, 1987; Eriksson *et al.*, 1994), is believed to be more involved in the postural control of the mandible (Eriksson *et al.*, 1984; Hannam & McMillan, 1994). During incisal biting the superficial masseter is active at 86% while the deep portion of the masseter is only active at 47%, compared to full activity in both during intercuspal clenches (Blanksma & van Eijden, 1995). This difference in activity highlights how the different regions of the masseter can act independently. Thus, intra-muscular electromyography (IM-EMG) may yield different results to surface electromyography (SEMG) as, depending on the position of the intra-muscular electrodes, IM-EMG can be biased more towards the deep masseter while SEMG will have a higher contribution from the superficial masseter.

While there is no doubt that IM-EMG has superior recording characteristics (Türker, 1993), almost all studies into human jaw reflexes to date have used SEMG to quantify the reflex response (Ottenhoff *et al.*, 1992a; Takada *et al.*, 1995; Türker *et al.*, 1997; Louca *et al.*, 1998). Reasons for this may include the fact that IM-EMG recordings give a more localized view, which is not

desirable if the whole muscle response is required. Intra-muscular electrodes are much more invasive than surface electrodes, and IM-EMG recordings require more sophisticated recording and analysis equipment due to the higher frequency content (Basmanjian & De Luca, 1985). The main benefit of IM-EMG is that it contains less artefacts (cross-talk, stimulus and movement) than SEMG (Türker *et al.*, 1999). Additionally, IM-EMG is less susceptible than SEMG to interference from facial mimic muscles (Stohler, 1999). This is of particular importance in the measurement of masseter activity due to the proximity of zygomaticus major, platysma and risorius to surface electrodes.

When applying mechanical stimulation it is not possible to deliver an exact stimulus profile without the use of a preload (static force) between stimuli (Brodin *et al.*, 1993b; Türker *et al.*, 1997); however this practice has been criticized (Linden *et al.*, 1995) and doubts have been raised about which receptors are stimulated (Türker, 2002). By recording from the inferior alveolar nerve in humans and stimulating the teeth two different receptor types have been identified (Trulsson *et al.*, 1992; Trulsson & Johansson, 1994). The 'saturating' group, which are more active in response to static forces less than 1N and lose there dynamic sensitivity at very low forces; and the 'non-saturating' group, which display a linear response to forces above 5N and keep their dynamic sensitivity even during the application of preloads of several Newtons. Similarly, research on the characteristics of perio-receptors also divided them into similar groups; i.e. slow and rapid rate responsive (reviewed in Linden, 1990) that may correspond to the 'saturating' and 'non-saturation' groups respectively (Türker *et al.*, 1994). Therefore, it is necessary for the effect of preload to be elucidated so that the receptors responsible for the reflex activity observed during tooth stimulation to be identified, as a pre-load of 1N or above would block the dynamic sensitivity of the 'saturating' receptors hence allowing the nearly exclusive stimulation of 'non-saturating' (rapid rate responsive) receptors.

To investigate the connection between receptor systems and the central nervous system in humans, the accepted procedure is to electrically or mechanically stimulate the selected receptor system and record the resultant changes in the muscle electromyogram (EMG) using surface and/or intra-muscular electrodes. The two most common analysis techniques for quantifying these responses are full-wave rectification and averaging of the EMG record around the time of stimulation (Hannam *et al.*, 1969; Jenner & Stephens, 1982) and compilation of peri-stimulus time histograms (PSTHs) from single motor unit records (Stephens *et al.*, 1976). These techniques are probability based and rely on the principle that a significant increase in the discharge probability at a time after the stimulus represents an excitatory

postsynaptic potential while a significant decrease indicates an inhibitory postsynaptic potential. This assumption has introduced serious errors into the description of connections between various parts of the central nervous system. The errors in the analyses arise from the fact that the peaks and troughs in response to a stimulus, which represent increases and decreases in discharge probability, can reflect not only direct synaptic effects, but also secondary effects arising from the discharge probabilities of the pre- and post-synaptic cells (Moore *et al.*, 1970). However, many researchers have not heeded this warning and a number of conclusions have been made from data analyses that may contain significant errors (Brooke *et al.*, 1999; Okdeh *et al.*, 1999; Sonnenborg *et al.*, 2000; Nadler *et al.*, 2004).

To overcome the errors caused by using probability-based methods a new method, the peri-stimulus frequencygram (PSF), has been proposed whereby the time between discharges is measured (Türker & Cheng, 1994). However, it was not until recently that the errors in the probability-based methods, and the ability of the PSF to overcome them, was proven (Türker & Powers, 1999). Nevertheless, the PSF has a number of drawbacks. Firstly, it is more difficult to perform than simply full-wave rectifying and averaging EMG data, and secondly it is significantly more time consuming. A typical rectifying and averaging experiment requires approximately 50 stimuli (Chapters 6, 7 and 8) while a traditional PSF (or PSTH) experiment may require many hundreds to correctly identify subtle reflexes. To overcome the problem of trial duration, a method to use multi-unit rather than single-unit records to create PSFs has been proposed. This approach has been proven to work in brain slice preparations where all parameters can be modified and tested under tightly controlled conditions (Türker & Powers, 2003). This new method is now called cumulative peri-stimulus dischargegram (CPSD), in recognition that it is the cumulative discharge rates of multiple units that are being analysed, rather than the discharge frequency of a single unit.

The hypothesis considered here was that by using the CPSD it would be possible to determine whether the cumulative discharge rates of the underlying motor units were altered in the same way as indicated by the rectified averaged EMG. Or if some of the changes seen in the EMG were artefacts of the averaging process (Moore *et al.*, 1970), without the need for complex spike-recognition software or hundreds of stimuli in each experimental condition.

In Chapter 7 it was shown that axial stimulation of the upper left central incisor evokes a reflex response in the masseter, but not in the digastric. The in the masseter response is triphasic and consists of an early peak, a trough and then a late peak. The results indicated that the rate of force delivery, and to a lesser extent the level of muscle activity, influenced the

characteristics of the responses evoked. It was also shown, through the use of local anaesthetic, that the early peak was a genuine excitatory reflex and possibly generated by the muscle spindles. The trough was a genuine inhibitory reflex, this time inhibitory, and was mediated by the periodontal mechanoreceptors. While the late peak was likely to be an artefact caused by the clustering of action potentials following the inhibition rather than the results of an excitatory post-synaptic potential to the motoneuron, although this could not be proven with the probability-based analysis methods used.

The present study extends the previous work by determining the changes evoked by altering stimulus intensity, preload (static force on the tooth between stimuli) and the level of muscle activity when controlled axial stimuli are applied to the human central incisor. Furthermore, the minimum reaction time for both increasing and decreasing voluntary muscle activity was established to facilitate discrimination between reflex activity and activity that may be a deliberate subject reaction. In addition to recording the total force generated by the jaw, recordings were taken from the left masseter using both intra muscular (IM-) and surface (S) EMG to determine whether there were any differences between the two recording types using traditional analysis methods. Finally the IM-EMG was analysed using the CPSD method, adapted from the literature (Türker & Powers, 1999 & 2003), to find the cumulative discharge rates underlying the EMG and determine whether the reflexes seen in the EMG corresponded to a change in the discharge rates of the underlying motor units.

9.2. METHODS

9.2.1. SUBJECTS

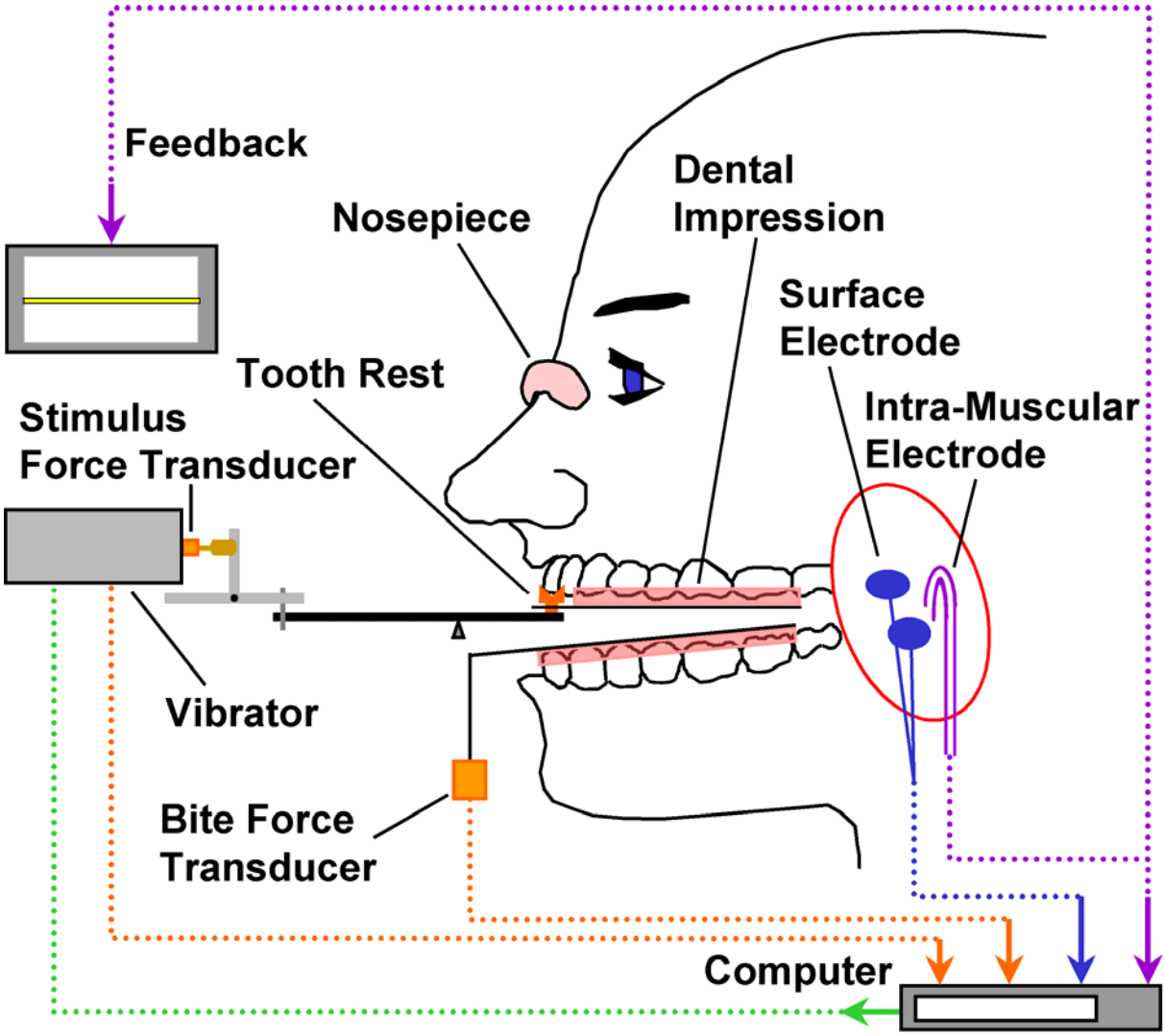
Written informed consent was obtained from twelve adult volunteers (ten males and two females) with healthy teeth and gums, and no history of orthodontic treatment or dysfunction. The age range of the subjects was 19 to 39 years with the average being 23. The experiments were approved by the Human Ethics Committee of The University of Adelaide and conformed to the Declaration of Helsinki. All subjects were blind to the outcome. Since the general details of the experimental set-up have been given in Chapter 7 only a brief description is provided below.

9.2.2. PROCEDURE

Subjects bit into impression material (Formasil II, Heraeus Kulzer-Wehrheim) mounted on two bite plates with their upper left central incisor on a tooth rest. The mean separation distance of the plates was set to 12mm (Türker, 1988), and an axial tooth stimulator, shown in Figure 9.1, was used to stimulate the incisor along the long axis of the tooth via the tooth rest. Movement of the subject's head was minimized by the use of a nosepiece, which also counteracted the axial forces applied to the tooth. White noise was played through headphones to mask any sound made by the tooth stimulator (Meier-Ewert *et al.*, 1974; van der Glas *et al.*, 1988; Sato *et al.*, 1994).

Surface electrodes were placed on the left masseter to record the SEMG. In order to record multi-unit intra-muscular activity, two Teflon® insulated silver wires (100μm diameter) were inserted approximately 1cm into the left masseter to ensure recordings from the deep masseter muscle. Both EMG methods were recorded for all subjects. The EMG signals were amplified (3000x) and high-pass filtered (5Hz) before recording. The IM-EMG was full wave rectified and low-pass filtered (0.1Hz), then presented to the subject on an oscilloscope screen for feedback. The force applied to the incisor was recorded for future reference. The bite force was recorded via a transducer on the lower bite bar in order to study the net mechanical response of the masticatory muscles to the stimulus. All data channels were sampled at 12-bits and 5kHz using specially designed software (LabView®, National Instruments) described in detail in Appendix 1. There was negligible aliasing as the frequency content of the recorded signals over 2.5kHz was minimal.

Figure 9.1: Experimental Set-Up – Discharge Rate Experiments



The subject bite into impression material mounted on two bite plates with their upper left central incisor resting on a tooth rest. Movement of the subject was further minimized by the use of a fixed nosepiece, in addition the nosepiece counteracted against axial forces applied to the tooth. A computer produced the desired force profile randomly between one and three seconds. Two force transducers, one located in the vibrator arm and the other below the lower bite plate, picked up the stimulus force applied to the tooth and the total force generated by the jaw respectively. The master EMG activity was recorded from both surface and intra-muscular electrodes as well as the outputs of both force transducers; in addition, the intra-muscular EMG activity from the masseter was fed into a filter box and used for online feedback.

The stimulus profile used was a half-sinusoid with time to maximum of approximately 20ms followed by a constant force held for 100ms. The tooth was stimulated at random intervals of between 1 and 3 seconds. Throughout the experiment, two levels of muscle contraction (5% and 10% of maximum voluntary contraction - MVC), three different stimuli (1, 2, 3N) and three different preloads (0.5, 1, 1.5N) were used. In total, 18 different conditions were employed in the study, in addition, three trials at 0% MVC were utilised to establish background noise levels.

Nine of the subjects participated in trials to determine the minimum reaction time. Two reaction conditions were tested: an increase in muscle activity in response to the stimulus and a decrease in muscle activity in response to the stimulus. Subjects were asked to bite at a constant 10% MVC until they felt a stimulus, at which time they were either to stop biting or bite harder, depending on the trial condition. To give the subject sufficient time to react the inter-stimulus interval was increased to a random period of between 8 and 10 seconds.

9.2.3. DATA ANALYSIS

Offline analysis involved zero-phase band-pass filtering the EMG signals (surface 5-500Hz, intra-muscular 50-1500Hz), rectifying the signals, extracting a defined time period from around the stimuli (for reflex trials pre- and post-stimulus times were set at 100ms; for reaction trials the pre-stimulus time was 100ms and the post-stimulus time, 400ms), averaging them for each trial (n=50 for reflex and n=20 for reaction) and finally zero-phase low-pass filtering (surface 200Hz, intra-muscular 500Hz).

Cumulative sums (CUSUMs) of the normalised averaged EMG data were constructed (Ellaway, 1978), from which a specially written computer program extracted various reflex characteristics such as latency, duration and strength. The EMG and force analysis procedures are detailed in Chapters 6 and 7 respectively, hence are only covered briefly below.

When averaged over a sufficiently large number of trials, the noise in a rectified EMG signal that is not time locked to the stimulus becomes approximately constant. This noise was estimated for each subject and each recording method by finding the minimum pre-stimulus value in the average of the three no contraction trials. This value was then subtracted from all other EMG levels to give an almost noise free signal.

EMG traces were full wave rectified and normalised to the pre-stimulus average. All EMG reflex strengths were calculated as a percentage of the

theoretical maximum inhibition (Chapter 6) i.e. complete cessation of activity was defined as -100%. Due to the need to overlook reflexes in the reaction time experiments, the error box (minimum deflection classed as significant) was increased from 200% of the maximum pre-stimulus variation to 300%. This ensured only the largest variations in the CUSUM were used.

Averaged bite force records were assembled in the same way as EMG records but normalisation consisted of subtracting the average pre-stimulus bite level from the trace to obtain a change in bite force that could be compared between trials that had different pre-stimulus averages. The bite force was averaged, then zero-phase low-pass filtered at 150Hz. A change in bite force was classified as a reflex only if the strength was greater than twice the largest pre-stimulus variation (similar to the error box approach for the EMG records).

9.2.4. DISCHARGE RATE ANALYSIS

The method used for the discharge rate analysis of the multi-unit IM-EMG was adapted from the literature (Türker & Powers, 2003). The details are described below and illustrated in Figure 9.2.

The multi-unit IM-EMG trace was zero-phase high-pass filtered at 1kHz (differentiation) and extracted around the time of the stimulus as described above (Figure 9.2A). Individual traces were then passed through a threshold detector to produce acceptance pulses. The threshold used depended on the amplitude distribution of the individual trace and was set between the 90th and 97th percentile values (approximately three times the mean of the rectified pre-stimulus values). This method selected a small number of large amplitude motor units for analysis. Figure 9.3 shows a typical amplitude distribution and the effect of selecting different threshold levels on the corresponding CPSDs.

After the threshold had been selected, and threshold pulses generated (Figure 9.2B), acceptance spikes were calculated by removing pulses that were closer to the previous pulse than the desired minimum time limit (10ms for trough analysis, 100 impulses/s limited, Figure 9.2C; and 2ms for peak analysis, 500 impulses/s limited, Figure 9.2D). The instantaneous discharge rate of each acceptance spike was determined by comparing the time of the current acceptance spike with the previous one. Instantaneous discharge rates belonging to each trial were then superimposed and averaged traces were generated by calculating the mean value for all data points at each time bin (Figure 9.2E and 9.2F). The averaged traces were then low-pass filtered

and smoothed (Figure 9.2G and 9.2H) so that the reflexes could be clearly identified.

To determine whether the changes seen in the CPSD were large enough to be classified as reflexes, CUSUMs were constructed and analysed in the same way as with averaged rectified EMG traces. According to the literature, for a peak to be identified as an excitation the cumulative discharge rate underlying the peak in the rectified averaged EMG must display a significant increase in the 500 impulses/s limited CPSD. Alternatively, for a trough to be identified as an inhibition the cumulative discharge rate underlying the trough in the rectified averaged EMG must display a reduction in the 100 impulses/s limited CPSD (Türker & Powers, 1999 & 2003).

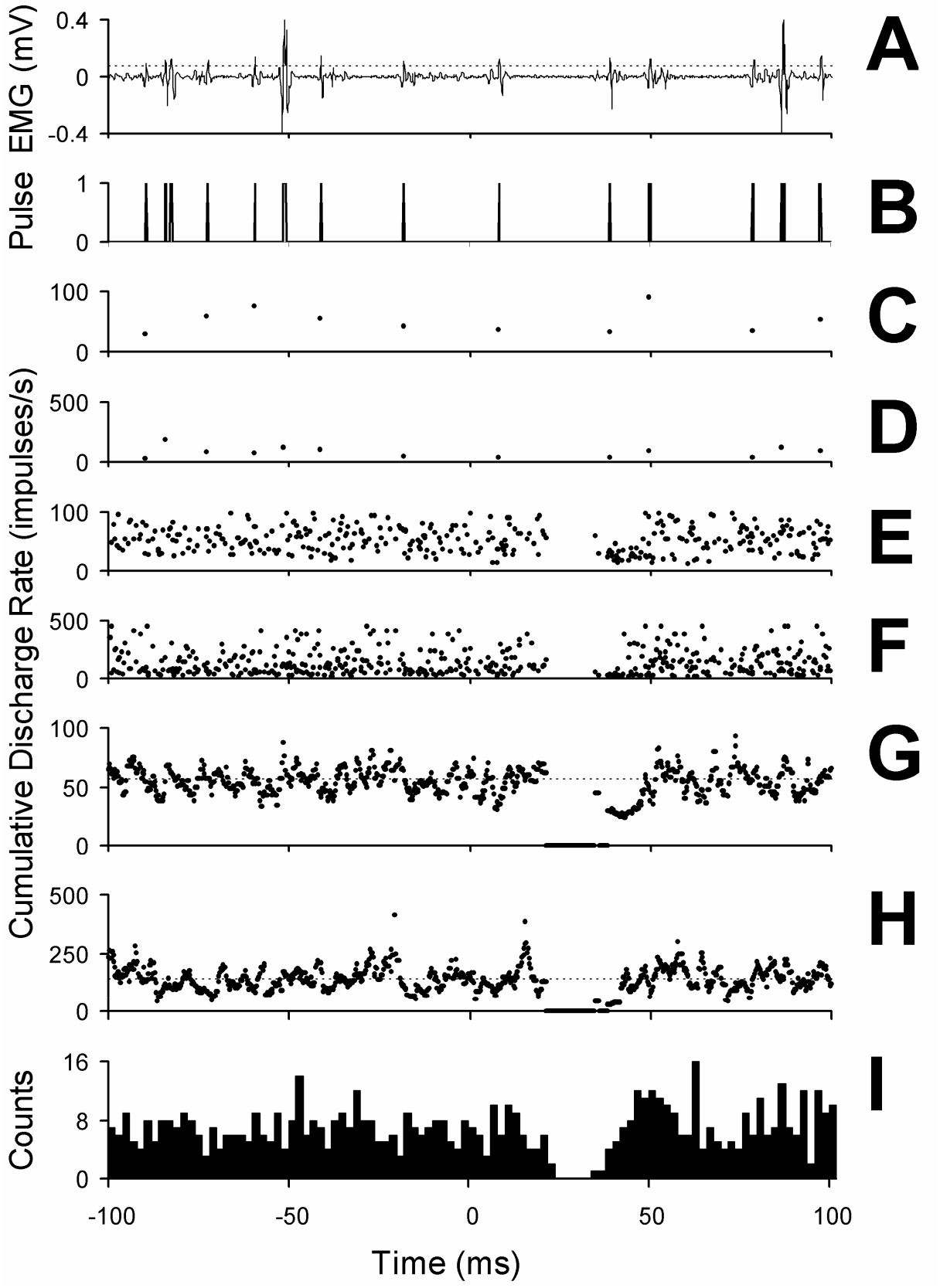
9.2.5. STATISTICAL ANALYSIS

To establish what, if any, effect the stimulus parameters had on the recorded values, an analysis of variance (ANOVA) was performed. If a parameter was determined to be significant then a Bonferroni post-hoc test was performed. The model used looked for main effects as well as two-way, three-way and four-way interactions between experimental parameters (analysis/recording type, bite level, stimulus force and preload). In addition to the experimental parameters a subject parameter, unique to each participant, was included in the statistical model to ascertain whether there were significant differences between subjects.

Binary logistic regression was performed to ascertain whether any of the experimental parameters affected the number of reflex occurrences. Chi squared tests were used to discover where any differences were.

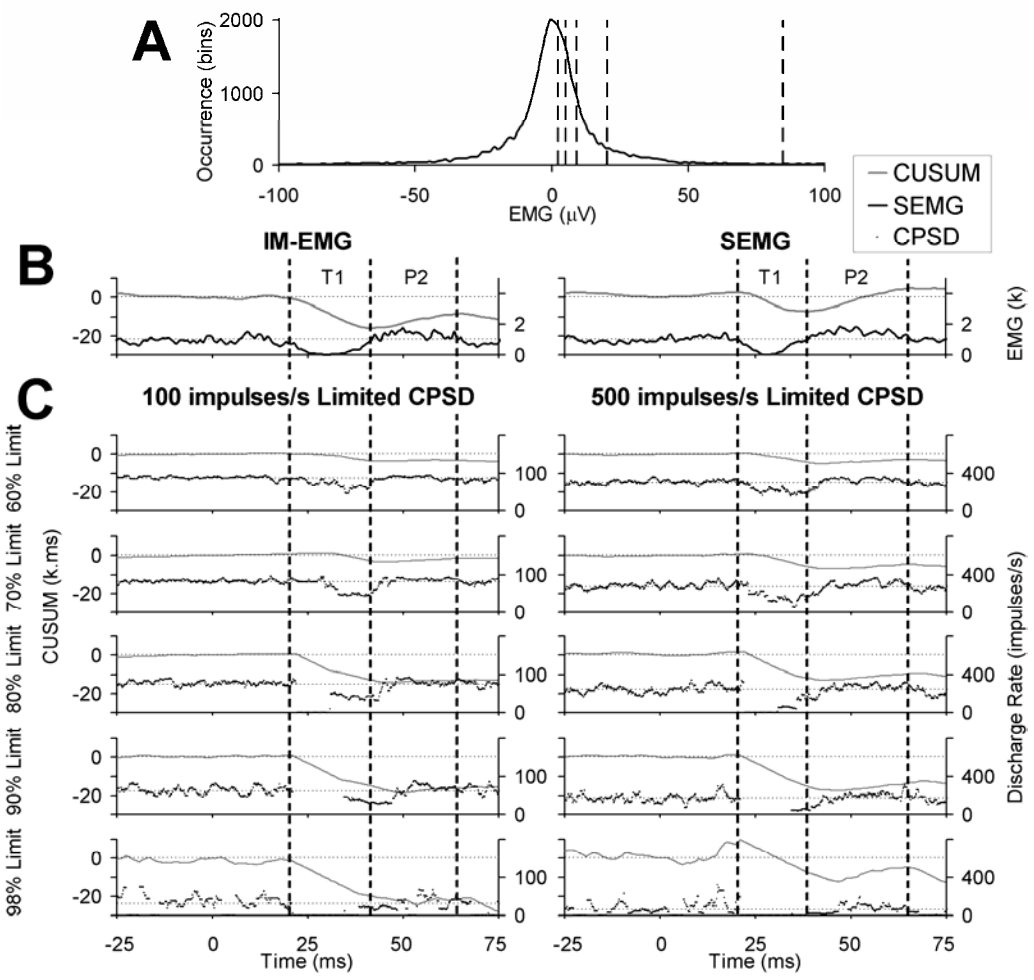
For all tests, the level of significance was 5% (i.e. $p<0.05$) and only significant results are reported. Results are given in the form mean ± 95% confidence interval; time calculations are given to the nearest 0.2ms; strength and occurrence rate calculations are given to 0.1% accuracy.

Figure 9.2: Calculating Cumulative Peri-Stimulus Dischargegrams



Steps involved in calculating cumulative peri-stimulus dischargerams (CPSDs). A) Extract the desired pre- and post- stimulus times from around the commencement of the stimulus (time 0). A longer time should be extracted than is to be analysed to ensure the discharge rates of the units at the start of the analysis time can be found. Zero-phase high-pass filter the trace (1kHz) and compare the trace with a threshold (97^{th} percentile value used). B) Create acceptance pulses whenever the high-passed trace crosses the threshold. C) To facilitate the detection of troughs remove all pulses that are closer than 10ms to the previous pulse and calculate the instantaneous cumulative discharge rate at the time of each of the remaining acceptance pulses. D) For peak detection, proceed as with troughs but use a 2ms window. Superimpose the instantaneous cumulative discharge rate values from all traces in the condition for E) trough detection and F) peak detection. Filter the averaged traces to produce CPSDs for G) trough detection and H) peak detection. I) The multi-unit peri-stimulus time histogram (bin width 2ms), included to show the increased number of counts following the inhibitory reflex during which the CPSD illustrated continued inhibition G). The stimulus parameters were 5%MVC, 2N stimulus and 0.5N preload, the number of averaged stimuli traces was 51.

Figure 9.3: Cumulative Peri-Stimulus Dischargegram Threshold Limits



Effect of different threshold limits on the cumulative peri-stimulus dischargegram (CPSD). A) Amplitude distribution of multi-unit intra-muscular EMG (IM-EMG) trace showing 60%, 70%, 80%, 90% and 98% cut-off limits. B) Rectified and averaged intra-muscular (IM-) and surface (S) EMG. C) 100 impulses/s limited CPSD in left column and 500 impulses/s limited CPSD in right column. The areas of the graphs corresponding to a trough (T1) and late peak (P2) as seen in the EMG records have been marked. The trough is easier to see in the CPSD at higher threshold limits, however if the threshold is too high then the reduced number of detected spikes increases the variability in the trace making it difficult to reliably detect reflex changes. At no threshold limit is the late peak seen in both EMG records mirrored in any of the CPSD traces. In fact, as shown in the CPSDs at high threshold limits, the duration of the trough is much longer than seen in the rectified and averaged EMG. Thus indicating that the late peak is really a clustering of low discharge rate units occurring at the same time in every trace, i.e. the rising phase of an inhibitory postsynaptic potential, not an excitatory postsynaptic potential. The subject and conditions are the same as Figure 9.2.

9.3. RESULTS

Previous experiments have identified three groups of significant diversions in the CUSUM (i.e. post-stimulus deflections that are larger than the error box): early peak, trough and late peak. However, while a number of traces showed what may have been the start of an early peak, it was almost always cut-off by a trough before it was classified as significant, and the overall occurrence rate was only 3.5%; hence, only the trough and late peak are discussed in detail below. Only one mechanical reflex condition was observed: change in bite force due to reflex, which was always negative (i.e. a reduction/relaxation in bite force).

Troughs that were seen in both the rectified averaged EMG analysis and the CPSD analysis corresponded to true reflex events and have been labelled 'inhibitions', while those seen in the rectified averaged EMG but not in the CPSD have been classified as 'artefactual troughs' as they were not true reflex events (i.e. not caused by a change in the motoneuron activity). Similarly, peaks seen in both analysis methods have been labelled 'excitations' while those not found in the CPSD have been labelled 'artefactual peaks'. In only one case was an event observed in the CPSD but not in the rectified averaged EMG; this late peak was excluded from the analysis.

Table 9.1 shows the reflex parameters and the experimental parameters that had a significant effect on the measured values. Only EMG reflexes where the reflex was identified simultaneously in one of the EMG records and in the CPSD were included (i.e. a change in the rectified averaged EMG corresponded to a change in the discharge rate of the underlying units).

Table 9.1: Results of Analysis on Reflex Data – Firing Rate

Change	Type	Statistic	Affected By	Significance
Trough	Reflex	Occurrence	Bite*Preload	†
			Subject	†††
			Stimulus	†††
		Latency	Bite*Stimulus	†
			Bite*Recording	†
			Subject	†††
		Strength	Subject	†††
			Stimulus	†††
			Recording	†††
		Duration	Subject	†††
			Recording	†††
	Artefact	Occurrence	Bite*Stimulus	††
		Latency	Bite*Stimulus	†††
			Bite*Preload	†
			Subject	†††
		Strength	Subject	††
		Duration		
Late Peak	Reflex	Occurrence	Subject	†††
		Latency	Subject	††
		Strength		
		Duration	Stimulus	†
	Artefact	Occurrence	Bite*Stimulus	†††
			Subject	†††
		Latency	Subject	†††
		Strength	Subject	†††
			Stimulus	††
		Duration	Subject	†
Bite Force		Occurrence	Subject	††
			Stimulus	†††
		Latency	Subject	†††
		Strength	Subject	†††
		Duration	Subject	†††
			Preload	††

The results of the statistical analysis on changes found in the EMG records of the masseter in response to axial stimulation of the incisor. Early peak is not included due to the low occurrence rate. Type refers to whether an event was found in both the EMG and discharge rate analysis (Reflex) or only in the EMG (Artefact), it does not relate to the reflex in the bite force. * indicates an interaction between two conditions. † represents $p<0.05$, †† and ††† represent $p<0.01$ and $p<0.001$ respectively. Almost every reflex statistic measured was found to be significantly different between subjects, illustrating that person-to-person variation is the source of the majority of differences observed. Some statistics were not influenced by any parameters and are identifiable by blank cells.

9.3.1. OCCURRENCE RATES OF EVENTS

The factors that influenced occurrence rates of events are illustrated in Figure 9.4. The occurrence rate of inhibition was unchanged with preload during a 5%MVC, with an average of 47.2 ± 6.7%. However, during a 10%MVC increasing preload caused a decrease in the occurrence rate from 54.2 ± 11.5% when the preload was 0.5N to 32.4 ± 11.1% when the preload was 1.5N. The difference with stimulus force was much greater, with an occurrence rate of 23.2 ± 6.9% at 1N, 50.0 ± 8.2% at 2N and 63.9 ± 7.8% at 3N. The occurrence rate of late excitation was low (8.4 ± 2.7%) and was unaffected by any of the experimental parameters. The reflex reduction (relaxation) seen in the bite force was influenced by the stimulus force: larger stimuli were more likely to elicit a response, with an occurrence rate of 23.4 ± 9.9% at 1N, 67.6 ± 10.9% at 2N and 75.0 ± 10.0% at 3N.

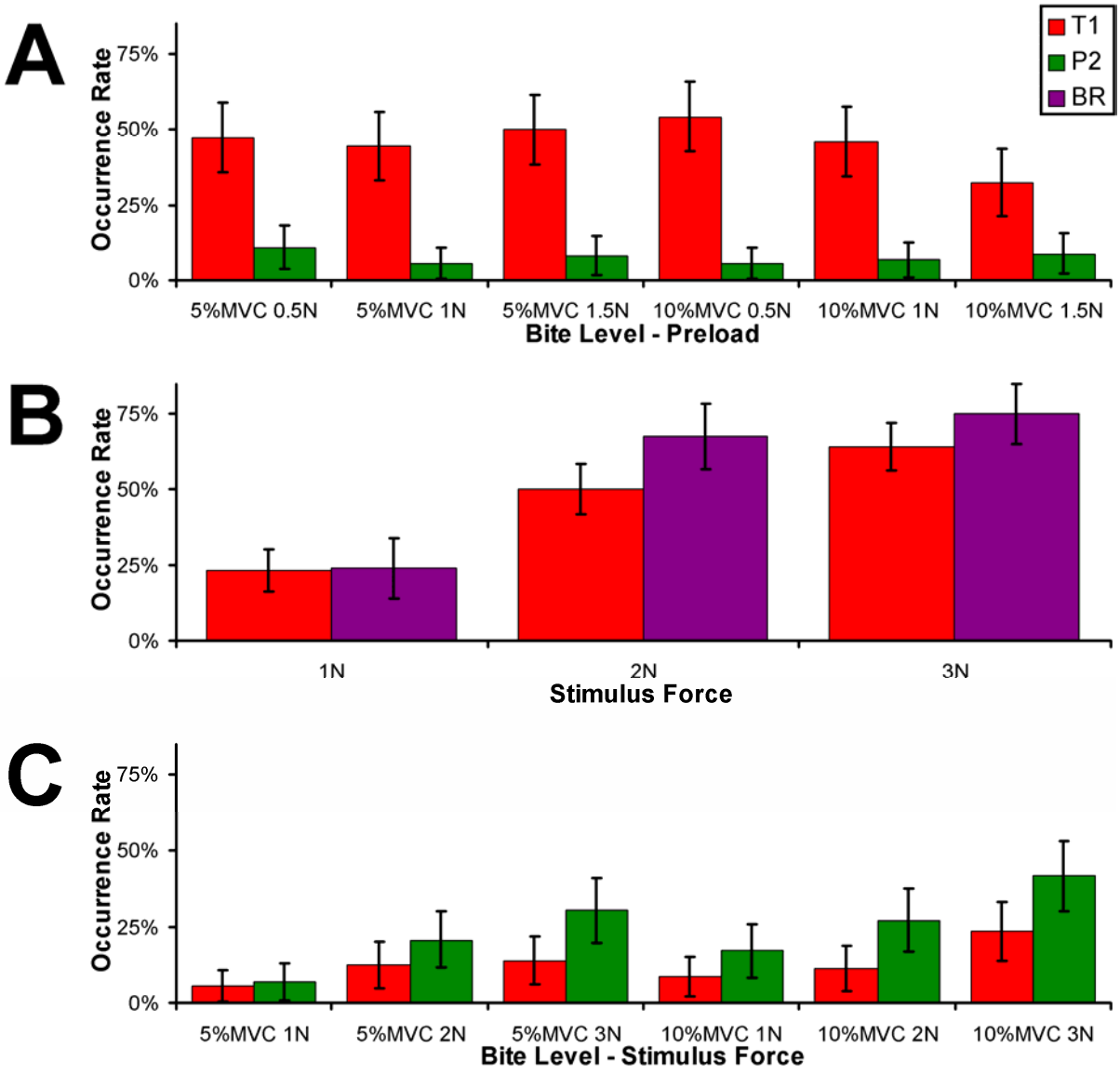
The occurrence of both artefactual troughs and late peaks was influenced by the combination of bite level and stimulus force. An increase in the stimulus force made it more likely that an artefact would be observed, and artefacts were more common at 10%MVC than at 5%MVC.

The overall occurrence rate of inhibitions was much greater than that of artefactual troughs (45.8 ± 4.7% versus 12.6 ± 3.1%) while the reverse was true for late peaks (genuine reflexes were observed 8.4 ± 2.7% of the time compared to 24.1 ± 4.0% for artefacts). No significant difference was found between the occurrence rates of reflexes seen in the rectified averaged IM-EMG compared with the rectified averaged SEMG records.

9.3.2. LATENCY

The average latency of the inhibition was found to be 20.8 ± 0.4ms, and while it was affected by the interaction of bite level and stimulus force, the difference was at most 1.6ms. There was also a difference between the latency found in the CPSD when compared with both the IM-EMG and the SEMG but only at 10% MVC: 19.8 ± 0.6ms for IM-EMG, 20.0 ± 0.6ms for SEMG and 21.6 ± 1.0ms for CPSD.

Figure 9.4: Reflex Occurrence Rates – Discharge Rate Experiments



Factors influencing occurrence rates of events in the masseter EMG. A) inhibition and late excitation occurrences at different masseter contraction levels and preloads. B) Occurrence rate of inhibition and reflex in bite force at different stimulus forces. C) Artefactual trough and artefactual late peak occurrence rates with bite level and stimulus force. The occurrence of inhibition was larger at stronger stimulus intensities, but decreased with increasing preload at 10% MVC. Late excitation reflexes did not vary with any stimulus parameter but are included to illustrate the low occurrence rate. As with inhibition, the occurrence of the reflex in the bite force increased with stimulus strength however it was not affected by contraction level or preload. Artefacts in the EMG were more likely to occur at higher stimulus intensities and levels of muscle activity. T1, P2 and BR indicate trough, late peak and reflex in the bite force respectively.

The average latency of the artefactual troughs was found to be 21.8 ± 1.4ms, not significantly different from that of the inhibition. The late excitation and artefactual late peak latencies were not significantly different at 46.0 ± 2.2ms and 43.0 ± 1.6ms respectively. The average latency of the reflex seen in the bite force was 29.6 ± 0.6ms, significantly longer than the inhibition latency with an average EMG/force delay of approximately 8.8ms. Neither the late excitation or artefactual late peak latency nor the latency of the reflex seen in the bite force were significantly influenced by any of the experimental parameters.

9.3.3. STRENGTH

The strength of the inhibition was significantly larger in the IM-EMG (-48.3 ± 2.6%) and CPSD (-48.8 ± 2.8%) recordings than in the SEMG (-43.9 ± 2.3%). It was also found that increasing the size of the stimulus increased the strength of both the inhibition (-44.4 ± 3.5% at 1N, -46.2 ± 2.4% at 2N and –48.8 ± 2.3% at 3N) and the artefactual late peak (27.44 ± 5.1% at 1N, 25.4 ± 2.7% at 2N and 32.6 ± 3.2% at 3N).

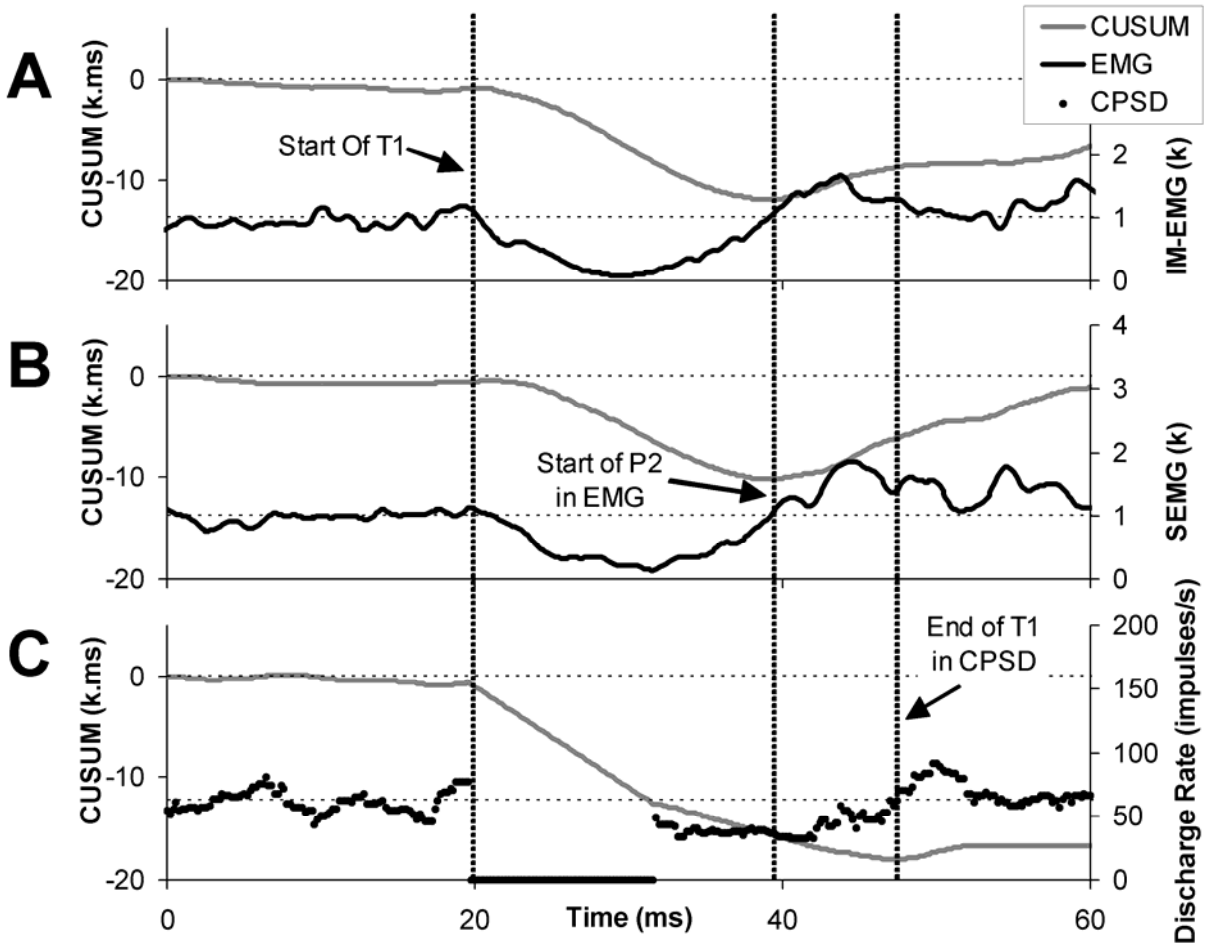
None of the experimental parameters affected the strength of the artefactual trough, late excitation or reflex seen in the bite force. At an average value of 30.3 ± 3.2%, the late excitation strength was not significantly different from that of the artefactual late peak, however the artefactual trough, with an average strength of –29.0 ± 2.7%, was significantly smaller than the inhibition. With an average decrease of 2.35 ± 0.2% from the pre-stimulus bite level the inhibition seen in the bite force was small but still distinguishable from the variability in the signals.

9.3.4. DURATION

The duration of the inhibition was significantly longer in the CPSD record (26.8 ± 1.0ms) than in either the IM-EMG (21.0 ± 0.8ms) or the SEMG (20.2 ± 0.8ms). It was also common to see the CPSD showing a reduced discharge rate at the same time that the intra-muscular and surface EMG showed an increased probability of firing. An example of this is shown in Figure 9.5.

The duration of late excitation in response to a 3N stimulus (19.4 ± 1.8ms) was significantly shorter than that in response to a 1N (26.4 ± 4.2ms) or 2N (27.8 ± 2.2ms) stimulus.

Figure 9.5: Probability Versus Discharge Rate Analysis



Difference between probability and discharge rate analysis. A) Averaged rectified intra-muscular EMG (IM-EMG) and the associated CUSUM. B) Averaged rectified surface EMG (SEMG) and the associated CUSUM. C) 100 impulses/s limited cumulative peri-stimulus dischagegram (CPSD) and the associated CUSUM. Both the intra-muscular and surface EMG show an increased probability of spikes following the trough (T1) commonly referred to as late peak (P2). However the CPSD shows that at the same time there is an increase in the EMG, the discharge rate of the underlying units is reduced. Thus indicating that this period actually represents the rising phase of hyperpolarization (i.e. the final phase of an inhibition), not the result of a depolarization in the associated motoneuron membranes (i.e. not the start of an excitation). The commencement of the stimulus was at time 0 and the trial conditions were 10%MVC and a 3N stimulus with 1.5N preload, the number of averaged traces was 44.

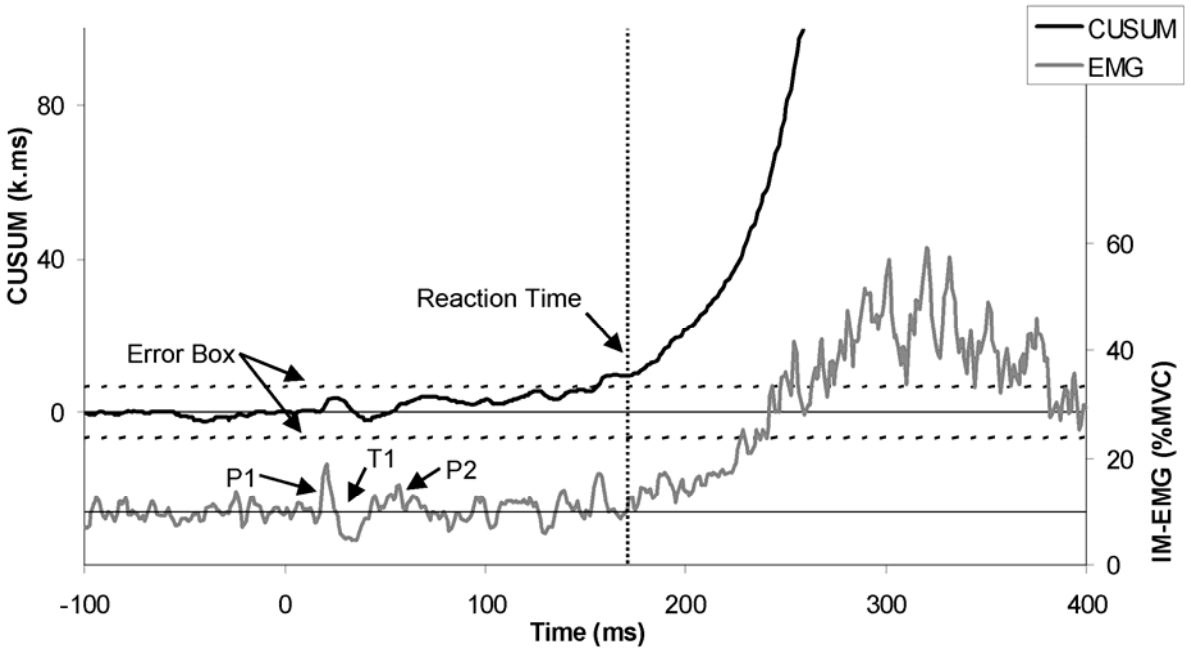
The durations of the EMG artifacts were not affected by any of the stimulus parameters, with the artefactual trough having an average duration of 18.8 ± 1.2ms and the artefactual late peak 20.8 ± 1.2ms. Both of these were shorter than found for the corresponding reflexes.

The reflex reduction in bite force was found to decrease in duration with increasing preload. The duration was 23.8 ± 1.6ms at a preload of 0.5N, 22.4 ± 1.4ms at 1N and 20.6 ± 1.6ms at 1.5N.

9.3.5. REACTION TIME

There were only minor (not significant) differences between 'increase' and 'decrease' experiments, individuals and recording methods. The average reaction times for IM-EMG, SEMG and bite force were: 193 ± 33ms, 198 ± 30ms and 196 ± 39ms respectively. Figure 9.6 shows a typical 'increase' reaction time experiment result. All three reflexes are visible before 75 ms but are too small to be detected owing to the increase in the error box level (see 9.2.3 Data Analysis). The reaction time for this trial was approximately 170ms, while the minimum reaction time for all subjects was established as 140ms for EMG and 150ms for bite force by taking the minimum of the averaged reaction time trials from each subject.

Figure 9.6: Reaction Time – Discharge Rate Experiments



Result of an 'increase' reaction experiment for one subject from the intra-muscular EMG (IM-EMG). It should be noted that the reaction time is not the time the CUSUM crosses the error box, but rather the time from the turning point (EMG = pre-stimulus mean) where the CUSUM continues to move in the same direction for a vertical distance equivalent to the size of the error box. The bite force record was similar to the CUSUM but not as steep. In addition to the reaction, early peak (P1), trough (T1) and late peak (P2) events are evident in the record, the number of averaged stimuli traces was 20.

9.4. DISCUSSION

The possibility that increased activity following silent periods in EMG recordings is due to the delay (clustering) of action potentials has been postulated for some time (Moore *et al.*, 1970; Miles *et al.*, 1987; Awiszus, 1988; Türker & Cheng, 1994); however, it has not previously been proven in the human masseter following mechanical tooth stimulation. By using a newly developed method for the construction of cumulative peri-stimulus dischargegrams (Türker & Powers, 2003) the present study has, for the first time, been able to show that the majority of late peaks found in the EMG of the human masseter following axial stimulation of the incisor are not associated with an increase in the cumulative discharge rate of the underlying motor units, and hence are artefacts of the averaging process.

The most significant experimental factor affecting the reflex evoked following axial stimulation of the incisor was the size of the stimulus: larger stimuli produced reflexes that were stronger and more frequent. The level of static force applied to the tooth between stimuli had an effect but only at a muscle contraction level of 10% MVC, not at 5% MVC (Figure 9.4A). However, the preload affected the net response of all jaw muscles, as seen in the bite force, at both contraction levels, by reducing the duration of the reflex when larger preloads were used. This suggests that not only the preload but also the level of muscle contraction determines the discharge rate of the PMRs. This result also indicates that the PMR output would be almost fully saturated when both the bite force and preload are strong (Trulsson, 1993). When a stimulus is delivered to the tooth while the output of the PMRs is nearly saturated, the discharge rate would change only a small amount, hence generating a weaker reflex.

The temporal characteristics of the masseteric reflexes were not altered by the EMG recording method (intra-muscular or surface EMG); however, the discharge rate analysis (CPSD) showed that the duration of inhibition was significantly longer than indicated by probabilistic analysis. It was also shown that the responses seen in intra-muscular recordings were significantly larger than those seen in the surface recordings.

9.4.1. SUBJECT DIFFERENCES

As with previous research (Lobbezoo *et al.*, 2001), there were highly significant inter-subject differences for all reflex statistics, indicating that the choice of subject is a critical factor in the determination of each reflex parameter. While it has been hypothesized that factors such as tooth

separation, tooth angle, periodontal health and even recent tooth usage may be important (Chapter 7), the effects that these factors have on the reflexes in the jaw are currently unknown.

9.4.2. TROUGHS

A trough was the most common, and strongest, response seen in the masseter. Prior experiments have used a local anaesthetic block to show that this reflex is the result of PMR activation (Brodin *et al.*, 1993b). Its latency corresponded well with other findings of 20ms for both horizontal loading (Yang & Türker, 1999) and unloading (Türker & Jenkins, 2000), and with the axial stimulation findings from Chapter 7 where the average latency was 19.5ms. However, the latency did not correspond to the latencies found with horizontal tapping, between 6 and 13ms (van der Glas *et al.*, 1984; Türker *et al.*, 1994). The discrepancy between tapping and pushing is likely to be due to the shape of the stimulus, tapping has a shorter rise time than pushing, hence should stimulate the receptors faster and more synchronously. The duration of the trough found in this study from the EMG traces, was similar to the 21ms found in Chapter 7 but not the same as the 37ms for orthogonal tapping (Türker *et al.*, 1994). This indicates that the direction and/or speed of stimulus application on the tooth are important factors in reflex duration. It was also found, by using CPSD analysis, that the true duration of the trough was almost 27ms, significantly longer than is found when analysing the EMG using probabilistic methods (rectifying and averaging around the stimulus) on both intra-muscular and surface recordings.

While a number of artefactual troughs were found in this study (changes seen in the EMG but not CPSD), both the strength and duration were significantly smaller than found for the genuine reflex inhibition. This indicates that the lack of detection in the CPSD may not be because the troughs is not real but may simply be that the CPSD method is not as sensitive to small reductions as the probability based analysis, or that the probability based analysis is classifying small troughs as significant when they are not.

9.4.3. LATE PEAKS

Previous reports on late peaks indicate a latency of approximately 40ms (van der Glas *et al.*, 1984) compared with 46ms found in the current study. There has been little consensus as to the nature of the late peak (reviewed in

Türker, 2002). The rectified averaged EMG analysis shows that a late peak rarely occurs without being preceded by a trough, that there is a significant difference between the start of the late peak and the end of the trough, and that the duration of the trough is not affected by the occurrence of the late peak. Furthermore, there is no reflex increase in the bite force record underlying the late peak, merely a partial recovery from the trough. The discharge rate analysis of the results showed that almost two-thirds of the late peaks found using traditional EMG analysis techniques were artefactual. In addition, unlike the artefactual troughs, the strength of the artefactual late peaks was not significantly smaller than the strength of the real late peaks indicating that the reduced detection rate in the CPSD was not the result of a decreased sensitivity in the analysis method.

9.4.4. Reflex in the Bite Force

It is clear that the troughs seen in the masseter EMG are, at least in part, responsible for the reflex in the bite force. While the latency of the reflex was longer in the bite force than in the EMG, the average delay of 8.8ms was similar to the EMG/force delay of 8-9ms found between an EMG inhibitory period and the reduction in bite force in a previous experiment (Türker & Jenkins, 2000).

While the difference was not significant, the occurrence rate of the reflex in the bite force was higher than the real inhibition under some conditions. This may be caused by errors in the analysis method; it can be difficult to identify the latency of the reflex as the method of stimulation causes an artefact in the bite force record (Figure 7.3). It is also possible that: (a) some of the smaller troughs classed as artefactual were in fact real (as discussed above) and they contributed to a decrease in the bite force, or (b) a muscle that was not recorded in this study, such as the temporalis, contributed to the observed changes in the bite force.

9.4.5. Reaction Time

The minimum reaction time to the stimulus was found to be approximately 140ms for the EMG and 150ms for the bite force, while no difference was found to exist between increase and decrease trials. This is the same as reported for tooth stimuli applied in the horizontal direction (Brodin *et al.*, 1993a; Yang & Türker, 1999). This result casts doubt on earlier studies that attributed responses at latencies greater than the reaction time, in some

cases up to 3 seconds (Yamamura *et al.*, 1993), to reflex pathways (Abbink *et al.*, 1998a). It should be noted that any activity (or lack of activity) in the EMG after the reaction time could be the result of an intentional change by the subject and hence should not be defined as a reflex.

In some reaction time experiments, the bite force changed before the associated masseter EMG, this confirms the results found in Chapter 8 that another muscle with a shorter reaction time (possibly the temporalis) contributes to the change in bite force.

9.4.6. RECORDING METHODS

It had been claimed that if the constant level of noise in averaged EMG traces were to be removed, there would be little difference between the strength of a reflex detected using IM-EMG and that detected using SEMG (Chapter 6). This was found not to be the case since the strength of the reflexes determined from the IM-EMG recordings were significantly larger than that found using SEMG. However, there were no differences between IM-EMG and SEMG in the latency, duration or number of reflex occurrences detected. A similar result has been found when analysing the temporal results of IM-EMG and SEMG recordings made from cervical muscles (Wittek *et al.*, 2001).

The differences in reflex strength between the two recording methods could come from a number of sources. Cross talk from facial muscles that underlie the surface electrodes (such as the zygomaticus major, platysma and risorius) will affect SEMG recordings much more than IM-EMG (Türker, 1993). This will cause a constant offset in the rectified averaged SEMG traces that will not be reduced by the noise removal process utilised in this study. The result will be an overestimation of the background level of activity in the SEMG and hence an underestimation of any reflex changes. A second possible source of difference is the two parts of the masseter, which have different contributions to the recordings. Both the fibre composition (Eriksson, 1982) and recruitment (Blanksma & van Eijden, 1995) are distinct between the deep and superficial masseter indicating the response to stimuli may also be different. By using eight different electrode locations, one study has found that the IM-EMG/force relationship in the human vastus lateralis muscle does not vary with location (Onishi *et al.*, 2000). This implies that localized recording made using intra-muscular EMG may correspond to the total muscle activity just as well as the overall recording made using surface electrodes. However, it is known that the masseter has a multi-pinnate structure, and functionally differentiated portions (Hannam &

McMillan, 1994) and so this finding may not hold for the masseter. It is suggested that when the strength of a reflex is of paramount importance, intra-muscular EMG should be used, however if discovering reflex occurrence or latency is the aim, surface EMG will produce results that are just as good, provided there are minimal movement artefacts or cross talk. It is not possible to perform a CPSD analysis on surface recordings, so identification of artefactual reflexes, and an accurate measurement of reflex duration, are only possible if intra-muscular recordings are used.

Multi-unit recordings were chosen over single motor-unit recordings in this study for a number of reasons. Firstly, single motor-unit recordings are so selective that they do not represent the connection of the stimulated afferent to the entire motoneuron pool. Secondly, single motor-unit recordings require a much larger number of stimuli to be delivered owing to their reduced signal content compared with multi-unit recordings. Finally, it is rarely possible to follow the same unit in a single-motor unit trace for sufficient time to complete an experimental protocol covering more than one or two trial conditions.

Unlike single-unit PSFs, CPSDs uses the time between multiple units to determine the frequency of action potentials arriving at a section of a muscle, rather than at a single motor-unit. While this method is dependent on the number of units being recorded it can be used to see periods of reduced (inhibition) or increased (excitation) discharge rates in multi-unit recordings. The advantage CPSD has over the traditional PSF is that multi-unit recordings contain much more information than those of a single-unit. Hence the number of trials required for a reliable picture of a reflex is significantly lower, while still being less susceptible to the synchronisation and count related errors common in probability-based EMG analyses (Moore *et al.*, 1970; Türker & Powers, 1999).

9.4.7. METHODOLOGICAL SETTINGS

The minimum time limits for the CPSD analysis are required because after high-pass filtering at 1kHz secondary spikes are produced in the multi-unit EMG record (see Figure 9.2A at approximately –80ms). While these secondary spikes are smaller than the primary spike they can sometimes cross the detection threshold and cause false threshold pulses. To overcome this problem, and still be able to indicate the cumulative discharge rates of the underlying units, the minimum time limits for peak and trough analysis were adapted from recommendations following experiments on brain slices (Türker & Powers, 2003). These limits are a trade off between type I and type

II errors in the acceptance spikes. Type I errors, or rejection of a true unit firing, must be avoided when looking for peaks, especially since the rising phase of the peak is brief. Hence the minimum time between acceptance spikes must be short. Type II errors, or the generation of an acceptance spike when there has been no unit firing, must be avoided when looking for troughs as delayed discharges of various units occurring in a short time period, corresponding to the rising phase of an inhibitory postsynaptic potential (Türker & Powers, 1999), will make it impossible to see the underlying genuine low discharge rate. Hence the minimum time between spikes must be relatively long, but not too long as to generate large numbers of type I errors. Ten milliseconds was chosen as the minimum time between pulses when looking for troughs as this value has previously proven successful at indicating troughs in isolated brain slice preparations (Türker & Powers, 2003). It is also important to note that for a change in the CPSD to be classified as a reflex at least part of the event must also be accompanied by a simultaneous change in the rectified averaged EMG record. Hence the two analysis methods work together to provide a check for the each other (Türker & Powers, 1999).

9.4.8. PERIODONTAL MECHANORECEPTORS

Despite the difficulty in stimulating the PMRs in isolation, a number of findings have been made in regards to the neuronal wiring that may exist in the jaw. Animal studies (Linden & Millar, 1988b; Byers & Dong, 1989; Linden, 1990) have shown that the majority of PMRs are located close to the apex of the tooth root; they respond to tension, but not compression (Cash & Linden, 1982), they have low activation thresholds, and they adapt slowly to changes in force. The second, less numerous, class of receptor is located between the gingiva and the apex, have a high activation threshold, and are rapidly adapting. These slow and fast adapting receptors are likely to correspond to the 'saturating' and 'non-saturating' receptors respectively as seen in human microneurography studies (Trulsson *et al.*, 1992; Trulsson & Johansson, 1994).

In the present study, by using three different preloads, it was possible to determine the effect of the 'saturating' PMRs on the reflexes evoked by axial stimulation of the incisor. At low preloads (0.5N) the receptors would respond to a stimulus, at medium preloads (1N) there would be a minimal change in activity due to an increase in the applied force and at high preloads (1.5N) there would be no change in their firing as a result of the stimulus. When the level of muscle contraction was low (5% MVC) the occurrence rate of the inhibitory reflex was constant, indicating that output

of the 'saturating' receptors was not yet saturated, even when a 1.5N preload was used. At a higher level of background muscle activity (10% MVC), the occurrence rate decreased to some extent with increasing preload (Figure 9.4 A), indicating that the saturating PMRs might have been saturated, so that the stimulus can only generate a small change in their discharge rate. Only low levels of muscle activity were used as higher levels reduce the inhibitory reflex and can cause fatigue in the jaw muscles (reviewed in van der Glas & van Steenberghe, 1989).

However, it was the level of stimulus that was the main factor involved in the occurrence rate of inhibition, with more inhibition occurring at higher stimulus levels (>1N), where the 'saturating' receptors would have lost their dynamic sensitivity. It is also important to note that preload had no effect on the strength or duration of the inhibitory reflex. Hence, while the 'saturating' receptors are more numerous, the present results suggest that it is the function of the 'non-saturating' receptors that provides the dominant inhibitory reflex seen in response to mechanical stimulation of the incisors.

9.4.9. LIMITATIONS OF THE STIMULATION TECHNIQUE

There is a delay inherent in the form of mechanical stimulation used in this study. The time for the vibrator to move to 5% of the maximum stimulus value after receiving the start of the stimulus profile and the movement to be picked-up by the strain gauges was measured as 3ms while the 5% rise time for the stimulus was 2ms. This indicated a mechanical delay in the system of approximately 1ms, caused by the inertia of the motor. In addition, the stimulus profile did not have a sharp edge, hence not all receptors would be stimulated at the same time and the integration of multiple receptor inputs before a threshold could be reached would take some time. However, as previously indicated, stimuli with sharp edges were avoided as they increased the involvement of the muscle spindles in the elicited reflex (Chapter 7). Hence the real speed of the inhibitory reflex loop might be somewhat less than the 21ms indicated in this study.

9.4.10. IMPLICATIONS

This study shows that the main response to axial stimulation of the incisor is inhibition, most likely generated by rapidly-adapting, non-saturating PMRs predominantly located around the middle of the tooth root. Since larger stimuli produced a larger and more frequent response, this reflex is

assumed to have a protective function, with stimuli that are more likely to damage the masticatory apparatus producing a stronger reflex response. It has also been shown that a clustering of action potentials following a trough, rather than an excitatory postsynaptic potential reaching the motoneuron pool, causes approximately two-thirds of the late peaks observed in the EMG; and that higher levels of muscle activity, and larger stimuli, are associated with larger numbers of these artefacts. However, care must be taken when interpreting these findings as the experiments were conducted under static conditions and the same results may not hold during movement of the jaw.

10. A Device For Investigating Neuromuscular Control In The Human Masticatory System

A new apparatus has been developed to study the control of mastication in humans. The subject places their teeth on fixed upper and mobile lower bite plates; the device then enables opening and closing movements of the lower jaw against a controlled resistance. It is also possible to vary the number of teeth in contact with the device during an experiment from the entire dental arcade to a single tooth. The specially designed lower bite plate is dynamic and allows for both rotation and translation of the lower jaw during movement, thus permitting the normal curvilinear trajectory of the jaw.

The lower bite plate can follow chewing initiated by the subject without resisting the movement ('no force' mode) via a dedicated microprocessor controlled compensation mechanism. Another function of the device is to inject a constant predetermined load onto the lower bite plate so that the subject chews against a fixed resistance or rapidly yielding food bolus ('fixed force' mode). The device can be programmed to increase or decrease the force during the closing or opening phase of chewing by feeding the position information into the force compensation system so both position and force change in parallel, hence simulating a bite onto a non-yielding, or sticky, food bolus ('normal chewing' mode). By use of a jaw position compensation mechanism the device can actively move the lower jaw, following any imposed position pattern ('position controlled' mode). The chewing simulator also has a mode that holds the position at a fixed level and allows the force to change ('position hold' mode). Furthermore, the device can inject additional rapid or slow forces or displacements onto the lower bite plate in order to elicit reflexes so that the response of jaw muscles to such stimuli can be examined at various jaw positions, force levels, phases of motion and velocities.

The different modes of the apparatus can be used to study the operation and feedback control of human mastication; in particular whether modulations in jaw muscle activity and reflexes are due to changes in force, velocity, position, chewing cycle phase or a combination of these factors.

This chapter is an edited version of the manuscript "A Device For Investigating Neuromuscular Control In The Human Masticatory System" by K.S. Türker, R.S.A. Brinkworth, P. Abolfathi, I.R. Linke, and H. Nazeran, which has been published in the Journal of Neuroscience Methods 136(2): 141-149 (2004).

10.1. INTRODUCTION

Mastication is a rhythmic act, the purpose of which is to break down food for digestion. This process involves the coordinated activity of jaw muscles, tongue, cheeks and lips to prepare the food for swallowing. There are two fundamental mechanisms of mastication: the central pattern generator and the peripheral control mechanisms. The central pattern generator sets the pattern of chewing and alternately stimulates jaw opening/tongue thrusting and jaw closing/tongue retracting muscles (Dellow & Lund, 1971). The peripheral control modulates the output of the central pattern generator and associated motoneurons so that optimal bite forces are developed during chewing, and masticatory organs are not damaged (Jüch *et al.*, 1985; Liu *et al.*, 1993).

The current information regarding the contribution of individual receptors within the jaw feedback system is incomplete, and the existing data is at times incongruous. For example, although most of the periodontal mechanoreceptors reach their maximum discharge rate at very low force levels (Trulsson & Johansson, 1994), and mechanical stimulation of the teeth typically initiates inhibition (van der Glas *et al.*, 1985); reviewed in (Türker, 2002), the feedback from these receptors make a large contribution to the chewing force in anaesthetized animals (Lavigne *et al.*, 1987; Inoue *et al.*, 1989; Morimoto *et al.*, 1989). The most likely answer to this apparent contradiction is that the contribution of various receptors to the motoneurons changes during chewing compared with static conditions, which is the condition used in most previous human work (Hannam *et al.*, 1970; Bailey *et al.*, 1979; van der Glas & van Steenberghe, 1988; Brodin & Türker, 1994; Sato *et al.*, 1994; Louca *et al.*, 1996; Louca *et al.*, 1998). There is a large body of evidence from animal experimentation on the masticatory system suggesting that movement-induced modulation of the strength of synaptic connections between receptors and motoneurons is a rule, rather than an exception (Appenteng *et al.*, 1982a); reviewed in (Lund, 1991). However, in human subjects, the extent of modulation in the receptor input to jaw muscle motoneurons during mastication is largely unknown.

Human mastication is a complex process that involves movement of the lower jaw, tongue and the head in concert so as to optimize the process and minimize damage. During opening movement, the lower jaw rarely goes straight down, but usually deviates to the chewing side. During closing, on the other hand, the lower jaw often crosses the midline before reaching the intercuspal position where the force is exerted on the bolus (reviewed in Matthews, 1975). Therefore it is ideal to study mastication where the movement of the jaw, head and tongue are unrestricted and chewing occurs naturally. However, as in all controlled studies, some restrictions to the

system need to be imposed in order to study the importance of each of the variables before attempting to investigate how they interact when the jaw moves. Therefore, to date the attention of most investigators has been on the synaptic connection of individual afferent systems to the jaw muscle motoneurons under the static (isometric) conditions of the jaws (reviewed in Türker, 2002) or strictly-controlled jaw movements (Ottenhoff *et al.*, 1992b; Abbink *et al.*, 1999a; Abbink *et al.*, 1999b).

The aim of this work was to build a system that goes one step further, to move the jaw more naturally in one plane (the mid-sagittal plane) permitting vertical displacement and rotation as well as protrusion and extrusion of the jaw. The new device is more flexible and better controlled than the only other similar device currently in use (Ottenhoff *et al.*, 1992b & 1994; Ottenhoff *et al.*, 1996). These advancements will help investigations into the mode of operation, and feedback control, of mastication under restricted conditions where the jaw moves only in the mid-sagittal plane.

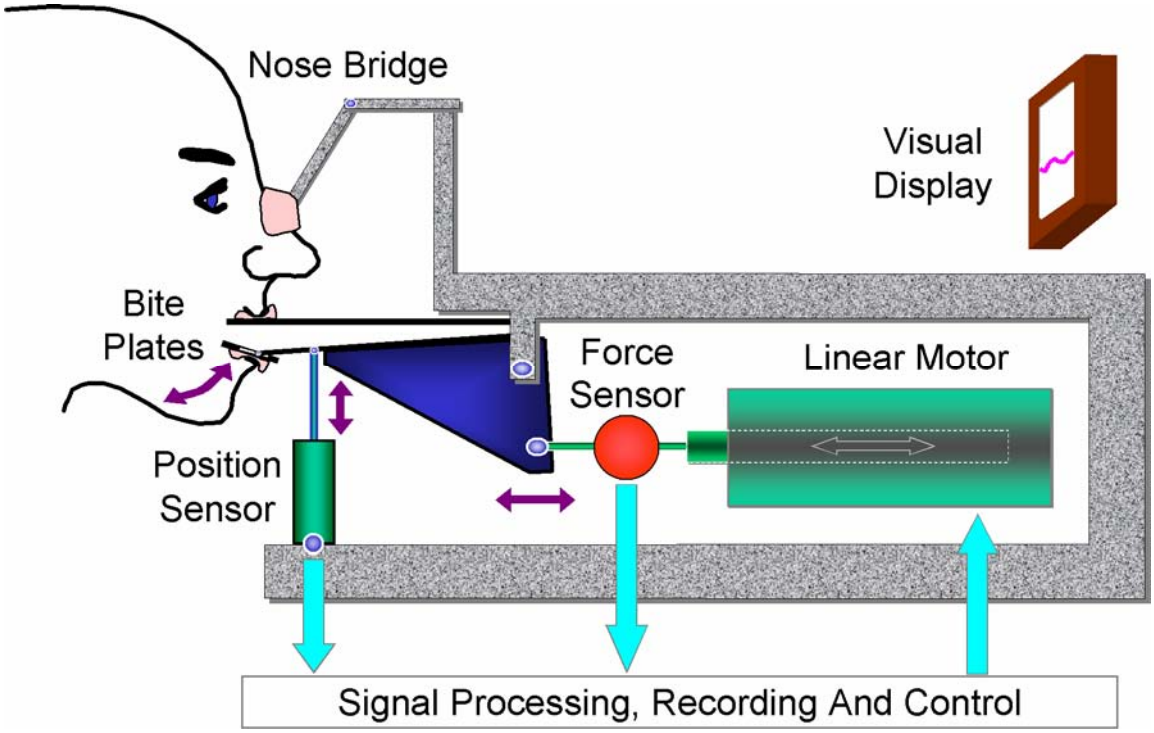
10.2. METHODS

An experimental platform has been designed and implemented in which subjects undergo simulated mastication within a controlled system. Figure 10.1 illustrates the system and how it functions. Figure 10.2 is a block diagram showing how the major components of the system interact with each other. Figure 10.3 shows, in detail, the operation of the dynamic lower bite plate and how the design allows for translation and rotation of the jaw.

10.2.1. BITE PLATES

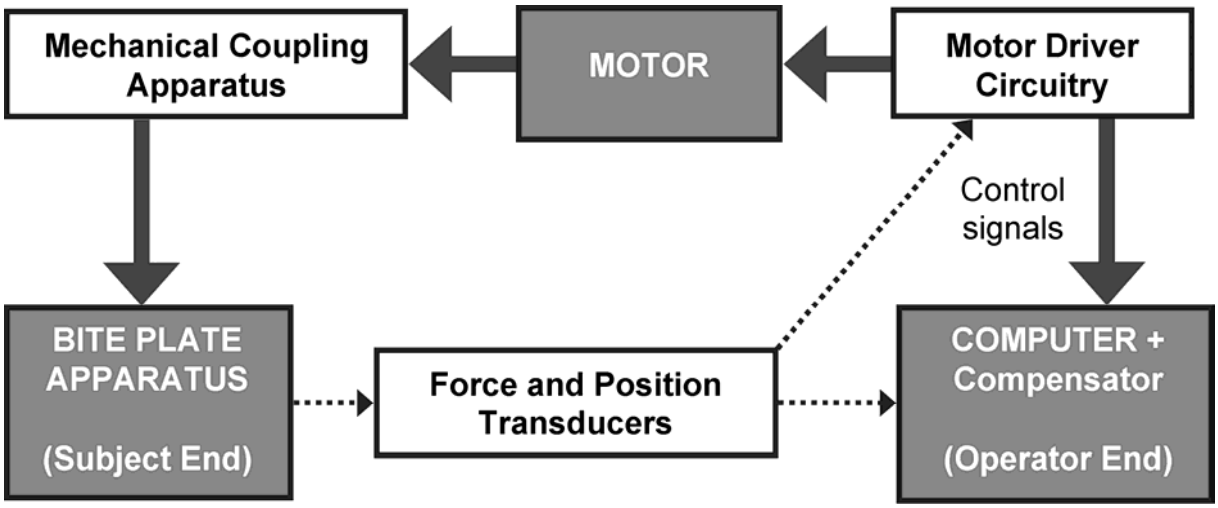
The subjects bite into dental impression material (3M; Express™ STD: Vinyl Polysiloxane Impression Material Putty) mounted onto two titanium bite plates (upper and lower) on the end of bite shafts. Since the impression material covers the teeth up to the gingiva it keeps the jaws firmly in place, so that once the subject bites into the impression and starts to 'chew', the lower bite plate follows the jaw precisely. The mould's firm grip on the teeth is necessary so that the bite plate follows the jaw movements without any delay, the force application to the lower bite plate is delivered almost instantly, and bite force changes are immediately monitored by the transducer/compensation system. The use of impression material also makes it possible to vary the number of teeth that are in contact with the bite plates, for example the impression can be cut away from one side of the jaw to study how the jaw reacts to unbalanced forces. As a precaution, the subjects are clearly informed that they can come out of the impression at any time during an experiment if they feel discomfort, without concern about damage to the mould, or loss of data. Further safety measures have been built into the device and are mentioned in section 10.2.7 below.

Figure 10.1: Experimental Set Up – Dynamic System



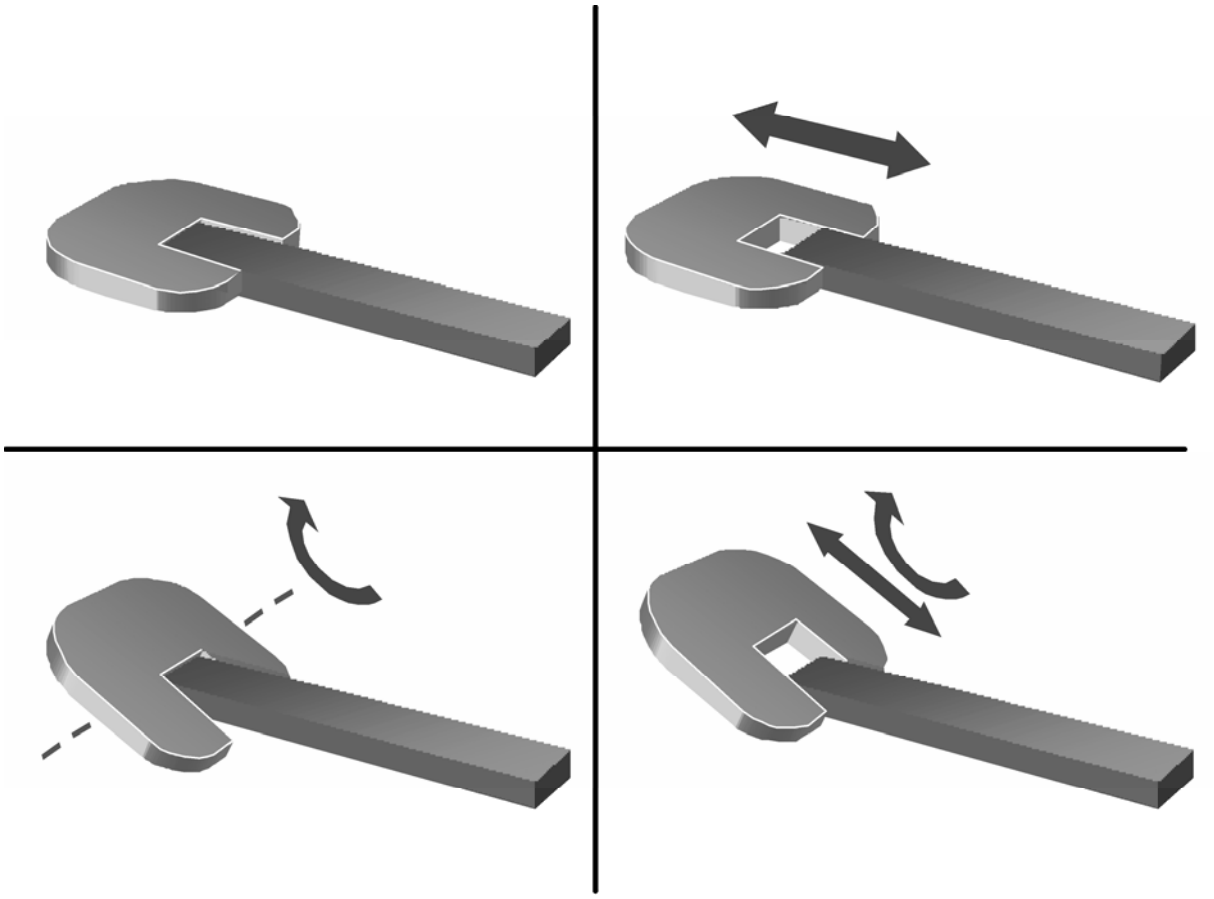
The subject bit into impression material mounted on the upper (UBP) and lower (LBP) bite plates located on bite shafts. Movement of the subject's head was minimized by use of a fixed nose bridge. The lower jaw can freely perform movements in the mid-sagittal plane, i.e., posterior/anterior open/close movements. The linear motor was coupled to the dynamic lower bite plate and controlled by a microprocessor based proportional-integral-derivative compensator. The position (P) and force (F) feedback information were used within the compensator and waveform generating computer program to achieve the modes mentioned in the text. A visual display could be utilised when the experimental protocol required the subject to perform predetermined levels of activity.

Figure 10.2: Major Components Of The Dynamic System



The main physical components of the device are: the bite plates, the various signal transducers, the computer (including the compensator), the DC motor (with the amplifier and driver circuitry system) and the mechanical coupling connecting the motor to the bite plates. The DC motor and associated amplifier system moves the lower bite plate on which the subject's lower teeth are placed, the computer and stand alone microprocessor compensator, along with the associated hardware and software, generate or compensate for force and position variables.

Figure 10.3: Dynamic Lower Bite Plate And Bite Shaft



The lower bite plate is dynamic in nature. While fixed to the teeth of the lower jaw, the lower bite plate allows the jaw to move freely in the sagittal plane; i.e. posterior, anterior and rotational movements. The lower bite plate is mounted onto a titanium bite shaft that is coupled to a linear motor. A controller is used to compensate for disturbances in the force or position, as well as inject various forces or positional changes on to the lower jaw in order to study the control of human mastication and the role of reflexes in the jaw muscles.

The thickness of the upper bite plate is approximately 3mm (with impression) and it is fixed in place to secure the upper jaw so that the head position is maintained throughout the experiment. To increase the rigidity of the head a nose bridge is used to further prevent movements. The lower bite plate is dynamic in the sense that while fixed to the mandible it allows jaw rotation in addition to anterior-posterior movements (see Figure 10.3). This specific feature of the lower bite plate allows for the complex curvilinear nature of the mandibular trajectory in the sagittal plane to be followed without resistance. The thickness of the lower bite plate is 5mm (with impression), however, during experiments where the change in jaw position is minimal, the plate can be removed so that the subject bites directly onto impression material on the lower bite shaft, the width of which is approximately 2.5mm (with impression). When the lower bite plate is removed the remaining bite shaft permits only vertical displacement of the jaw, the ability to rotate and translate the jaw is lost. Normally 2mm free space between the plates is allowed for uninterrupted bite force measurements. Hence the minimum interocclusal distance when the device is fully assembled is 10mm, however if the rotational and translational components of the device are not required this distance can be safely reduced to 7.5mm and recordings can still be made with as little as 6mm between the subject's upper and lower jaws.

10.2.2. DC MOTOR

The lower bite plate is coupled to a linear motor (LDS model V406) and a force sensor via a triangular hinged link. This triangular link translates vertical movements of the lower jaw into horizontal movements controlled by the linear motor. This allows for direct application and measurement of forces on the lower jaw. Using the motor, the maximum deliverable force is ±35N at a maximum rate of approximately 850N/s.

10.2.3. COMPUTER PROGRAM AND COMPENSATOR

The data acquisition and waveform generation are programmed and implemented in LabView® 6.02 (National Instruments) on a standard desktop computer fitted with a 12-bit DAQ card (National Instruments, NI PCI-6040E). The compensator is controlled by a microprocessor (Mitsubishi Electric, MSA0654) with a clock speed of 20MHz, the type of control paradigm is proportional-integral-derivative (PID) control with a loop rate of 1kHz; and is used to ensure the force and/or position of the lower bite plate

performs as required. During experiments, the computer and associated compensator allow several different modes of option:

10.2.3.1. NO FORCE MODE

When set to this mode, the compensator minimizes the resistance encountered during movement of the jaw by counteracting the weight of the lower bite plate as well as forces exerted by the subject. Hence, when the subject moves his/her lower jaw no force develops during the movement. This allows the lower jaw to move freely and reflexes to be studied during various stages of jaw movement. To achieve this mode, the force on the lower jaw (simulated food resistance) is measured using a Wheatstone bridge strain gauge mounted in series with the motor actuator (see Figure 10.1). During the closing phase of chewing, when the lower jaw is elevated voluntarily, the subtle increase in force is detected and the lower bite plate is raised accordingly. The reverse is done for the opening phase, where the lower bite plate is lowered along with the jaw. This compensation method also counterbalances the weight of the lower bite plate and associated connections. Hence the set up exhibits very little, if any resistance, to the simulated voluntary chewing of the subjects. This feature is important in order to investigate the activity of the proprioceptor input to jaw motoneurons under 'no load' conditions.

10.2.3.2. FIXED FORCE MODE

Similar to the 'no force' condition above, a predetermined force is chosen and, using the feedback controller (compensator), kept constant during movement (see Figure 10.4). This feature is important to investigate how different amount of loading affects the neuromuscular system in the jaw.

10.2.3.3. POSITION CONTROLLED MODE

Via a position compensation mechanism, the device can move the lower jaw to follow a predefined movement pattern (see Figure 10.5, PC traces). Thus permitting investigations into the passive movement of the jaw, and the role of muscle spindles.

10.2.3.4. NORMAL CHEWING MODE

The device can be set to vary the force during different phases of chewing by feeding in the position information into the force compensation system so that position and force change in parallel (see Figure 10.5, NC traces). In this way investigations can be made into the response of the human jaw system to (relatively) slow changes in the resistance to movement.

10.2.3.5. POSITION HOLD MODE

When in this mode the position is fixed at a predetermined level and the force permitted to change. This is mode is similar to the traditional static experiments but is necessary so that both static and dynamic experiments can be performed on the same subject, using the same equipment, on the same day.

Furthermore, during any of these modes the device can inject controlled rapid or slow force or position changes onto the lower jaw in order to generate reflexes so that the response of jaw muscles to such stimuli can be studied at various jaw positions, force levels and movement velocities (see Figure 10.6).

10.2.4. JAW ROTATION

One of the limitations of the current system is that the force sensor can only measure the force on the lower bite plate directed perpendicular to the lower bite shaft. However the dynamic nature of the lower bite plate means that rotation of the plate with respect to the shaft occurs in order to accurately follow the natural trajectory of the jaw. Hence the measured force will not be the true force on the lower jaw, the true bite force will be the measured force divided by the cosine of the angle between the shaft and the bite plate. During a typical experiment, there is no transducer on the system to record jaw rotation, as the presence of any electrical transducer would add bulk and interfere with the smooth operation of the plate. Since there is a transducer to record vertical displacement of the lower jaw, a series of measurements were taken to ascertain if there was a relationship between jaw angle (as measured between the shaft and plate) and vertical jaw gape.

Subjects were instructed to make rhythmic open-close movement of their jaw while in the device. At random times a measurement was taken of both the

gape and the jaw rotation. A line of best fit was found for the results from each subject to serve as the conversion between jaw gape and angle. The cosines of the angles were then calculated to find the relationship between jaw gap and the recorded force as a percentage of the total force on the lower jaw.

10.2.5. SUBJECTS

Only adult volunteers with healthy teeth and gums, and no history of temporomandibular dysfunction or orthodontic treatment are recruited for experiments. Written informed consent is always obtained from volunteers and all experiments are approved by the Human Ethics Committee of The University of Adelaide and conform to the Declaration of Helsinki.

Six subjects were used to test the different modes of operation for the device; the results derived from one subject are included for illustrative purposes. Four subjects were used to determine the relationship between jaw gape and jaw angle.

10.2.6. DATA RECORDING

Bipolar electrodes are placed to record the electromyogram (EMG) of the masseter, temporalis or digastric muscles bilaterally in the bandwidth of 20-1000Hz for surface recordings or 20-5000Hz for single motor units. Frequencies below 20Hz are not recorded due to the possibility of large mechanical artifacts during movement of the jaw. The bite force is recorded using a custom built strain gauge mounted on the leaver arm of the linear motor, while the position of the lower jaw is monitored using a linear variable distance transducer (LVDT; Solartron, Metrology). All data is digitized at 12-bits and recorded on a computer for off-line analysis. Off-line analyses can be done using a number of systems available in the laboratory (for example, the SPS-8701 template matching system for spike recognition of single motor unit traces, or the custom designed LabVIEW® analyses system described in Appendix 1). Surface EMG data are typically full wave rectified and low-pass filtered (100Hz) before graphing (Figures 10.4-6).

10.2.7. SAFETY ISSUES

The device includes the following precautions to reduce the risk of damage to subjects:

- The maximum possible opening of the jaw is set to the maximum comfortable jaw separation of the subject using a large adjustable bolt that once set is impossible to move by an opening force generated by the system.
- The maximum force deliverable by the motor and the system is set to ±35N and the maximum rate of force delivery is set to 850N/s.
- The device is electrically isolated from the mains by use of an isolation transformer so that any change in the mains supply current will not influence the workings of the device.
- If the power suddenly and unexpectedly cuts out, the lower bite plate returns to the rest position of the device (25mm separation between the plates) or the subject's maximum comfortable gape (limited by the large bolt as mentioned above), using the spring system of the motor. The motor spring constant is approximately 1N/mm, hence if the power were cut with a jaw separation of 7.5mm (minimum jaw separation) the maximum force delivered to the jaw would be 17.5N and would decrease with increasing separation between the plates.

10.3. RESULTS

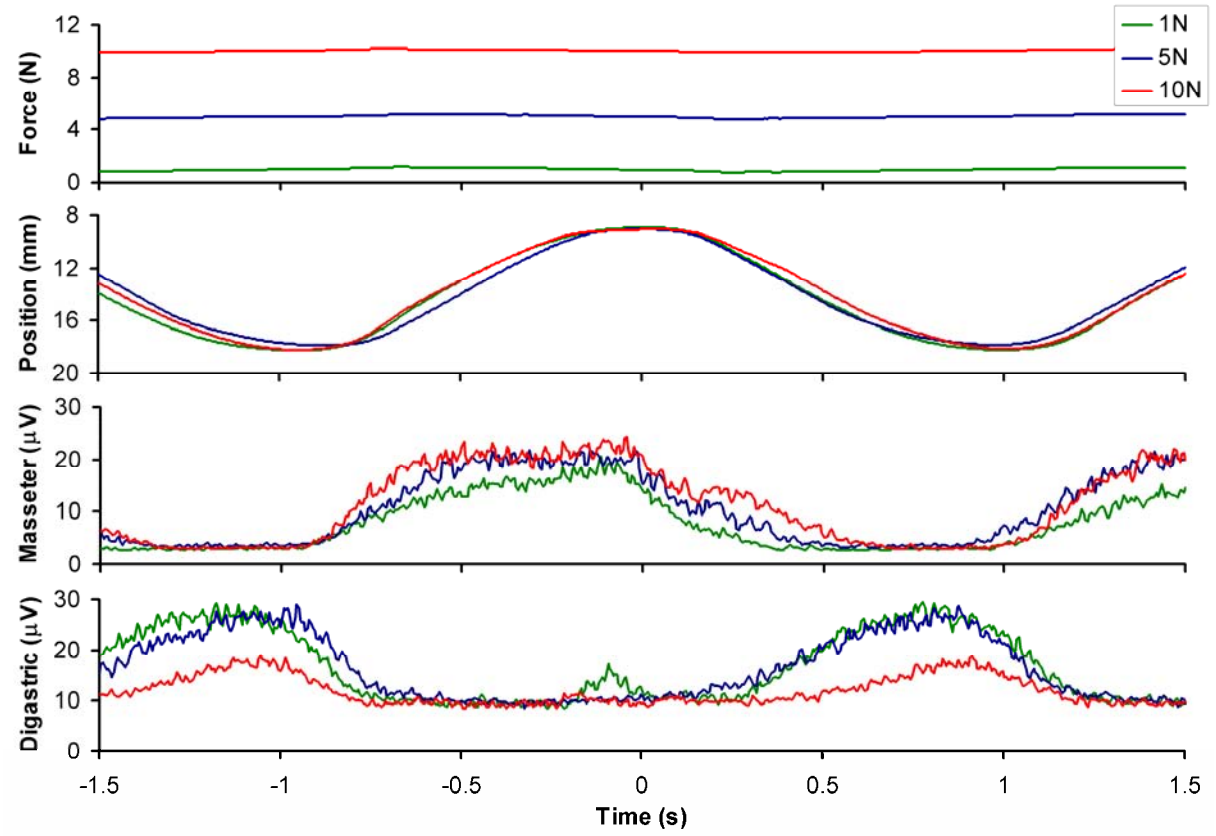
10.3.1. DIFFERENT MODES OF OPERATION

Results from an experiment are shown in Figures 10.4, 10.5 and 10.6. Figure 10.4 illustrates the jaw position, bite force and rectified filtered surface EMG when a fixed force resisted the movement of the lower bite plate. Note that the EMG of the closer muscle (masseter) increases and the opener muscle (digastric) EMG decreases as the force level is increased from 1N to 10N.

Figure 10.5 illustrates the two modes of the system where both force and position change. Position controlled mode, where the jaw is moved passively by the motor and the subject instructed only to exert a small amount of force with their lower jaw; and normal chewing mode, where force on the jaw is a function of the position and the subject instructed to make rhythmic opening and closing movements. Note that when the position of the jaw is moved by the motor the EMG levels of both muscles are lower than when the force and position move simultaneously and in the same direction. During both subject initiated and machine initiated movement, the masseter EMG rises and the digastric EMG falls as the jaw gape decreases, however in the different modes during the time of jaw closing the force on the mandible is changing in opposite directions. Although both the force and position recordings are symmetrical about occlusion for the normal chewing mode, both the masseter and digastric EMG are not, with the rising phase of activity longer than the falling phase.

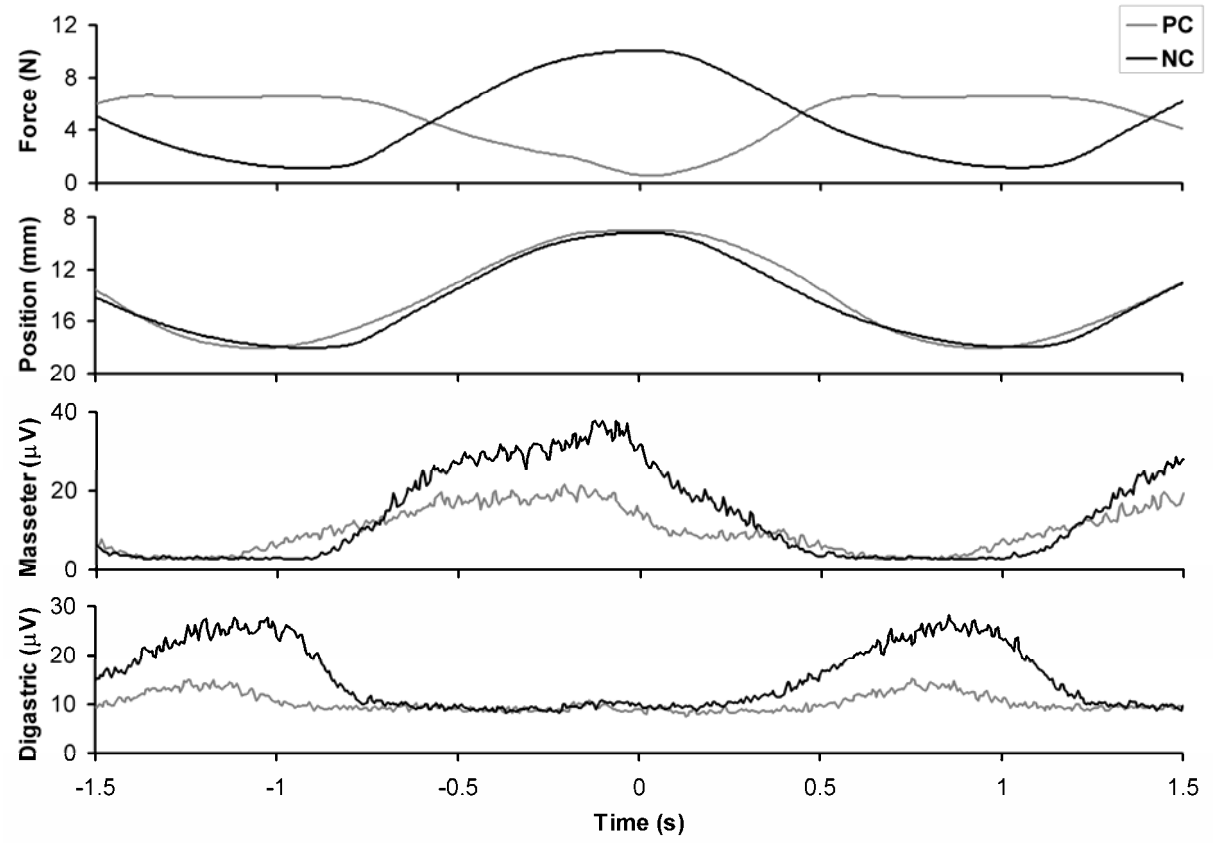
Figure 10.6 illustrates how predefined, controlled stimuli can be injected into the system. Note that masseter and digastric EMGs are alternating as the jaw moves while the force change is limited to levels set by the compensator. When a fast response is needed by the system, such as in the case of rapid stimuli, the compensator has to be adjusted to 'expect' rapid forces and hence is set to a very responsive level (high frequency or under-damped response; see also Figure 10.7). This is different to the level used when no rapidly developing forces are required and hence the compensation level is adjusted to a low frequency or over-damped response setting (as in Figures 10.4 and 10.5). When the system is under-damped, the variability of the lower frequency forces is larger than when the system is over-damped, but the system is able to deliver larger and faster stimuli (Figure 10.7).

Figure 10.4: Fixed Force Modes



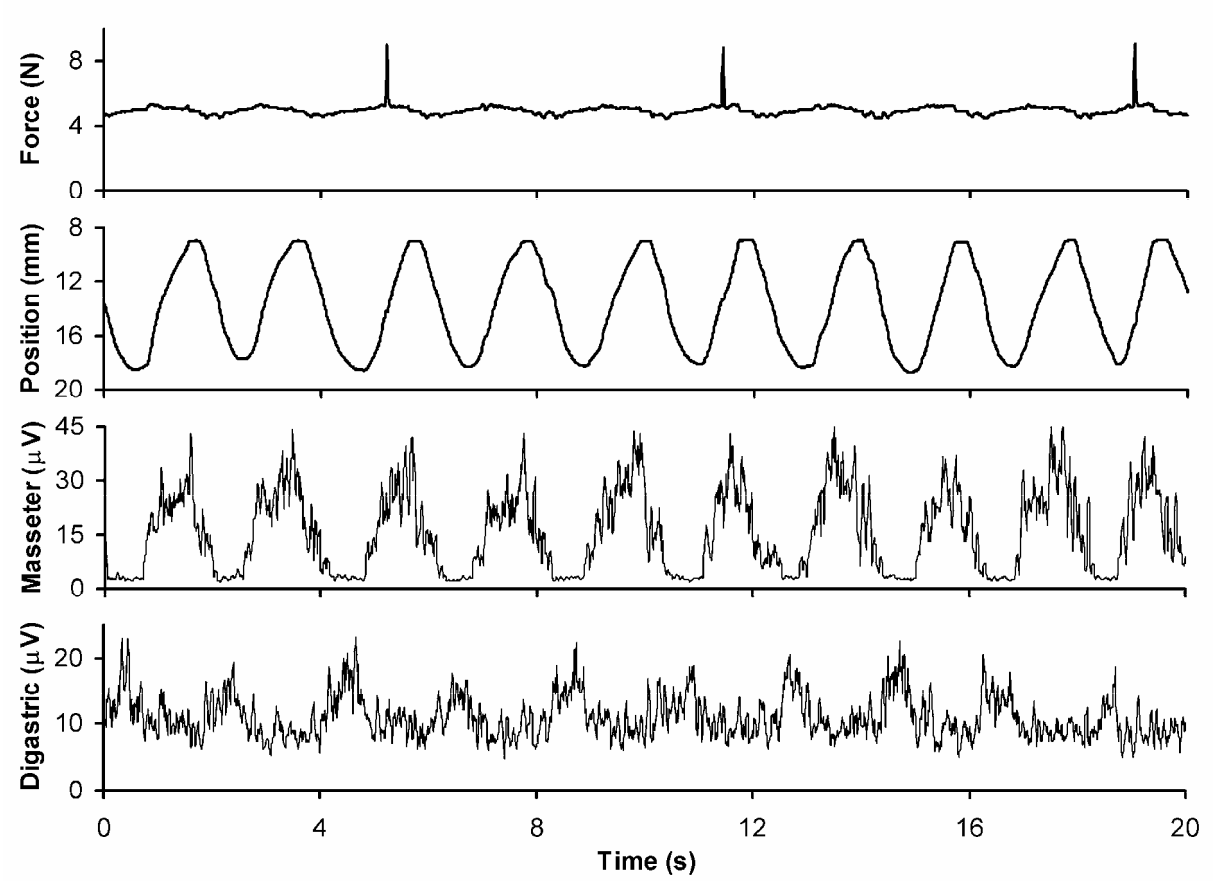
Three levels of constant force trials superimposed: 1N (light trace), 5N (medium trace) and 10N (dark trace). The subject moved their jaw at a constant rate and hence position changed smoothly while the force was kept at a constant level via the operation of the compensator. The data represent the average of 84 recorded cycles for each force level. Traces from top: force on lower jaw; jaw position; masseter EMG; digastric EMG. The EMG traces were full-wave rectified and low-pass filtered as described in the text. The force shown is the force measured by the system and represents only the component of the force directed perpendicular to the lower bite shaft. The position traces are vertical displacements between the fixed upper bite plate and the rotation point of the dynamic lower bite plate. Jaw angular rotation is not shown.

Figure 10.5: Variable Force Modes



The two variable force and position modes: position controlled mode (PC), and normal chewing mode (NC). In PC the pattern of jaw movement is defined by the system and the jaw is moved passively. In NC the movement of the jaw is under the control of the subject but the force on the lower jaw is a function of the position, in this case decreasing the jaw gape increases the force on the jaw. The data are average of 80 recorded traces for each mode. Trace information is the same as Figure 10.4.

Figure 10.6: Stimuli Applied During Jaw Movement



Force pulse stimulus applied to the lower jaw while the subject is 'chewing' against a fixed load: Force pulses of various amplitudes can be delivered to the lower bite plate at any time during jaw movement (although only closing is shown above) in order to investigate the modulation of reflexes during chewing. Trace information is the same as Figure 10.4.

10.3.2. CONTROL PARADIGMS

The use of under- and over-damped control settings is illustrated in Figure 10.7. The compensator used to control the movement of the lower bite plate can be altered depending on the experimental paradigm required. Figure 10.7 shows both an under-damped response and an over-damped response. Either control modes (force or position controlled) can be programmed for the type of response required.

10.3.2.1. UNDER DAMPED

In the under-damped condition the delay between the start of the stimulus and the commencement of the response is low and the rate of rise is high, however there is a small amount of instability in the step response. This setting is used when the speed of the response is the most important factor, an example of which shown in Figure 10.6. In this condition the rapid stimuli are very accurate but there is extra variability in the baseline.

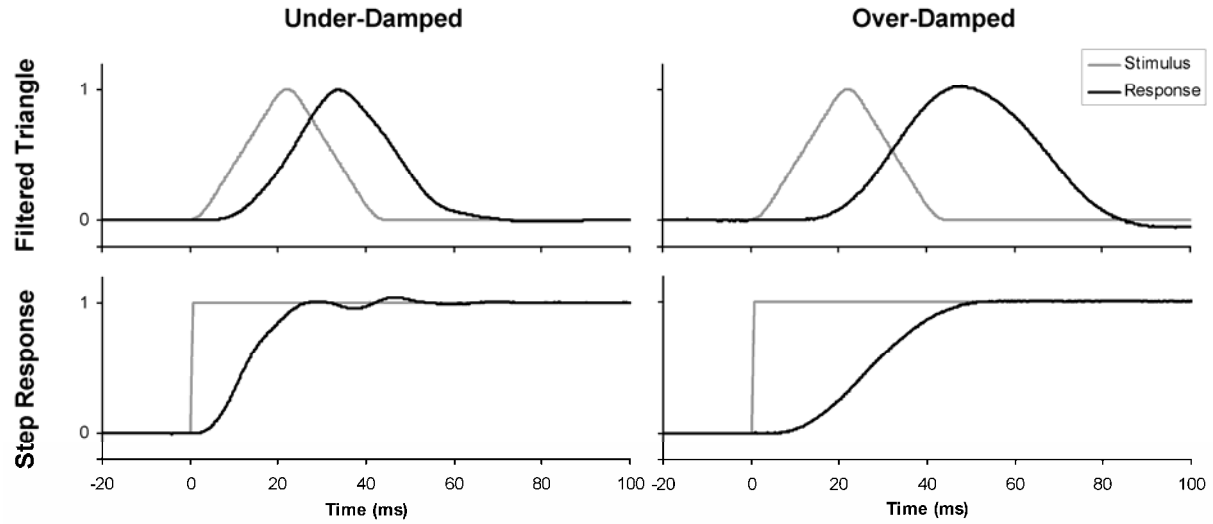
10.3.2.2. OVER DAMPED

In the over-damped condition the delay between the start of the stimulus and the commencement of the response is high, while the rate of rise is slower, the sharp peak of the triangular stimulus is smoothed and there is a small but long duration overshoot after the stimulus, however there is no instability in the step response. This setting is used when stability is the most important experimental factor and there are no high frequency stimuli, experimental examples of this setting are shown in Figures 10.4 and 10.5.

10.3.3. JAW GAPE VERSUS MEASURED FORCE

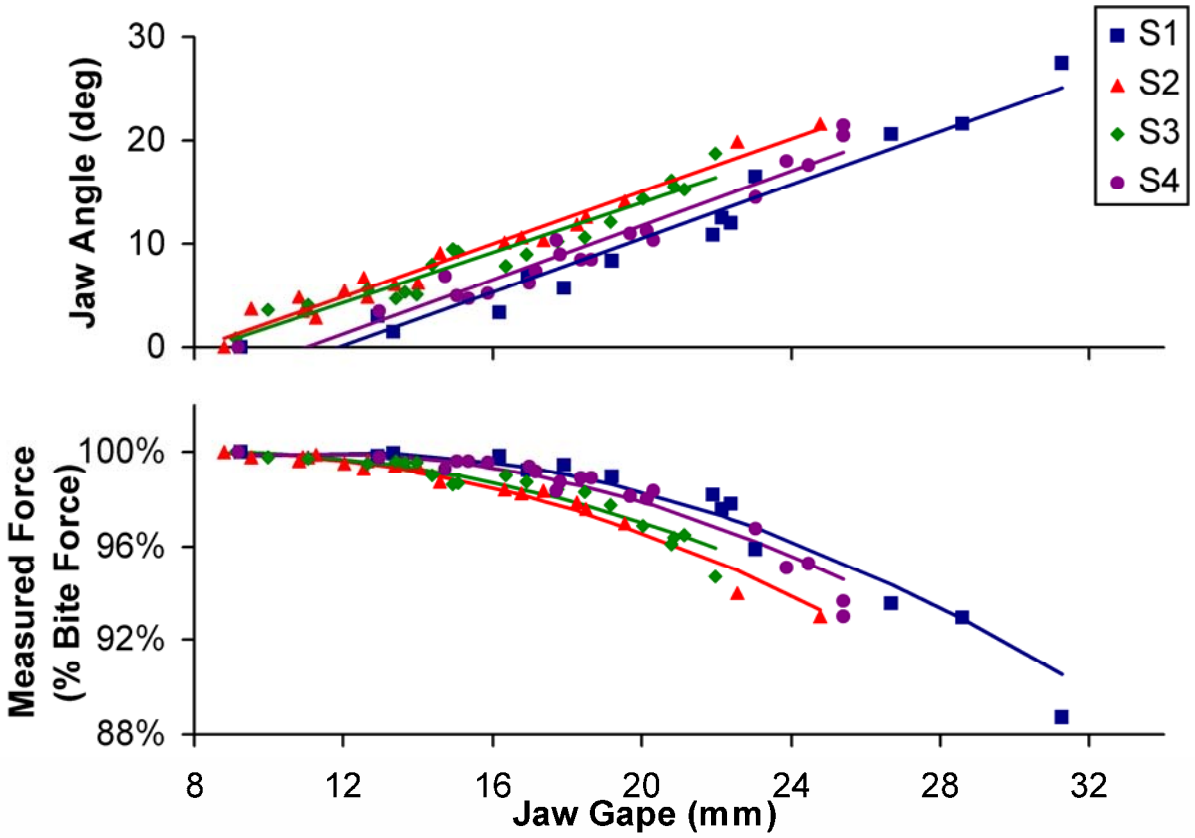
A high linear correlation (R^2 values of 0.95, 0.97, 0.93 and 0.94) was found to exist between jaw gape and jaw angle for each of the subjects tested while the slopes of the lines of best fit were: 1.61, 1.54, 1.46 and 1.60 deg/mm. This showed that with only a few measurements of gape and angle a reliable transformation could be derived to convert the force measured by the system into the force experienced by the lower jaw. In this way a simple mathematical transform can be applied to the force record to convert the measured force (perpendicular to the lower bite shaft) into bite force (perpendicular to the lower jaw).

Figure 10.7: System Response – Under- And Over-Damped



Response of the system to input stimuli utilising both under-damped and over-damped control conditions. The under-damped example is from a force controlled experiment while the over-damped response is from a position controlled experiment, however either paradigm can be chosen for both modes. The graphs have been normalised so a comparison of the responses can be made.

Figure 10.8: Jaw Angle And Measured Force Versus Jaw Gape



How the angle of the lower jaw varies with jaw gape (upper graph) and how the force measured by the system changes as a percentage of the bite force on the lower jaw with jaw gape (lower graph). Symbols represent the measured value while lines represent the modelled or predicted value. Results from four subjects are shown.

10.4. DISCUSSION

10.4.1. SYSTEM ADVANTAGES

The device described in this paper incorporates several important advancements to the only other device that is currently in use (Ottenhoff *et al.*, 1992a & b & 1994; Abbink *et al.*, 1999a). These advancements are as follows:

Firstly, the new device allows the lower jaw to move freely in the sagittal plane. This is achieved by the dynamic lower bite plate on a shaft that can move not only up and down but also slide in the anterior posterior plane and permit rotation of the jaw as illustrated in Figure 10.3.

Secondly, the new device, through a high-speed microprocessor driven proportional-integral-derivative compensator, controls the force on the lower bite plate, which is measured directly by strain gauges located in the motor lever arm. This controller allows a range of forces to be applied to the lower bite plate and any change in force due to subject's chewing is compensated for. Under the 'no force' mode, the lower bite plate exerts very little resistance to the simulated voluntary chewing of the subjects. This condition is an important feature since it allows the investigation of the effect of jaw movement on the jaw reflexes. Movement alone may generate changes in the activity of the proprioceptors hence affect the receptor input to jaw motoneurons via pre-motoneuronal mechanisms. Only this 'no load' experiment will bring out whether changes observed in the reflex responses during chewing are due to movement or due to the presence of force developed between the jaws when a resistance is encountered.

Thirdly, and similar to the previous device, the new system can inject a predetermined load onto the lower bite plate to which the lower jaw works against during closing movement and where the force varies as the jaw gape changes (normal chewing mode). However, the present system, using a feedback controller (compensator), can also keep the injected force at a constant level even when the subject is exerting force to overcome the resistance of the lower bite plate (fixed force mode; Figure 10.4). Again the changes in the reflexes originating from various receptors can be studied during these phases. Using a fixed force simulates the conditions where the food bolus yields as the jaw closes on it while the normal chewing mode studies conditions where the food bolus resists the bite force and hence the force changes as the lower jaw moves.

Fourthly, with the hold function of the device, the position of the jaw can be kept at any degree of jaw separation while allowing the force to change (position hold mode). This function is necessary to study whether it is movement or position that influences the modulation of reflexes.

Fifthly, via a position compensation mechanism, the device can move the lower jaw to follow any desired movement pattern (position controlled mode). This is necessary so as to determine how the reflexes are altered as the jaw is moved passively, without descending commands from the cortex to the jaw muscles. This mode brings in the possibility to study the passive tensions in the masticatory system.

Finally, in all modes there is the capability of injecting controlled, rapid or slow, force or position changing, stimuli to the lower jaw in order to generate reflexes so that the response of jaw muscles to such stimuli can be studied at various jaw positions, force levels and movement velocities (see Figure 10.6).

10.4.2. SYSTEM LIMITATIONS

The current configuration of the system has some limitations that prevent it from being an ideal mastication simulator. While protraction and retraction, vertical movement and jaw rotation in the mid-sagittal plane are included in the system, there is little room for lateral excursions of the jaw, which are part of the normal mastication cycle (Matthews, 1975; Koolstra & van Eijden, 1997). Furthermore, no provisions are in place to control for the position or movement of the tongue within the mouth. The thickness of the bite plates limits jaw closure to 8-10mm when the device is fully assembled and 6-7.5mm when the dynamic lower bite plate is removed. Although restrained by the nose bridge, downward forces that may develop as a result of neck muscle activation on the upper bite plate are not recorded. The limitation regarding the measurement of the actual bite force has been mentioned in the Methods (section 10.2.4), and a way to overcome the problem has been presented in section 10.3.3. Despite the limitations, the potential of this device is high and it is likely to form the platform through which modulation of most of the receptors in the masticatory system can be studied and peripheral control of mastication further investigated.

11. JAW MOVEMENT ALTERS THE REACTION OF HUMAN JAW MUSCLES TO INCISOR STIMULATION

The changes in the minimum time to consciously react (reaction time) and the order of jaw muscle recruitment to precisely controlled axial stimulation of the incisors during controlled jaw movements are not known. To this end, ten subjects were recruited to investigate the reaction time of bilateral temporalis and masseter muscles as well as bite force. Stimuli were delivered axially to the upper central incisors during active jaw closing and opening, as well as under static conditions.

The results showed that the reaction time was increased an average of 35% during both jaw opening and closing movements when compared to static jaw conditions. The left temporalis was recruited approximately 10ms before the right temporalis, while no significant side differences were found between the masseter muscles. The masseter muscles were recruited an average of 20ms before the temporalis muscles during jaw closing, but no difference existed during opening. Under static conditions the reaction time in the bite force was approximately 16ms longer than the left temporalis, but was not significantly different from the reaction time of any of the other muscles, indicating that, under the conditions tested, the left temporalis was more often responsible for the initiation of the mechanical reactions in the jaw.

This study is a prerequisite for investigations into the modulation of reflexes during jaw movement, since a response to a stimulus commencing after the minimum reaction time may not be entirely reflex in origin.

This chapter is an edited version of the manuscript “Jaw Movement Alters The Reaction Of Human Jaw Muscles To Incisor Stimulation” by R.S.A. Brinkworth, and K.S. Türker, which is currently under review.

11.1. INTRODUCTION

The events that occur in the muscular skeletal system in response to stimuli can be broken down into two main groups: reflexes and conscious reactions.

Reflexes involve relatively few synapses between the receptor and the motoneuron and hence have short latencies compared to conscious reactions. Although parameters can be altered by the characteristics of the stimulus (Smith *et al.*, 1985; Yamada *et al.*, 1985; Brodin *et al.*, 1993b), reflexes are generally stable and their study can lead to an understanding of the neuronal networks present in biological systems. Examples of reflexes in the human jaw include the H-reflex induced by direct stimulation of the Ia nerve (Godaux & Desmedt, 1975a; Riedo & Ruegg, 1988; Burke *et al.*, 1989; Scutter & Türker, 2000), the jaw-jerk reflex caused by activation of muscle spindles (Hannam & Matthews, 1968; Lamarre & Lund, 1975; McNamara *et al.*, 1977; Poliakov & Miles, 1994; Fukuyama *et al.*, 2000) and the periodontal receptor induced reflex following mechanical stimulation of the incisors (Sessle & Schmitt, 1972; van der Glas *et al.*, 1984; van der Glas *et al.*, 1985; Louca *et al.*, 1994; Türker & Jenkins, 2000).

Conscious reactions involve some form of decision by the subject to perform an action in response to a stimulus; they involve many more synapses than reflexes (Welford, 1988) and hence are slower. In reflex studies, a direct link between stimulus and response can be inferred, i.e. stimulation of incisor teeth by rapidly rising mechanical stimulation causes a reduction in the level of jaw muscle activity (Chapter 7). However the same link does not hold for conscious reactions. Thus it is imperative that reaction-time events are not classified as reflexes lest incorrect assumptions be made of the neuronal networks that exist in the system under investigation. Therefore it is essential to establish the minimum conscious reaction time of the system to a given stimulus before the reflex responses to the same stimulus can be established.

Previous attempts to define the minimum reaction time (time of earliest conscious reactions) of the human jaw to mechanical stimulation have involved stimulation of the labial surface of the incisors (Brodin *et al.*, 1993a), or stimulation of the entire lower jaw (Ottenhoff *et al.*, 1992b), both under static conditions. The current study aims to establish the reaction time and reaction recruitment order of the jaw muscles to controlled axial stimulation of the upper central incisors under both static and dynamic conditions. Since chewing alters the strength of the synaptic input to the masticatory muscles (reviewed in Lund, 1991), and the cortical excitability of the masseter is changed during different biting tasks (Butler *et al.*, 2001), it

was hypothesised that the both the reaction time and the order of muscle recruitment would change during jaw movement.

It is known that the basic pattern of movement is produced in the central pattern generator (reviewed in Delcomyn, 1980), which sets the pattern of chewing and alternately stimulates jaw opening and closing muscles (Dellow & Lund, 1971; Chandler *et al.*, 1985; Nozaki *et al.*, 1986; Westberg *et al.*, 2000). Recruitment of jaw muscles and portions of jaw muscles have been studied and established during a chewing cycle (Møller, 1966) and the recruitment order of motor units inside the masseter does not appear to be effected by the rate of contraction (Desmedt & Godaux, 1979). However it is not known if the pattern of jaw muscle activity changes in reaction time experiments where subjects are asked to bite as soon as a stimulus is felt. We hypothesised that the pattern would change since the reaction time experiments require a sudden and explosive contraction of the jaw muscles, which is different than the orderly recruitment of muscles during normal cyclic chewing.

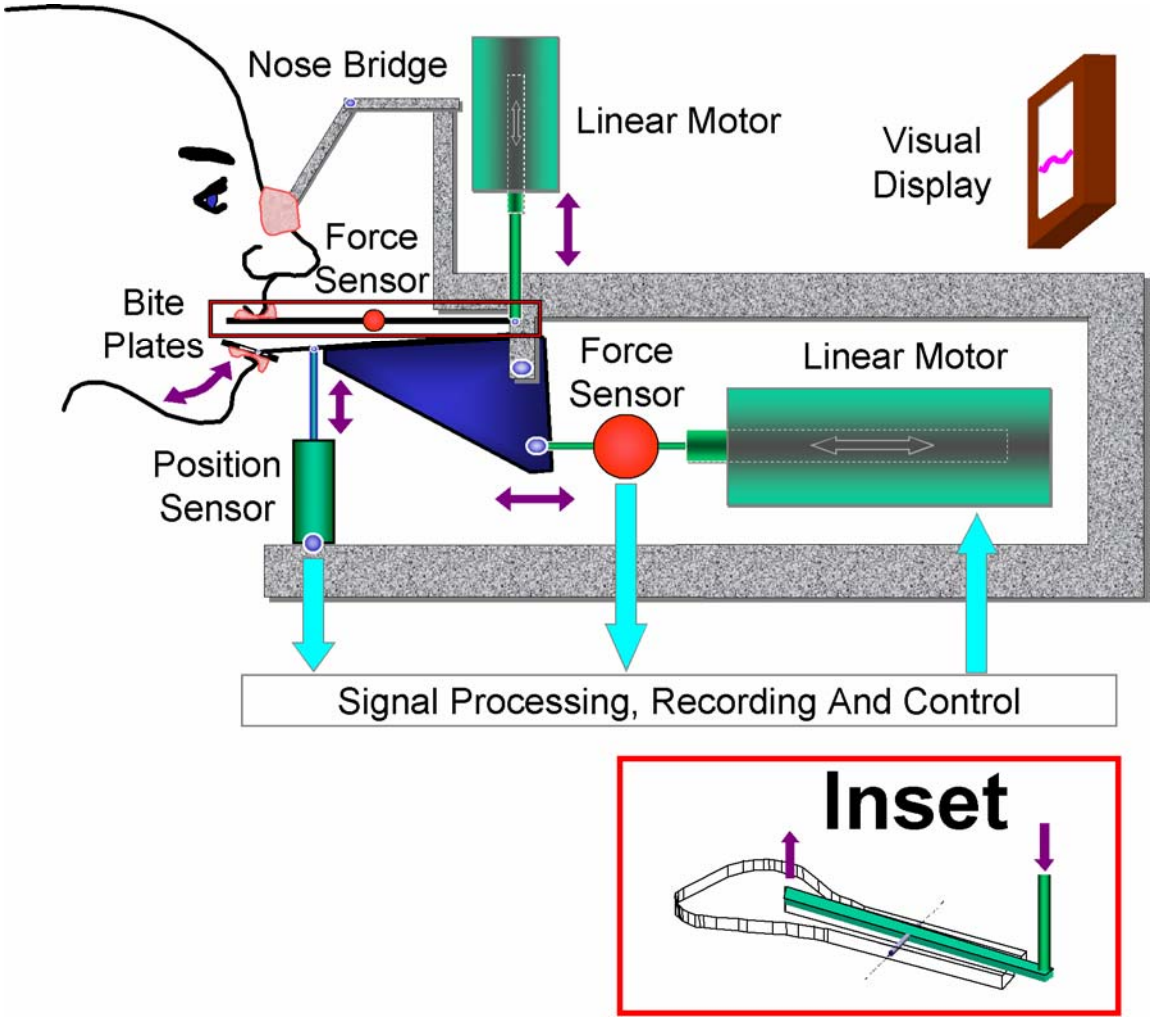
11.2. METHOD

Written informed consent was obtained from ten adult volunteers (six males and four females) with healthy teeth and gums, and no history of orthodontic treatment or dysfunction. The age range of the subjects was 19 to 26 years. The experiments were approved by the Human Ethics Committee of The University of Adelaide and conformed to the Declaration of Helsinki. The experimental setup is shown in Figure 11.1. It is similar to the device described in Chapter 10 but with an axial tooth stimulator installed, analogous to one described for molar stimulation in Chapter 8 but adapted for incisal stimulation.

11.2.1. PROCEDURE

Subjects bit into impression material (3M; Express™ STD: Vinyl Polysiloxane Impression Material Putty) mounted onto two titanium bite plates. The impression material covered the teeth up to the gingiva across the entire dental arch so that once the subject bit into the impression and started to 'chew' during the dynamic trials the lower bite plate followed the jaw precisely. A section in the middle of the upper bite plate was not connected to the rest of the plate. This titanium strip had a linear motor (LDS model V201), controlled by a custom designed microprocessor-driven control system, connected to one end. The other end held the impression of the subject's central incisors (cut away from the rest of the impression) and was attached by means of dental adhesive (3M; VPS Tray Adhesive). A hinge in the middle of the strip converted the downwards force produced by the linear motor into an upwards force on the incisors (Figure 11.1 inset). In this way the force on the upper central incisors could be controlled independently of the force on the rest of the dental arch. The direction of force on the incisors was perpendicular to the upper palate. Hence while the majority of the force was directed axially along the long axis of the tooth, there was an orthogonal component. This orientation was chosen as it best represented the direction of force applied to the incisors during normal mastication. Movement of the subject's head was minimised by the use of a nose bridge, which also counteracted the axial forces applied to the tooth (Figure 11.1).

Figure 11.1: Experimental Set Up – Reaction Time



The subject bit into impression material mounted on two titanium bite plates. The upper plate had a section cut away from around the central incisors that allowed a small linear motor to apply controlled forces directly to the teeth (inset). The lower plate was dynamic and permitted rotation, translation and vertical displacement of the lower jaw. The force on the lower jaw was controlled by a large linear motor. Both motors were controlled by independent, custom-built microprocessor controlled compensators. Relevant sensors, depicted in the diagram, transduced incisor force, bite force and jaw position, the values of which were digitised and recorded on computer. A visual display was used during static trials so the subject could maintain the desired level of muscle activity.

Surface electrodes were placed over the temporalis and masseter muscles on both sides of the jaw in order to record the surface electromyogram (SEMG) of the muscles. The SEMG signals were amplified (typically between 3 000 and 10 000x) and high-pass filtered (20Hz) before recording to minimise possible movement artefacts. The force applied to the incisor, and the force on the lower jaw, were transduced by custom-built strain gauges. This data were then fed into two independent custom-built compensators and were used to ensure that the forces were tightly controlled. A position sensor (LVDT; Solartron, Metrology) connected to the equipment as shown in Figure 1 measured the jaw gape. All data channels were sampled at 12-bits and 2kHz using a specially designed (LabVIEW® National Instruments) computer program detailed in Appendix 1.

The stimulus profile used was generated online via a custom designed computer program and had a maximum value of 4N with a half-sinusoid rising edge and a time to maximum of approximately 30ms, this force was then held constant for 190ms then followed by a falling phase, which was identical in shape to the rising phase only in reverse. This, along with a constant 1N force held between stimuli (preload), minimised the high frequency components in the stimulus (Türker *et al.*, 1997). The value of 4N was selected, as it is not known how, or if, movement alters the detection threshold for these stimuli, and 4N was sufficient to be detected by all subjects under both static and dynamic conditions. From the results of the experiments described in Chapter 7 it was known that a force of 2N on a single incisor was sufficient to cause a strong reflex response in the masseter during static conditions, hence twice the force was chosen as both upper central incisors were to be stimulated.

Four different conditions were tested: dynamic jaw opening, dynamic jaw closing, static with feedback from the right temporalis and static with feedback from the right masseter. Sixty stimuli were applied for each condition in blocks of two, the order of which was randomised. The time between each stimulus depended on the condition (see below) while the time between conditions was approximately 5 minutes.

The maximum voluntary contraction (MVC) of the subject was determined over a period of three maximal efforts of 5s duration with at least 20s between attempts. Subject bit into the impression mounted on the bite plates locked 12mm apart. Before each maximal attempt the subject was instructed to close their eyes to minimise visual feedback queues. The experimenter then counted to three before instructing the subject to bite as hard as they could onto the impression. The experimenter then counted to five after which the subject was instructed to stop contracting but remain on the bite plates. The recorded EMG was rectified and low-pass filtered at 1Hz.

The maximal value over the course of the three contractions was then defined as 100%MVC.

11.2.1.1. STATIC TRIALS

During the static trials the lower bite plate was locked in place 12mm below the upper bite plate. Feedback was supplied to the subjects on a computer screen showing a rectified and filtered (1Hz) SEMG trace from either their right temporalis or right masseter muscles. Subjects were instructed to contract their jaw muscles such that the level of activation was 5%MVC; they were not informed as to the origin of the feedback. Sixty stimuli were applied to the upper central incisors randomly between 8 and 12 seconds. The subjects were instructed to substantially increase the level of activity as soon as they feel a stimulus, then return to the nominated contraction level after approximately ½ a second.

11.2.1.2. DYNAMIC TRIALS

During the dynamic trials, the lower bite plate was free to move and track the lower jaw while the compensator was programmed to ensure a constant 5N was applied to the jaw at all times (see Chapter 10). This force was the same during both opening and closing of the jaw and was directed downward. The level of 5N was selected as it was sufficient to induce SEMG activity in the jaw muscles (for baseline readings) but not large enough to cause fatigue over the duration of the experiment.

The subjects were instructed to make large, rhythmic jaw opening and closing movements at their natural rate. No feedback was supplied to the subject regarding their level of muscle activity or speed of movements. The amplitude of the chewing cycles was such that the subject had to open larger than 75% of their maximum opening every cycle. If this amplitude was not reached in a number of consecutive cycles then the experimenter informed the subject they must open their jaw wider. This large value was chosen to ensure a large range of movement and to slow down the passage of the jaw ensuring that the opening or closing cycle was not completed before a stimulus could be delivered and detected by the subject.

The computer generated an internal digital reference pulse randomly on every second opening or closing cycle when the lower jaw passed through a set gape that was determined for each subject and approximately equal to mid-range. Due to the need for measurements of normal SEMG activity as well as reaction time measurements, a stimulus was applied to the upper

central incisors, on average, once in every three times a reference pulse was generated. The other reference pulses were used to identify the average SEMG activity that normally occurs during opening and closing when the jaw is passing through the mid-open gape (see Figure 11.2). The subjects were instructed to close their jaws as fast as they could once they felt a stimulus and hold their jaw closed for approximately ½ a second before recommencing simulated mastication. In this way both opening and closing reaction time recordings were performed in the same trial and separated during offline analysis. The duration of one trial was 250 open/close phases or 10 minutes, which ever was faster, and each subject performed two dynamic trials.

11.2.2. OFFLINE ANALYSIS

Offline analysis involved zero-phase band-pass filtering the SEMG (20-500Hz), rectifying the signal, then extracting a defined time period from around the trigger (pre-trigger time was 200ms and post-trigger 500ms). The detection of the reaction time was similar to that described in Chapter 6 except, as with previous reaction time experiments (Chapters 8 and 9), the size an EMG change needed to be before it was classified as a reaction was 300% of the maximum pre-trigger deviation. This ensured only large events were recognised. Also, since reactions are much larger than normal jaw reflexes it was not necessary to average the SEMG and bite force traces. Hence up to 60 readings of reaction time for each person and each condition were available depending on the number of stimuli the subject missed, although this number was generally low (<5 per condition), and the rate of stimulated chewing, slower movement resulted in fewer stimuli being delivered (dynamic only).

Unlike static trials, where the baseline post-trigger value is the same as the pre-trigger value, the SEMG during dynamic trials changed, not in response to the stimulus but rather as a function of jaw movement. Hence the chewing cycles where no stimuli were applied to the jaw, but a reference pulse was generated, were averaged and used to set the baseline for the dynamic trials (see Figure 11.3).

The average level of activity in the 100ms preceding the reference triggers was calculated to determine if there were any differences in the level of activity between the masseter and temporalis muscles or the different phases of movement. This duration was selected as it was large enough to reduce random fluctuations seen in EMG recordings but small enough to be an accurate representation of the pre-trigger level. To permit a comparison

between muscles, the SEMG activity was normalized to the maximum voluntary contraction of the respective muscle.

11.2.3. STATISTICAL ANALYSIS

11.2.3.1. REACTION TIME

Due to the active compensation of the force on the lower jaw during dynamic trials it was only possible to determine the reaction time as seen in the bite force during static trials, as changes in the force were masked during movement. Hence the data were analysed in two groups. Firstly, the bite force reaction times were ignored and the four experimental conditions (dynamic jaw opening, dynamic jaw closing, static with feedback from the right temporalis and static with feedback from the right masseter) compared to determine the reaction times of the two sides (left and right) and two muscles (temporalis and masseter). Secondly, the reaction time in the bite force was compared to the feedback muscle and side combinations using only the static protocols.

The assumptions made by the statistical model were as follows. The subjects were randomly chosen from a population with a mean of zero and a variance of σ_B^2. The residuals were normally distributed with a mean of zero and a variance of σ_R^2. The reaction time had an overall mean. The side, muscle and condition shifted the mean and possibly interacted such that:

Reaction Time = Mean + (Side*Muscle*Condition) + Subject + Residual

An analysis of variance was used to identify the factors and interactions that were significant at the 5% level ($p<0.05$). Following this, Tukey's multiple comparison procedure was used to identify which levels of the factors and their interactions were significant (Xu, 1996).

The mean reaction time is not a good indication of the fastest reaction time in the jaw, so for the purposes of this experiment the overall reaction time was defined as the 5th percentile value, i.e. the value smaller than 95% of the data. Since the subjects and residuals were both independent and normally distributed, the 5% lower percentile was given by the following formula, where $z_{0.05}$ is the lower 5th percentile value of the standard normal distribution (1.645).

5% Reaction Time = Mean + (side*muscle*condition) + $z_{0.05}\sqrt{\sigma_R^2 + \sigma_B^2}$

All results are given in the form: result ±CI, where CI is the 95% confidence interval (1.96 times the standard error) and represents the range in which the true mean is 95% likely to occur. Reaction times are given to the closest 1ms while CIs and standard deviations are given to 0.1ms resolution. Only results that were statistically significant ($p<0.05$) are reported in the text.

11.2.3.2. REACTION RECRUITMENT ORDER

To determine how the reaction recruitment order varied between muscles and conditions, the reaction times for each muscle, condition, subject and trial were converted to ranks. The muscle with the shortest reaction time was given a rank of 1, the second fastest a rank of 2, the third fastest 3 and the slowest muscle 4. If the reaction time for one or more muscles could not be determined for a given trial then that trial was removed from further analysis (this occurred approximately 17% of the time).

Since the ranked data were non-parametric, Kruskal-Wallis tests for several independent samples were used to determine if there were any changes in the reaction recruitment order in a given muscle with changing test conditions, or if under a given condition there was a difference between muscles. Post-hoc Mann-Whitney U tests were used to determine where the significant differences were if the Kruskal-Wallis test was significant.

As a consequence of the repeated statistical tests on the same set of data, the level of significance was set to 1% ($p<0.01$) to reduce the possibility of type 1 errors. All results are given in the form: result ±CI, average ranks are provided to 2 decimal places and, while not used to determine significant differences, CIs are reported (2 decimal places) to give an indication of the spread in the data. Only results that were statistically significant ($p<0.01$) are reported in the text.

11.2.3.3. BACKGROUND SEMG

To investigate the affect of background muscle activity on the reaction time the background EMG values were plotted against the reaction time for the corresponding trials for each subject and correlation coefficients (r^2 values) calculated.

To determine if the background level of muscle activity was different between the temporalis and masseter muscles, or between the different phases of movement (opening and closing), an analysis of variance (ANOVA) was employed. The model looked for an interaction and main effects between the

recorded muscle and the phase of jaw movement and was blocked for subjects. Since the results had a large positive skew, logarithmic transforms were employed to ensure normality in the results (Brinkworth *et al.*, 2004).

Since the application of logarithmic transforms causes confidence intervals that are larger on one side of the mean the results of the background EMG analysis are presented in the form: average (upper limit, lower limit) where upper limit and lower limit correspond to the mean plus CI and mean minus CI respectively. Results are supplied once a reverse transform was applied and only results that were statistically significant ($p<0.05$) are reported in the text.

11.3. RESULTS

The large amplitude chewing cycles specified for the experiment resulted in a slow rate of jaw movement. Frequency analysis of the position data showed that the average chewing rate selected by the subjects was 0.32 ±0.046Hz (mean ±CI).

Figure 11.2 shows the position and stimulus information from one subject during dynamic trials. The histogram (Figure 11.2 B) shows the position of the jaw at the time the internal reference trigger was generated. As with all subjects there was no difference between the distribution of the jaw positions with and without stimulus but the positions during opening was set to be slightly smaller than those during closing so that on average the jaw positions would be the same just after the application of force (Figure 11.2 A).

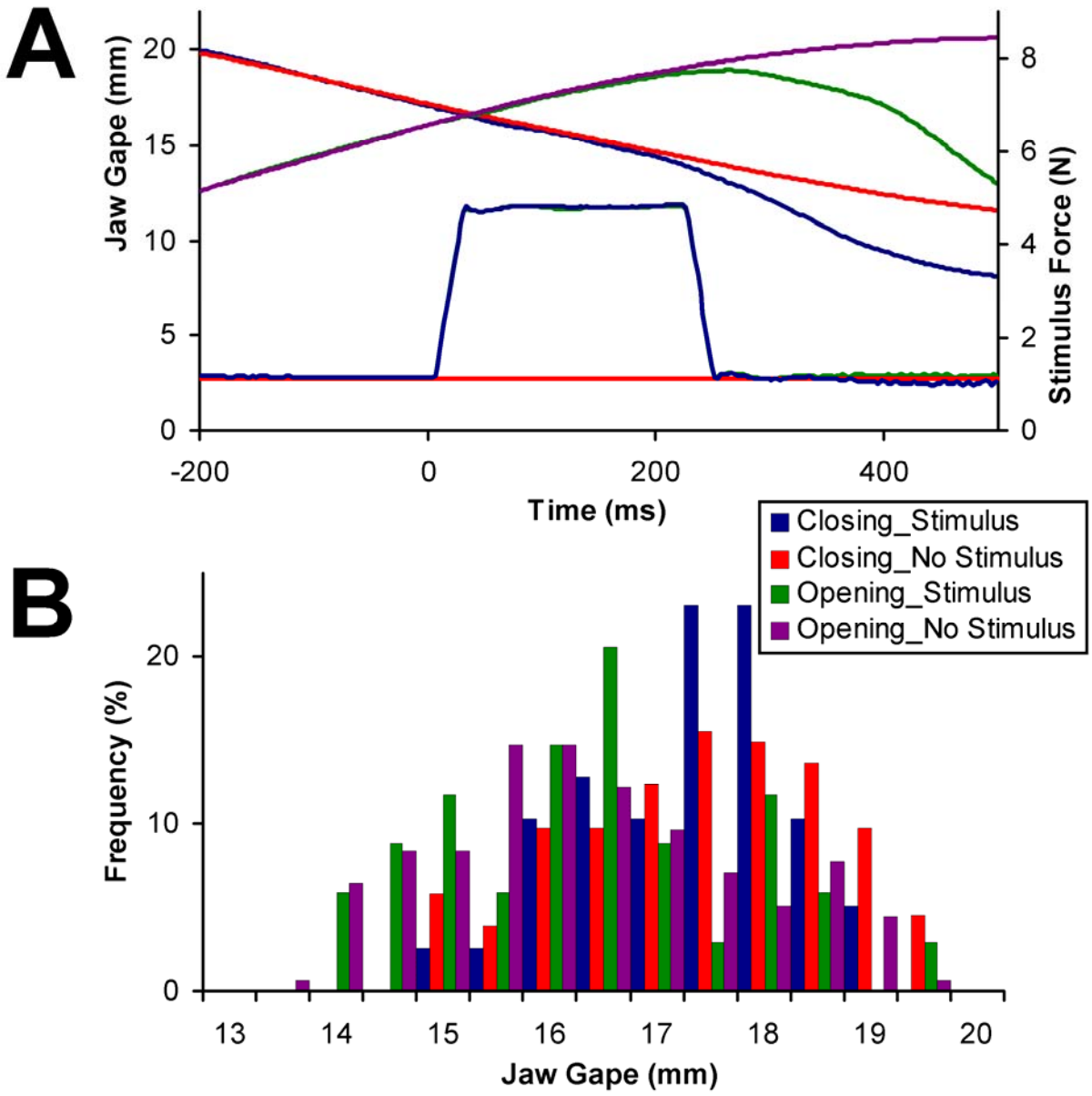
Figure 11.3 shows the SEMG and bite force data obtained from one subject during both dynamic and static trials. Variations from the no-stimulus trials (dynamic) or from the pre-trigger mean (static) are clearly visible making the determination of reaction time relatively clear.

11.3.1. REACTION TIME

11.3.1.1. RESULTS WITHOUT BITE FORCE

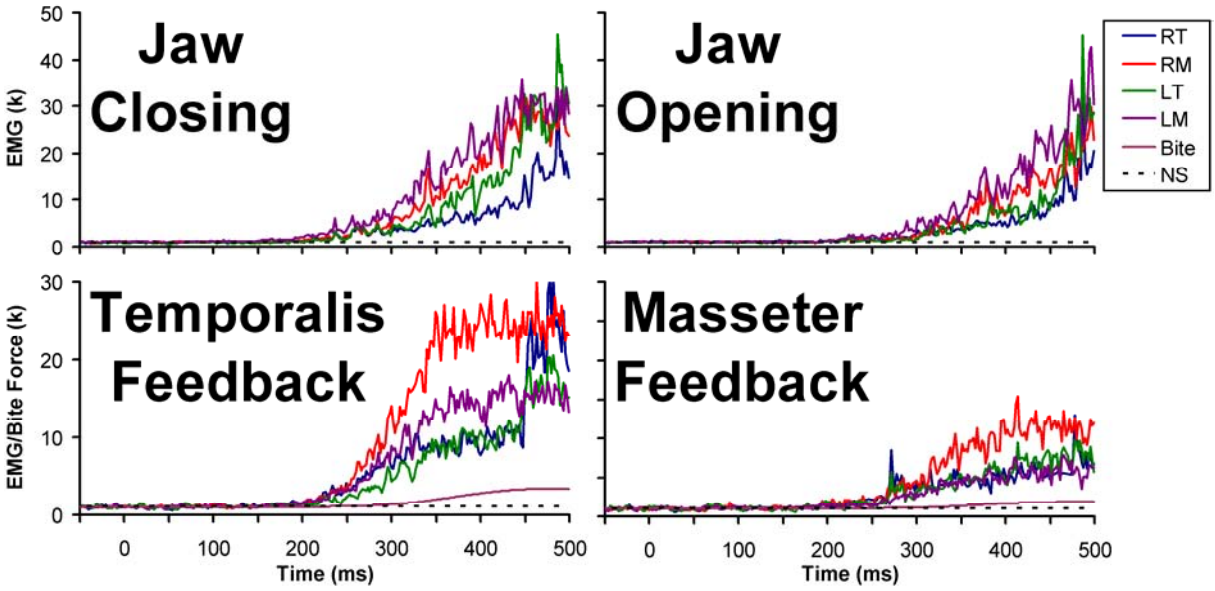
Due to the inability to measure a reaction-induced change in the bite force under dynamic conditions (Methods), the data were first analysed using only the SEMG recordings, the results of which are shown in Figure 11.4. Significant interactions between muscle and condition, as well as side and muscle were identified. The between subject standard deviation was estimated to be 44.1ms and the residual standard deviation 68.4ms. Hence the combined estimate for the standard deviation of the system was 81.4ms.

Figure 11.2: Jaw Position and Incisal Force During Dynamic Trials



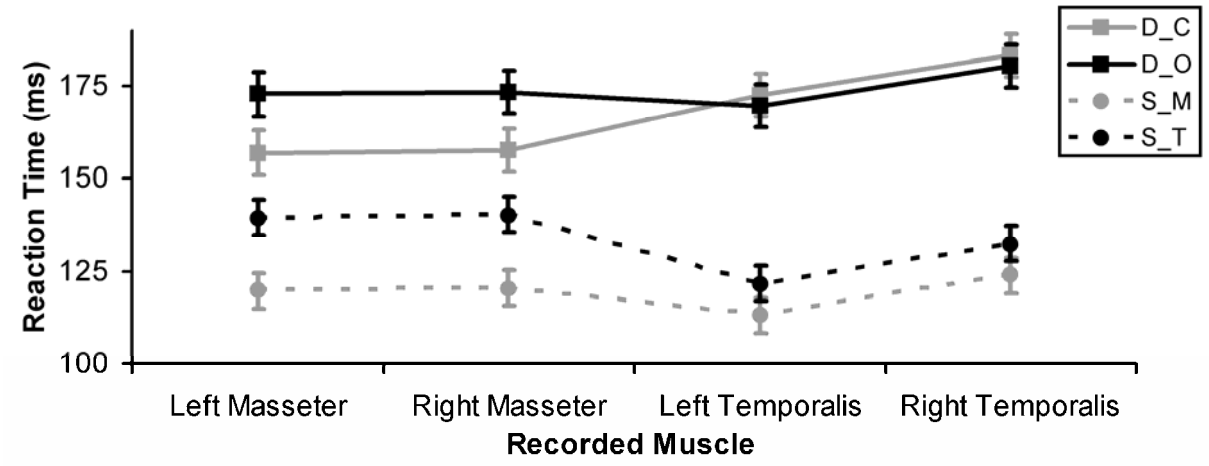
Results from one subject showing all used dynamic trials. A) Average jaw gape and force on the upper central incisors with reference to the internally generated trigger, occurring at time 0. B) Histogram of jaw positions at time of internally generated trigger. The number of averages were: 39 for jaw closing with a stimulus, 154 for jaw closing without a stimulus, 34 for jaw opening with a stimulus and 156 for jaw opening without a stimulus. The number of averages without stimuli was larger than those with a stimulus due to the need for the stimuli to be spaced out.

Figure 11.3: SEMG and Bite Force Results vs Time



Results from one subject showing all used dynamic and static trials. RT: right temporalis, RM: right masseter, LT: left temporalis, LM: left masseter, Bite: bite force record, NS: no stimulus level. Data have been averaged to facilitate viewing. The no stimulus level (k) has been normalised to one so the change due to the subject's detection of a stimulus can be clearly seen. In the static conditions the reactions are larger (compared to the no stimulus level) when the temporalis muscle was used for feedback than when the masseter was the feedback muscle. The reason for this is because 5% of maximum temporalis activation corresponded to a smaller level of activity in all muscles than when 5% of the masseter activity was used as the desired reference point for feedback, hence the reactions are proportionally larger. A number of subjects showed similar activity while the rest showed the opposite, higher levels of masseter activity when the temporalis was contracting at 5% of maximal contraction; an example of a subject showing this behaviour is depicted in Figure 11.7.

Figure 11.4: Reaction Time of Jaw Muscles



Estimated reaction times (5th percentile value) of the main jaw closing muscles under different conditions. D_C: dynamic jaw closing, D_O: dynamic jaw opening, S_M: static with feedback from the right masseter, S_T: static with feedback from the right temporalis. Error bars are the 95% confidence intervals (1.96 times the standard error of the mean). Static trials resulted in reduced reaction times, and while the masseter reaction times were shorter than those of the temporalis during dynamic jaw closing, it was longer when the jaw was static and the temporalis muscle was used for feedback.

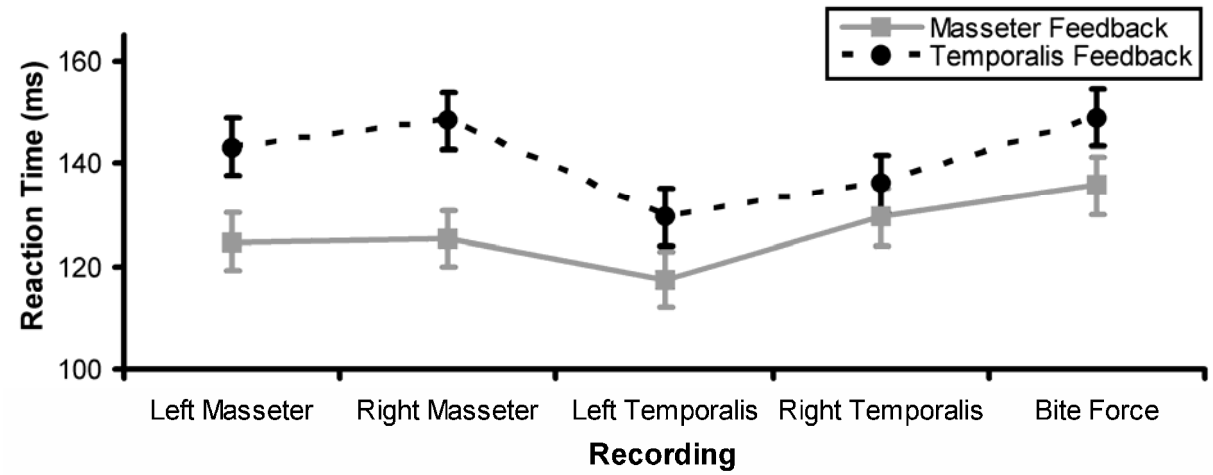
The static protocols resulted in significantly shorter reaction times than the dynamic protocols for all muscles. Left masseter: 164 ±5.9ms dynamic, 129 ±4.8ms static; right masseter: 165 ±5.9ms dynamic, 130 ±4.8ms static; left temporalis: 171 ±5.9ms dynamic, 117 ±4.8ms static and right temporalis: 182 ±5.9ms dynamic, 128 ±4.8ms static. In addition, with a latency of 157 ±5.9ms the reaction time of the masseter muscles were significantly shorter during jaw closing than the 173 ±5.9ms found during jaw opening or the reaction time of the temporalis muscles under either of the dynamic protocols, 177 ±5.9ms.

During the static protocols using the right masseter muscle as feedback resulted in an overall reaction time of 119 ±4.8ms with no significant difference found to exist between the muscles (118 ±4.8ms for temporalis muscles and 120 ±4.8ms for masseter). In contrast, when the right temporalis muscle was used for feedback the temporalis muscles were recruited earlier than the masseter muscles, reaction times 127 ±4.8ms for the temporalis muscles and 140 ±4.8ms for the masseter muscles. Finally, the pooled results showed that the reaction time of the left temporalis muscle was, on average, 11ms shorter than the right temporalis, however no difference was found between the left and right masseter muscles.

11.3.1.2. RESULTS WITH BITE FORCE

The results of the analysis performed on the recorded data containing the bite force recordings are shown in Figure 5 and cover only the static protocols. A significant interaction was found to exist between the reaction time in the recorded channel (muscle and bite force) and the condition (origin of feedback). Although similar to the estimates made in the analysis without bite force, the standard deviations in the statistical model with bite force were not the same. The between subject standard deviation was estimated to be 47.2ms and the residual standard deviation 64.6ms. Hence the combined estimate for the standard deviation of the system was 80.0ms.

Figure 11.5: Reaction Time of Jaw Muscles and Force



Estimated reaction times of the main jaw closing muscles and the total bite force of the lower jaw under static conditions with 5% maximum voluntary contraction of either the right masseter or temporalis muscles. The reaction times were reduced when feedback was supplied from the masseter muscle however this change was only significant in the masseter muscles and the bite force record, not in the temporalis muscles. For details see Figure 11.4.

Due to differences in the statistical model, the inclusion of bite force and the exclusion of the dynamic protocols, the estimates for the SEMG reaction times were slightly different than predicted in the previous section. The reaction time as seen in both masseter muscles, and the bite force, increased when the temporalis muscle was used as feedback compared to the masseter, however there was no change in the reaction time of the temporalis muscles under the two feedback conditions. When the right masseter muscle was used for feedback the average reaction time of all muscles was 124 ±5.5ms, with no statistical difference between the different muscles. However at 136 ±5.5ms, the reaction time as seen in the bite force record was only significantly longer than the reaction time in the left temporalis muscle, 117 ±5.5ms. When the right temporalis muscle was used for feedback the reaction times of the masseter and temporalis muscles were found to be different, 146 ±5.6ms and 133 ±5.6ms respectively. During feedback from the temporalis muscle the reaction time as seen in the bite force increased to 149 ±5.6ms and was found to be significantly longer than that of the left temporalis muscle, 130 ±5.6ms but not significantly different to the reaction time of any other muscle.

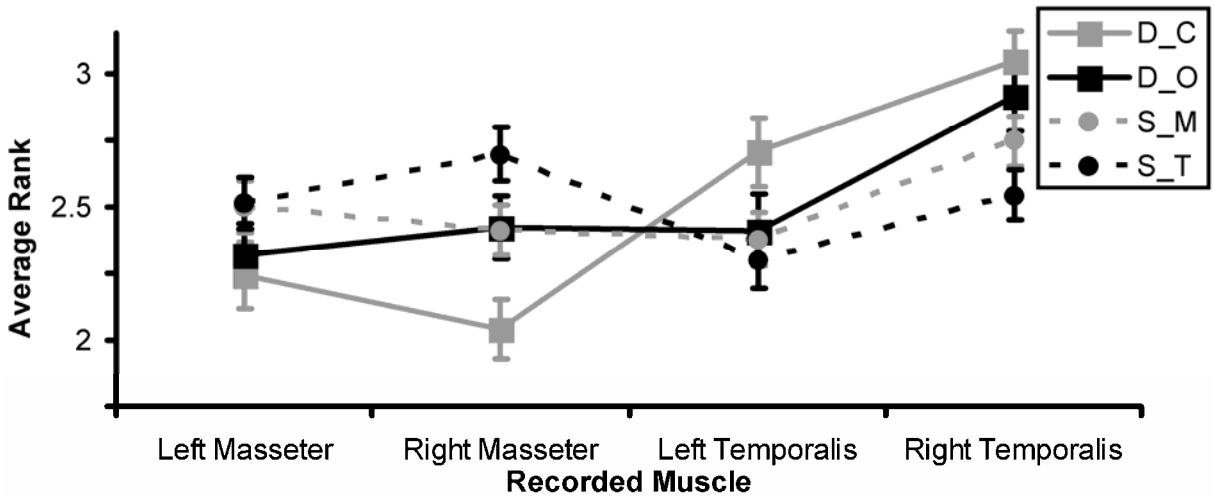
11.3.2. REACTION ORDER

The reaction recruitment order of each muscle, under each condition, is shown in Figure 11.6. Approximately 17% of trials were removed due to the inability to determine the reaction time from one or more muscles. Significant differences were found to exist between conditions for each muscle and between muscles for each condition.

11.3.2.1. MUSCLES

In all muscles tested there was a significant change in the reaction recruitment order when dynamic conditions were used compared to static conditions. Both the left and right masseter muscles were recruited earlier during movement, ranks of 2.28 ±0.12 and 2.23 ±0.12 respectively, than during static trials, ranks of 2.51 ±0.10 and 2.55 ±0.10. While the opposite was true for the left and right temporalis, the recruitment was later during movement than when the jaw was stationary, 2.56 ±0.13 dynamic and 2.34 ±0.10 static for the left temporalis and 2.98 ±0.20 dynamic and 2.64 ±0.10 static for the right temporalis.

Figure 11.6: Reaction Order of Jaw Muscles



The average ranks of the main jaw closing muscles under different conditions. D_C: dynamic jaw closing, D_O: dynamic jaw opening, S_M: static with feedback from the right masseter, S_T: static with feedback from the right temporalis. During jaw closing a sudden conscious bite recruited the masseter muscles before the temporalis muscles (i.e. the masseter muscles had a lower rank). However, when the jaw was not moving and the temporalis was used for feedback the temporalis muscles were recruited before the masseter muscles. For details see Figure 11.4.

Neither of the muscles on the left of the jaw was recruited differently under the two static conditions. However using the right masseter for feedback resulted in a reduction in the rank (earlier recruitment) of the right masseter muscle, 2.41 ±0.09 compared with 2.70 ±0.10 when the right temporalis was used for feedback. Similarly, using the right temporalis for feedback resulted in an earlier recruitment of the right temporalis muscle, 2.54 ±0.10 compared with 2.75 ±0.10 when the right masseter was used for feedback.

Significant differences with the reaction recruitment order were found to exist between jaw opening and closing for the left temporalis (2.41 ±0.14 opening, 2.70 ±0.13 closing) and the right masseter (2.42 ±0.12 opening, 2.04 ±0.11 closing). However no differences were found between the left masseter (2.32 ±0.12 opening, 2.24 ±0.13 closing) and the right temporalis (2.91 ±0.13 opening, 3.04 ±0.11 closing).

11.3.2.2. CONDITIONS

In all conditions tested, except for when the jaw was not moving and the right masseter was used for feedback, there was a significant difference between the reaction recruitment order of the masseter and temporalis muscles. This difference was most marked during dynamic jaw closing where the average rank of the masseter muscles was 2.14 ±0.12 while the average rank of the temporalis muscles was 2.87 ±0.12. During jaw opening this difference was reduced, but was still significant, with the masseter muscles having an average rank of 2.37 ±0.12 and the temporalis muscles 2.66 ±0.13. When the jaw was not moving and the right temporalis was used for feedback it was the temporalis muscles that had the lower average ranks: 2.42 ±0.10 compared with 2.60 ±0.10 for the masseter muscles. During stationary trials when the right masseter was used for feedback there was no significant difference between the reaction recruitment order of the masseter and temporalis muscles with the masseter muscles having an average rank of 2.45 ±0.09 and the temporalis muscles an average rank of 2.56 ±0.10.

The only time there was a difference between the reaction recruitment order in the masseter muscles was when the jaw was stationary and the right temporalis was used for feedback, in this case the left masseter was recruited before the right masseter. In contrast, the left temporalis muscle was recruited before the right temporalis muscle under all conditions tested.

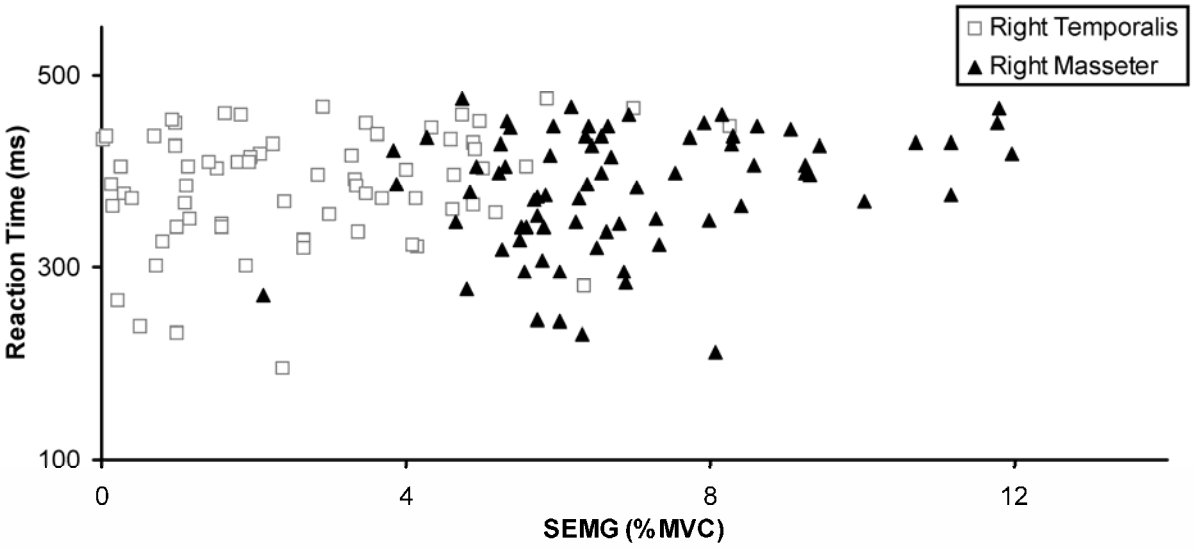
11.3.2.3. BACKGROUND SEMG

Analysis of the level of muscle activity preceding the reference trigger in the dynamic conditions showed that there was no interaction between the recorded muscle and the phase of the movement (i.e. both temporalis and masseter altered in the same way with movement) and that the level of activity in the masseter was not significantly different to that of the temporalis. However, there was a significant difference between the levels of muscle activity during jaw closing, 2.13 (3.05, 1.49)%MVC, compared to opening, 1.09 (1.60, 0.74)%MVC.

In the static conditions the muscle activities were: 6.22 (9.15, 3.29)%MVC in the temporalis and 6.02 (6.46, 5.65)%MVC in the masseter during masseter feedback and 5.71 (6.19, 5.49)%MVC in the temporalis and 6.42 (10.64, 3.73)%MVC in the masseter during temporalis feedback. The proportion of muscle activity was higher than during the dynamic conditions and, on average, slightly higher than the desired 5%MVC in the target muscle. While there was no significant difference between the average activity in the controlled versus uncontrolled muscles, the variability (as seen by the size of the confidence interval) was much greater in the uncontrolled muscles.

No significant correlations between reaction time and background EMG levels were found. Figure 11.7 shows a typical example of the background EMG level versus reaction time in one subject.

Figure 11.7: Background EMG Versus Reaction Time



Results from one subject during static conditions. The r^2 values for this subject were 0.079 for the right temporalis muscle and 0.082 for the right masseter muscle. While a preponderance of data is found close to the 5%MVC (maximum voluntary contraction) level, as this was the desired level during static trials when the EMG was controlled, there is also a large amount of outliers. These outliers represented the conditions when the EMG of that particular muscle was not controlled. In this subject, unlike the subject depicted in Figure 11.3, the EMG of the temporalis muscle when the masseter was controlled was lower than the activity of the masseter muscle when the temporalis was controlled, i.e. 5%MVC of the temporalis corresponded to >5%MVC in the masseter and 5%MVC of the masseter corresponded to <5%MVC in the temporalis. No significant correlation was found to exist between the level of EMG activity in a muscle and the reaction time of that muscle.

11.4. DISCUSSION

While previous attempts to define the minimum reaction time of the human jaw to mechanical stimulation have been undertaken (Ottenhoff *et al.*, 1992b; Brodin *et al.*, 1993a), they have been conducted only under static conditions. This is the first study to determine the reaction time and reaction recruitment order of jaw muscles during simulated mastication. The results showed that the reaction time increased by an average of 35% during jaw movement and that the left temporalis was recruited approximately 10ms before the right temporalis, regardless of the experimental condition. However, there was no significant side-to-side difference between the masseter muscles. The masseter muscles were recruited an average of 20ms before the temporalis muscles during jaw closing, but no difference existed during opening.

Four aspects of this study are of interest. Firstly, simulated mastication increased the minimum reaction time significantly in all muscles and in all subjects when compared to static conditions. Secondly, the left temporalis muscle was recruited earlier than right temporalis in all static and dynamic conditions. Thirdly, the masseter muscles were recruited earlier than the temporalis muscles during jaw closing but not during opening. Finally, under static conditions, the reaction time and hence the reaction recruitment order varied depending on the muscle that was used for feedback.

It should be noted that the reaction recruitment order found in the current set of experiments was an average trend only; it was not a fixed rule with differences not only between subject and conditions but also between each attempt in the same subject.

11.4.1. METHODOLOGICAL CONSIDERATIONS

In the present study a technique similar to that used in Chapter 8 was used to determine the minimum reaction time, which incorporated every response for each of the subjects. Using the cumulative sum of the SEMG and an error box of 300% of the maximum pre-stimulus variation, reaction times for each stimulus were determined and used to generate a histogram of raw reaction time values. The data were then analysed to determine which, if any, of the experimental parameters (muscle and/or movement) caused a change in the distribution of the reaction times. The lowest 5^{th} percentile value was taken as the minimum reaction. This value was used deliberately since the mean, mode or median values of the raw reaction time values could

not indicate the fastest reaction time for the entire group, hence classifying half of the reactions as occurring before the reaction time, i.e. they would be classified as reflexes in future reflex studies.

Reaction times are influenced by both the type of stimulation used as well as the rate of stimulus delivery (Brodin *et al.*, 1993a). Hence while inter-conditional differences, such as the longer latency found during dynamic conditions when compared to static, will remain the same future research utilising vastly different experimental paradigms should use the reaction time values as a guide only.

11.4.2. STATIC CONDITIONS

Experiments performed on the jaws of monkeys have shown that with many days of extensive training the average reaction time to a visual stimulus is 150-170ms (Blair-Thomas & Luschei, 1975), however reaction times of 120-130ms have also been sited (Luschei & Goodwin, 1975). While this is longer than the reaction time for the jaw under static conditions found in the current study, the form of stimulation was different, so too was the definition of reaction time (crossing a threshold compared to the start of a sustained change) and the results were an average, not the 5th percentile value used in the current study. The reaction time of human jaw muscles to mechanical stimulation of single tooth (Brodin *et al.*, 1993a) and the entire lower jaw (Ottenhoff *et al.*, 1992b) have been determined previously, but only under static conditions. These studies have reported masseter reaction times of 140ms for orthogonal incisal stimulation and a range of 104-283ms for lower jaw stimulation.

Previous experiments on the minimum reaction time of the masseter and jaw force to axial stimulation of the incisor under static conditions showed that the force generated by the lower jaw sometimes increased before the increase in the SEMG of the masseter muscle (Chapter 9). This was partially explained by the study on the molar teeth that showed the reaction time of the temporalis muscles was less than that of the masseter muscles, and that there was no difference between the reactions times of the two masseters and the jaw force (Chapter 8). However since the experiments used different muscles for feedback, and different teeth were stimulated, the exact reaction recruitment order for tooth stimulation could not be determined. The results of this experiment clearly show that under static conditions, and the incisor teeth stimulated, the left temporalis muscle is preferentially recruited before any of the other muscles, and that it was the likely cause of the commencement of change in the jaw force. In the present study, the delay

between the activation of the left temporalis and the change in the bite force was found to be 19ms, similar to the EMG/force delay reported to exist in the biceps of monkeys (Wolpaw & Carp, 1990).

It is known that when the trigeminal nerve is stimulated, the direct motor responses in the masseter and temporalis muscles occur at the same time (Cruccu, 1986). Hence there is no latency difference between the masseter and temporalis muscles if the signals reached the trigeminal nerve simultaneously. The finding that there was no significant difference between the reaction times in the various muscles when the right masseter was used for feedback is consistent with the idea that the conscious reaction is generated in the motor cortex and sent to the trigeminal nerve at the same time. However, the finding that the reaction time of the masseter muscles were delayed (by approximately 20ms) when the right temporalis muscle was used for feedback indicates that the reactions were not generated at the same time, or that a different biting strategy was employed, one that favoured the temporalis muscles at the expense of the masseters.

11.4.3. DYNAMIC CONDITIONS

This study has shown that the minimum reaction time increased by as much as 35% during simulated chewing. This might partially be due to the perception threshold of stimuli changing during jaw movement, as has been found to occur in both the hand (Schmidt *et al.*, 1990) and the leg (Brooke *et al.*, 2000) in addition to the jaws (Chapman *et al.*, 1987; Kemppainen *et al.*, 1993; Kemppainen *et al.*, 2001; Andreatta & Barlow, 2003). However, a large stimulus (4N) and a relatively fast delivery (30ms rise time) were deliberately used in order to reduce the number of missed reactions in all conditions and minimise the impact of a changing perception threshold. In fact, the number of missed reactions was very few and did not relate to the conditions tested. Moreover, the rate of stimulus application was 30ms during both conditions and the average difference between static and dynamic conditions in the temporalis muscles was over 50ms (i.e. much longer than the stimulus delivery time). Hence the difference between the reaction times in the two conditions was not merely due to a change in the perception threshold.

During chewing, large numbers of trigeminal afferents monitor jaw and tongue movements, as well as the bite force (reviewed in Lund, 1991). This information generates a barrage of action potentials that are sent to the central nervous system. This barrage may then overcome the importance of a small stimulus applied on only two teeth hence delaying the subjects' reaction to the stimulus. The possibility of the reaction time of muscles being

altered by the presence of addition stimuli has been investigated with the use of both transcranial magnetic stimulation (Ziemann *et al.*, 1997; McMillan *et al.*, 2004) and experimental muscle pain (Ervilha *et al.*, 2004). In all cases it was found that the inclusion of additional stimuli (magnetic pulses or experimental pain) caused a significant delay in the reaction time of the subject.

The difference in the reaction time of the masseter during closing compared to opening is not due to a small (but significant) change in the level of muscle activity, as a similar change in muscle activity was found in the temporalis but without the associated change in reaction time. This indicates that the change was due to other factors. It may be that during jaw closure the pattern of activity from the central pattern generator is stronger to the masseter than to the temporalis thereby facilitating the activity generated by the reaction of the subject to the stimulus to a greater extent in the masseter than in the temporalis. However, as it stands this is only speculation.

11.4.4. REACTION RECRUITMENT ORDER

During voluntarily biting tasks, the bite force is generated by the activity of masseter, temporalis and medial pterygoid muscles (Warwick & Williams, 1973). Since the level of activity in each of the jaw muscles, and even different components of the same muscle (Blanksma & van Eijden, 1990; Blanksma *et al.*, 1992; Blanksma & van Eijden, 1995), changes from one bite to the next, even when the task is identical (Scutter & Türker, 1998), the masticatory system has been described as mechanically redundant. This means that a particular bite force could be generated by an almost infinite combination of muscle forces from various jaw muscles (van Eijden *et al.*, 1990). It can be suggested therefore that the reaction recruitment order of jaw muscles may not be fixed but changes depending on the task. The present study has shown that on average the basic pattern of the reaction recruitment order is relatively steady, some muscles are recruited preferentially before others and that this reaction recruitment order changes depending on the condition.

Another finding from the reaction recruitment order analysis was that there is a general tendency for the muscles on one side of the jaw to be recruited before the other. The type of feedback did not cause this side difference as no feedback on muscle activity was provided to the subjects during jaw movement. There have been reports regarding left-right asymmetry in jaw reflexes that tied this difference to the chewing side preference (Murray &

Klineberg, 1984) and claimed that that the asymmetry was the rule rather than exception (Hoogmartens, 1986). However, others demonstrated that there was no side-to-side difference in anterior temporalis or in the masseter during chewing or during rapid jaw closing tasks (Nielsen & Miller, 1988). The reasoning underlying the earlier activation of the left temporalis therefore needs further investigation.

11.4.5. BACKGROUND SEMG

As large variations in the level of background EMG were expected in both muscles, particularly when only one muscle was controlled during static tasks and none during dynamic, it was necessary to determine if there was any correlation between the background EMG level and reaction time. While a number of studies have shown that the level of background activity alters a muscles reflex response (De Luca *et al.*, 1982; Matthews, 1986; Miles & Türker, 1986; Türker, 1988; Toft *et al.*, 1989; Cathers *et al.*, 2004) experiments presented previously have shown that at low levels of muscle activity there is little change (Chapters 8 and 9). It has also been shown that the reaction time of human elbow-flexors is unchanged with varying loads (Ervilha *et al.*, 2004), a finding backed up by the results of the current study. Hence, while the level of EMG activity may be an important factor in the elicitation of reflexes, particularly at high levels, it has little or no impact on the reaction time.

11.4.6. FUTURE DIRECTIONS

Now that the reaction time and reaction recruitment order of the human jaw muscles to mechanical stimulation of incisal teeth under dynamic conditions has been determined, the next step is to study the modulation of periodontally induced jaw reflexes during simulated mastication. The current study is a prerequisite for investigations into the modulation of reflexes during jaw movement since a response to a stimulus commencing after the minimum reaction time may not be entirely reflex in origin.

12. Conclusion

There have been a number of important findings derived from the work contained in this thesis.

Firstly, a new method for the identification, classification and measuring of reflexes has been presented. This method, based on the area between the EMG and the pre-stimulus mean (or cumulative sum; CUSUM), it is simple to implement, reliable, accurate and free from observer bias.

Secondly, the response of the human jaw muscles to axial stimulation of both the incisor and the molar teeth under static conditions have been found. The results show that the responses of the two are fundamentally different indicating a disparity between how the human jaw reacts to incisal and molar stimuli.

Thirdly, it has been shown that the majority of increases in the rectified and averaged EMG following the reflex inhibition caused by axial stimulation of the incisor are not true reflexes but rather 'synchronised activity', artefacts of the averaging process.

Finally, a new device has been described that permits controlled movements of the human jaw. This device was then used to determine the change in EMG of the main jaw closing muscles and bite force reaction times between static conditions, when the jaw was closing and when the jaw was opening. The findings were a dramatic increase in the reaction time when the jaw was moving compared to when it was stationary, and faster reaction time in the masseter muscles during jaw closing compared to opening.

12.1. REFLEX MEASUREMENT AND DETECTION

The method for the measurement of reflex occurrence, latency, strength and duration from EMG records described in Chapter 6 and used in Chapters 7, 8 and 9; and the increase in the error box size to ignore reflexes but identify reactions (Chapters 8, 9 and 11), is accurate, repeatable and free from observer bias. However it does have a number of variables to be set depending on the responses to be measured. The two main variables are the number of stimuli to be delivered and the pre-stimulus analysis time to be used. The decision of what values to use requires not only some prior knowledge of the size and latency of the desired response but also the conditions under which it will be evoked.

If the response is very large with respect to the background variability (several times larger), such as with a conscious reaction, H-reflex or motor evoked potential, then the selection of variables is not that critical. Averaging 20 traces together (Chapter 9) will give a good result but reliable measurements can also be taken from individual traces (Chapters 8 and 11). The same is true for the duration of the pre-stimulus period. Provided it is long enough to cover the entire frequency content of the signal (approximately 100ms) then the exact duration is not crucial.

However if subtle reflexes are to be investigated then the selection of variables become more critical. Of most importance is the size (both amplitude and duration) of the response. For example if the average amplitude of the response is approximately 25% above, or below, the pre-stimulus mean and the duration is expected to be 20ms then the error box must be less than 5k.ms (product of amplitude and duration). Once this is known then experimental conditions that may have an impact must be taken into account. High levels of muscle activity are associated with larger variability in the pre-stimulus period and may reduce reflex durations (McNamara *et al.*, 1977; Fung *et al.*, 1982; Miles & Türker, 1986) hence lower levels are recommended to study small reflexes. It is also important to make the pre- and post-stimulus times the same so that a reliable estimation of the signal over the entire analysis time can be made (Chapter 6). To do this the temporal statistics (latency and duration) of the reflex must be approximated. Once all these parameters have been estimated (reflex size, pre-stimulus variability and length of analysis) the number of stimuli required can be derived, where by the error box size will be directly proportional to the variability in the signal and the square root of the analysis time, and inversely proportional to the square of the number of stimuli.

The results from the reflex experiments (Chapters 7, 8 and 9) show that 50 stimuli and a pre-stimulus analysis period of 125 or 100ms is sufficient for most studies. Although early excitation may have been more easily identifiable from the background variability if more stimuli, or a smaller pre-stimulus time, were used.

When discussing the limits used for the detection of a significant event the 'error box' was used in preference to the Poisson distribution suggested in the literature (Davey *et al.*, 1986) for a number of reasons. Firstly, the curved limit of the Poisson distribution is much better at detecting a gradual, sustained change from the background than a number of rapid events as seen in reflex studies. Secondly, it is possible that a negative reflex event could occur after a random increase in the background level. This reflexes event may then not cross the threshold, but if the random background level were negative then the level would be crossed. Hence it is better to apply the limits between every two turning points, regardless of the absolute value of the CUSUM. In the concept of a limit that increases with time is largely unnecessary when using the method discussed in Chapter 6 due to the detection method looking for 'relative' (between turning points) rather than 'absolute' (between a turning point and the 0 level) changes.

12.2. EARLY EXCITATION

Due to the latency, duration, excitatory nature, and the increased incidence during a local anaesthetic (LA) block of incisal teeth to remove the periodontal reflex component (Chapter 7), the early excitation reflex is most likely to be caused by the activation of muscle spindles in the jaw-closers, which are known to respond to stretch and vibration (Granit & Henatsch, 1956; Crowe & Matthews, 1964; Fukuyama *et al.*, 2000). Table 12.1 compares the findings from a number of studies into jaw reflexes with regards to early excitation.

In the experiments described in the preceding text (Chapters 7, 8 and 9) conditions that may have activated muscle spindles were deliberately avoided. If a loading or unloading stimulus stretches the jaw muscles (Matthews, 1975) or large vibrations are present (Orchardson & Sime, 1981) then the muscle spindles will be activated and hence an early excitation reflex is likely to be generated. By fixing the head using tooth moulds and a nosepiece to reduce jaw stretch, and by using a stimulus with preload to remove high frequency components in the stimulus, the possibility of stimulating the muscle spindles is reduced. This was confirmed in Chapter 7 by the findings showing the occurrence of early excitation was minimal and only increased when fast force rates (which have larger frequency components) were used. The fact that early excitation occurrences increased during LA application to the incisors points out that axial stimulation of incisors induces simultaneous activity in muscle spindles and periodontal mechanoreceptors (PMRs) and that part of the spindle response is suppressed by the stronger inhibitory response from the PMRs. During the LA block PMR related inhibition dramatically reduced hence exposing the early excitation originating from spindles. Therefore, during normal mastication these two pathways may be competing to regulate the activity in the jaw-closers.

Table 12.1: Summary Of Findings Regarding Early Excitation

Researchers	Stimulus	Occurrence	Latency	Notes
(Hannam *et al.*, 1970)	5-10N tapped with light hammer with 2ms duration, delivered to the labial surface of upper central incisor	Consistently observed	Similar to jaw-jerk	Not removed by LA. Concluded to be the result of spindles
(Goldberg, 1971)	Labial surface of maxillary central incisor via stick and reflex hammer	Consistently observed	7ms	Was attenuated by LA. Force of tap and bite level not controlled
(Sessle & Schmitt, 1972)	4N dynamic and 2N static force delivered axially to right maxillary central incisor with duration of 10ms, rising phase of 3ms stimulus rate 2Hz	Frequently evoked	N/A	Not analysed due to limitations in analysis/ stimulus method
(Orchardson & Sime, 1981)	10-30N axial force delivered manually	Consistently evoked	8ms	Not removed by LA. Subjects jaws were relaxed
(van Steenberghe *et al.*, 1989)	Electronically driven pendulum system	Not always evoked	6ms	Small compared to following inhibition
(Brodin *et al.*, 1993b)	3N and 2N slow and fast forces applied orthogonal to labial surface of incisor. Preload used to remove vibrations from start of stimulus	Rarely, if ever, evoked	N/A	Some, or all, could be attributed to artefacts of the rectification process.

Researchers	Stimulus	Occurrence	Latency	Notes
(Türker & Jenkins, 2000)	2N unloading stimuli applied orthogonally to maxillary incisor	40% occurrence rate before LA and 60% during	13ms	Size increased during LA
Incisor Study (Chapter 7)	2N stimuli with preload applied at three different rates applied axially to left maxillary incisor	Very low but increased with increasing rate and after LA. 3% slow stimulus before LA, 28% fast during LA	13ms	Nose piece used to stop movement of the head
Molar Study (Chapter 8)	3, 6 and 8N stimuli with preload applied to 1st right upper molar	Rarely evoked (16%)	9.5ms	More common when jaw active
2nd Incisor Study (Chapter 9)	1, 2 and 3N stimuli with three different preloads applied axially to left maxillary incisor	Almost never evoked (<5%)	N/A	Steps taken to reduce incidence

The combined findings from these researchers suggests that the cause of the early excitatory reflex is the stimulation of muscle spindles either by stretch or vibration and that its presence can be masked by the periodontal mechanoreceptor induced inhibition that commonly follows stimulation of incisor teeth. By using relatively small forces, a preload to reduce vibration and by fixing the head to reduce stretch the incidence of early excitation is reduced. LA represents local anaesthetic.

Although the reflex occurrence rate in the studies described in this thesis (Chapters 7,8 and 9) was found to be low, studies in animals clearly show the existence of the neural structures for the early excitatory reflex. In decerebrate cats (Dessem, 1995) it has been shown that tooth displacement induces short-latency depolarisations in spindle cell bodies in the mesencephalic nucleus of the trigeminal nerve and in the motoneurons of the jaw elevator muscles. Similar short-latency excitatory reflexes in the masseter have been observed following intra-oral stimulation in rats (Funakoshi & Amano, 1974). It has also been shown that orthogonal unloading of an upper incisor tooth briefly stretches the jaw-closers and hence generates the early excitatory response much more successfully (Türker & Jenkins, 2000). With a 60% occurrence rate during the application of LA compared to less than 30% found in one set of experiments utilising axial loading of incisors (Chapter 7), less than 5% in another (Chapter 9) and approximately 16% when the stimuli were applied to molar teeth (Chapter 8).

12.3. INHIBITION

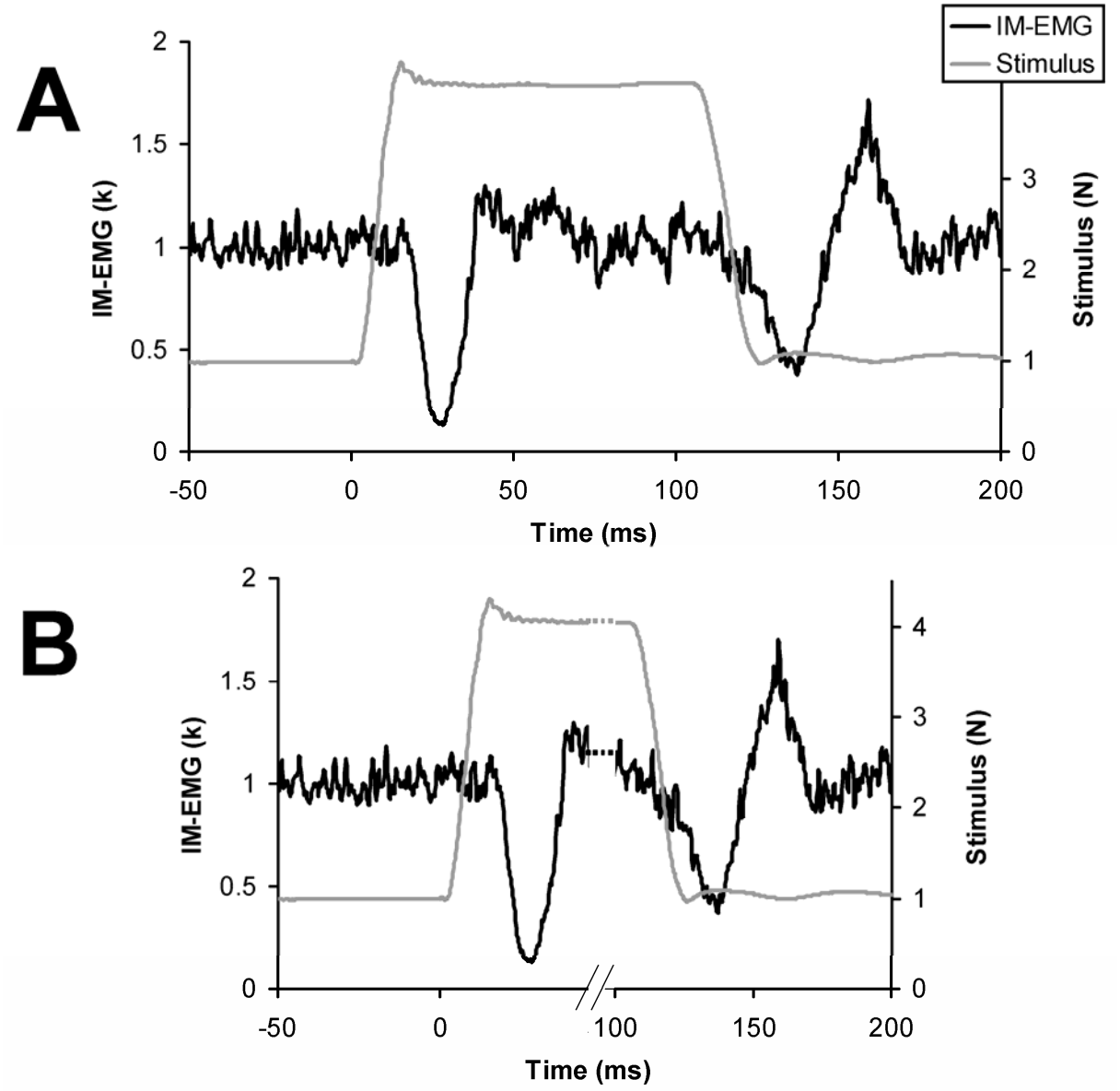
While many researchers have found that the primary response to rapid mechanical stimulation of the incisor teeth is an inhibition of the jaw closing muscles (Sessle & Schmitt, 1972; Matthews, 1975; van der Glas *et al.*, 1984; van der Glas *et al.*, 1985; Brodin *et al.*, 1993b; Louca *et al.*, 1994 & 1996; Türker *et al.*, 1997) produced by the activation of PMRs (Louca *et al.*, 1998; Türker & Jenkins, 2000) the evidence for reflexes following stimulation of the molar teeth was less convincing (Yamamura *et al.*, 1993). The research presented in Chapter 8 shows that there is little, if any, inhibition produced when the molar teeth are stimulated with forces of 8N or less and that PMRs are not responsible for the reflex activity.

The current findings show that the inhibitory reflex following axial stimulation of the incisor is real, as it is associated with a decrease in the firing rates of the underlying motor units (Chapter 9), and that it is produced by the stimulation of PMRs, as the application of a local anaesthetic block removed the reflex (Chapter 7). The inhibition is modulated by the size of the stimulus, with larger stimuli producing larger and more frequent responses (Chapter 9). It overwhelms the smaller early excitation produced by the stimulation of muscle spindles (Chapter 7). It is dependant on the rate of stimulus application with fast stimuli more likely to elicit a response than slow stimuli (Chapter 7), while the level of voluntary muscle activity (assuming less than 20%MVC) and the size of the preload have little or no affect on the reflex parameters (Chapters 7 and 9). Finally, it was shown, using the cumulative peri-stimulus dischargegram (CPSD) analysis method, that the duration of the inhibition duration was longer than indicated in both the rectified and averaged intra-muscular and surface EMG traces (Chapter 9).

Direct stimulation of the inferior alveolar nerve in anaesthetised cats has indicated that there may be two phases of reflex inhibition in the masseter (Kidokoro *et al.*, 1968); and it has been proposed that two separate inhibitory pathways reflexly control the masseter muscle (Sumino, 1976). Experiments utilising electrical stimulation in humans has also found evidence for two inhibitory phases (Godaux & Desmedt, 1975b; Miles & Türker, 1987; Miles *et al.*, 1987) while mechanical stimulation has elicited both single and dual phase responses (Sessle & Schmitt, 1972; van der Glas *et al.*, 1984). Using the stimulation procedures described in the various experimental chapters within this thesis no two phase inhibitions were found. While this could be due to a difference between orthogonal (all previous studies) and axial (current studies) stimulation of incisors previous orthogonal experiments employing similar force profiles have found only a single inhibitory phase

(Türker *et al.*, 1994; Yang & Türker, 1999). The most likely reason for the discrepancy is that mechanical stimuli often include both a loading and an unloading phase, both of which are capable of eliciting similar reflex responses in the masseter (Türker & Jenkins, 2000). This is shown in Figure 12.1 where the post-stimulus analysis time has been increased to include both the rising and falling phases of the mechanical stimulation, then the hold phase reduced to show how the close temporal proximity of the rising and falling phases might produce a response that could be mistaken for a two phase inhibition.

Figure 12.1: Reflex Response To Loading And Unloading Of Incisor



A) Averaged (n=140) reflex response to axial stimulation of the incisor, post-stimulus period has been increase to show both the loading and unloading responses. B) What the responses might look like if the 'hold phase' of the stimulus was reduced. Unloading of the incisor produced a reflex inhibition in the intra-muscular (IM-) EMG of the masseter muscle which was similar to the one produced by loading. If the rising and falling phases of the stimulus were brought closer together then the unloading response would occur immediately after the loading response, and it would appear that the stimulus produced a two phase inhibition.

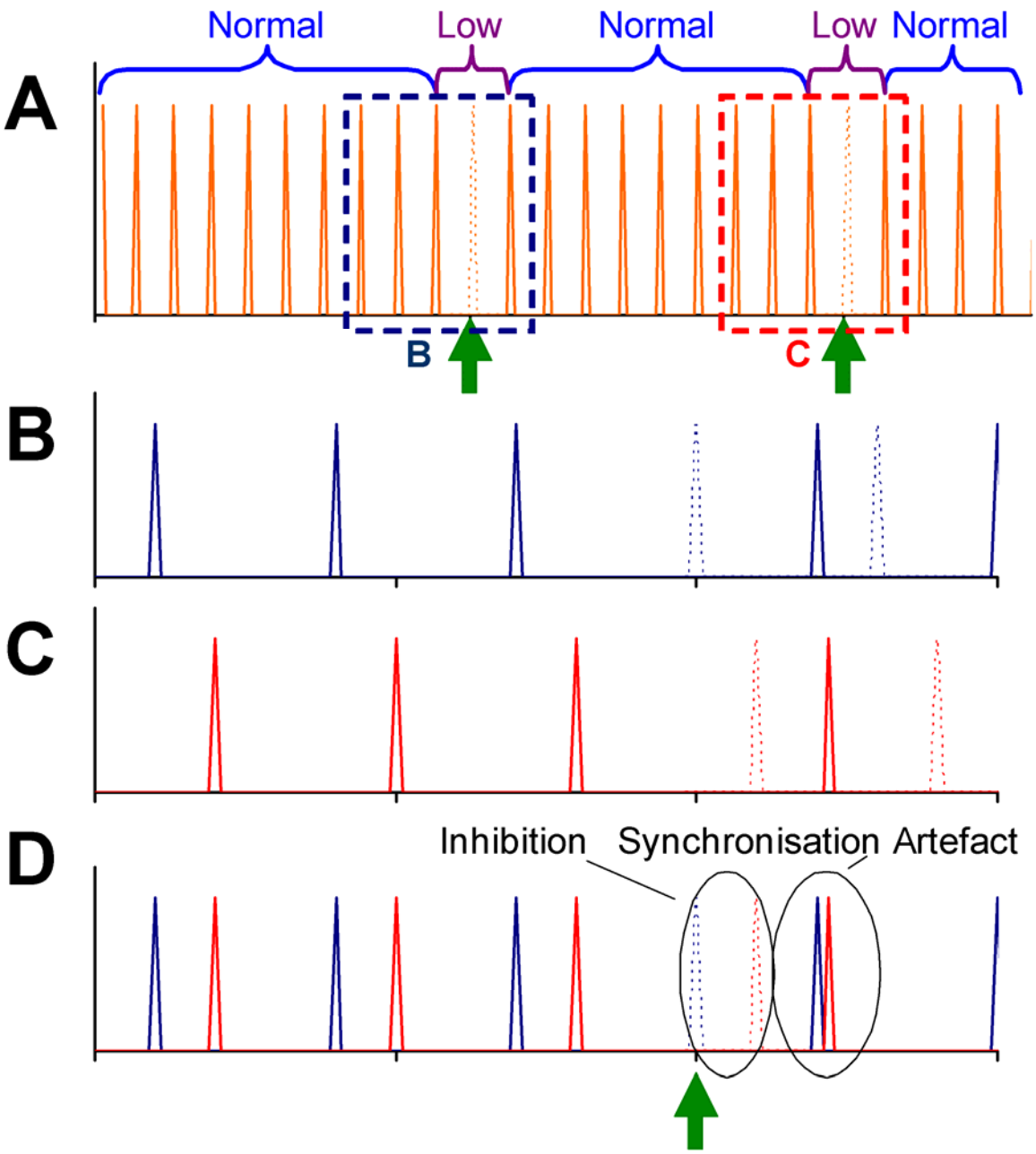
12.4. LATE EXCITATION

Previous experiments reporting the response of human masseter muscles to orthogonal stimulation of the incisor have found transient decreases and increases in the EMG activity (Beaudreau *et al.*, 1969; Hannam *et al.*, 1969; Brodin *et al.*, 1991). In full-wave rectified and averaged EMG records the series of downward going waves interposed or followed by upward going waves was labelled the post-stimulus EMG complex (van der Glas *et al.*, 1984). While the main response to rapid incisor stimulation was inhibition (Bjornland *et al.*, 1991) a subsequent increase in the EMG was always observed. While some researchers explained this increase as a muscle spindle response to the preceding inhibition (Yemm, 1972b; Mitchell, 1991), or as a polysynaptic reflex response to the stimulus (van der Glas *et al.*, 1985), others thought it may be an artefact caused by the delay (clustering) of action potentials following the inhibition (Moore *et al.*, 1970; Miles *et al.*, 1987; Awiszus, 1988; Miles *et al.*, 1989b).

All information regarding the level of muscle activity in a skeletal muscle is frequency encoded (Bray *et al.*, 1994; Sherwood, 2004), that is the force generated by the muscle is a function of the frequency of action potentials (motor unit firings) received by the muscle. If the time between receiving action potentials is long then the muscle will be relaxed, however if the time between successive action potentials is short then the muscle will contract at a level related to the time between events. The problem with traditional probability based analysis procedures is not that they forget this basic information but that they ignore that fact that when EMG traces are averaged around a stimulus some time information is lost, specifically that events occurring at the same time after a stimulus, but in response to different stimuli, do not occur at the same time. Rather they are separated by several seconds and have no impact on one another, hence will not be transferred to an increase in muscle force.

To over come this problem a theory was devised whereby after every stimulus the motor units that would have fired during the period of an inhibitory reflex were delayed. Hence, after each stimulus there would be a period of no unit firings followed by an occurrence marking the end of the inhibitory period. When looking at the response to a single stimulus no excitation would not be apparent, as the unit is simply inhibited. However, if a number of stimuli were averaged together, and the end of the inhibitory period was roughly the same time after the stimulus, the motor unit occurrences marking the end of the inhibition would overlap and appear to indicate an excitation. This concept is illustrated in Figure 12.2.

Figure 12.2: Artefacts In Probability Based Analysis



A) Unit firing with constant frequency until delayed by a stimulus (arrow) applied at a random time. 'Normal' represents a background firing rate while 'Low' represents a period of reduced firing rate. As can be seen there is no period of increased firing rate. Zoom in of units around stimulus 1 (B) and stimulus 2 (C). D) Superimposition of units averaged around the time of stimulus delivery. Solid lines indicate unit occurrence while dashed lines indicate time of firing if no stimulus was applied. The effect of the stimulus was only to delay the occurrence of unit firings a brief time. However, probability-based analysis methods, which look only at the number of units to fire in a given time period, would classify the clustering of units after the inhibition as an excitation even though it is purely an artefact of the averaging process (synchronisation error).

Further research was conducted utilising controlled rapid and slow pushes to the labial surface of incisor teeth (Brodin *et al.*, 1993b). The results showed that rapid pushes invoked a short latency inhibition and late excitation but also the EMG tended to increase slowly over a long period of time, while slow stimuli only produced the slow increase in EMG, the rapid inhibition and excitation were absent. To investigate the cause of these responses a study was conducted to find the inter-spike intervals underlying the EMG following stimulation of the incisor (Türker *et al.*, 1994). The results indicated that while the slow increase over time was a real event there was no reduction in the inter-spike interval underlying the rapid increase (late excitation) following the inhibitory reflex.

It should be noted that an attempt by another group to replicate the results of Brodin et al (1993) to slow orthogonal loading of the incisor failed (Louca *et al.*, 1996) to elicit the long latency, slow increase in EMG over time. However, in response a study was conducted to determine the conditions most amenable to eliciting the slow excitatory response (Türker *et al.*, 1997). The latter study discovered that the rise time of the stimulus needed to be 100ms or longer, which was more that four times the duration used by Louca et al (1996), and it was critical that high accelerations in the force profile be avoided, thus half-sinusoids rather than trapezoidal waveforms should be used. Despite these constraints the occurrence rate of the slow reflex excitation was still only about 50%. This was yet more evidence of the variability between subjects.

In the study reported in Chapter 7 it was shown that axial stimulation of the incisor produced the rapid inhibition and late excitation responses observed in response to orthogonal stimulation, but the slow increase in EMG was not found. In Chapter 8 it was discovered that axial stimulation of the molar did not induce a significant inhibitory response and the occurrence of late excitations was much lower than when the incisor was stimulated and an inhibition produced. Hence, it was unclear if the late excitation following the inhibition in response to axial stimulation of teeth was real or artefactual.

In 1994 a method was proposed to determine the instantaneous discharge rates of single motor units (Türker & Cheng, 1994) later termed peri-stimulus frequencygram (PSF). While the method was good, the validity and superiority of the results over traditional analysis, including rectification and averaging and peri-stimulus time histograms, was proven by experiments performed on rat brain slice preparations (Türker & Powers, 1999), there were a number of limitations. Of primary concern was the time required to perform the analysis. Single motor unit studies require hundreds of stimuli to be delivered during a single trial condition before an accurate picture could be established. This time frame means that it was impossible to test

more than a few conditions in an experiment, severely limiting scope. It also introduces other factors such a fatigue that can make it difficult to interpret the results.

To overcome this problem a new method, this time utilising multi-unit rather than single-unit recordings was put forward and tested using brain slice preparations (Türker & Powers, 2003). This method, cumulative peri-stimulus dischargegram or CPSD, was adapted for use with human reflex studies and the results from experiments where the incisor was axially stimulated are presented in Chapter 9. It was found that approximately two-thirds of the increases in the EMG following a reflex inhibition were not associated with an increasing discharge rate of the underlying motor units. Hence the late excitations were not a true reflex but rather 'synchronised activity', an artefact of the averaging process.

12.5. PERIODONTAL MECHANORECEPTORS

By recording from the inferior alveolar nerve using tungsten electrodes, two different receptor types have been identified in the human periodontium (Trulsson *et al.*, 1992; Trulsson & Johansson, 1994). The 'saturating group' are more active in response to static forces less than 1N and loose their dynamic sensitivity above this level, and the 'non-saturating' group which display a linear response to forces over 5N and keep their dynamic sensitivity even during the application of static forces above this level.

A number of studies have shown that the majority of receptors are located around the apex of the tooth root (Pfaffmann, 1939a & b; Cash & Linden, 1982; Byers & Dong, 1989). It has also been reported that PMR afferents enter both the mesencephalic nucleus (Jerge, 1963; Funakoshi & Amano, 1974; Linden, 1978) and the trigeminal ganglion (Corbin & Harrison, 1940). These findings appear to support the conclusion that there are two different types of receptor, or that the different populations of receptors function differently.

The trigeminal ganglion receptors project to the main sensory nucleus of V and the spinal trigeminal complex (Kruger & Michel, 1962) and are thought to be in the conscious perception of oral sensation via thalamic and cortical pathways (Byers, 1985). Single unit studies of trigeminal ganglion cell bodies from periodontal receptors showed that they have the same kind of directional sensitivity and respond to touch, pressure, and tooth movement as the mesencephalic mechanoreceptors (Beaudreau & Jerge, 1968). Previous researchers have claimed that the trigeminal ganglion receptors were often very slowly adapting (Beaudreau & Jerge, 1968; Appenteng *et al.*, 1982a), compared with medium to rapid rates for the mesencephalic neurons (Jerge, 1963). However, other research has indicated that there may only be one type of PMR, slowly-adapting (Trulsson *et al.*, 1992), and the rapid adaptation rates seen during some experiments may be because some of the PMRs were not optimally stimulated (reviewed in Linden, 1990).

There are conflicting reports regarding the location of PMR cell bodies within the brain depending on the type of analysis used. Histochemical studies have injected radioactive dyes directly into the trigeminal ganglion or mesencephalic trigeminal nucleus in animal brains and traced all the receptors coming from individual teeth (Byers *et al.*, 1986; Byers & Dong, 1989). These studies have shown that the receptors closest to the apex of the tooth root have cell bodies in the mesencephalic trigeminal nucleus while those located in the middle of the root have cell bodies in the trigeminal ganglion. However a physiological study (Linden & Scott, 1989) has shown the opposite, receptors in the apex of the tooth root have connections to the

trigeminal ganglion while those in the middle of the root have cell bodies in the mesencephalic trigeminal nucleus. This disparity has lead to confusion and a number of different, conflicting, wiring diagrams of the human masticatory system being proposed (Yang, 1999; Türker, 2002) regarding the location of the PMR cell bodies.

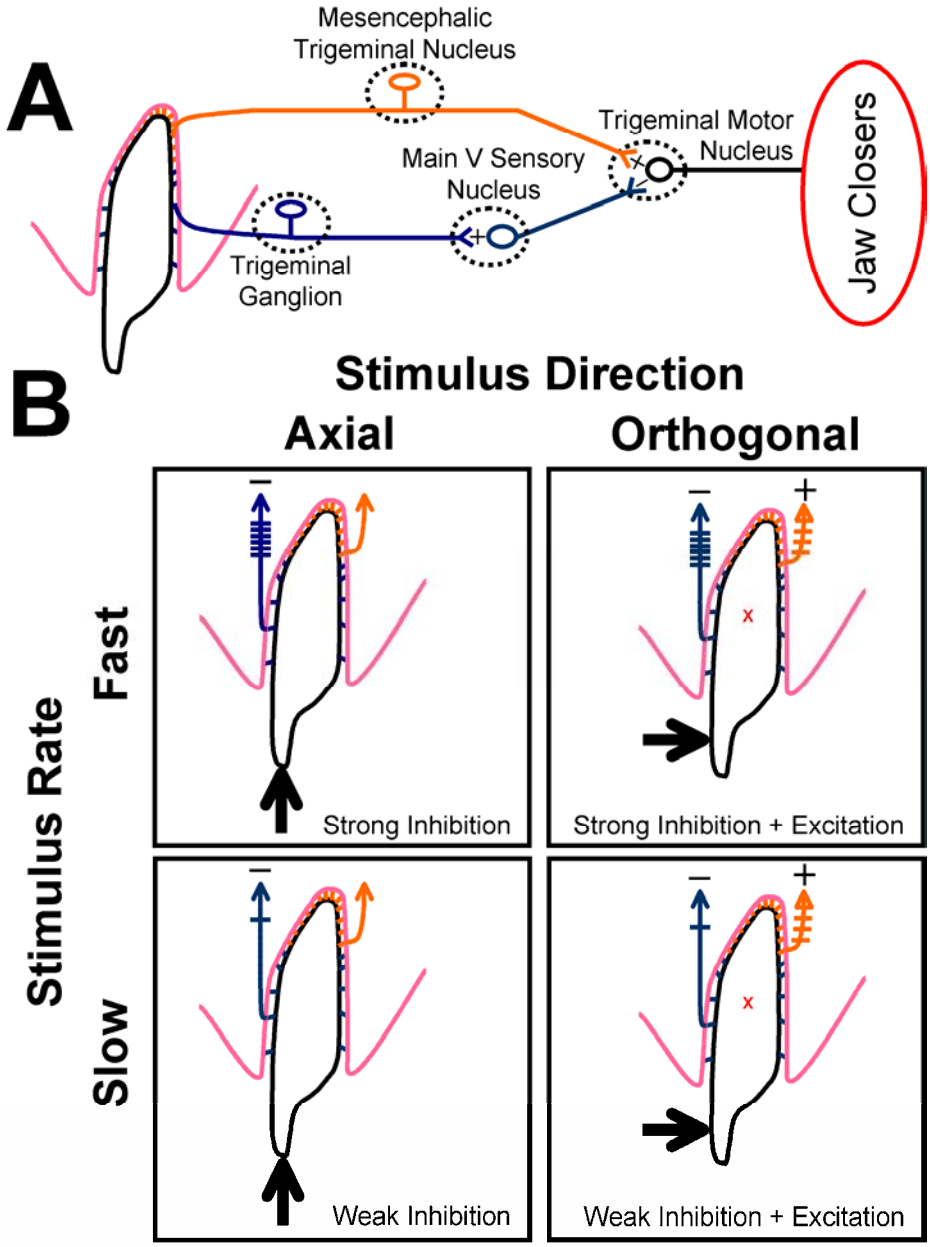
In a set of histochemical experiments conducted in the cat the ultrastructural characteristics of both muscle spindles and slowly-adapting (saturating) PMRs were studied (Bae *et al.*, 1996). The results showed that both made monosynaptic connections to nerves in the trigeminal motor nucleus, that were presumed to be jaw-closing α-motoneurons, and the afferents contained clear, spherical, synaptic vesicles indicating an excitatory connection. However, the connections of the PMRs were different to those made by the muscle spindles and could possibly indicate a slower, indirect influence rather than a direct excitatory connection. No mention was made of the location of the PMR cell bodies within the brain.

Animal studies have shown that PMRs respond to tension, but not compression (Cash & Linden, 1982). Hence, orthogonal stimulation will stimulate receptors both at the apex and in the middle of the tooth root; however stimulation along the long axis of the incisor root (as described in Chapters 7 and 9) will crush the receptors at the apex and stimulate only the receptors in the middle of the tooth root. Previous orthogonal (Brodin *et al.*, 1993b; Türker *et al.*, 1994; Türker *et al.*, 1997) stimulation experiments and an axial experiment described in Chapter 7 have shown that slowly rising stimuli elicit excitatory reflex activity if applied orthogonally but little or no response if applied axially. While rapidly rising stimuli elicit strong inhibitory activity regardless of the direction of the applied stimulus. These results show that the saturating receptors are located at the apex of the tooth and produce excitation in the jaw closing muscles, while the non-saturating receptors are located around the middle of the tooth root and inhibit jaw-closers if stimulated.

Using this information regarding the saturation rates of the receptors at various points along the tooth root. The effect that these receptors have on the jaw-closers from orthogonal (Brodin *et al.*, 1993b) and axial (Chapter 7) experiments. The finding that saturating receptors make excitatory connections to the jaw-closers (Bae *et al.*, 1996). The discovery that trigeminal ganglion receptors project to the main V sensory nucleus (Kruger & Michel, 1962), with excitatory connections (Bae *et al.*, 1993). Together with the conclusion that PMR afferents project to both the mesencephalic nucleus and the trigeminal ganglion (Funakoshi, 1981) with the majority going to the mesencephalic nucleus (Byers & Dong, 1989), it is possible to propose a wiring diagram for the incisor/masseter system, shown in Figure 12.3. Non-

saturating receptors are located near the middle of the tooth root, the activation of which has a proposed inhibitory effect on the masseter. These PMRs have cell bodies in the trigeminal ganglion and make excitatory connections to neurons in the main V sensory nucleus, which then have an inhibitory effect on the jaw-closers. The saturating receptors, which are located at the apex of the tooth root, have a proposed excitatory effect on the jaw-closers via monosynaptic connections with α-motoneurons, however the nature of this connection is not exactly the same as the muscle spindles (Bae *et al.*, 1996) indicating that it could be a weaker, slower connection.

Figure 12.3: Proposed Model Of The Neural Wiring In The Jaw



A) Proposed model of the affect periodontal mechanoreceptors have on the jaw closing muscles. B) The response during different stimulation conditions. Slowly adapting receptors (orange) are located at the apex of the tooth, and rapidly adapting receptors (blue) located closer to the gingiva. The fulcrum (X) is the point about which the tooth rotates during orthogonal stimulation. Below the fulcrum the force applied to the tooth, and surrounding tissue, is in the same direction as the stimulus, above the fulcrum the force is in the opposite direction. Slowly adapting receptors are activated by orthogonal stimuli and have a proposed slow or indirect excitatory effect on the jaw-closers. These receptors do not respond to axial stimuli as they are compressed. Rapidly adapting receptors, which have a proposed inhibitory effect on jaw-closers, are activated by fast stimuli in either the axial or orthogonal direction.

12.6. REACTION TIME

Static experiments on the human jaw have shown that the reaction time of the masseter muscle to controlled orthogonal stimulation of the incisor is approximately 140ms (Brodin *et al.*, 1993a) while stimulation of the entire lower jaw produces a large range of reaction times, 104 to 283ms (Ottenhoff *et al.*, 1992b).

The results from Chapter 9 shows that the reaction time of the masseter to controlled axial stimulation of the incisor under static conditions is approximately 140ms, the same as during orthogonal and while the change in bite force was, on average, 10ms later some reactions in the bite force record were earlier than those of the masseter. This discrepancy was partially explained when the temporalis, masseter and bite force reaction times were determined in response to molar stimulation in Chapter 8. Under such conditions the bite force sometimes changed before the EMG of the masseter but the reaction time of the temporalis was earlier. However it was unclear if the difference was due to changes in the site of stimulation, or of the type of feedback used (masseter or temporalis). Hence further studies were needed where the incisors were stimulated, both the masseter and temporalis muscles were recorded and the type of feedback varied. These experiments were carried out and the results, presented in Chapter 11, indicate that while the type of feedback did have an impact, the temporalis muscle often reacted faster than the masseter. It was also found that when the jaw was rhythmically opened and closed, the reaction time went up by approximately 35% compared to when the jaw was stationary and that the masseter reacted faster during jaw closing than during jaw opening but no mastication phase differences were found to exist in the temporalis muscles.