THE CONTRIBUTION OF PERIODONTAL MECHANORECEPTORS TO PHYSIOLOGICAL TREMOR IN THE HUMAN JAW

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DOCTOR OF PHILOSOPHY

by

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Author's Declaration

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Paul F. Sowman

November, 2007

Abstract

The human jaw, like all other articulated body parts, exhibits small oscillatory movements during isometric holding tasks. These movements, known as physiological tremor, arise as a consequence of the interaction of various factors. One of these factors is reflex feedback from peripheral receptors. In the human jaw, receptors that innervate the periodontium are able to transduce minute changes in force. This thesis examines the contribution of these periodontal mechanoreceptors (PMRs) to the genesis of physiological tremor of the human jaw.

By using frequency domain analysis of time series recorded during isometric biting tasks, the character of physiological jaw tremor can be revealed. Physiological jaw tremor was observed in force recorded from between the teeth as well as from electromyograms recorded from the principal muscles of mastication. These recordings have shown us that jaw physiological tremor consists of a frequency invariant component between 6 and 10Hz. This frequency remains unaltered under various load conditions where the mechanical resonance of the jaw would be expected to vary greatly (Chapter 2). Such findings indicate a 'neurogenic' origin for this tremor. A possible candidate for this neurogenic component of physiological tremor in the jaw is the reflex feedback arising from the PMRs.

Using local anaesthetisation, it has been shown in this thesis, that by blocking outflow from the PMRs, the amplitude of neurogenic physiological jaw tremor can be reduced dramatically. This procedure caused a dramatic reduction in not only the mechanical recordings of tremor but also in the coupling between masseteric muscles bilaterally (Chapter 3) and between single motor units recorded from within a homonymous muscle (Chapter 4).

The obvious mechanism by which periodontal mechanoreceptor anaesthetisation could reduce the amplitude of physiological tremor in the jaw would be by reducing the amplitude of the oscillatory input to the motoneurones driving the tremor. This interpretation remains controversial however as physiological tremor in the jaw can be observed at force levels above which the PMRs are supposedly saturated in their response. In light of this knowledge, the saturating characteristics of these receptors in terms of reflex output were examined. To do this, a novel stimulation paradigm was devised whereby the incisal teeth were mechanically stimulated with identical stimulus waveforms superimposed upon increasing tooth preloads. This necessitated the use of a frequency response method to quantify the reflexes. An optimal frequency for stimulation was identified and used to confirm that the hyperbolic saturating response of PMRs observed previously, translated to a similar phenomenon in masticatory reflexes (Chapter 5).

These data reinforced the idea that physiological tremor in the jaw was not just a consequence of rhythmic reflex input from PMRs, as the dynamic reflex response uncoupled from the input as the receptor-mediated reflex response saturated. An alternative hypothesis was then developed that suggested the effect of PMR suppression in physiological tremor was via tonic rather than rhythmic effects on the masseteric motoneurone pool.

By utilising a novel contraction strategy to manipulate the mean firing rate of the motor neuron pool at a given level of force production, data contained in Chapter 6 shows that population motor unit firing statistics influence the expression of physiological tremor, and such manipulations mimic, to an extent, the changes in firing statistics and tremor amplitude seen during anaesthetisation of the PMRs. This thesis therefore posits a

mechanism whereby periodontal input influences the firing rate of motoneurones in such a way as to promote tremulous activity (Chapter 5). However, as this proposed mechanism did not explain the full extent of tremor suppression seen during PMR anaesthetisation it can therefore only be considered a contributing factor in a multifactor process.

Acknowledgements

Dedication

I'd like to dedicate this thesis to my Mum and Dad.

To Mary-Anne and Fred, who nurtured my interest in learning from the beginning.

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Chapter 1
Literature Review

1.1 Publication Acknowledgement

Parts of the following review have been published in "The role of periodontal mechanoreceptors in mastication." Arch Oral Biol. 2007 Apr;52(4):361-4. Epub 2007 Jan 12. Türker KS, Sowman PF, Tuncer M, Tucker KJ, Brinkworth RS

1.2 Introduction

The main function of mastication is to break food down into small enough pieces for swallowing. Mastication is performed by jaw muscles that generate large forces across very short distances and apply them via rigid teeth. Such large forces could easily damage the teeth or the supporting tissues of the oral cavity such as the tongue and cheeks if they were not controlled precisely and effectively.

In this literature review, the importance of peripheral feedback control during masticatory behaviour will be outlined. Most attention will focus on the position of the periodontal mechanoreceptors (PMRs) in this control mechanism and particularly their reflex actions on the main jaw closing muscle, the masseter. The second focus of this literature review will be physiological tremor. This basic phenomenon of neuromuscular control will be discussed in terms of its hypothesised origins. Specific attention will be paid to the neurogenic component of physiological tremor and the

extent to which exteroceptive reflexes could influence its emergence and/or extent of expression. Lastly, the existence of physiological tremor in the jaw will be discussed.

1.3 The Muscles of Mastication

The masseter muscles act in concert with the temporali and medial pterygoids to elevate the mandible, bringing the teeth into opposition. These are the jaw-closing muscles and they are characterized by a morphology that suits the development of large forces which, during normal mastication in the human, average around 27 kgs (Gibbs *et al.*, 1981). Conversely, their antagonists, the jaw-opening muscles (lateral pterygoid and the suprahyoid group), are better designed to produce velocity and displacement (van Eijden *et al.*, 1997). Together, these muscles are known as the 'muscles of mastication' (Luschei and Goldberg, 1981).

1.3.1 Masseter

The masseter muscle has a complex structure. Its muscle fibres are arranged in a multi-pennate pattern that specialises different portions for different functions. Whilst complex in architecture, a gross division based on compartmental function is made between the deep and superficial regions (Hannam and Mc Millan, 1994). The superficial portion, originating from a single strong tendon arising from the anterior two thirds of the zygomatic arch, inserts into the inferior third of the mandible. The muscle fibres in this division of the masseter are generally directed downwards and slightly backward (Scott and Dixon, 1972), and are most active during strong force production, mandibular elevation and jaw retrusion on the side ipsilateral to the muscle (Hannam

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and Mcmillan, 1994). In the deep portion, fibre direction is vertical and the muscle bulk is richly supplied with muscle spindles (Eriksson and Thornell, 1987; Eriksson *et al.*, 1994). Its function is believed to be primarily postural control of the mandible (Eriksson *et al.*, 1984; Hannam and McMillan, 1994).

1.4 Supraspinal Control of Mastication

1.4.1 Cortical Control of Mastication

The corticomotoneuronal cells that supply the motoneurones that control the musculature of the face and head project their fast-conducting efferent fibres in the corticobulbar tract. Those that project onto trigeminal motor nuclei project both ipsilaterally and contralaterally (Kuypers, 1958a; Iwatsubo *et al.*, 1990) though their relative distribution is asymmetrical. The corticobulbar fibres that project onto masseter motoneurones follow an approximately 80:20 contralateral bias (Pearce *et al.*, 2003), which is evident, albeit subclinically, as a reduction in the level of voluntary EMG in the masseter contralateral to the lesioned hemisphere of stroke patients (Cruccu *et al.*, 1988). Conversely, the suprahyoid motoneurones, also supplied by fast-conducting corticobulbar fibres, project more equally bilaterally (Cruccu *et al.*, 1989; Gooden *et al.*, 1999).

Although neuroanatomical evidence, arising from both human and animal studies (Kuypers, 1958b, a) suggests a role for the motor cortex in the control of mastication and other voluntary jaw movements, studies in monkeys where these areas of the motor cortex are cooled to temporarily inhibit their function, have failed to impair force

control in the masticatory apparatus (Murray *et al.*, 1991). These findings agree with earlier work by Luschei and Goodwin (1975) who reported that following bilateral lesioning of similar cortical areas in monkeys, phasic, forceful bite development was maintained. However, this study also reported a lasting disability to maintain low, steady bite forces. The conclusion of such studies suggests that the motor cortex plays little part in ongoing masticatory function but instead is important for the conscious initiation and termination of mastication, or for the performance of deliberate "extra-masticatory" tasks such as holding or carrying. It has been suggested also, that such projections may be important for the fine control that is required for speech production [Nordstrom (2004), Unpublished].

1.4.2 The 'Central Pattern Generator'

Supraspinal control of masticatory motor function, particularly that giving rise to the rhythmic jaw movements that characterise normal masticatory function, is thought to arise primarily from a central pattern generator (CPG) (Dellow and Lund, 1971) located in the medial bulbar reticular formation of the brainstem between the motor root of the trigeminal nerve and the inferior olive. The CPG rhythmically and alternately excites and inhibits the antagonistic muscles of mastication such that rhythmic opening and closing occurs without conscious executive input from the motor cortex (reviewed in Lund, 1991). Whilst the CPG dictates the underlying rhythm of mastication, the entire process seems to be dependent upon the amplification of depolarizing potentials in motoneurones by peripheral sensory feedback (Kubo *et al.*, 1981; Lund, 1991). This schema suggests that the initial conscious descending command from the motor cortex

creates both the phase reset for the CPG and the initial afferent 'kickstart' that allows perpetuation of masticatory cycling until conscious termination or the disappearance of peripheral stimuli (when the bolus is swallowed) occurs. This idea is bolstered by experimental results that show that rhythmic jaw movements that arise from the CPG can be elicited in anaesthetised animal preparations by stimulation of an area in the cortical precentral gyrus (Lund and Lamarre, 1974) that has become consequently known as the cortical masticatory area.

1.5 Masticatory Neuroanatomy

An authoritative description of the neuroanatomical organisation of the masticatory system's reflexes is available in (Taylor, 1990). The following is a brief overview. The motor nucleus of the fifth cranial nerve (N.V) contains the γ- and α- motoneurones of the muscles of mastication. The axons of these motoneurones combine with proprioceptive afferents in the root of N.V. However, the cell bodies of these afferents are located within the brain rather than in the trigeminal ganglion. This collection of cells is referred to as the mesencephalic nucleus of the trigeminal nerve (Mes V). Studies of the response properties of Mes V cells by natural stimulation have shown that some cells can be activated by the stretch of jaw muscles. Others have been shown to respond to mechanical stimulation of one or more teeth, and are therefore presumed to terminate on periodontal receptors. The spindle afferent cell bodies are found throughout the length of the nucleus, and those from the periodontal tissue are found only in the caudal half (Cody *et al*., 1972). A schematic summary of these afferents is presented in figure 1.1

Figure 1.1. Schematic of the proposed neuroanatomy underlying reflexes elicited by physiological stimulation of trigeminally supplied receptors. Activity in pathway 1 is direct to the trigeminal motor nucleus and results in excitation of jaw-closing motoneurones. Pathway 2 inhibits jaw-closing motoneurones via interneurones in the supratrigeminal nucleus, reticular formation or trigeminal brainstem sensory complex. Receptors are indicated as: a) cutaneous; b) periodontal; c) mucosal or periosteal; d) temporomandibular joint; or; e) muscle. Diagram adapted from Dubner *et al*. (1978).

1.6 Peripheral Control of Mastication

Although experiments on animals have established the existence of a rhythm for mastication produced by a CPG (Dellow and Lund, 1971), there is evidence that masticatory forces are controlled precisely by peripheral feedback [reviewed in Türker (2002)] and that these forces can change from bite-to-bite depending on the consistency of the food bolus being chewed (Thexton, 1992). In lightly anaesthetised chewing rabbits it has been illustrated that, by disabling the maxillary and inferior alveolar nerves and thereby removing sensory feedback arising from the PMRs, the facilitation of the masseter muscle, observed when a test strip is inserted between the teeth, is greatly reduced (Lavigne *et al.*, 1987; Morimoto *et al.*, 1989). When spindle cell bodies are also destroyed, the facilitation of the jaw closers disappears almost completely (Morimoto and Nagashima, 1989). These experiments convincingly demonstrated that peripheral receptors, especially the PMRs and the muscle spindles, play important roles in modulating the activity of motoneurones in 'chewing' animals.

1.6.1 Periodontal Mechanoreceptors

The PMRs are so named because of their location in the supporting tissues that surround the tooth root and their primary method of activation: mechanical stimulation of the periodontium. The response characteristics of these receptors suggest that they are similar to Ruffini endings in the skin. They respond maximally when the part of the periodontal ligament in which they lie is put under tension (Linden, 1990). The magnitude of response elicited by these receptors corresponds directly to the amplitude,

rate and direction of force applied to the teeth (Trulsson *et al.*, 1992; Hannam and Mcmillan, 1994; Trulsson and Johansson, 1994; Türker *et al.*, 1997).

Animal studies suggest the division of PMRs into two main subgroups: rapidly- and slowly-adapting receptors. It is further suggested that these subgroups follow a group-specific spatial distribution within the periodontium. The most common subgroup of PMRs exists near the apex of the tooth root (Ness, 1954). These apical receptors respond to tension, but not compression (Cash and Linden, 1982), have low activation thresholds, and they adapt slowly to changes in force. There also exists a second class of receptor located below the apex closer to the fulcrum of rotation, these also respond only to tension but have a higher activation threshold, and are rapidly-adapting (reviewed in Linden, 1990). It has been assumed that differing responses observed from PMRs corresponds to the existence two morphologically distinct receptor groups, i.e. slowly-adapting Ruffini endings and more rapidly-adapting Meissner corpuscles. Histologically however, there is little evidence to support this division (Maeda *et al.*, 1990). Rather, as the response of receptors is positionally graded in this way, from the apex to the fulcrum of the tooth, a purely biomechanical explanation for the different response characteristics based on the location of a single receptor type within the periodontal ligament (Cash and Linden, 1982; Linden and Millar, 1988a) [reviewed in Linden (1990)] seems more likely.

When a force is applied to the crown of a tooth, rotation of that tooth occurs about a fulcrum. Given that the PMRs respond only to tension, the various degrees of displacement of the tooth root relative its distance from the fulcrum will dictate the

degree of tension the receptor is subjected to and hence the relative gradation of its response. Experimentally this response has been observed to be largest for those receptors lying closest to the apex and is seen to diminish closer to the fulcrum. Such observations fit well with a biomechanical explanation for divergent receptor responses to a given stimulus, as the tooth root at the apex would undergo the greatest displacement and so exert the greatest tensioning onto those receptors in that locale. As a result they would appear to have a lower force threshold and be more slowly-adapting than those receptors closer to the fulcrum of the tooth.

This phenomenon may also be responsible for the saturating and non-saturating division of receptor groups identified by Trulsson and Johansson (1994,1995,1996). In their schema, saturating receptors lose sensitivity to changes in force above 1N. Conversely, the non-saturating receptors display a linear increase in discharge rate to forces up to and beyond 5N and do not lose their dynamic sensitivity to small changes in force above and below a high DC level. It seems that the biomechanical properties described to explain the different response characteristics of PMRs in animals could equally be applied to saturating and non-saturating receptors; whereby, saturating receptors are analogous to rapidly adapting receptors and non-saturating analogous to slowly adapting receptors.

The afferent projections of the PMRs, along with those of other oral receptors, travel to the brainstem in the mandibular and maxillary divisions of the trigeminal nerve. The cell bodies of the PMRs are located in the semilunar ganglion, and in the mesencephalic

nucleus of the trigeminal nerve. Histochemical studies have shown that the distribution of receptor cell bodies between these ganglia reflects their distribution around the tooth root. The receptors closest to the apex of the tooth root i.e. rapidly adapting receptors, have cell bodies in the mesencephalic nucleus while those located in the middle of the root i.e. slowly adapting receptors, have cell bodies in the semilunar ganglion (Byers *et al.*, 1986; Byers and Dong, 1989). This neat distribution pattern is contradicted by a number of other studies (reviewed in Linden, 1990) that show that most (over 70%) of the receptors whose cell bodies reside in the trigeminal mesencephalic nucleus congregate in the labial to mesial aspects of the teeth. On the other hand, those receptors whose cell bodies lie in the trigeminal ganglion are distributed equally throughout the periodontal space.

1.7 Reflexes Elicited in the Jaw Muscles by Mechanical Stimulation of the Teeth

1.7.1 The "Early Excitation" Reflex

A mechanical tap stimulus to the tooth usually induces an early masseteric excitatory reflex, which precedes the inhibitory reflex response (Hannam *et al.*, 1970; Goldberg, 1971; Orchardson and Sime, 1981). This reflex, termed the "periodontal-masseteric reflex" may originate from the periodontal space, since, in some experiments, it has been observed to disappear during periodontal local anaesthesia. It is also possible that this early excitatory reflex may originate from PMRs that excite spindle cell bodies in the trigeminal mesencephalic nucleus (Baker and Llinas, 1971). It is most likely however, that this reflex originates from vibration sensitive muscle spindles in the jaw

closers, though the latency of this reflex appears shorter than the spindle mediated jaw

jerk reflex (Carels and Van Steenberghe, 1985). In decerebrate cats, Dessem (1995) has

shown that tooth displacement induces short-latency depolarizations in spindle cell

bodies in the mesencephalic nucleus of the trigeminal nerve and in the motoneurones of

the jaw elevator muscles. Similar short-latency excitatory reflexes in the masseter have

been observed following intra-oral stimulation in rats (Funakoshi and Amano, 1974).

In the decerebrate cat, tooth tap stimulation activated the masseter motoneurones (both

α and γ) at a latency of about 6 ms (Sessle, 1977).

1.7.2 Inhibition

Other than the early excitatory reflex, which becomes more obvious when local

anaesthetic block is applied to the stimulated tooth, tooth tap stimuli induce a prominent

inhibition of the jaw closing muscles at a latency of 12 ms, presumably by stimulating

highly rate-sensitive PMRs (Van Der Glas *et al.*, 1985; Louca *et al.*, 1994). Recently,

the importance of this rate sensitivity to stimulus force in eliciting excitatory or

inhibitory responses from the masseter has been illustrated experimentally. Slowly

rising stimuli, i.e. stimuli with a 40N/s or lower rate of rise, mainly induced an

excitatory reflex while the rapidly rising stimulus, i.e. stimuli with a 1000N/s or greater

rate of rise, usually induced inhibition (Türker *et al.*, 1994). There were also other

variables that affected the success of the stimulus in inducing a certain type of reflex

response, such as the presence of a preload and the exact stimulus force profile. A

slowly rising mechanical stimulus is likely to stimulate only the PMRs, as the reflex

response to a 3N force disappeared when local anaesthetic was infiltrated around the

stimulated tooth (Brinkworth *et al.*, 2003). Reflex work, where slowly-rising mechanical stimuli were applied to the incisor teeth in an orthogonal direction suggested that, at low levels of background activity, the stimulus generates a net closing force, whereas at high levels of background activity, it induces a net reduction of the closing force (Yang and Türker, 1999). This may be due to the differential activation of motor units by PMRs, whereby small motor units receive excitatory input and large motor units receive inhibitory input (Yamamura and Shimada, 1992). Therefore, at low bite force levels, reflex activation of small-sized motor units would be relatively intense, helping to keep food between the teeth (Trulsson and Johansson, 1995). However, when the background bite force is already high, the same periodontal input may inhibit the larger motor units and hence limit further increases in bite force, which could damage the teeth and supporting structures.

1.7.3 The Unloading Reflex

The unloading mechanism in the jaws is experimentally studied by introducing, then suddenly and unpredictably removing, a load between the jaws. In response to such stimuli, unloading reflexes have been recorded for both jaw opener and closer muscles (Lamarre and Lund 1975; Miles and Wilkinson 1982). The most prominent component of the jaw-unloading reflex is a short latency (10-20 ms) reduction in the surface electromyogram (SEMG) of the jaw closing muscles. In addition, an increase in the SEMG of the anterior digastric muscle is observed at a slightly longer latency (approximately 25 ms) (Hannam *et al.* 1968). A number of receptors are affected by the fracturing of a brittle object between teeth and thus could be responsible for

initiating this reflex. These receptors include muscle spindles, temporomandibular joint (TMJ) receptors, PMRs, and also acoustic receptors (Duncan *et al*. 1992). However there has been little direct investigation into which receptors actually control this reflex.

There have been several attempts to determine whether the PMRs contribute to the unloading reflex (Türker and Jenkins, 2000). Blocking periodontal input by infiltration of local anaesthetics around the roots of the teeth, failed to alter the unloading reflex (Poliakov and Miles 1994; Miles *et al*. 1995). Therefore, it was concluded that PMRs were of minimal importance. Instead, it was put forward that muscle spindles are the principal contributors to the unloading reflex, primarily because the latency of the onset of SEMG reduction is consistent with a monosynaptic pathway.

However, the masticatory system is quite unique in that not only is it possible to exclude PMRs with the use of local anaesthetics, but to also destroy the spindle cell bodies (in animals) that originate from the jaw muscles. Goodwin and Luschei (1974) showed that destruction of the spindle cell bodies in the trigeminal mesencephalic nucleus of monkeys did not alter the jaw-unloading reflex. Therefore, disfacilitation due to the removal of spindle input cannot be fully responsible for the jaw-unloading reflex.

Recently, a new technique for inducing the unloading reflex by the withdrawal of orthogonally applied forces to teeth has been trialled. This approach does not move the

jaws appreciably, but induces an inhibitory response in the jaw closers which is totally abolished by the local anaesthetic block of the stimulated teeth (Türker and Jenkins 2000). This study showed that PMRs do contribute to the jaw-unloading reflex, since the net response of tooth unloading was a substantial inhibition of the jaw closers. This cannot be appreciated when the contribution of the PMRs are assessed by unloading the entire jaw, since as well as the PMRs, other receptors such as the spindles, TMJ receptors and skin receptors would then contribute to halting the closing of the mandible.

The results of several studies (Ottenhoff *et al.* 1992a; Ottenhoff *et al.* 1992b) indicate that, during chewing, PMRs are predominantly responsible for eliciting the additional muscle activity that is required to overcome resistances encountered between the teeth. If this excitatory pathway is stimulated by pressure on the teeth during biting, then the removal of this pressure that occurs with tooth unloading would result in the removal of the excitatory input, and hence a reduction in masseter muscle activity. Thus, the inhibition seen with periodontal unloading could be due to both an activation of the inhibitory pathway and also a removal or reduction of the activity in the excitatory pathway.

The size of the inhibitory responses seen in the orthogonal tooth unload experiments is likely to be an underestimation of the PMR mediated inhibition that occurs under normal jaw unloading situations. Fracturing of an object between the jaws would

unload several teeth in the upper and lower jaw and stimulate many more receptors than unloading of a single tooth in the orthogonal direction. Furthermore, the overlap of the early excitatory response would reduce and delay the impact of the inhibitory response further.

1.8 Physiological Tremor

All active positions of the human body are characterised by small, approximately sinusoidal, involuntary movements (Elble and Koller, 1990). These small movements, usually unresolvable by the human eye, are common to all body parts and are present during postural or isometric conditions and also during the performance of otherwise smooth slow movements (Wessberg and Vallbo, 1995). It is these 'micro' non-pathological movements that are known as physiological tremor. The genesis of these movements falls into two main categories; those that are the consequence of inherent mechanical characteristics of the body part in question, and those that are of 'neurogenic' origin.

1.8.1 Mechanical Resonance

The mechanical resonance hypothesis for physiological tremor is based on the premise that body parts can be thought of as second order mechanical systems that, when perturbed, tend to exhibit damped oscillation at their natural frequencies. Sources proposed for such ongoing perturbations include the unfused twitches of newly recruited motor units or the pressure pulse associated with blood circulation. The frequency of this oscillation is determined by the system's moment of inertia and its

stiffness. Because of these relationships, the frequency of this component of tremor is not invariant, rather, it can be decreased with the addition of a load (an increase in the moment of inertia) and increased when a movement or hold is performed against an elastic load such as a spring (an increase in elasticity). It has been comprehensively demonstrated that a large proportion of those oscillations that constitute physiological tremor are due to passive mechanical properties of the body part under study (Stiles and Randall, 1967; Joyce and Rack, 1974; Brown *et al.*, 1982; Elble, 1986; Takanokura and Sakamoto, 2001). Furthermore, due to the significant divergence in the mechanical properties of different body parts, their natural frequencies, calculated to be as high as 25 Hz for the finger, the jaw 3 Hz (Cooker *et al.*, 1980) and the arm as low as 2 Hz (Stiles and Randall, 1967; Joyce and Rack, 1974), would dictate that physiological tremors fill a broad frequency band. For this reason, the common concept that physiological tremor occurs at ~10Hz is misleading and may account for much of the perceived disagreement regarding physiological tremor that exists in the literature. The oft reported ~10Hz or 6-12Hz physiological tremor which has received much literature attention, is the component thought to represent neurogenic physiological tremor. In many cases however, notably resting states, the mechanical component of tremor, driven by the pressure pulse of the heart or unfused motor unit firings, may be much larger than the neurogenic component.

1.8.2 Neurogenic Tremor

In contrast to that frequency dictated by their natural resonances, a similar frequency of tremor can be observed across many body parts e.g. a component of physiological

tremor is seen in the finger (Mcauley *et al.*, 1997), arm (Raethjen *et al.*, 2002) and in the jaw (Van Steenberghe, 1982; Junge *et al.*, 1998) circa 8Hz (hereafter "8Hz"). The frequency invariance of this component across different body parts and in the presence of changes in load (Fox and Randall, 1970; Allum *et al.*, 1978; Arihara and Sakamoto, 1999) and elasticity (Sutton and Sykes, 1967; Elble and Randall, 1976), suggests the presence of a common driving oscillator. The existence of a supra-spinal oscillator that drives oscillations in motor output has long been postulated e.g. Marshall and Walsh (1956); Armstrong (1974). Recently, the existence of cortical oscillatory activity that is coherent with motor output has become detectable with the advent of high resolution electroencephalography (EEG) and magnetoencephalography (MEG). Many reports of coherent oscillations between cortical signals and motor output now exist e.g. (Salenius *et al.*, 1997; Gross *et al.*, 2000; Gross *et al.*, 2002; Pohja *et al.*, 2002; Raethjen *et al.*, 2002; Baker and Baker, 2003; Grosse *et al.*, 2003; Salenius and Hari, 2003; Hansen and Nielsen, 2004; Pollok *et al.*, 2004; Riddle *et al.*, 2004; Timmermann *et al.*, 2004; Riddle and Baker, 2005; Schnitzler and Gross, 2005; Riddle and Baker, 2006; Schnitzler *et al.*, 2006) however the ability of these methods to reliably demonstrate that these cortical sources are efferent generators of tremor remains debated (Mcauley, 2001; Riddle and Baker, 2005; Rivlin-Etzion *et al.*, 2006).

1.8.3 Reflex Oscillations

Reflex loop oscillation is another source of rhythmic activity thought to be a possible origin of the neurogenic component of physiological tremor. Any body part subject to reflex feedback is susceptible to oscillation at a frequency determined by the sum of the

delays in the reflex response pathway. These delays include the activation time of the receptors initiating the reflex, the following delay in the afferent and efferent pathways, including synaptic time, and the delay in the subsequent force output of the effector muscles that subserve the reflex response. For reflex feedback to account for the frequency of oscillation, the reflex delay should be equivalent to half the oscillation period –approximately 60ms for a tremor frequency of 8Hz. While it is often argued that this is a reason against the reflex hypothesis, loops being longer in the arm than in the jaw for example, the contribution to the loop time made by the length of the nerves is very small relative to the time taken up by electromechanical delay and the synaptic transit. Therefore, the total reflex loop time can be similar across all systems as it is mostly dependent on electromechanical delays. Accordingly, it has been expounded by many authors that the stretch reflex loop is primary responsible for normal physiological tremor (Lippold, 1970; Matthews and Muir, 1980; Burne, 1987; Jacks *et al.*, 1988; Durbaba *et al.*, 2005). Whether the stretch reflex in isolation could be responsible for the initiation of physiological tremor remains controversial however (Elble and Koller, 1990), as the very low gain in this reflex loop is unlikely to generate the level of instability necessary to sustain oscillation. Nevertheless, there remains a significant quantity of evidence that links the stretch reflex to physiological tremor. Techniques that enhance the gain in the stretch reflex loop are known to enhance the expression of physiological tremor (Cussons *et al.*, 1978; Young and Hagbarth, 1980) while decreasing the activity in the stretch reflex loop by limb restraint (Burne, 1987) greatly diminishes it. Furthermore, the well documented enhancement of physiological tremor normally seen during fatigue is blocked by limb ischemia (Cresswell and

Loscher, 2000). Studies such as this last one provide convincing evidence of an interaction between the stretch reflex loop and an independently existent physiological tremor, that, in some cases promotes tremor expression (Lippold, 1970; Joyce and Rack, 1974; Young and Hagbarth, 1980) whilst in other cases acts as a tremor limiting factor (Goodwin and Luschei, 1974; Neilson and Neilson, 1978; Cooker *et al.*, 1980).

As the above demonstrates, reflex contributions to physiological tremor have sustained interest for some time. Interestingly, this seems to largely have been focussed on proprioceptive feedback, especially that arising from muscle spindles. Although other reflex pathways have been mentioned (Stein and Oguztöreli, 1976), other than a few studies, e.g. van Steenberghe and de Vries (1980), Fisher *et al.* (2002), little attention has been paid to the possible contribution of exteroceptive reflexes to the genesis of physiological tremor. Both of the aforementioned studies showed that local anaesthetic blockade of pertinent mechanoreceptors during isometric holding significantly diminished the neurogenic component of physiological tremor. Additionally, another study has shown a positive correlation between exeroceptor reflex gain and tremor amplitude (Mcfarland *et al.*, 1986).

1.9 Synchronisation of Motor Units: The Final Common Pathway for Physiological Tremor

Motor units (MU) are the smallest elements of neuromuscular control, and activation of motor units is the final common path for all common neural control strategies. During voluntary contractions, there is a weak tendency for pairs of motor units in a given human muscle to fire within a few milliseconds of one another. This phenomenon has been termed 'short-term synchrony' and is believed to result from common excitatory (Sears and Stagg, 1976) or inhibitory (Türker and Powers, 2002) inputs which branch widely to innervate some or all motoneurones in the motoneuronal pool of a given muscle.

If this input is oscillatory, there will be a tendency for rhythmic synchrony between motoneurones to occur at the frequency of this input. Applying this theory to the case of physiological tremor, the rhythmic input that is responsible for the neurogenic component, whether that be a supraspinal generator or a reflex loop oscillation, will be expressed at the motor unit level as rhythmic synchrony. Although there remains some contrary opinion to this thesis (Freund, 1983; Semmler and Nordstrom, 1998), a considerable weight of empirical evidence now supports the idea that the neurogenic component of physiological tremor is the result of rhythmic motor unit synchronisation (Elble and Randall, 1976; Erimaki and Christakos, 1999; Halliday *et al.*, 1999; Wessberg and Kakuda, 1999; Kilner *et al.*, 2002; Christakos *et al.*, 2006). It is important to note that this effect is independent of motor unit firing rate, in that tremor

is not the result of motor units firing synchronously at their intrinsic firing rates, rather, that their firing rates are being modulated at a frequency determined by the tremorgenic input. In this way, two motor units each firing at above 15Hz, may show synchrony at 8Hz if they share a common input at this frequency. While the occurrence of this synchrony is not based on synchronous entrainment of intrinsic firing rates and is therefore often said to be 'rate independent' (Wessberg and Kakuda, 1999), the strength of synchrony will be related to firing rate according to the carrier frequency principle (Matthews, 1997). This states that as the carrier frequency [dictated by the intrinsic firing rate of the motor unit(s) in question] approaches the frequency of the input, the gain at the frequency of that input will increase. This principle, explained in terms of the modulation of single motor unit firings, applies equally to synchrony occurring in motor unit pools (Harrison *et al.*, 1991; Lowery and Erim, 2005) though the relationship may not be apparent when only a narrow range of physiological firing frequencies are examined (Christou *et al.*, 2007).

1.10 Physiological Tremor in the Jaw

The study of physiological tremor has only extended to the human mandible in a few cases. The principle factors determining physiological tremor in the mandible are analogous to those in other body parts however and many parallels can be made. Like the upper limb the mandibular physiological tremor spectrum is dominated by its mechanical characteristics as a critically damped second order linear system. The natural frequency of this system is considerably lower than the upper limb though; it has been calculated that the jaw muscles convey a corner frequency of around 3Hz onto

the masticatory apparatus. This filter characteristic dictates that sources of displacement, including neural inputs will tend to give rise to an envelope of oscillatory jaw activity at the very low end of the power spectrum i.e. between 0 and 3Hz. Consistent with this, McCarroll and De Vries (1988) have shown that the resting physiological jaw tremor spectrum consists at rest, almost entirely of a ~1Hz component driven by the cardio-ballistic effect of blood ejection. As the jaw becomes active, a greater proportion of this tremor becomes correlated with neuromuscular activity levels, whereas the cardio-ballistic component remains constant, thereby decreasing in its relative contribution. These early reports do not discuss the possibility of a neurogenic component of physiological tremor that may be independent of mechanical resonances, however close examination of figure 1B in de Vries *et al.* (1984) shows that a peak is evident in the power spectra at 8Hz that doesn't seem harmonically related to the 'discrete' cardio-ballistic mechanical frequencies. This area of the mandibular physiological tremor, analogous to that in other body parts thought to represent neurogenic physiological tremor was investigated previously by van Steenberghe and de Vries (1980). By using a tooth mounted transducer that could measure the intrusion of a tooth into the periodontium, they measured physiological tremor during very low force occlusion where the incisors were brought into contact in such a way that the subject could perceive the intermittent contact that was a consequence of the variations in contraction force caused by physiological tremor. They describe the physiological tremor spectrum as occupying a frequency band between 1 and 20Hz with a large local peak between 3 and 8Hz. Of especial significance to the subject considered in this thesis, van Steenberghe and de Vries

(1980) included anaesthetisation of the periodontium in their experimental protocol. The effect this had was to lower the frequency of the physiological tremor. Regrettably, as there was no force-feedback available to the subjects who participated in this experiment, it is likely that in the anaesthetised condition, the subjects' ability to maintain the minimal occlusal force needed to allow comparable fluctuations in tooth intrusion between conditions to occur, would have most likely been abolished. In this way the loss of the measured 8Hz tremor peak may have been a result of the reduction in tooth mobility caused by the higher intrusive force resulting from increased biting force. Under anaesthetised conditions the subjects would most likely have bitten harder in order to establish tactile sensations from deeper structures, an effect that has been described empirically by Trulsson and Johansson (1996) who showed that holding forces applied during periodontal anaesthesia increased by up to 400%. This interpretation was in part that made by the authors. They expected higher bite forces during anaesthetisation (though not measured) and attributed the reduction in tremor frequency to the tendency, observed by Allum *et al*. (1978), for tremor to shift to lower frequencies with increasing contraction force. The authors rule out the reflex loop hypothesis though their justification for doing this is unclear. They do however discuss the possibility that anaesthetisation may have caused lengthening of the reflex loop (an explanation that would require acceptance of the reflex loop hypothesis) or that the removal of periodontal feedback increased the loop gain in a proprioceptive reflex pathway. This last hypothesis seems feasible as several authors suggest that the spindle reflex minimizes the amplitude of physiological tremor (Goodwin *et al.*, 1978; Neilson and Neilson, 1978; Cooker *et al.*, 1980; Matthews, 1997), therefore any increase in its

gain that was caused by removal of PMR input could act to lessen physiological tremor amplitude. Presently however, experimental evidence suggests that lessening the gain in the PMR reflex pathway causes no change in the spindle reflex gain (Hoogmartens and Caubergh, 1988; Miles and Poliakov, 1996).

The neurogenic component of mandibular physiological tremor has been investigated in several other studies. Notably, the report of Junge *et al*. (1998) that showed it was unlikely to arise from either mechanical resonance or from the stretch reflex. They showed that the mean frequency of physiological tremor that was coherent with a driving rhythm in the EMG that occurred at ~7.5Hz. There was no evidence of the damped oscillation that would be expected following a chin tap if the stretch reflex was to be implicated. Additionally, loading the jaw did not change the physiological tremor frequency thereby ruling out pure mechanical resonance phenomena. A limitation of this method in ruling out a mechanical basis for the physiological tremor exists however. Whilst an increased moment of inertia resulting from the mandibular load would have lowered the resonant frequency, the coincident increase in stiffness created by the increased muscle activity in the jaw muscles working to counteract this load would have increased the resonant frequency, perhaps counteracting the effect of loading. Nevertheless, this study provides reasonably convincing evidence of a frequency invariant component to the physiological tremor of the mandible and rules out the stretch reflex as its origin. Additional recent work has reinforced these findings, showing that the neurogenic component of mandibular physiological tremor is driven by reciprocal alternating activation of the jaw openers and closers (Jaberzadeh *et al.*, 2003a) which, in light of the paucity of evidence suggesting a synaptic link between

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jaw opening and closing motoneurones [see Matthews (1975) for discussion], furthers the idea that the rhythm for this activity is centrally generated. Subsequent work from this group (Jaberzadeh *et al.*, 2003b) suggests that the amplitude decrement in mandibular physiological tremor seen during experimentally induced muscle pain is further evidence of such a central origin for physiological tremor. However, these findings ignore the known enhancement of γ drive that occurs during masseteric muscle pain (Wang *et al.*, 2000), a mechanism that would act to reduce tremor by enhancing muscle spindle sensitivity as the previously discussed work of Goodwin and Luschei (1978) shows.

While it seems that the evidence for a stretch reflex origin for the neurogenic component of physiological jaw tremor can be ruled out, a supraspinal origin for physiological tremor in the mandible remains possible. Furthermore, as McFarland *et al.* (1986) showed that the neurogenic mandibular physiological tremor was positively correlated in amplitude with the gain in reflexes evoked by intraoral mechanical stimulation, an exteroceptive reflex origin of physiological tremor of the mandible also remains a possibility, even though the authors of the report indicating its involvement, (Van Steenberghe and De Vries, 1980), rule this out.

Chapter 2

Methods of Time and Frequency Domain Analysis of Physiological Tremor in the Human Jaw

2.1 Publication Acknowledgement

The following is a modification of "Methods of time and frequency domain examination of physiological tremor in the human jaw" Hum Mov Sci. 2005 Oct-Dec;24(5-6):657-66. Epub 2005 Oct 11. Sowman PF and Türker KS

2.2 Abstract

This chapter discusses techniques for examining physiological tremor. The EMG component driving mandibular physiological tremor at 8Hz can be revealed in the time domain manifestation of EMG by demodulation. The co-occurrence of 8Hz physiological tremor in force and EMG can also be seen in the frequency domain representations of these signals and coherence analysis provides a method by which the degree of co-occurrence can be statistically investigated. Additionally, estimation of time lags between the signals by phase and cumulant density analysis provides evidence of the direction of dependence. Data presented herein using these techniques illustrates that for the human jaw, physiological tremor arises from a rhythmic component of EMG. This component is frequency and amplitude invariant across a range of bite forces indicating that it is not due to interaction between the stretch reflex and the mechanical resonance of the system.

2.3 Physiological Tremor in the Jaw

Physiological tremor defined as normally occurring low-amplitude rapid back and forth movement of a body part, is usually observed in the frequency range of 6-12Hz [for review see (Elble and Koller, 1990; Mcauley and Marsden, 2000)]. This phenomenon, most commonly investigated during slow movements and isometric contractions, has been reported in many body parts. The human masticatory system is also characterized by physiological tremor yet this has been reported on in only a few instances (Van Steenberghe and De Vries, 1980; Mcfarland *et al.*, 1986; Junge *et al.*, 1998; Jaberzadeh *et al.*, 2003a). These investigations have revealed a peak in the tremor spectrum at around 8Hz, which is consistent with that frequency of tremor observed in other motor systems.

It has been proposed that physiological tremor arises from activity perpetuated in reflex loops involving feedback from muscle spindles (Lippold, 1970; Joyce and Rack, 1974; Joyce *et al.*, 1974; Matthews and Muir, 1980; Jacks *et al.*, 1988; Prochazka and Trend, 1988), however more recently this has been contested and the hypothesis that physiological tremor is generated centrally by either central pacemakers or central oscillatory circuits (Farmer *et al*. 1993; Farmer 1999; Wessburg and Kakuda 1999; Gross *et al*. 2000; McAuley and Marsden 2000; Gross *et al*. 2002), has been widely accepted. Furthermore, the contribution of the stretch reflex to physiological tremor has been discounted (Wessburg and Kakuda 1999). The discounting of the stretch reflex remains controversial however and recently, Duruba *et al*. (2005), restated the position

put forward by Matthews and Muir (1980) that, under true isometric conditions with the body part fixed against a rigid transducer, the 8Hz component of tremor is abolished. Furthermore, Duruba and colleagues showed this to be the case in 7 of 9 subjects tested in their experiment, arguing that the use of acceleration, velocity or displacement to measure tremor inevitably includes stretch reflex mechanisms, as these measures require the segment to be free in its movement at the end of the lever opposite the hinge, a requirement that in turn allows the segment to oscillate in space and increases the gain in the stretch reflex loop.

This chapter argues that when measuring tremor via force recordings in most body parts, the significant compliance introduced by soft tissues when that segment is coupled to a transducer, may dampen the frequency components of interest to such an extent that they become insignificant. The teeth however provide the opportunity to fixate a body part (the mandible) to a rigid transducer providing an almost direct coupling to the bone, thus providing the opportunity to minimise the contribution of the stretch reflex to jaw tremor whilst still being able to measure minimally damped force fluctuations representative of oscillatory activity. Moreover, under such conditions, increasing the stiffness of the muscle is possible thereby allowing examination of a further mechanical variable.

The rhythmic EMG activity that gives rise to tremulous mechanical output (Elble and Randall, 1976) can be described as amplitude modulated noise; such a description

describes the relatively broadband nature of the EMG 'carrier' spectrum upon which is superimposed a narrowband oscillation responsible for the more easily measured mechanical tremor (Journee, 1983). Because the broadband and narrowband components of EMG activity overlap, it is almost impossible to recognise physiological tremor visually in raw EMG recordings. For this reason, the process of 'demodulating' EMG, described in a series of papers (Fox and Randall, 1970; Journee, 1983; Journee and Van Manen, 1983; Journee *et al.*, 1983) was developed to provide a method by which the narrowband modulation signal can be revealed and visualized.

2.4 Demodulation of EMG

Demodulation of EMG is usually achieved by full-wave rectifying high-pass filtered EMG (10-50Hz) and then low pass filtering (5-20Hz) to smooth the signal. The high pass filter prior to rectification removes the low frequency artefact caused by movement and returns a zero-meaned amplitude-modulated signal. As any modulation of the signal is assumed to occur at all frequencies, high-pass filtering at this stage will not destroy low-frequency modulation information (Raethjen *et al.*, 2002); Timmer, *et al*., 1998). Full-wave rectification of the signal then performs the demodulation, as the negative components of the signal are made positive revealing the modulation of amplitude. Low-pass filtering can now proceed utilizing a zero-phased smoothing window. This technique, often referred to as obtaining the 'envelope' of the EMG signal, is often used to visualize the low frequency on/off patterns of EMG during the gait cycle. However, by altering the filtering characteristics with regard to the higher frequencies involved in

physiological tremor, this technique can be used to visualize the bursting pattern of EMG that underlies physiological tremor.

2.5 Frequency Domain Analysis of Physiological Tremor

The technique of demodulating EMG provides a good way of visualizing EMG physiological tremor in the time domain and provides the starting point for analysis in the frequency domain, the difference being that no smoothing is performed post rectification. Frequency domain analysis of tremor usually involves transformation of time series data into the frequency domain by means of the Fast Fourier Transform (FFT). Using the most common method seen in the literature with regard to EMG, individual power spectra are estimated using a periodogram method described by (Brillinger, 1981) and (Amjad *et al.*, 1997) as: $d_x^T(\lambda,l) = \int_{(l-1)T}^{lT} x(t)e^{-i\lambda t}dt \approx \sum_{t=(l-1)T}^{lT-1} e^{-i\lambda t}x_t$,

where the discrete Fourier transform $d_x^T(\lambda,l)$ of the *lth* segment from time series x(t) at frequency λ is constructed from a series of disjoint sections L of length T. A distinct advantage of this method is that due to the non-overlapping nature of the segments used to estimate the power spectrum, their independence allows a relatively simple estimation of the variance and subsequently confidence intervals for the comparison of power spectral estimates (Chatfield, 1996). Where comparison of spectra between subjects or between levels of contraction intensity (where the power at all frequencies is greater) is desired, it is necessary to normalize the spectra to obtain an estimate of the distribution of power between frequencies bounding the full bandwidth. One way to do

this is to divide the intensity in each of the frequency bins of the spectral estimate by the total power in the estimate (Vaillancourt and Newell, 2000; Sosnoff *et al.*, 2004; Sosnoff *et al.*, 2005). Spectra presented in the following figures have been normalised by this method.
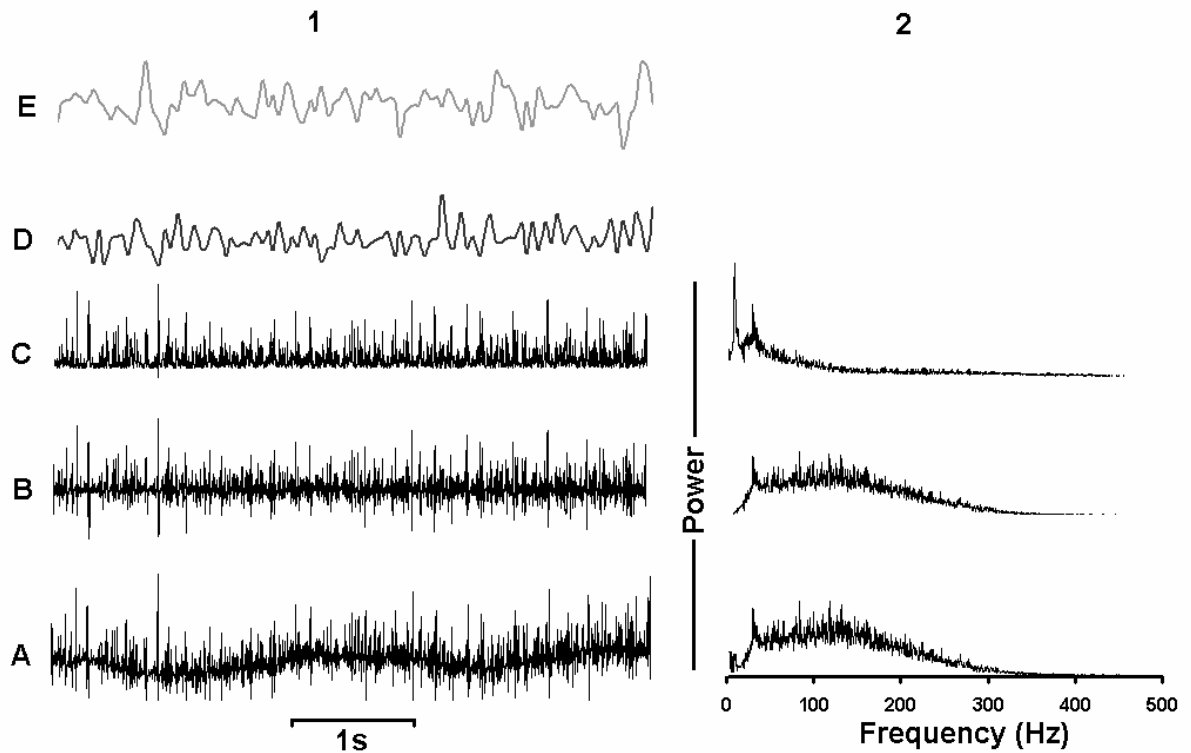
Figure 2.1. Column 1. Time domain realisations of force and EMG (five-second segments) taken from a two-minute recording. A. Raw EMG. B. High-pass filtered EMG (20Hz). C. High-pass filtered and full-wave rectified EMG. D. EMG "envelope" high-pass filtered at 20Hz, full wave rectified, then low pass filtered to 20Hz using a zero-phase smoothing window (Hanning). E. High-pass filtered force (1Hz) recorded from between the incisors during an isometric biting task. Column 2. Frequency domain estimates of the EMG represented in column 1 (A-C). Oscillations occurring at ~8 per second are evident in both time domain representations D and E and are reflected in the large 8Hz peak evident in the frequency domain estimate of the rectified EMG (C) but not in power spectra of the signals prior to rectification (A and B).

The peak in the masseteric EMG power spectrum evident in Figure 2.1 is transmitted, via the teeth, to the object being bitten on. In the case of these experiments this was a force transducer mounted on rigid steel bite bars. Figure 2.2 shows that in 5 out of seven subjects examined, a peak in the force power spectral record between 6 and 10Hz is evident.
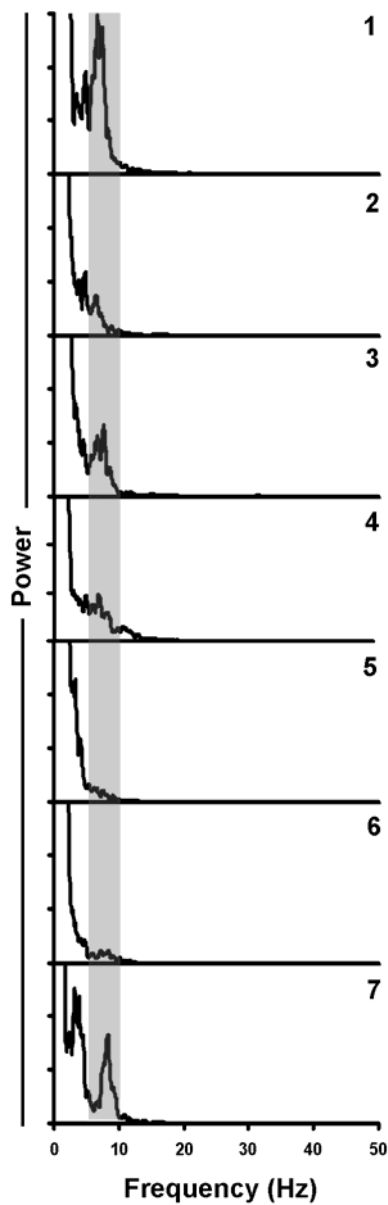
Figure 2.2. Normalised power spectra for the force record obtained during 2 minutes of continuous isometric biting at 2.0N in 7 subjects. All but 2 of the subjects, 5 and **6, have a distinct peak in the 6-10Hz area and of those, subject 6 has a small peak in this frequency band.**

2.6 Coherence Analysis

While the above example shows obvious interrelationship between the two processes of masseteric EMG and mandibular force in the frequency domain, it is possible to formally investigate this relationship using cross-spectral methods [see (Rosenberg *et al.*, 1989; Christakos, 1997; Halliday and Rosenberg, 2000) for a formal discussion of such methods]. The measure most often used to quantify this relationship is coherence. Coherence spectra give an estimate of linear coupling between two time series in the frequency domain, from which significant coherence at a given frequency infers rhythmicity at that frequency in both signals (Hansen and Nielsen, 2004). The application of coherence analysis can be summarized as follows: individual power spectra are estimated using a periodogram method discussed above, where the discrete power spectral estimate is constructed from a series of disjoint segments, denoted here in accordance with (Amjad *et al.*, 1997) as L. Following Halliday *et al.* (1995), this chapter has used $f_{xx}(\lambda)$ and $f_{yy}(\lambda)$ to represent the power spectra of processes x and y, respectively. The magnitude of the cross spectrum between x and y at each frequency λ is denoted by $f_{xy}(\lambda)$. The coherence function between the two signals is defined at each frequency λ as:

$$\left|R_{xy}(\lambda)\right|^2 = \frac{\left|f_{xy}(\lambda)\right|^2}{f_{xx}(\lambda)f_{yy}(\lambda)}$$

Here, the coherence estimate provides, at each Fourier frequency λ, a measure of the coupling between signals in the frequency domain. Coherence estimates provide a

normalized measure of linear coupling on a scale from 0 to 1 where 0 indicates no

linear association and 1 complete association (Halliday and Rosenberg, 2000). As with

the estimation of power spectra, the use of the periodogram method allows the

construction of confidence limits. These limits will be directly influenced by the

number of non-overlapping segments (L) used, as by increasing L, the variance is

decreased. The estimation of a 95% confidence interval can be made by the following

equation from Amjad *et al*. (1997): $1 - (0.05)^{1/(\Sigma L - 1)}$ where $\sum$L is the total number of

segments used in the construction of the coherence estimate. Of course increasing L

will have the additional effect of decreasing the length of L which in turn decreases the

frequency resolution of both the power spectral estimate and the coherence estimate.

The frequency resolution of the estimate is a function of segment length defined by

$r = \dfrac{1}{2T}$ where T is the length of L in time. Therefore, in deciding the number of

segments to use in the analysis it is important to consider this trade-off.
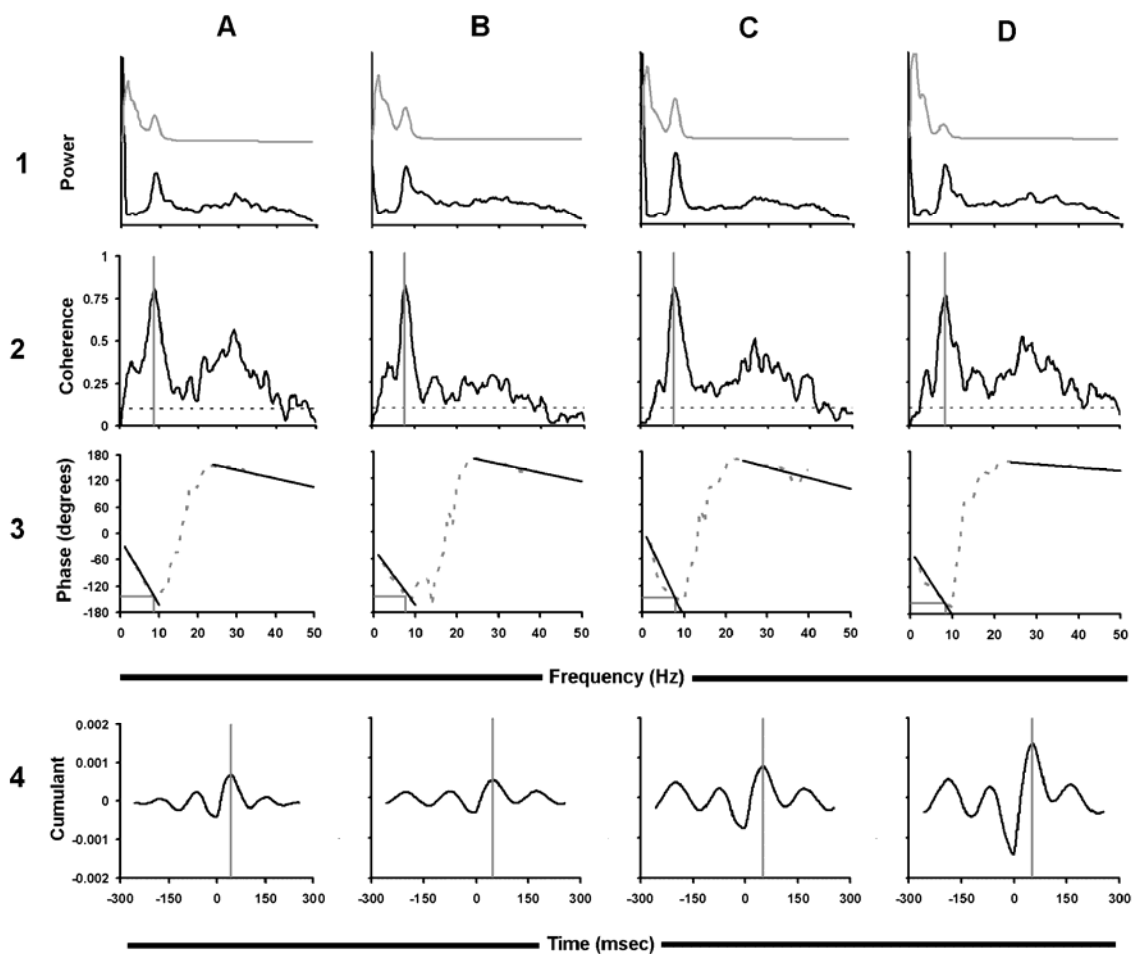
Figure 2.3. Frequency domain analysis of physiological jaw tremor at 4 different isometric bite levels: A. 0.5N, B. 2.0N, C. 5.0N and D. 10.0N. 1. Normalised power spectra, top grey trace is the force spectrum while the black trace below is the corresponding masseteric EMG spectrum. 2. Coherence estimates for the demodulated EMG and force spectra from 1. These estimates are constructed from 40 3-second segments returning a frequency resolution of 1/3Hz. The horizontal dotted line represents the 95% confidence interval (0.1). The large peak at 8Hz is indicative of the correlation between the 2 signals at this frequency. 3. The phase relationship between EMG and force for the frequency band of

significant coherence. Regression lines have been fitted to those regions of constant phase relationship (black lines). This relationship indicates a constant time lag between masseteric EMG and mandibular force production equivalent to ~55ms. Solid grey lines indicate the phase lag at the frequency of peak coherence. The lag is ~160°. 4. Cumulant density functions for the coherence relationships in 2. The large positive peaks show, in good agreement with the phase plot, that force lags EMG by ~55ms. Furthermore the period of correlation is ~125ms equating to a frequency of 8Hz which is equivalent to the frequency of peak coherence. Exact values for the parameters described in this figure are presented in Table 2.1.

Bite Force	0.5N	2.0N	5.0N	10.0N
Frequency at peak coherence	8.6Hz	7.6Hz	8Hz	8.3Hz
Phase lag at peak coherence	143.6°	143.8°	148.5°	157.0°
Time lag estimated from phase relationship	46ms	52ms	52ms	52ms
Cumulant time lag	44ms	50ms	50ms	53ms
Period of cumulant	110.5ms	126.0ms	117.0ms	109.0ms
Frequency of correlation estimated from period of cumulant	9.0Hz	7.9Hz	8.5Hz	9.2Hz

Table 2.1. Summary values for Figure 2.2.

2.7 Investigating Time Delays between EMG and Force

While the existence of physiological tremor and its relationship to EMG rhythmicity is incontrovertible, the origin of the oscillation is contested. Frequency domain analysis of physiological tremor may be able to shed light on these controversies. It could be argued that oscillations observed in EMG are as a result of cyclical activation of muscle, via muscle spindle excitation, in response to oscillation arising from the resonant characteristics of the body part being measured. In such a case the force component of tremor should precede that of the EMG. To analyse this hypothesis we can, from estimation of the complex valued coherency function (coherence is the square of the absolute value of the coherency, (see (Amjad *et al.*, 1997); eqn. 2.6), determine the phase relationship between the two signals. Where a straight line can be fitted to the phase representation associated with significant coherence, a constant time lag (or advance) is inferred. A positive value in the y-direction obtained from the phase plot indicates a phase advance at a particular frequency, whereas a negative value indicates a phase lag. The time lag associated with the phase relationship can be estimated with the relationship $t = \dfrac{\phi(\lambda)}{2\Pi\lambda}$ where $\phi(\lambda)$ is the phase value in radians at frequency λ.

An alternative or auxiliary method of estimating time delay between processes is to use the cumulant density function. Given that cross-spectral analysis essentially represents cross correlation in the frequency domain it can be considered analogous to the frequency-domain derived estimate of cross correlation. Therefore, the inverse FFT of the cross spectrum, known as the cumulant density function, is analogous to the time-

domain derived cross correlation and can provide information about the magnitude of correlation at given time lags between related processes.

2.8 Discussion

As the coherence estimates and their associated phase plots in Figure 2.2. illustrate, it can be shown that tremulous activity around 8Hz in force records during isometric biting is dependent on EMG activity. While this may not fully rule out the contribution of reflexes to the enhancement of tremor (Elble and Koller, 1990; Timmer *et al.*, 1998b) it shows that for the most part, EMG oscillations give rise to force oscillations and that EMG oscillations associated with tremor are not solely a consequence of a mechanical resonance phenomena. Furthermore, in the case of the experimental data presented here and previously McFarland *et al* (1986); Sowman *et al* (2005), the occurrence of physiological tremor around 8Hz in the jaw can be shown consistently under true isometric conditions.

Another suggested origin of physiological tremor is as a result of the unfused twitches of newly recruited motor units. Since motor units generally begin firing at frequencies consistent with the frequency range of physiological tremor, and the fact that newly recruited units contribute a proportionately large amount to the force output (Allum *et al.*, 1978), such a hypothesis is plausible. There are, however, arguments that mitigate the validity of this hypothesis. Firstly, physiological tremor can be observed at contraction levels above where it is expected that all motor units would be active and firing at frequencies above that of recruitment (Mcauley *et al.*, 1997), and secondly,

significant coherence is observed between motor units at the frequency of tremor independently of their firing rate (Wessberg and Kakuda, 1999). These factors have led to the general hypothesis that the frequency peak corresponding to physiological tremor in EMG and that signal that represents the mechanical output of tremor, are representative of synchronization of motor unit firings resulting from a common oscillatory input to the motoneurone pool (Halliday *et al.*, 1999). Figure 2.3. illustrates that the frequency and amplitude of oscillatory activity in the EMG remain stable over a range of bite force intensities assumed to have different underlying motoneurone firing statistics. Furthermore, if it is assumed that the 8Hz peak in these spectra represents the extent of underlying short-term synchronisation (Halliday *et al.*, 1999), it can be said that the level of synchronization remains stable across a broad range of EMG activation levels. These observations are consistent with the idea that a significant contribution to physiological tremor comes from a frequency-fixed input to the motoneurone pool.

2.9 Conclusion

The techniques developed for investigating physiological tremor discussed in this chapter have allowed a greater understanding of this fundamental motor phenomenon. For the jaw, that component of physiological tremor at 8Hz, common to other motor systems, is a direct result of a narrow frequency band in the EMG. This 8Hz component is present under true isometric conditions indicating little contribution from the stretch reflex loop, rather, a common input to the masseteric motoneurone pool is indicated. While the contribution of intra- and perioral mechanoreceptors cannot be ruled out, the phase relationship between force and EMG at the frequency of tremor

suggests that any reflex activity arising from such loops is likely to be modulatory as opposed to generative.

Chapter 3

Periodontal Anaesthesia Reduces Common 8Hz Input to Masseters during Isometric Biting

3.1 Publication Acknowledgement

3.2 Abstract

During isometric contractions of the jaw muscles, oscillations in the rectified masseteric EMG record that are coherent with the mandibular force output are evident at 8Hz. This chapter has investigated the load dependence of these oscillations under both force and EMG feedback conditions and the extent to which these oscillations are coupled bilaterally in the jaw muscles. This chapter further investigated the extent to which afferent information arising from the periodontium during biting influenced the extent of 8Hz EMG tremor and the bilateral coupling between masseters at this frequency. Using coherence analysis this chapter has shown that a significant load independent coupling of EMG between the closing muscles of the jaw occurs at 8Hz as a result of a common 8Hz input to the masseters. This common input is significantly reduced when afferent information from the periodontium is blocked. These results suggest that afferent information arising from the periodontium enhances the expression of

peripheral tremulous activity, which may be important for optimising the response of the jaw to changes in forces occurring between the teeth.

3.3 Introduction

Significant controversy surrounds the origin of physiological tremor. A current theory, based on the observations of rate independent motor unit (MU) modulation (Elble and Randall, 1976; Erimaki and Christakos, 1999; Halliday *et al.*, 1999; Wessberg and Kakuda, 1999; Kilner *et al.*, 2002) and corticomuscular coherence (Salenius *et al.*, 1996; Salenius *et al.*, 1997; Gross *et al.*, 2000; Kilner *et al.*, 2000; Gross *et al.*, 2002; Hansen and Nielsen, 2004) suggests that physiological tremor arises from either central pacemakers or centralised oscillatory circuits (Farmer *et al.*, 1993; Farmer, 1999; Wessberg and Kakuda, 1999; Gross *et al.*, 2000; Mcauley and Marsden, 2000; Gross *et al.*, 2002) which modulate MU firing rates causing a tremor at the frequency of this common input (for discussion see (Farmer, 1999)). Other evidence suggests that tremor arises from activity perpetuated in muscle spindle reflex loops causing rhythmic synchronisation of MUs relative to the delay time around the loop (Lippold, 1970; Joyce and Rack, 1974; Joyce *et al.*, 1974; Matthews and Muir, 1980; Jacks *et al.*, 1988; Prochazka and Trend, 1988; Cresswell and Loscher, 2000; Fisher *et al.*, 2002; Durbaba *et al.*, 2005).

Relatively few studies have considered the involvement of receptors involved in the transmission of tactile information but of those that have, van Steenberghe and de Vries (1980) and Fisher *et al*. (2002), showed that impairment of tactile sensation reduced the component of physiological tremor around 6-10Hz. van Steenberghe and de Vries

(1980) propose that the effect they observed in the mandible was due to multiple muscle reflex pathways, however they did not measure the concurrent EMG activity that may have shown this. Conversely Fisher and colleagues proposed that the decreased coupling between muscles they observed was due to a decoupling of centralised oscillators, as there was no decrease in the EMG power at that frequency. This chapter sought to examine the effect of periodontal anaesthesia on mandibular tremor with respect to the underlying changes in EMG. Specifically this chapter sought to examine the extent to which any alteration of physiological tremor was a result of a change in the extent of coupling between the masseters bilaterally or as a result of a change primarily in the spectral power content of the EMG. Given that frequency analysis of mandibular physiological tremor during slow movements and isometric contractions has revealed a peak in the tremor spectrum at around 8Hz that is coherent with a component of the rectified EMG signal from the jaw closing muscles (Junge *et al.*, 1998; Jaberzadeh *et al.*, 2003a) it would seem likely that the rhythmic motor activity that underling physiological tremor in the jaw system would be coherent and in-phase bilaterally, indicative of a common input to both muscles. Furthermore, it was hypothesised that any change in mandibular tremor after periodontal mechanoreceptor (PMR) anaesthetisation will be as a result of decreased common input from the PMRs to the masseter muscles. To eliminate the possibility that any changes in the tremor spectrum are due to changes in exerted bite force or masseteric activation following PMR anaesthetisation, force feedback and EMG feedback paradigms at three contraction levels were used to confirm that the component of tremor investigated here was

analogous to the load-independent component of physiological tremor established in previous studies (Halliday *et al*. 1999).

3.4 Methods

Experiments were conducted according to the regulations of, and with approval from, the University of Adelaide human ethics committee and conformed to the Declaration of Helsinki. A total of 9 volunteers (7 females and 2 males; age range 18-34 years) were recruited for these experiments, all of whom provided written informed consent. All subjects had natural and healthy dentitions and were free of dental symptoms at the time of the experiments.

3.4.1 Subjects

Subjects sat upright in a dental chair adjusted for height such that they could bite onto fixed metal bite plates (inter-incisal distance of 12mm) whilst maintaining a neutral cervical position. The repeatability of this position was established by the use of a fixed nosepiece that was set to contact the subject's nose when the required position was attained (method previously described in (Brinkworth *et al.*, 2003)). The metal bite plates were coated with a semi-rigid dental impression material (3M Express™, Michigan) moulded to each subject's teeth individually. Most of this impression was then cut away so that only the impression that surrounded the upper and lower incisors remained. This ensured that only the incisor teeth were used in biting during the experiment. Adhesive EMG electrodes (Duotrode®, Myo-Tronics Inc, Western Australia) (2 cm interelectrode distance), were affixed to the skin over the masseter

muscles in a superior inferior configuration along the longitudinal axis of the muscle fibres, 0.5 cm posterior to the to the anterior borders. Prior to affixation of recording electrodes the skin was prepared by abrasion followed by an alcohol scrub to ensure an interelectrode impedance of less than 5kΩ in all experiments. Force was measured from a transducer mounted on the lower bite plate and recorded at a sampling rate of 2000Hz. Bipolar EMG was amplified (1000 – 10 000x) and filtered (20-1000Hz) and recorded at a sampling rate of 2000Hz for 2 minutes for each condition via a specially designed data acquisition program (National Instruments, LabVIEW®, Texas, (Brinkworth, 2004). Prior to further analysis, the DC level was removed from the EMG signal and it was full wave rectified (for discussion on the use of this method for EMG demodulation and spectral analysis see (Journee, 1983; Journee and Van Manen, 1983; Journee *et al.*, 1983; Timmer *et al.*, 1998a)). Subjects were grounded using a lip-clip electrode (Türker *et al.*, 1988) and presented with filtered feedback via a horizontal target line on a dedicated feedback monitor screen (15 inch, refresh rate 60Hz). EMG signals used for feedback were full-wave rectified before low pass filtering at 1Hz.

Subjects were required to bite until a required target feedback level (2, 5 or 10% maximal voluntary contraction (MVC)), was reached and then maintain this level as accurately as possible for a period of 2 minutes. Feedback was given as a percentage of MVC from either left masseteric EMG or from the bite force. MVCs were obtained prior to the start of the experiment by having the subjects perform three maximal bites of five seconds duration in the position described above. Force and EMG feedbacks were scaled to the maximal force and EMG values obtained during this procedure. The feedback monitor screen was scaled to display levels from 0-13% MVC, with the y-axis

measuring 210mm. Subjects eyes were a horizontal distance of 900mm away from the

monitor. This experimental set-up is illustrated in Figure 3.1.
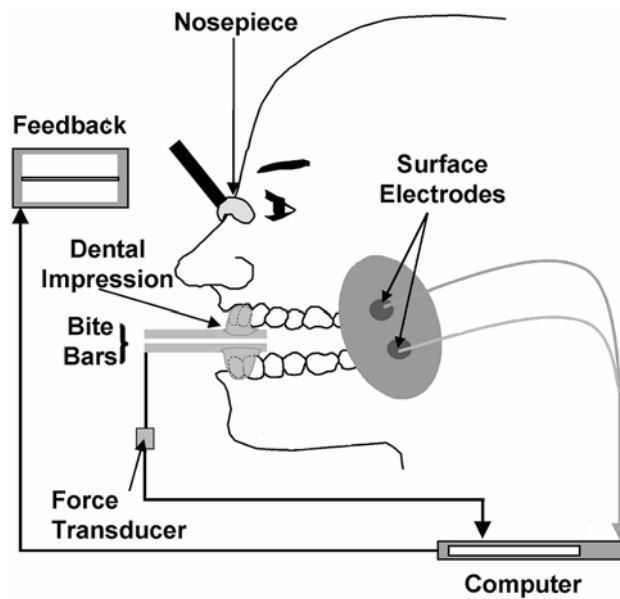
Figure 3.1. General experimental set-up. Bite plates and nosepiece were connected to the same rigid framework ensuring repeatable head positioning between trials. Metal bite plates were coated with a dental impression material large enough to cover only the incisors. EMG from the masseter muscles and force from the transducer incorporated in the bite plates was recorded by a computer and could be presented as feedback on a dedicated monitor.

Biting tasks for each of the feedback conditions at each of the feedback levels were randomised and interspersed with 30-second rest periods. The first segment henceforth referred to as "Control 1", consisted of 6 biting tasks (3 with force feedback, 3 with EMG feedback (2, 5 and 10% MVC). Once the biting tasks for Control 1 had been completed, the subjects rested for 20 minutes. The protocol was then repeated for a second segment, henceforth referred to as "Control 2" with a different randomisation of biting tasks. After completion of Control 2, subjects had their upper and lower peri-incisal periodontium anaesthetised by local anaesthetic (LA) infiltration (4ml, Xylocaine 2%®, Astra Pharmaceuticals Pty. Ltd. NSW, Australia). Adequate anaesthesia was determined to have been achieved when the subject no longer had tactile or vibratory sensation in either labial or buccal aspects of the upper or lower incisor teeth canine to canine (Türker and Jenkins 2000; Brinkworth *et al*. 2003). The above biting tasks were then repeated with a different randomisation 5 minutes after completion of the anaesthetisation procedure (about 15 minutes after the initial dose of the LA). This third segment will be henceforth referred to as "LA".

3.4.2 Data Analysis

Coherence analysis was performed to determine correlation in the frequency domain of the simultaneously recorded EMG signals and the subsequent relationship of force tremor to these. This analysis was performed in accordance with the methods of (Rosenberg *et al.*, 1989) and (Halliday *et al.*, 1995). Coherence spectra give an estimate of linear coupling between two time series in the frequency domain, from which significant coherence at a given frequency infers rhythmicity at that frequency in

both signals (Hansen and Nielsen, 2004). The application of coherence analysis to EMG data is described in detail by Halliday *et al*. (1995) and has been implemented here using a custom designed LabVIEW® (National Instruments, Texas) based computer program. Briefly, individual power spectra are estimated using a periodogram method, where the discrete Fourier transform is constructed from a series of disjoint sections, denoted here in accordance with (Amjad *et al.*, 1997) as L. Following Halliday *et al*. (1995), this chapter has used $f_{xx}(\lambda)$ and $f_{yy}(\lambda)$ to represent the power spectra of processes *x* and *y*, respectively. The cross spectrum between *x* and *y* at each frequency λ is denoted by $f_{xy}(\lambda)$. The coherence function between the two signals is defined at each frequency λ as:

$$\left| R_{xy}(\lambda) \right|^2 = \frac{\left| f_{xy}(\lambda) \right|^2}{f_{xx}(\lambda) f_{yy}(\lambda)}$$

Here, the coherence estimate provides, at each Fourier frequency λ, a measure of the coupling of EMG signals in the frequency domain. Additionally, from estimation of the complex valued coherency function (see (Amjad *et al.*, 1997); equation 2.6), the associated phase relationship between the two signals can be assessed to give an indication of the direction of temporal association, a positive value indicating a phase advance at a particular frequency, or a negative value indicating a phase lag.

To summarize coherence estimates, individual coherence functions can be combined across subjects, thus creating a "pooled coherence" estimate. Pooled coherence is defined by Amjad *et al*. (1997) as:

$$\frac{\left|\sum_{i=1}^{k} \hat{f}_{a_i b_i}(\lambda) L_i\right|^2}{\left(\sum_{i=1}^{k} \hat{f}_{a_i a_i}(\lambda) L_i\right)\left(\sum_{i=1}^{k} \hat{f}_{b_i b_i}(\lambda) L_i\right)}$$

where $\hat{f}_{a_i b_i}(\lambda)$ denotes an estimate of the second order spectrum $f_{a_i b_i}(\lambda)$ estimated from L_i disjoint sections. All signals in the present study were divided into L disjoint segments such that the final estimate of coherence would have a frequency resolution of 0.25Hz. As with individual coherence estimates, pooled coherence estimates provide a normalized measure of linear coupling on a scale from 0 to 1 where 0 indicates no linear association and 1 complete association (Halliday and Rosenberg, 2000).

3.4.3 Statistics

The estimation of a 95% confidence interval for coherence estimates can be made by the following equation from Amjad *et al*. (1997): $1 - (0.05)^{1/(\Sigma L - 1)}$ where $\sum L$ is the total number of segments used in the construction of the coherence estimate. With regard to this, any portion of the coherence estimate exceeding this limit can be deemed large enough to reject the null hypothesis that the coherence estimate is not significantly different from zero.

To determine the effect of feedback type, force level and then LA on the functional coupling of EMG and force, individual coherence records for the masseteric EMG from all nine subjects were pooled within condition (Control 1, Control 2, LA) as per the

method of (Amjad *et al.*, 1997). Subsequently, the pooled coherences of the controls were compared using the χ^2 method of comparison, for which a computation of the test statistic is defined by (Amjad *et al.*, 1997) as:

$$2\left(\sum_{i=1}^{k} L_i \hat{z}_i^2 - \frac{\left(\sum_{i=1}^{k} L_i \hat{z}_i \right)^2}{\sum_{i=1}^{k} L_i} \right)$$

where $\hat{z}_i$ denotes the Fisher transformed coherency estimate of the ith pair of processes. This method was also used prior to pooling to ensure equivalence of individual records. This analysis technique is further elaborated on in (Halliday and Rosenberg, 2000), for its application to pooled data. Significance was set at the 5% level for both pooled coherence and the difference of coherence tests.

In order to illustrate the relevance of pooled data as a representation of the underlying individual data, individual data were further summarised by using a histogram approach similar to that used by (Farmer *et al.*, 1993) and (Mattei *et al.*, 2003). Individual coherence estimates were divided into 2Hz frequency bins from 0 to 20Hz, the average coherence for that bin was then calculated and if it exceeded the level of significance a value of 1 was assigned. If significance was not attained a value of 0 was assigned. In this way a representation of the individual contributions to the pooled data could be visualised as the percentage of individual subjects attaining significant coherence in each frequency bin. Additionally, a further measure of the percentage of subjects with

at least one significant frequency bin between 6 and 12Hz was made in order to incorporate a bandwidth within which physiological tremor was expected to occur.

Prior to analysis of power spectra, these were first transformed such that the power in each 0.25Hz frequency bin was expressed as a percentage of the total power. EMG spectra were tested for difference by repeated measure ANOVA (SPSS®) by integrating the individual normalised EMG spectra in the bandwidth of 6-12Hz thus deriving an estimate of power in the frequency range of interest. A priori difference contrasts were set up to test the within subjects effect of condition, comparing firstly the controls and then the controls pooled against LA. Mauchly's test was used to assess the sphericity of data prior to further analysis of results.

3.5 Results

3.5.1 Load Independence of EMG Tremor

Prior to LA, masseteric EMG for all feedback levels was characterised by a spectral peak at 8Hz. To examine the load independence of this EMG peak, the individual normalised power spectra were integrated between 6 and 12Hz and any change of power in this frequency band due to different loading assessed by repeated measures ANOVA. This showed that there was no difference between the feedback levels (2, 5 or 10% MVC) suggesting that in the range of contraction levels assessed here, increasing the load on the mandible or the muscle activation required to maintain these loads, did not affect the expression of 8Hz tremor.

3.5.2 Effect of LA on Force Tremor

Prior to LA, force records were characterised by small amplitude high frequency fluctuations superimposed on larger amplitude low frequency oscillations. During LA, the high frequency oscillations were noticeably diminished. This is illustrated in figure 3.2a. Spectral analysis revealed that the high frequency oscillations were centred around 8Hz. After anaesthetisation however the 8Hz peak was greatly diminished (Figure 3.2b).
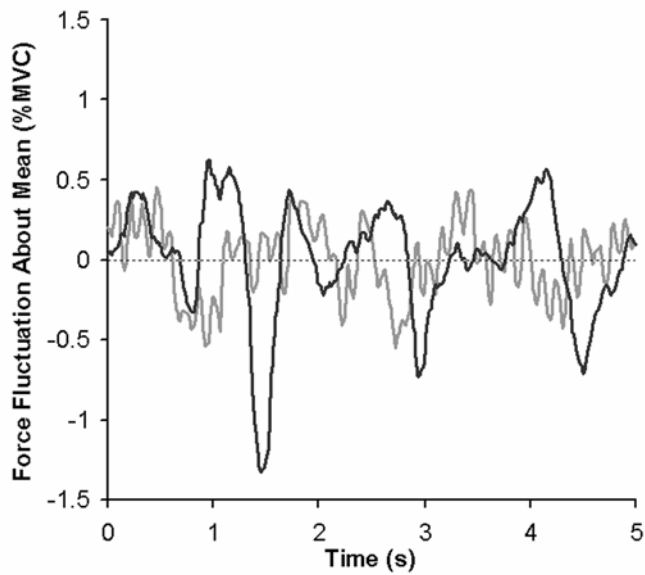
Figure 3.2a. Raw force records for a subject biting at 5% maximal voluntary contraction (MVC) with force feedback. Grey trace =Control 1, Black trace =Local Anaesthetic. Mean force level has been removed. The control condition force record is characterised by high frequency oscillations superimposed over low frequency oscillation. The application of LA greatly reduced the high frequency oscillation.
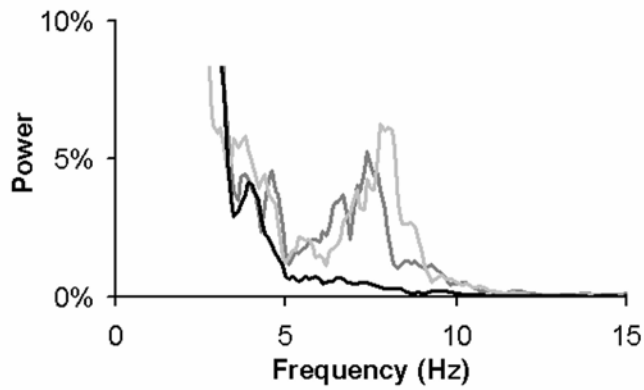
Figure 3.2b. Force power spectra. This figure shows the power spectra for the full 2-minute force recordings corresponding to the data presented in figure 3.4a. Grey traces =Controls 1 and **2. Black trace =Local Anaesthetic. The reduction of the 8Hz component is clearly visible.**

3.5.3 Effect of LA on EMG Power Spectra

Upon examination of the normalised EMG spectra, it was apparent that there was a power peak at 8Hz prior to LA that was attenuated bilaterally during LA. This is illustrated in Figure3.3.
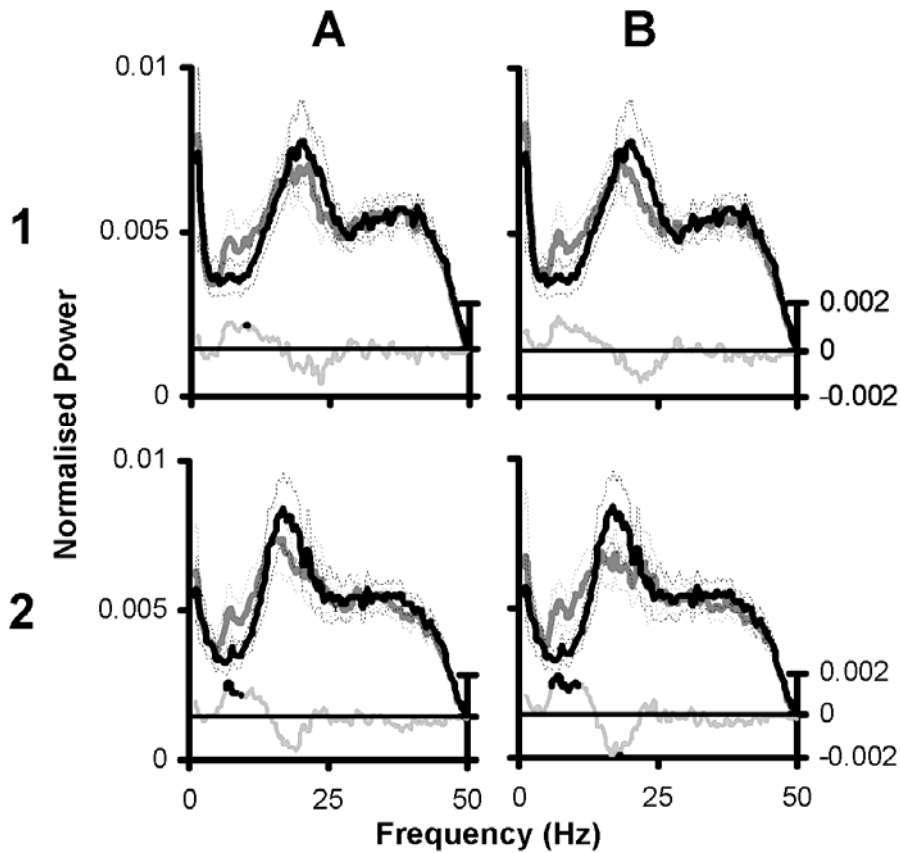
Figure 3.3. Average normalised power spectra for: 1) Left masseter EMG and 2) Right masseter EMG. Column A shows the control condition 1 vs. LA and Column B the control condition 2 vs. LA. Controls are in dark grey while the LA condition is in black, respectively coloured dotted lines represent the 95%CI for the power spectra. The light grey trace at the bottom of the graphs represents the difference between the controls and LA; the superimposed black trace on top of the difference represents those bands of difference where there is no overlap of 95%CIs, i.e. the spectra are significantly different. The control traces were characterised by a peak at 8Hz which was reduced during LA, in addition all EMG spectra were characterised by a second, larger peak at ~20Hz.

Examining the influence of LA on the individual normalised power spectra between 6 and 12Hz showed a significant effect ($p < 0.01$) of condition (controls 1,2 or LA) that was due to the LA condition being significantly different to the controls as assessed by a priori established difference contrast. While there was no difference between the controls for either muscle (left or right masseter) or feedback level (2, 5 or 10% MVC) there was a significant interaction between feedback type and condition (controls 1 and 2 vs. LA) with the reduction in power being greater during LA for the force feedback condition than for the EMG feedback condition. This is illustrated in figure 3.4.
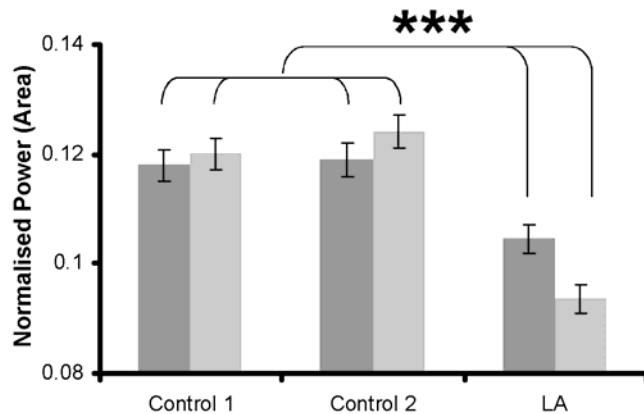
Figure 3.4. Difference in 6-12Hz range in masseter EMG power between conditions for 2 different modes of feedback. Dark grey = EMG feedback, light grey = force feedback. There was a significant within-subjects difference as tested by a priori defined contrast between the controls and LA ($p<0.01$), while there was no difference between controls. These data include left and right masseters and all 3 levels of contraction, as these factors were not significant. There was a significant interaction between feedback type and condition that is evident in the larger reduction in power during LA for the force feedback condition.**

Mauchly's test of sphericity was not significant (p=0.43) indicating that the data satisfied the requirements for the analysis without the need for adjustment of degrees of freedom.

3.5.4 Effect of LA on Force to EMG Coherence

Coherence analysis revealed that prior to LA the force was coherent with masseter EMG at 8Hz and lagged the EMG by ~145º, equivalent to a time delay of ~50ms. A comparison of the pooled EMG to force coherences showed no significant difference between the control conditions, however, when the difference between all three conditions was tested, significant differences were evident. A single very large decrease in coherence centred on 8Hz was evident in both EMG and force feedback pooled coherences. An example of the pooled coherence between the left masseter and force and the corresponding χ^2 tests for differences of coherence are shown in Figure 3.5.
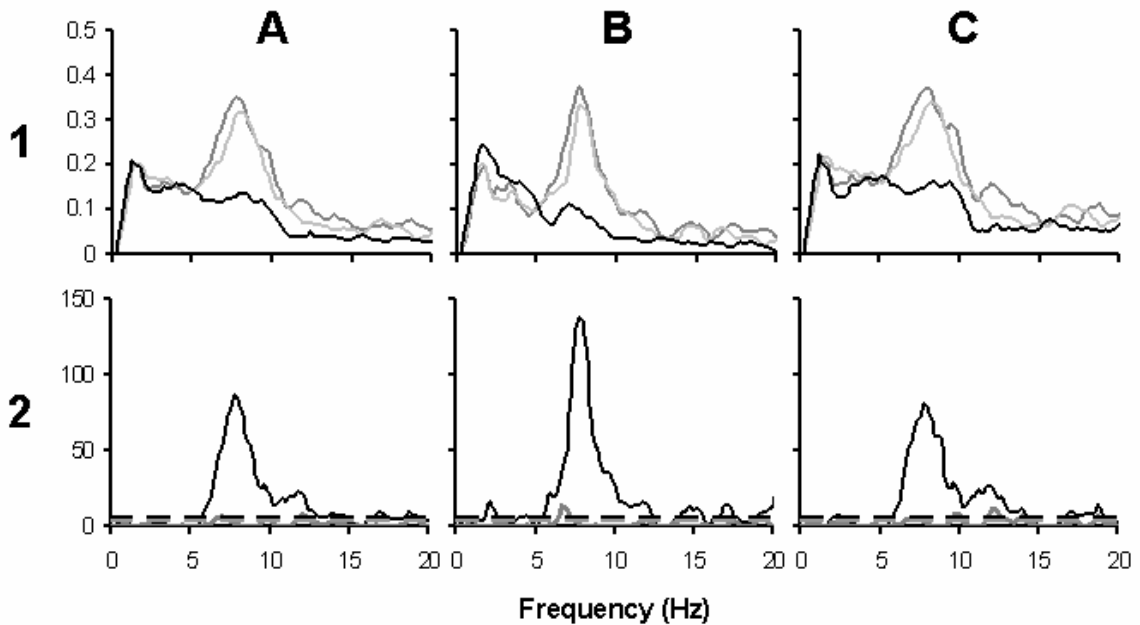
Figure 3.5. Left masseter EMG to force coherence, force feedback. 1) Coherence spectra for the coherence between the left masseter EMG and force recorded simultaneously from between the incisors, dark grey trace Control 1, light grey trace Control 2 black trace LA. 2) χ^2 test for difference of coherence, grey trace = difference between controls, dark grey horizontal dotted line is the respective 95% CI. Black trace = difference between all 3 conditions (2 controls and LA), the horizontal black dotted line is the respective 95% CI. A, B and C represent the feedback levels 2, 5 and 10% MVC respectively. Prior to LA, coherence traces were characterised by a large 8Hz peak which was greatly attenuated during LA, this is reflected in the difference of coherence (2).

3.5.5 Bilateral Coupling between Jaw Muscles.

Prior to anaesthetic block of the front teeth, significant coupling between the left and right masseter muscles could be seen at 8Hz. The phase relationship between the 2 sides showed that this coupling was in-phase indicating synchronous oscillatory activity. The 8Hz coherence was considerably reduced for all feedback levels during the period following LA administration and the incidence of significant coherence occurring between 6 and 12Hz was reduced in the same pattern.

This effect was also evident in the pooled coherence data with the χ^2 difference of coherence showing a significant difference centred on 8Hz at all feedback levels. There was no significant difference between the pooled coherence during the 2 controls for any feedback level or feedback condition, though the control conditions were more consistent under the condition of EMG feedback. A noticeable feature reflected in both the population summary and the pooled coherence is the re-emergence of a coherence peak at 8Hz during LA at the highest contraction level under both feedback conditions albeit at a drastically reduced level (figures 3.6 and 3.7).
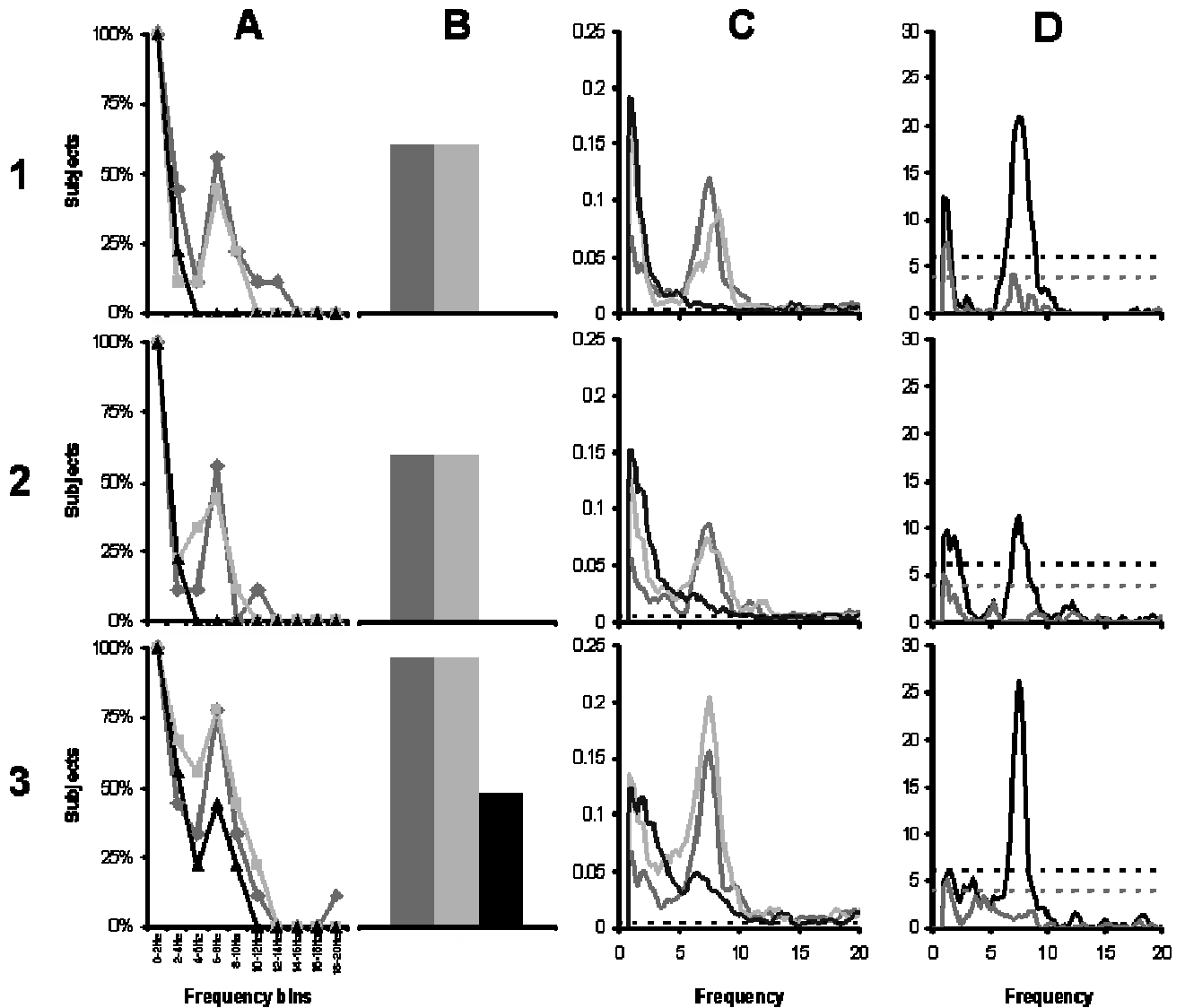
Figure 3.6. Masseter to masseter coherence EMG feedback. Dark grey = Control 1, light grey = Control 2, black = LA. 1) 2% MVC; 2) 5%MVC; 3) 10%MVC. A) Percentage of subjects with significant average coherence in a given frequency bin. B) Percentage of subjects with at least one significant average coherence bin between 6 and 12Hz. C) Pooled coherence, horizontal dashed line = 95% CI. D) χ^2 difference of coherence, black trace is overall difference between all 3

conditions (2 controls and LA), grey trace difference between controls. Horizontal

black dashed line is 95%CI for difference between all 3 conditions; horizontal grey

dashed line is 95%CI for difference between controls.
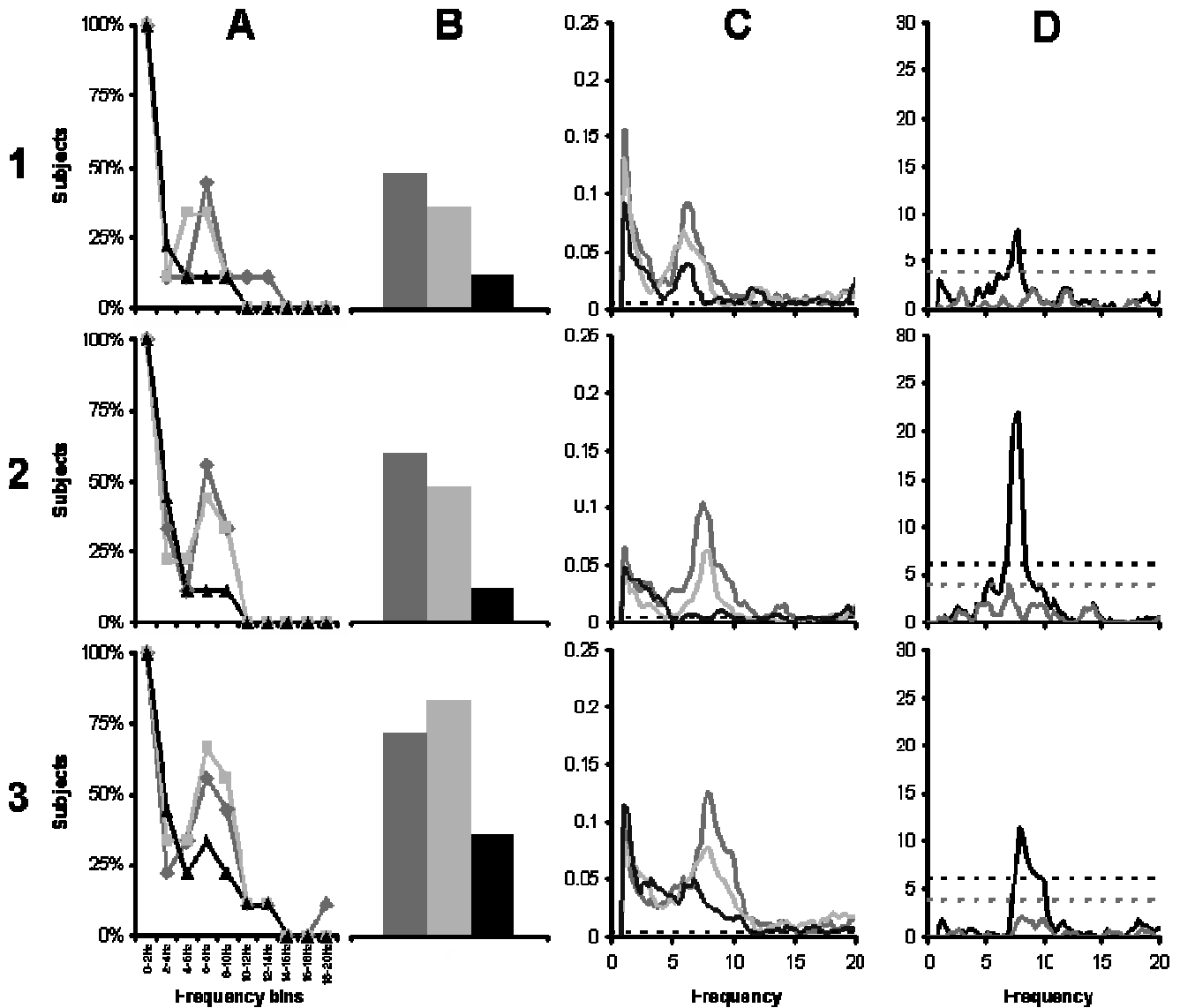
Figure 3.7. Masseter to masseter coherence Force feedback. Dark grey = Control 1, light grey = Control 2, black = LA. 1) 2% MVC; 2) 5%MVC; 3) 10%MVC. A) Percentage of subjects with significant average coherence in a given frequency bin. B) Percentage of subjects with at least one significant average coherence bin between 6 and 12Hz. C) Pooled coherence, horizontal dashed line = 95% CI. D) χ^2 difference of coherence, black trace is overall difference between all 3 conditions, grey trace difference between controls. Horizontal black dashed line is

95%CI for difference between all 3 conditions; horizontal grey dashed line is

95%CI for difference between controls.

3.5.6 Correlation between 8Hz EMG Power Diminution and EMG-EMG Coherence

To assess the extent to which the decrease in EMG-EMG coupling seen during LA was directly related to the decrease in EMG power at the same frequency, the difference between the average of the controls 6-12Hz EMG power and 6-12Hz EMG power during LA was calculated and the extent to which it was correlated with the difference between the average control 6-12Hz EMG-EMG coherence and the 6-12Hz EMG-EMG coherence during LA for each subject was assessed. The relationship between the two was strong ($R = 0.78$, $p = (0.017)$) indicating a link between the decrease in masseteric 6-12Hz EMG power and the decrease in coupling seen in the same frequency band.

3.6 Discussion

3.6.1 Spectral Components of Jaw Tremor Masseteric EMG

The force output spectrum of the mandible during isometric contraction is characterised by a single peak at around 8Hz. Contrastingly, the rectified masseteric EMG spectrum recorded concurrently is characterised by a broad dominant peak at around 20Hz and a smaller early peak at 8Hz that is coherent with, and phase advanced on the 8Hz peak in the force spectrum. Previous investigations of jaw tremor have shown that EMG to jaw tremor coherence is strongest between 6 and 12Hz and occurs predominantly below 20Hz (Junge *et al.*, 1998; Jaberzadeh *et al.*, 2003a; Jaberzadeh *et al.*, 2003b). Presumably modulations ~20Hz, evident in EMG are not reflected in force records or EMG to force coherence because the jaw system mechanically damps out these higher

input frequencies. The two distinct areas of EMG frequency activity this chapter has observed, one at 8Hz and another area ~20Hz is consistent with previous observations made during isometric contractions in other muscle groups (Farmer *et al.*, 1993; Mcauley *et al.*, 1997; Halliday *et al.*, 1999). Farmer (1999) proposed that this might reflect two distinct descending rhythmic components, the higher frequency acting as a carrier frequency for the lower frequency component. The lack of bilateral coherence seen in this experiment at ~20Hz seems to indicate that for the jaw this frequency band is not the result of a common descending component, other experimenters however have shown significant intermuscular EMG coherence in two distinct bands that would support this hypothesis (Fisher *et al.*, 2002; Kilner *et al.*, 2004). Another explanation for this may be that low frequency peaks in the EMG spectra represent common modulations of firing rate that are independent of the intrinsic firing rate represented by a larger later peak (Elble and Randall, 1976; Halliday *et al.*, 1999). The pattern of the EMG spectra presented here agrees with the single MU spectra presented by (Elble and Randall, 1976), furthermore the dominant component of surface EMG power spectra is expected to be directly related to the average firing rate of the MU pool (Weytjens and Van Steenberghe, 1984a; Weytjens and Van Steenberghe, 1984b; Van Der Glas *et al.*, 1994; Myers *et al.*, 2003). It would follow then that the 20Hz component is a representation of the overall mean firing rate of the recorded masseteric MUs and the 8Hz component a modulation of this. Such an explanation would fit the pattern of bilateral EMG-EMG coherence existing at 8Hz, representing a common modulatory input, and the absence of significant EMG-EMG coherence existing in the higher frequency band where the greater power is a result of intrinsic firing rates. This

interpretation is likely to be overly simplistic however as there are significant factors that may confound interpretation of EMG power spectra in this way (Solomonow *et al.*, 1990) [for review see (Farina *et al.*, 2004)].

3.6.2 Reduction in Tremor and Changes in Force Production

Anaesthetisation of the peri-incisal periodontium causes a significant reduction in the 8Hz component of physiological mandibular tremor. This result confirms the findings of van Steenberghe and de Vries (1980) and shows that the cause of the reduced tremor is due to a decrease in a common input to the masseters bilaterally at this frequency. (Van Steenberghe and De Vries, 1980), suggested that the reduced mean frequency of the mandibular tremor observed during anaesthetisation in their study could be due to increased large MU contribution to force output as a result of harder biting caused by a lack of mechanoreception. (Trulsson and Johansson, 1996b) showed that without feedback this increased force output does occur. Their experiment showed that when subjects were asked to hold a small transducer between the incisors after the peri-incisal periodontium had been anaesthetised, the force exerted to perform the task was up to four times larger than that used to perform the same task prior to LA. However, by controlling to the same level the force production during biting, both pre and post LA, this chapter has shown that jaw physiological tremor driven by masticatory muscle activity, is reduced significantly at 8Hz independently of any increase in force. This suggests that the reduction in the 8Hz component of the force spectra is a direct consequence of an afferent contribution to physiological tremor, rather than an indirect effect produced by increased force output. Adding further to the argument that 8Hz

tremor decreased independently of an increase in force, at low bite force levels i.e. 2 and 5% MVC, the abolition of 8Hz tremor was most striking; an almost complete abolition of 8Hz coupling between the masseters during LA was evident along with a parallel diminution of coherence at the same frequency between EMG and force. At the highest bite level investigated here i.e. 10% MVC, a significant, albeit smaller, peak at the same frequency was observed during LA compared to controls. This was attributed to increased activation, via mechanical coupling, of neighbouring unanaesthetised PMRs, or due to an increase in the discharge rate of muscle spindles at increased levels of muscle activation (Taylor, 1990; Passatore *et al.*, 1996) or a combination of both. At lower bite force levels such mechanisms are less likely to be of consequence. Furthermore, while there was no significant effect of increasing force level on the 8Hz component of EMG, there was a noticeable increase in the incidence of significant 6-12Hz coherence between the masseters with increasing contraction level. At the lowest bite levels this was lower than 50%, whereas at the highest levels there was close to 100% occurrence of significant coherence. This result suggests that inter-individual differences in dental parameters influence greatly the force required to excite the periodontal mechanoreceptors (PMRs) to a level where there is a synchronous influence on tremor.

The size of the current sample precludes its reduction into groups based on classification of dental parameters such as differences in craniofacial morphology, functional patterns of mastication or occlusion and the pooled analyses used here to describe the response of the group as a whole to a gross intervention conceals such differences. Changes in the low frequency content of EMG, analogous to those seen

here, in patient groups with craniomandibular disorders (Van Der Glas *et al.*, 1994), suggest that variables that alter the mechanics of sensory transduction may be important in such disorders and therefore a study of a larger sample, where classification and grouping of subjects according to such variables is possible, may shed light on the extent to which the frequency content of EMG is related to such variables .

3.6.3 Tremor Arising From Feedback Loops

Although there is a significant quantity of research supporting the idea that physiological tremor is due to oscillation in reflex servo loops (Lippold, 1970; Joyce and Rack, 1974; Joyce *et al.*, 1974; Matthews and Muir, 1980; Jacks *et al.*, 1988; Prochazka and Trend, 1988), contradictory accounts of the existence or non-existence of physiological tremor in the presence of deafferentiation suggest some limitations with this hypothesis (Halliday and Redfearn, 1958; Lamarre *et al.*, 1975). Furthermore, several authors contest that differences in loop times rule out the existence of a common tremor frequency resulting from such mechanisms (Marshall and Walsh, 1956; Marshall, 1959, 1961; Elble, 1986). Our experiments show, however, that for the jaw, afferent feedback derived from the PMRs plays a significant role in the generation of physiological tremor.

It is possible that feedback, either excitatory (Lund and Lamarre, 1973; Brodin and Türker, 1994) or inhibitory (Brinkworth *et al.*, 2003), from the PMRs could cause oscillation within a reflex loop. It is well established that, in a closed loop system where there is a gain greater than unity at a feedback phase lag of 180 degrees, unstable oscillation will result (Matthews, 1997). In this experiment, the phase lag observed at

peak coherence frequency, which was consistent with that observed by Junge *et al.* (1998), only reached ~145 degrees and is unlikely to cause such instability. However, (Stein and Oguztöreli, 1976) present a model which shows that interactions between feedback and the physical properties of muscle can cause load independent self-limiting oscillations in the physiological tremor frequency range. They suggest that delays in reflex pathways in the range of 20 to 50ms will cause oscillation in the physiological tremor frequency range. Such a delay is in accordance with the reflex loop time established in the disynaptic PMR pathway (Brinkworth *et al.*, 2003) and the time delay estimated from the phase relationship in this experiment.

3.6.4 Central Origins of Tremor

Given that many authors support the hypothesis that physiological tremor is the result of centrally generated rhythms (Lamarre *et al.*, 1975; Farmer *et al.*, 1993; Mcauley *et al.*, 1997; Farmer, 1999; Wessberg and Kakuda, 1999; Gross *et al.*, 2000; Mcauley and Marsden, 2000; Gross *et al.*, 2002), it could be postulated that the PMRs may provide a tonic excitation to a central generator that drives jaw movement modulations. A similar mechanism of somatosensory dependence is proposed by (Jaberzadeh *et al.*, 2003b) to account for the decreased amplitude of peak tremor frequency (8Hz) during experimentally induced pain in the masseter muscle and by (Hansen and Nielsen, 2004) to account for enhanced ~10Hz tibialis anterior EMG tremor after peripheral stimulation. While there remains little evidence to support such a mechanism in the jaw at this stage, other authors (Lee and Stein, 1981; Elble, 1986; Timmer *et al.*, 1998b), have proposed that reflex loops and centrally generated rhythms may be interacting

mechanisms that contribute more or less to produce tremors. Elble (1986) suggests that a central oscillator may be responsible for the rhythm and a mechanical reflex system involved in governing tremor amplitude. In the jaws, feedback from the highly force sensitive PMRs entering the loop responsible for physiological tremor would allow for automatic load compensation in the amplitude of oscillation and could contribute to the load independence of physiological tremor seen here. While the existence of in-phase coupling seen in these experiments could support the existence of a common central input, it is expected that in-phase excitation of PMRs occurring in the type of symmetrical bite task used here, could cause an in-phase bilateral input to the opposite side of the feedback loop. Whether this situation would still result in the observed lagging of force behind EMG is unknown.

3.6.5 Decreased Tremor due to Decreased Coupling of Centralised Oscillators

This chapter cannot come to the same conclusion as Fisher *et al*. (2002), that the reduced bilateral coupling seen here is due only to a disruption of the coupling of central oscillatory mechanisms, as there was a concurrent decrease in the EMG power at the coupling frequency not seen in the experiment of Fisher and colleagues. Furthermore, the extent to which EMG power was reduced as a result of PMR anaesthetisation was strongly correlated with the extent to which coherence between the masseters was reduced. While a different frequency band was examined in this chapter, which may account for some of the differences, there are interesting parallels to be drawn to a subsequent report from the same group (Kilner *et al.*, 2004), in which they showed decreased coupling between hand muscles in a deafferented patient compared

to controls. This decreased coherence, in agreement with the findings of this chapter, was observed concurrently with reduced EMG power in both the coherence band reported on in Fisher *et al*. (2002) and a similar coherence band (7.8-12.5Hz) to that which is reported on here.

While the exact mechanisms by which PMRs contribute to jaw physiological tremor will only be revealed by further directed study, it seems that with regard to the jaw at least, the contribution of peripheral inputs play a distinct role in the expression of 8Hz physiological tremor. The conclusions drawn by Timmer *et al*. (1998) seem most likely to apply here, i.e. while physiological tremor is not arising solely from reflex loops, such loops (in this case the PMR inhibitory pathway) are able to enhance the amplitude of existing oscillations driven by synchronous EMG activity.

3.6.6 Methodological Issues

During LA of the upper and lower incisors, neighbouring soft tissues were also anaesthetised. It is possible that anaesthesia of soft tissues may affect the excitability of motoneurones or efficacy of synapses if they actively take part in the bite procedure or display tonic activity. In the injection paradigm used here, the tongue could not be anaesthetised since LA was only administered on the labial side of the lower teeth. Both upper and lower lips were however anaesthetised during the LA administration. Since the lips are not involved in our bite procedure and the mechanoreceptors in the

lips do not have tonic activity (Johansson *et al.* 1988), locally anaesthetising the lips should not affect the afferent traffic and the contribution of the mechanoreceptors in the lips to motoneurone excitability and/or synaptic efficacy should be minimal.

3.6.7 Functional Implications

The function of physiological tremor remains unclear although it has been proposed that it minimises the stickiness in motor systems, an under-damping that allows for rapidity of mechanical response (Lippold, 1970; Greene, 1972). In the jaw, rapidity of response is of especial significance. The teeth are a rigid interface, vulnerable to rapid changes in force that occur in such instances as when an object held between the teeth suddenly breaks (Miles and Madigan, 1983). In such cases, physiological tremor may optimise the transmission of reflex activity into mechanical action, minimising the possibility of tooth damage. The idea is therefore forwarded that PMR activity enhances oscillatory EMG activity to optimise the balance between the critical need for rapid response to changes in force, and intrinsic damping mechanisms that minimise the tendency of the system toward excessive tremor (Neilson and Neilson, 1978; Matthews, 1997).

3.7 Conclusion

This chapter has shown that the physiological force tremor of the mandible during isometric biting is characterised by an 8Hz peak. This peak, driven by EMG

modulations at the same frequency, is independent of load force or EMG activation levels up to 10% MVC. The blocking of afferent information from the PMRs by LA significantly reduces common bilateral input to the masseter muscles at 8Hz and significantly reduces tremor at this frequency. It is concluded that afferent input from the PMRs plays a significant role in either the genesis or maintenance of physiological tremor in the human jaw.

Chapter 4

Periodontal Anaesthetisation Decreases Rhythmic Synchrony between Masseteric Motor Units at the Frequency of Jaw Tremor

4.1 Publication Acknowledgement

The following is a modification of "Periodontal anaesthetisation decreases rhythmic synchrony between masseteric motor units at the frequency of jaw tremor." Exp Brain Res. 2007 Jan 10; [Epub ahead of print] Sowman PF, Ogston KM, Türker KS.

4.2 Abstract

This chapter links the reduction in jaw physiological tremor around 8Hz following periodontal mechanoreceptor (PMR) anaesthetisation to changes in coherence between masseteric motor unit discharges. Single motor unit activity presented in this chapter was recorded from two separate sites in the right masseter muscle during a low level tonic contraction, both prior to and during anaesthetisation of the peri-incisal PMRs. Anaesthetisation of PMRs decreased coherent activity between motor units circa 8Hz, and decreased synchrony between the same motor unit pairs. It is proposed that tremor-generating inputs that cause rhythmic synchronisation of masseteric motor units arise from, or are amplified by the PMRs.

4.3 Introduction

Slow movements and isometric contractions of the jaw muscles are characterised by small oscillations in jaw force (Mcfarland *et al.*, 1986), velocity (Van Steenberghe and De Vries, 1980) and acceleration (Jaberzadeh *et al.*, 2003a). These oscillations are evident at frequencies similar to the components of physiological tremor observed in other motor systems at 8Hz and are unchanged in frequency or normalised-amplitude by increased bite force (see chapter 2). Recently, Halliday *et al.*, (1999), demonstrated that physiological tremor arises from rhythmic single motor unit (SMU) synchronisation at the frequency of physiological tremor. If physiological tremor is the result of synchronous SMU activity, then common excitatory or inhibitory inputs to motoneurones, by enhancing or diminishing synchrony, may affect the resultant physiological tremor spectrum. Research in this regard has shown that afferent input may enhance components of physiological tremor measured in force (Cresswell and Loscher, 2000), velocity (Van Steenberghe and De Vries, 1980), the muscle-to-muscle electromyogram (EMG) coherence (Fisher *et al.*, 2002; Kilner *et al.*, 2004; Sowman *et al.*, 2006), and cortico-muscular coherence (Hansen and Nielsen, 2004). The previous chapter showed that the 8Hz component of the surface EMG (SEMG), suggested to be a good predictor of SMU synchronisation at that frequency (Halliday *et al.*, 1999), is markedly diminished during isometric biting following anaesthetisation of the PMRs (see chapter 3). The extent to which such physiological tremor-causing oscillations are resultant upon coherent activity in masseteric SMUs has not been investigated, and there has been little investigation of the extent to which exteroceptive input may impact

upon ongoing coupling between SMU firings. The first aim of this chapter was to test directly the hypothesis developed in our previous chapter (Sowman *et al.*, 2006); that the reduction in 8Hz mandibular physiological tremor during anaesthetic block of the PMRs is as a result of a decrease in the coherent activity between SMUs at the frequency of physiological tremor. The second aim was to test the hypothesis that SMU-to-SMU (UTU) coherence at physiological tremor frequency and time domain measures of SMU synchrony represent similar phenomena.

4.4 Methods

4.4.1 Subjects

A total of 7 consenting volunteers participated in the study (2 male, 5 female; age range 20-31), all were naïve and had complete and healthy dentition and were free from periodontal disease. These experiments were approved by the Ethics Committee for Human Experimentation of the University of Adelaide and follow the recommendations of the Declaration of Helsinki for Human Experimentation. All volunteers gave informed written consent.

4.4.2 Single Motor Unit Recordings

Recording of SMU activity from two points in the right masseter muscle was performed using custom made intramuscular electrodes. Each electrode had three Teflon[®]-insulated silver wires; two of the wires comprised the bipolar SMU electrode, and the third wire was the macro-EMG electrode (the reference electrode for the macro EMG was attached to the opposite earlobe). The SMU wires were completely insulated except for the tips, and the macro EMG electrode had the insulation stripped 15mm from the terminal (Scutter and Türker, 2000). Both electrodes were inserted, one at a time at least 1cm apart, and to a depth of approximately 2 cm into the anterior deep portion of masseter using 25G needles. Each needle was immediately withdrawn, leaving the three wires in the belly of the muscle. SMU signals were filtered at 200Hz - 5kHz, and macro EMG signals were filtered at 50Hz - 5kHz.

4.4.3 Surface EMG and Bite Force Recording

Subjects sat upright in a dental chair adjusted for height such that they could bite onto fixed metal bite plates (inter-incisal distance of 12 mm) whilst maintaining a neutral cervical position. The width of the bite plates was such that only the incisor teeth were used in biting during the experiment. The metal bite plates were coated with an acrylic dental impression material. Adhesive EMG electrodes (Duotrode™, Myo-Tronics Inc, Western Australia) (2 cm interelectrode distance) were affixed to the skin over the masseter muscles in a superior–inferior configuration along the longitudinal axis of the muscle fibres, 0.5 cm posterior to the anterior borders. Prior to affixation of recording

electrodes, the skin was prepared by abrasion followed by an alcohol scrub to ensure an interelectrode impedance of less than 5 kΩ in all experiments.

Force was measured from a transducer mounted on the lower bite plate and recorded at a sampling rate of 10,000 Hz. Bipolar EMG was amplified (1,000–10,000x), filtered (20–1,000 Hz) and recorded at a sampling rate of 10,000 Hz via a specially designed data acquisition program [National Instruments, LabVIEW®, Texas, (Brinkworth, 2004)]. Prior to further analysis, the DC level was removed from the EMG signal and it was full-wave rectified.

4.4.4 Trials

During all trials, subjects isometrically contracted the masseter muscle until stable SMU activity could be detected in each of the SMU electrodes. The subject maintained this approximate stable level of SMU firing throughout recording in all conditions. To keep both SMUs firing steadily, the subject was provided with visual feedback of the action potentials generated by the SMUs in both electrodes on a two-channel oscilloscope running at a slow sweep speed of 0.2s/cm (Figure 4.1 C, D) and asked to maintain the spikes in both channels throughout the experiment.

Surface EMG

A   0.2 mV

Force

B   5N

Multi unit EMG - 1

C   0.2 mV

Multi unit EMG - 2

D   0.6 mV

0.5secs
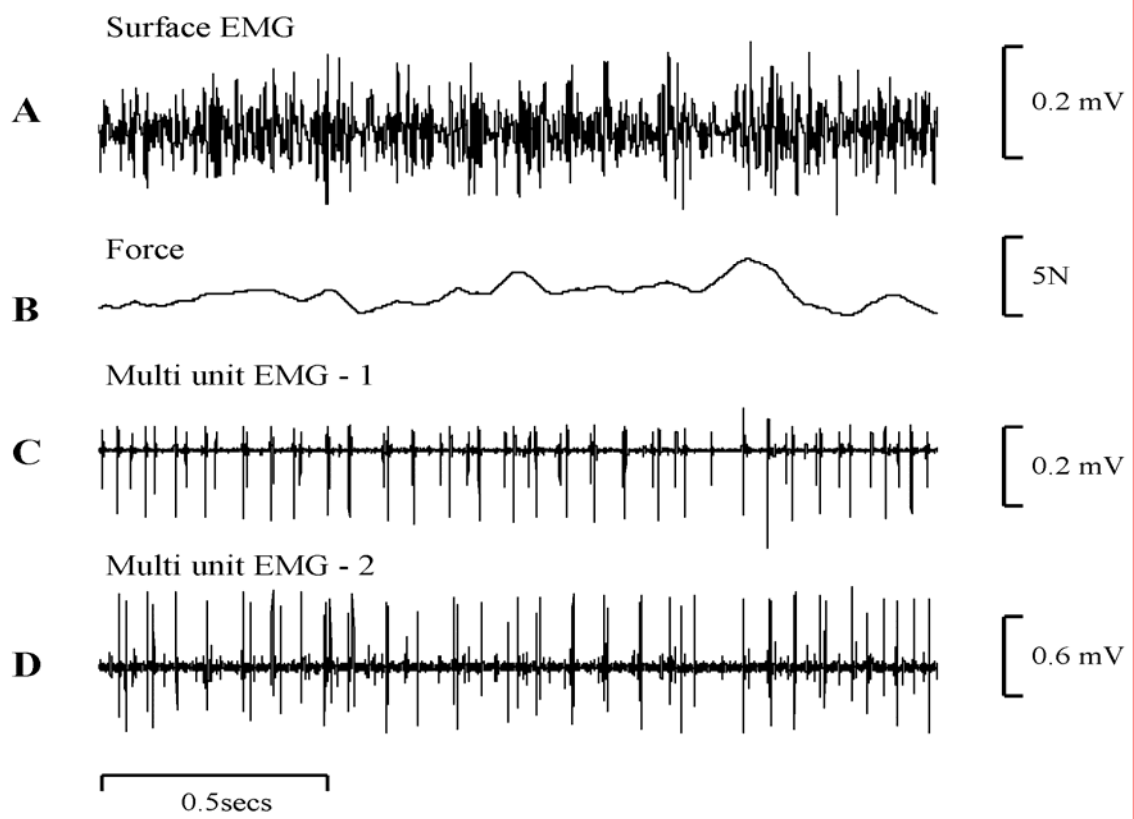
Figure 4.1. Example of the raw data obtained from a subject during the control condition. Trace A is the surface EMG from the masseter, trace B is the bite force record, and traces C and D are the multi unit data recorded from each of the SMU electrodes.

Each subject executed two biting conditions, using only the central incisal teeth top and bottom. The control condition involved the subject biting down on the bite bars (front teeth PMR activation). This was then repeated following the infiltration of local anaesthetic (LA) (4 ml, Xylocaine 2%®, Astra Pharmaceuticals Pty. Ltd. NSW, Australia) into the subject's upper and lower peri-incisal periodontium. Adequate anaesthesia was determined as the absence of tactile or vibratory sensation in either labial or buccal aspects of the upper or lower incisor teeth canine to canine (Türker and Jenkins, 2000; Brinkworth *et al.*, 2003).

4.4.5 SMU Discrimination

Analysis of each experiment was performed off-line. The data from the SMU channels (such as in Figure 4.1C, D) were played through a waveform discriminator (SPS-8701). During discrimination of the action potentials from a SMU, the stability of the SMU was continuously inspected using its representation on the Macro EMG record from the same site. This simultaneous representation of the unit on the Macro EMG was obtained using the acceptance pulses of the unit to trigger the averaging of Macro EMG. These representations that were unique to each unit, helped monitor the stability of the unit. Interspike interval (ISI) histograms for each SMU over the successive epochs were computed, and also used to confirm the accuracy of the discrimination of the SMU. The activity of each SMU was characterised in terms of its mean frequency (Hz), standard deviation (SD) and the coefficient of variation (CV = SD / mean ISI x

100) of discharge in each of the conditions. Only those records that contained at least 150 seconds of continuous firing were considered for further analysis.

4.4.6 Time Domain Analysis

SMUs that were successfully discriminated in separate electrodes during the same trial were paired for cross-correlation analysis, to assess the amount of SMU synchronisation present. Cross Correlation Histograms (CCH, see Figure 4.2 middle row) were computed and the cumulative sum (CUSUM) (Ellaway, 1978) technique was used to see if a central synchronous peak was apparent, and if so, its estimated location and width. Peak durations were estimated from the difference in the lags at which the minimum and maximum values (within the approximate range of –10 to + 10 ms) of the CUSUM occurred. A custom-designed computer program (implemented on IGOR$^{®}$) determined the lowest and highest point in the CUSUM within the –10 to 10 ms range. Each CCH was then examined by eye to confirm the validity of the peak start and peak end. Occasionally the peak would begin or end outside the 10ms limits, and when that happened the limits of the peak were manually adjusted.

The strength of synchronisation was quantified using CIS; the number of extra counts divided by the duration of the trial (Nordstrom *et al.*, 1992).

4.4.7 Frequency Domain Analyses

The rectified SEMG and the time series of SMU firings were transformed into the frequency domain using a periodogram method, whereby the discrete Fourier transform

is constructed from a series of disjoint sections (Halliday *et al.*, 1995; Sowman and Türker, 2005), implemented here using LabVIEW® (National Instruments, Texas), and normalised by dividing the power in each 0.5Hz frequency bin by the total power between 1 and 100Hz (Sosnoff *et al.*, 2005). Power spectra thus derived were used to identify the occurrence of a frequency peak in the SEMG indicative of physiological tremor.

4.4.8 Coherence Analysis

Coherence analysis was performed to examine the frequency domain correlation of the simultaneously recorded SMU signals and the concurrent frequency domain relationship of force and SEMG. Coherence analysis enables revelation of common frequency inputs to the motoneurones (Rosenberg *et al*. 1989; Farmer *et al*. 1993).

For each SMU pair, coherence spectra were computed separately, from 0 to 100 Hz with a resolution of 0.5 Hz. In each spectrum, a 95 % confidence level was computed under the assumption that the two spike trains were independent (Rosenberg *et al*. 1989). Any coherence value, which reached this level, was taken to reflect the existence of a significant correlation between the corresponding frequency components of the spike trains.

Coherence analysis has been implemented here using a custom LabVIEW® (National Instruments, Texas) program following the periodogram method described in detail by (Rosenberg *et al.*, 1989) and (Halliday *et al.*, 1995).

From estimation of the complex valued coherency function [see (Amjad *et al.*, 1997) eqn 2.6], an estimate of the phase relationship between the two signals can also be made. A positive value indicates a phase advance at a particular frequency, whereas a negative value indicates a phase lag.

Individual coherence functions can be combined to create a "pooled coherence" estimate. Pooled coherence is defined by Amjad *et al.*, (1997) as:

$$\frac{\left|\sum_{i=1}^{k} \hat{f}_{a_i b_i}(\lambda) L_i\right|^2}{\left(\sum_{i=1}^{k} \hat{f}_{a_i a_i}(\lambda) L_i\right)\left(\sum_{i=1}^{k} \hat{f}_{b_i b_i}(\lambda) L_i\right)}$$

In this equation $\hat{f}_{a_i b_i}(\lambda)$ represents an estimate of the second order spectrum $f_{a_i b_i}(\lambda)$ estimated from L_i disjoint segments. Pooled coherence estimates, as with individual coherence estimates, provide a normalized measure of frequency domain coupling on a scale from 0 to 1; 0 indicative of no linear association and 1, total association (Halliday and Rosenberg, 2000). This method was used in this chapter to provide a pooled estimated of SMU coherence for each subject where multiple SMU pairs were recorded within a condition (Control or During-LA) and, where a subject performed multiple contractions for a given condition, a pooled estimate of EMG to force coherence was also formed for that subject.

Subsequently, the difference in SMU-to-SMU (UTU) coherence between conditions was compared using the χ^2 method of comparison, for which a computation of the test statistic is defined by (Amjad *et al.*, 1997) as:

$$2\left(\sum_{i=1}^{k} L_i \hat{z}_i^2 - \frac{\left(\sum_{i=1}^{k} L_i \hat{z}_i \right)^2}{\sum_{i=1}^{k} L_i} \right)$$

where $\hat{z}_i$ denotes the Fisher transformed coherency estimate of the ith pair of processes. This analysis technique is further elaborated on in (Halliday and Rosenberg, 2000), for its application to pooled data.

Given the possibility that the large number of data points used in the pooled estimate may overestimate coherence if the variability is not constant (Baker, 2000) a 99% confidence interval for all pooled estimates and difference of coherence tests between pooled estimates was used.

The overall population coherence within condition was summarised by a histographic method (Mattei *et al.*, 2003). For each individual SMU pair, where the coherence estimate exceeded the level of significance for a given frequency bin (0.5Hz width), a value of 1 was assigned to that bin. If significance was not attained a value of 0 was assigned. In this way a representation of the overall data could be visualised as the percentage of individual UTU coherence estimates attaining significant coherence in each frequency bin.

To compare the magnitude of change in SMU coherence across the population the area of coherence between 5 and 10Hz was calculated for each SMU pair and then the means were compared between conditions. Independent samples t-tests were used to compare these changes and the changes hypothesised to occur in synchronisation.

The area of 5-10Hz coherence was also used to examine the presence of a correlation between coherence at the frequency of physiological tremor and the value for synchronisation.

4.5 Results

4.5.1 SEMG and Force

Prior to LA, an obvious 8Hz physiological tremor peak was evident in the EMG spectra of 4 of the 7 subjects who participated in these experiments (Figure 4.2A). In all of these subjects this peak was significantly coherent with the force record at the same frequency (Figure 4.2B) and in all 7 subjects, the phase relationship at 8Hz showed that force lagged EMG by around 150°. During LA the 8Hz EMG peak was notably diminished in all subjects that had a physiological tremor peak during control. The average bite force level increased following the application of LA from 17.6 to 22.9N but the increase did not reach the level of significance.
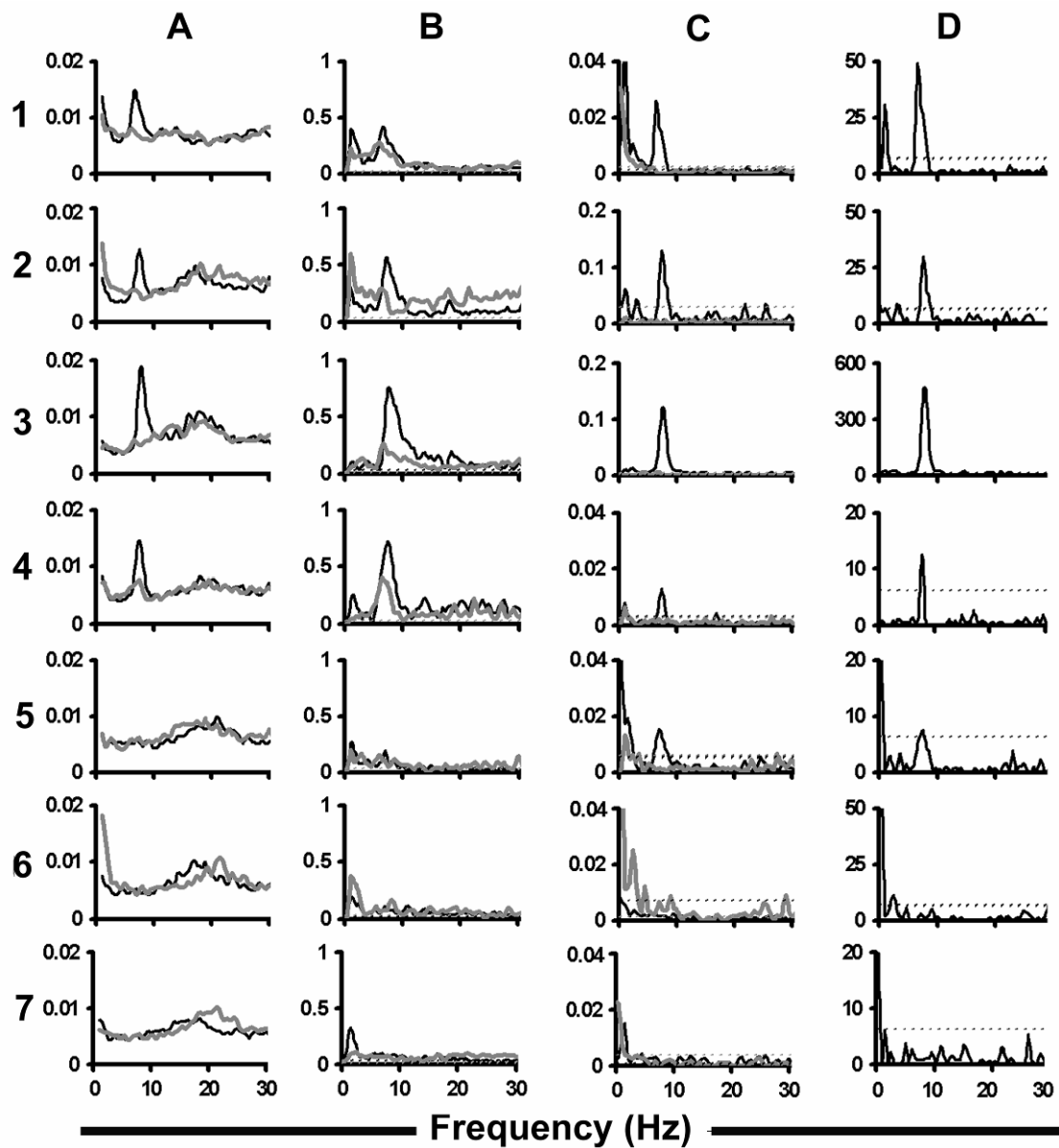
Figure 4.2. Spectral analysis of physiological tremor in 7 subjects. A, Normalised SEMG power spectra Control (black traces) and during LA (grey traces). B, Pooled SEMG to force coherence, Control (black traces) and during LA (grey traces). The dashed horizontal lines represent the respective 99% confidence intervals (CIs) (colour matched to the traces). C, pooled motor unit-to-motor unit (UTU) coherence Control (black traces) and during LA (grey traces). The dashed

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horizontal lines represent the respective 99% confidence intervals (CIs) (colour matched to the traces). D, χ^2 difference of coherence, horizontal dashed line in D represents the 99% CI for a significant difference between coherence estimates.

4.5.2 SMUs

A total of 54 SMU pairs during Control and 60 SMU pairs during LA were formed from 47 MUs discriminated from 20, 5-minute long, multi unit recordings during Control and 56 MUs discriminated from 28, 5-minute long, multi unit recordings during LA.

The means for the SMU firing rates, 12.9Hz during control and 13.3Hz during LA (see Figure 4.5) were not significantly different.

4.5.3 SMU Coherence

The UTU coherence spectra reflected the EMG spectra in all of the 4 subjects that had an obvious tremor peak prior to LA i.e. a significant 8Hz coherence peak was present in the pooled UTU coherence and this peak was abolished during LA (Figure 4.2C). The χ^2 test for difference of coherence revealed that the Pre-LA compared to LA UTU coherence estimates were significantly different around 8Hz (Figure 4.2D) for these 4 subjects. Additionally, one of the subjects (5) that did not have an obvious tremor peak in the SEMG spectrum, exhibited a significant 8Hz UTU coherence peak prior to LA that was abolished during LA. There was a significant difference between the conditions for UTU coherence in this subject also. In all of the subjects showing significant 8Hz UTU coherence, the phase relationship in this frequency band was always within 10° of completely in-phase.

The population analyses showed that overall, during the control period around 25% of all SMU pairs were significantly coherent at the physiological tremor frequency, this was reduced to around 5% during LA. For the 27 SMU pairs taken from those subjects with a significant UTU coherence peak at the frequency of tremor prior to LA, almost 50% were significantly coherent around 8Hz during the control contraction, and that percentage dropped to around 10% of the 34 SMU pairs during LA (See Figure 4.3). This result was reflected in a significant difference between the coherence area between 5 and 10Hz during control compared to during LA ($p<0.05$), when this was tested further on those subjects with a significant UTU coherence peak at the frequency of tremor prior to LA, the difference was even greater ($p<0.01$) (See Figure 4.4).
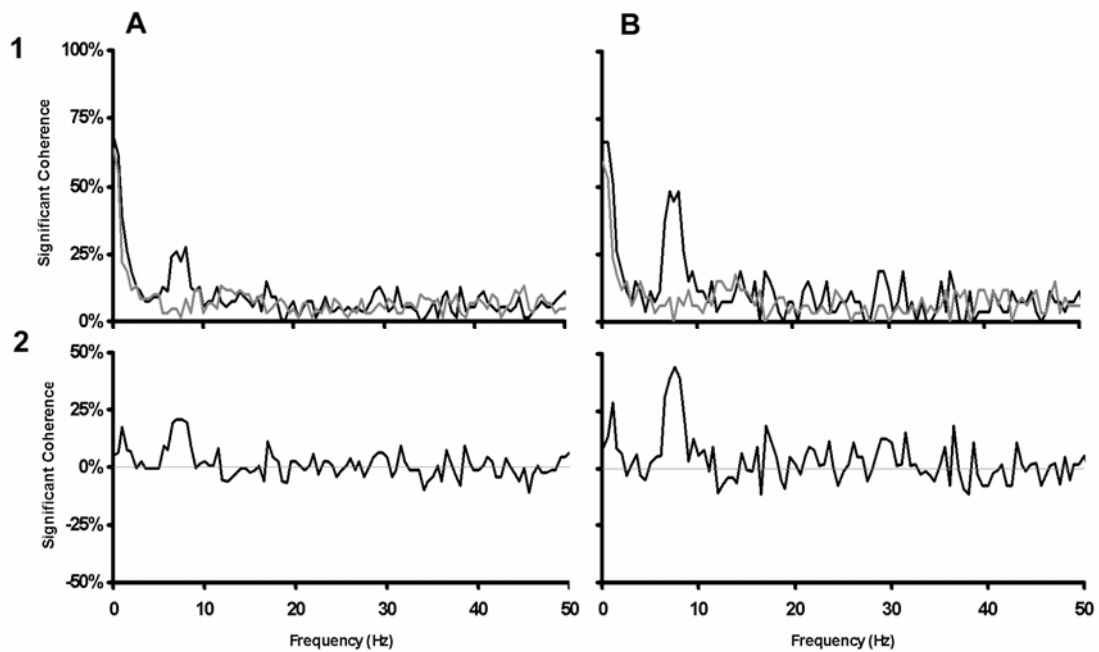
Figure 4.3. Population coherence summary. 1A. Population summary for the incidence of significant coherence for all SMU pairs and 1B, for the SMU pairs taken from the 5 subjects with a significant peak in the pooled UTU coherence at physiological tremor frequency, Control (black trace) and During LA (grey trace). 2. Difference between the Control and LA traces in 1.
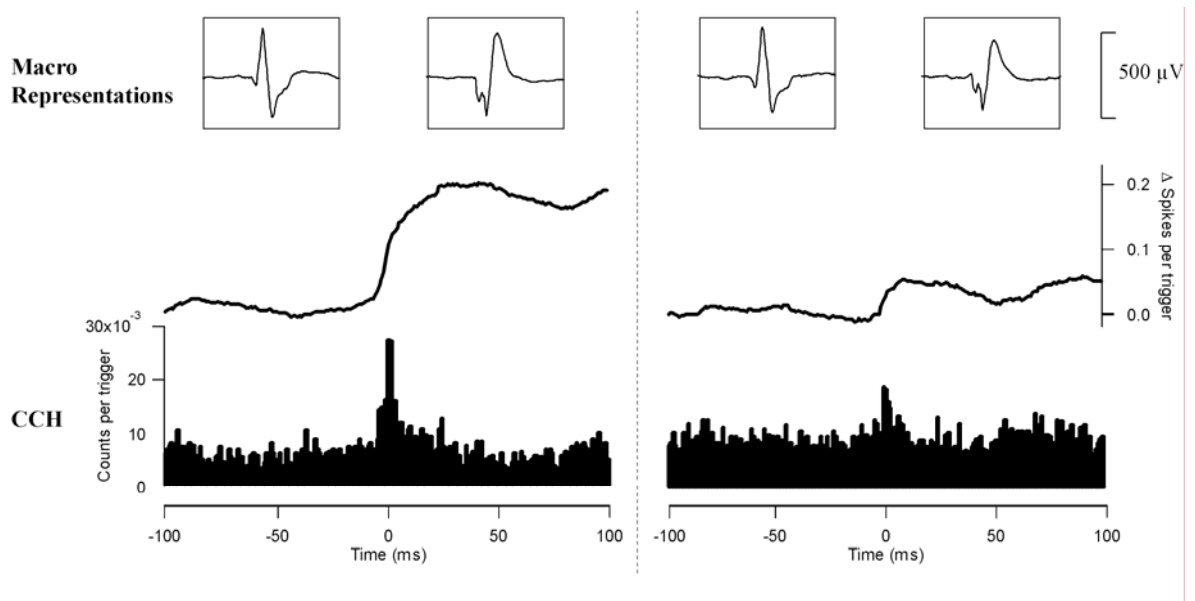
Figure 4.4. Example of the same motor unit pair and its Cross Correlation Histogram (CCH) in the Control and LA condition. Left panel is control and right LA. The top row shows the macro representation each motor unit, clearly showing that both Unit A and Unit B remain stable across the bite bar and LA + bite bar conditions. The middle row shows the CCH (bottom traces) and corresponding CUSUMs (top traces). This example shows the typical decrease in synchronisation between SMU pairs seen in those subjects who had physiological tremor prior to LA.

4.5.4 Synchronisation

During LA the strength of synchronisation (CIS) was decreased from 0.15 to 0.12 this change was not significant. However, when the 27 SMU pairs from the 5 subjects that had a significant peak in the UTU coherence at the frequency of physiological tremor were compared there was a significant reduction in the strength of synchronisation during LA (CIS) from 0.19 during control to 0.11. Furthermore, when the mean strength of synchronisation of the 27 SMU pairs from those 5 subjects with tremor was compared to the 27 SMU pairs taken from the 2 subjects that didn't exhibit tremor, the difference was again significant (CIS for subjects with tremor = 0.19 vs. subjects without tremor = 0.10, $p < 0.05$). There was no significant difference in peak width between the two conditions; 10.7ms during control and 10.6ms during LA.

4.5.5 Relationship between SMU Synchronisation and Coherence During Control and LA

In order to examine the relationship between the strength of SMU synchronisation and coherence at the frequency of physiological tremor, the strength of synchronisation was compared with the area of coherence between 5 and 10Hz for all SMU pairs both during control and LA. A significant positive association was observed during the control condition for all SMU pairs, this association was even stronger when the SMUs from the 2 subjects that didn't exhibit tremor were excluded (Figure 4.6).
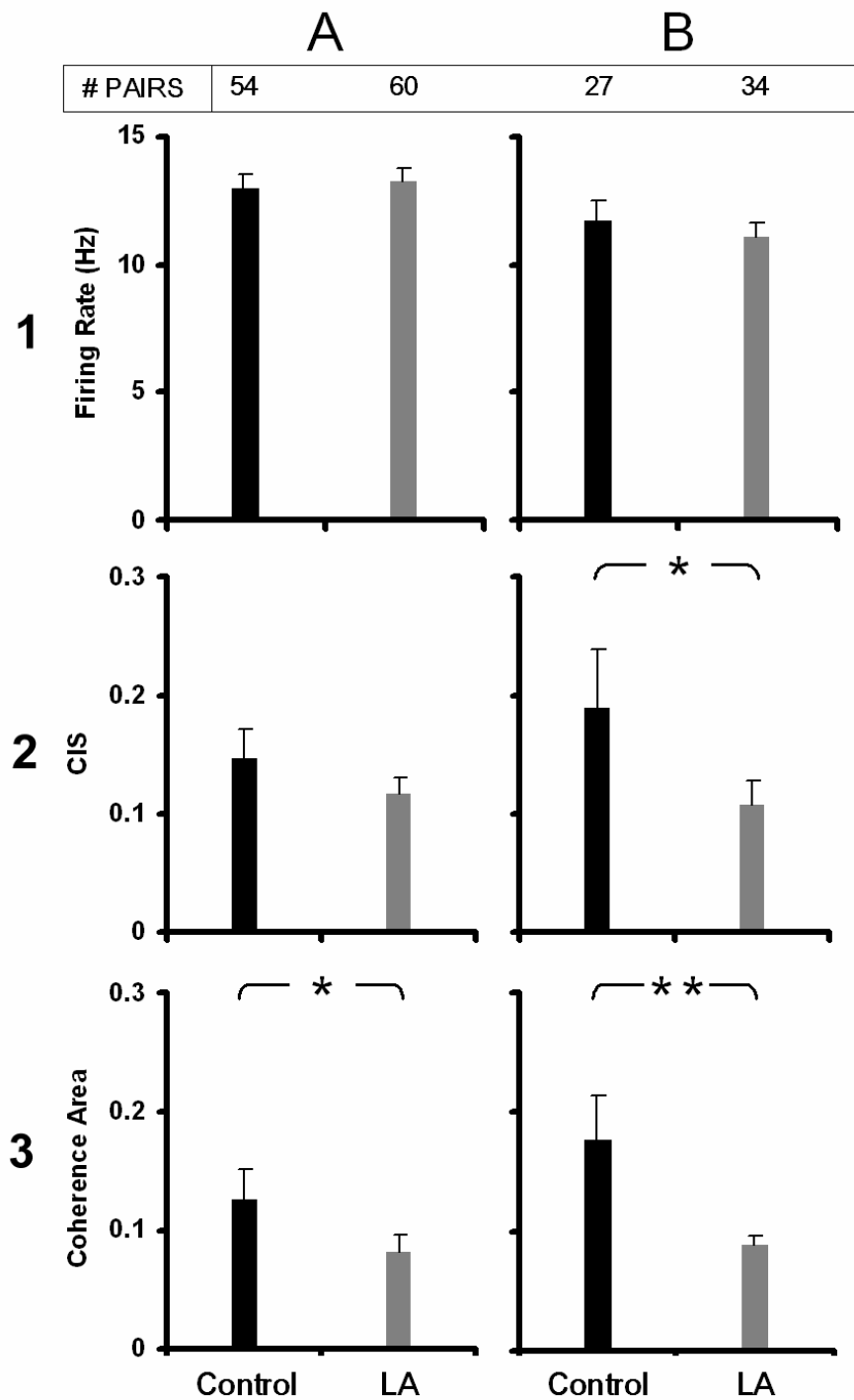
Figure 4.5. Mean differences in 1: Firing rate, 2:Synchronisation strength and 3:Coherence area 5-10Hz in A: Single motor unit (SMU) pairs and B: for the SMU pairs taken from the 5 subjects with a significant peak in the pooled UTU

coherence at physiological tremor frequency Control (black bars) and During LA (grey bars). Legend at top gives number of SMU pairs for each condition. * = $p<0.05$, ** = $p<0.01$.
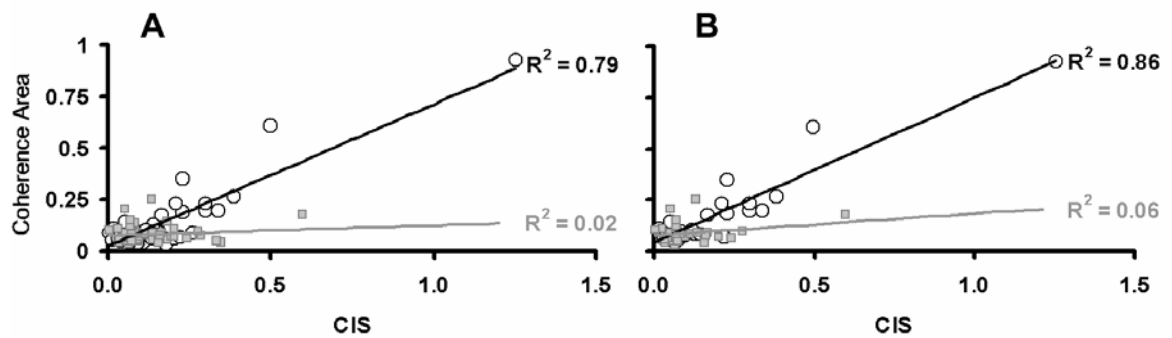
Figure 4.6. Relationship between single motor unit (SMU) synchronisation strength (CIS) and the area of coherence between 5 and 10Hz. A: relationship for all units, B: those units taken from the 5 subjects with a significant peak in the pooled SMU-to-SMU coherence at physiological tremor frequency. Black open circles represent the SMU pairs during control and the solid grey squares during LA. Relevant R^2 values are displayed in colour-matched text.

4.6 Discussion

Previous studies have examined the importance of PMRs in jaw muscle control and found that humans rely on signals from PMRs to regulate the activity in the jaw muscles (Trulsson and Johansson, 1996b). There are several lines of evidence that indicate that PMRs are strongly involved in human mastication (see Lund 1991; Türker 2002 for reviews), though to date the specific role PMRs play has not been clearly elucidated.

The present chapter investigated the changes in SMU coherence/synchronisation and jaw physiological tremor that occur during isometric contractions when the afferent information arising from PMRs is interrupted. This chapter has reinforced the evidence presented in previous work that shows that the load independent/neurogenic component of physiological tremor (Halliday *et al.*, 1999) can be reduced by blocking of exteroceptive afferent input (Van Steenberghe and De Vries, 1980; Fisher *et al.*, 2002; Sowman *et al.*, 2006) and confirmed our previous speculation (Sowman *et al.*, 2006) that the reduction in jaw physiological tremor during anaesthetic block of the PMRs is due to the reduction or removal of a common input to masseteric motoneurones.

4.6.1 SMU Synchronisation and Coherence

In this chapter, spectral analysis revealed a significant decrease in the coherence between MUs around 8Hz in all of those subjects that exhibited a clearly identifiable

physiological tremor prior to anaesthetisation. This decrease in UTU coherence was consistent with a decrease in 8Hz SEMG power and force physiological tremor spectrum confirming that 8Hz physiological tremor in the jaw is at least partly resultant from coherent activity occurring between MUs at this frequency (Erimaki and Christakos, 1999; Halliday *et al.*, 1999; Christakos *et al.*, 2006). Furthermore, the significant relationship found between coherence at the frequency of physiological tremor and the strength of synchronisation, as has been previously demonstrated by others (Semmler *et al.*, 2002a), shows that coherent, in-phase SMU activity represents a similar phenomenon to time domain measures of synchrony.

4.6.2 Cause of Decreased Synchronisation and Coherence

Two possible mechanisms for the decrease in SMU synchronisation seen at the frequency of jaw physiological tremor during anaesthesia of the PMRs are proposed. The first is that jaw physiological tremor arises as a result of reflex feedback from the PMRs or other mucosal mechanoreceptors. Such a hypothesis fits well with the work of McFarland and colleagues (1986) who showed that mandibular force physiological tremor was proportional in its magnitude to the gain of reflex response to mechanical perioral stimulation. They suggest that, rather than being due to feedback in the stretch reflex loop, jaw physiological tremor was more likely due to exteroceptive reflex loops. The authors of that study could not rule out the influence of the stretch reflex loop however and suggest that neural input from the stretch reflex loop, interacting with the filter properties of jaw closing muscles, could lead to the force instabilities seen there.

For the change in tremor amplitude seen in the current chapter to be due to changes in stretch reflex sensitivity there would have to be an interaction between PMR afferent input and stretch reflex sensitivity. This has been investigated by several authors. Both Lobbezoo *et al*. (1993) and Erkelens and Bosman (1985) found that reduced periodontal input increased the sensitivity of the masseteric stretch reflex. However, in the studies of Hoogmartens and Caubergh (1988) and Poliakov and Miles (1994) PMR anaesthetisation had no effect on the sensitivity of the masseteric stretch reflex. This disagreement is most likely due to the differences in tooth stabilisation in the two experimental protocols. Lobbezoo *et al*. (1993) suggest that the lack of antagonistic midline tooth support in their experiment and that of Erkelens and Bosman (1985) was the differentiating factor enhancing fusimotor drive and thus the stretch reflex. In our study, like that of Hoogmartens and Caubergh (1988)and Poliakov and Miles (1994) there was antagonistic midline tooth support throughout the experiment hence, like those researchers, it was expected that no change in stretch reflex activity would have occurred. Furthermore, Lobbezoo *et al*. (1993) propose that any increase in the gain of the stretch reflex is likely to increase the amplitude of tremor rather than decreasing it as this chapter has shown. The results presented here therefore concur with the proposal of McFarland and colleagues (1986). This idea garners further support from the fact that reflex delay times in the disynaptic PMR pathway (Brinkworth *et al.*, 2003), fit the model proposed by (Stein and Oguztöreli, 1976) which shows that interactions between feedback loops with delay times in the range of 20 to 50ms and the physical properties of muscle can cause load independent self-limiting oscillations in the physiological tremor frequency range. The removal of a significant amount of PMR

information in this pathway could therefore be expected to cause a significant diminution of physiological tremor.

Contraposed to the purely reflex based generation of physiological tremor, is mounting evidence for the existence of a central source for the various component rhythms of physiological tremor (Salenius *et al.*, 1996; Salenius *et al.*, 1997; Mima *et al.*, 1999; Gross *et al.*, 2000; Mima *et al.*, 2000; Gross *et al.*, 2002; Salenius and Hari, 2003). While it seems unlikely that interruption of input from the periodontium should interrupt a centrally generated loop, it is possible that PMR activation at the frequency of this rhythm has an amplifying action. This hypothesis follows the proposal of other authors (Lee and Stein, 1981; Elble, 1986; Timmer *et al.*, 1998b), that reflex loops and centrally generated rhythms may be interacting mechanisms that contribute more or less to produce tremors. Elble (1986) suggests that expression of physiological tremor arising from central oscillator may be governed in amplitude by a mechanically mediated reflex. In the jaws, feedback from the highly force sensitive PMRs entering or interacting with a loop responsible for jaw physiological tremor, may act to enhance this common rhythm.

4.6.3 Functional Basis

It is clear from previous studies that humans rely on signals from PMRs to regulate their jaw muscle activity. Particularly when they first contact, manipulate, and hold food substances between their teeth (Trulsson and Johansson, 1996b), and that patients

lacking PMR feedback have abnormal chewing action (Trulsson and Gunne, 1998) and bite force regulation (Haraldson, 1983). Our data provide insight into how rhythmic drive to masseteric SMUs is changed when PMR input is removed and suggests that PMR activity is important in the production of motor activity as fundamental as physiological tremor. While the existence of a function for physiological tremor is yet to be established, it may be that physiological tremor has no function per se, but is rather a by-product of other motor strategies. With this in mind it may be useful to consider the functional significance of the SMU synchrony that gives rise to tremor. In regard to this there are several lines of evidence that would suggest that synchronisation enhances power output. The level of synchronisation appears to be reduced between MUs in the hand muscles of individuals who require greater independent control of the fingers (such as musicians), whereas individuals who perform gross strength training activities, SMU synchronisation levels are higher (Semmler and Nordstrom, 1998). These authors believe their findings suggest that an enhancement of SMU synchronisation contributes to training-induced increases in muscle strength. It therefore follows that in the jaw; biting tasks requiring strength would benefit from a rhythmic synchronising input to motoneurones. This proposed function for rhythmic synchronous activity has been proposed previously by (Baker *et al.*, 1999) who showed that mean force output from a simulated motoneuronal pool increases with increasing synchronous activity. Whilst the above schema suggest tremor may be an emergent product of SMU synchronisation, several authors (Lippold, 1970; Greene, 1972; Goodman and Kelso, 1983) have suggested that tremor in itself may be a strategy employed or exploited by the motor system to minimise stickiness and maximise

rapidity of response. If either hypothesis proves to be true, the existence of tremor can be regarded respectively as the inevitable consequence of an advantageous strategy or advantageous in itself. The fact then that the PMR activity enhances the occurrence of physiological tremor suggests another role for these receptors that confers an advantage upon oromotor function.

Chapter 5

Saturation of Masseteric EMG Entrainment to Rhythmic Tooth Stimulation

5.1 Publication Acknowledgement

Chapter 6

Mandibular Physiological Tremor is Reduced by Increasing-Force Ramp Contractions and Periodontal Anaesthesia

6.1 Publication Acknowledgement

The following is a modification of "Mandibular physiological tremor is reduced by increasing-force ramp contractions and periodontal anaesthesia" Exp Brain Res. 2008 Jan;184(1):71-82 Epub 2007 Aug 8. Sowman PF, Brinkworth RS, Türker KS.

We have previously shown that the application of anaesthesia to periodontal mechanoreceptors (PMRs) dramatically reduces the 6 - 12Hz physiological tremor in the human mandible during constant feedback isometric contractions. This chapter shows that during a ramp contraction where force is slowly increased, the amplitude of mandibular physiological tremor is almost five times smaller on average than when the same force ramp is performed in reverse i.e. force is slowly decreased. This smaller tremor is associated with a higher mean firing rate of motor units (MUs) as measured by the sub-30Hz peak in the multi-unit power spectrum. The decrease in the amplitude of physiological tremor following PMR anaesthetisation is associated in some instances with a similar increase in the overall firing rate; however this change does not match the diminution of tremor. The authors postulate that the decrease in mandibular physiological tremor during increasing force ramps may be due to a change in the mean

firing rate of the MUs. The change in tremor seen during PMR anaesthetisation may in part be due to a similar mechanism; however other factors must also contribute to this.

6.1 Introduction

Physiological tremor is thought to consist of two interacting mechanisms. The first relates to the mechanical properties of the limb or body segment in question. The mechanical resonance of this segment dictates that when it is perturbed it will exhibit a decaying oscillation at its natural frequency. This frequency of tremor can be altered by loading or unloading of the segment or by changing the elasticity of that load. The second component of physiological tremor that occurs at 6 – 12Hz is frequency invariant with changes in load and elasticity and is therefore thought to be of neural origin [for review see Elble and Koller (1990)]. The expression of this component of tremor is manifest through rhythmic synchronisation of motor units (MUs) in response to a common input (Elble and Randall, 1976; Halliday *et al.*, 1999; Christakos *et al.*, 2006). Whether this 'neurogenic' component of physiological tremor is of primarily supraspinal origin, or arises from the propagation of oscillatory activity in reflex feedback loops remains contentious. A significant body of evidence exists to support both theories and furthermore, it is likely that the two are not mutually exclusive (Elble and Koller, 1990; Timmer *et al.*, 1998b).

Of primary interest to those studying reflex involvement in physiological tremor has been the involvement of the stretch reflex (Lippold, 1970; Matthews and Muir, 1980; Durbaba *et al.*, 2005); conversely, little attention has been paid to the involvement of exteroceptive reflexes. The evidence from those studies that have investigated the role of exteroception in physiological tremor suggests that afferent blockade or deafferentiation reduces its amplitude (Sanes, 1985; Fisher *et al.*, 2002; Kilner *et al.*, 2004). This phenomenon has been repeatedly demonstrated in the human jaw. When the afferent outflow from the periodontal mechanoreceptors are blocked by local anaesthetic the amplitude of neurogenic mandibular physiological tremor is greatly diminished (Van Steenberghe and De Vries, 1980; Sowman *et al.*, 2006; Sowman *et al.*, 2007). Such studies show that afferent feedback from tactile receptors play a role in the expression of physiological tremor, though the mechanism by which this might occur remains unknown.

The hypothesis that reflexes, given their relatively low gains, could independently give rise spontaneously to the oscillatory behaviour underlying physiological tremor remains controversial (Elble and Koller, 1990). We therefore set out to test an alternative hypothesis for this phenomenon; that afferent input from PMRs may alter global MU firing statistics in such a way as to favour the expression of a common input.

In the human jaw, the mean firing rate of masseter MUs during moderate contractions is known to be around 20-25Hz (Van Boxtel and Schomaker, 1983) whereas mandibular physiological tremor occurs between 6 and 12Hz (Junge *et al.*, 1998). We hypothesised

that the size of mandibular physiological tremor, assuming the amplitude of its source remained constant, should increase if the mean masseteric MU firing rate i.e. the carrier frequency for physiological tremor (Matthews, 1997) was reduced toward the frequency of physiological tremor.

This hypothesis was split into two parts; firstly a slowing of MU firing rates could enhance physiological tremor and secondly, PMR anaesthetisation would cause the opposite case i.e. a global increase in MU firing rates that accompanied a diminution of physiological tremor.

In order to manipulate the firing rate of the MU pool while maintaining a comparable level of PMR activation, we took advantage of the firing frequency hysteresis known to occur in motoneurones when recruitment/derecruitment paradigms are investigated (Button *et al.*, 2006). The use of ramped force targets under isometric conditions has shown that, for the relaxation phase of a given force ramp triangle, MUs de-recruit at lower force levels than that at which they were recruited (Person and Kudina, 1972; Denier Van Der Gon *et al.*, 1985; Christova and Kossev, 2001). Hence, for any given force during isometric relaxation compared to the same force in the ramp-up phase, the distribution of active MUs is different, and the mean firing rate lower.

This chapter sought to investigate the involvement of mean firing statistics on the expression of physiological tremor by using such a contraction paradigm to manipulate

these. We then sought to compare any changes seen to those when a LA block was administered to identify similarities or a lack thereof.

To test these hypotheses we examined the differences in physiological tremor under five different isometric contraction conditions. These conditions were; 1. During a continuous isometric ramp relaxation, 2. During a continuous, increasing-force isometric ramp contraction, 3. During a constant force isometric contraction contiguous to a ramp relaxation, 4. During a constant force isometric contraction contiguous to an increasing-force isometric ramp contraction and 5. During a constant force isometric contraction.

The specific aims of this chapter, were to establish whether any differences in physiological tremor during such tasks were related to changes in the mean firing rate of the underlying MUs as assessed by the sub 30Hz frequency peak in the EMG power spectrum (Gath, 1974; Agarwal and Gottlieb, 1975; Lago and Jones, 1977; Blinowska *et al.*, 1979; De Luca, 1979; Blinowska *et al.*, 1980; Christakos and Lal, 1980; Lago and Jones, 1981; Verroust *et al.*, 1981; Van Boxtel *et al.*, 1983; Van Boxtel and Schomaker, 1983, 1984; Weytjens and Van Steenberghe, 1984a; Weytjens and Van Steenberghe, 1984b; Blinowska and Verroust, 1987; Pan *et al.*, 1989; Harrison *et al.*, 1991; Van Der Glas *et al.*, 1994; Myers *et al.*, 2003) and to see if periodontal anaesthetisation during such tasks altered the expression of physiological tremor

6.2 Methods

6.2.1 Subjects

Experiments were conducted according to the regulations of, and with approval from, the University of Adelaide human ethics committee and conformed to the Declaration of Helsinki. A total of 10 volunteers (7 females and 3 males; age range 18-43 years) were recruited for these experiments, all of whom provided written informed consent. All subjects had natural and healthy dentitions and were free of dental symptoms at the time of the experiments.

Subjects sat upright in a dental chair adjusted for height such that they could bite onto fixed metal bite plates (inter-incisal distance of 12mm) whilst maintaining a neutral neck position. The repeatability of this position was established by the use of a fixed nosepiece that was set to contact the subject's nose when the required position was attained [method previously described in (Brinkworth *et al.*, 2003)]. The metal bite plates were coated with a rigid acrylic dental impression material. Only the incisor teeth were used in biting during the experiment. Custom made intramuscular multi-unit fine wire electrodes were used to record EMG from the left masseter. Each electrode had two Teflon®-insulated silver wires comprising the bipolar multi-unit electrode. The multi-unit wires had the insulation stripped 2mm from the terminal (Scutter and Türker, 2000). Electrodes were inserted to a depth of approximately 2 cm into the anterior deep

portion of masseter using 25G needles. Each needle was immediately withdrawn, leaving the two wires in the belly of the muscle. Multi-unit EMG signals were sampled at 5kHz, filtered (20-2500Hz) and recorded to a computer hard-drive via a specially designed data acquisition program (National Instruments, LabVIEW®, Texas, (Brinkworth, 2004). Prior to further analysis, the EMG signal was full wave rectified [for discussion on the use of this method for EMG demodulation and spectral analysis see (Journee *et al.*, 1983; Timmer *et al.*, 1998b)] and the DC level was removed. Subjects were grounded using a lip-clip electrode (Türker *et al.*, 1988) and presented with filtered feedback via a horizontal target line on a dedicated feedback monitor screen (refresh rate 60Hz). EMG signals used for feedback were full-wave rectified before low pass filtering at 1Hz. The feedback monitor screen was scaled to display levels from 0-30N, with the y-axis measuring 210mm. Subjects' eyes were a horizontal distance of 900mm away from the monitor. This experimental set-up has been illustrated in previous reports (Sowman *et al.*, 2006)

6.2.2 Biting Tasks

Five different biting tasks were investigated in this experiment. The first type consisted of simple isometric ramped contractions in either an increasing direction (UP) or a decreasing direction (DOWN). Feedback was given as a bite force in Newtons (N). For the UP task (Fig. 6.1D) subjects followed a linearly increasing force target that started at 0N, increased over 2 minutes and terminated at 20N. The opposite target

direction was used for the DOWN task i.e. the target declined continuously from 20-0N over 2 minutes. For the DOWN task (Fig. 6.1A) the subject was instructed to bite up to the initial target before the target began to move, this normally took approximately 1-2 seconds. The second type of task was a ramp-and-hold task, either UPHOLD or DOWNHOLD, during which, for the UPHOLD task (Fig.6.1E), subjects followed a linearly increasing force target that started at 0N and increased over 1 minute and terminated at 10N, they then held this level constantly for a further 1 minute. The opposite target direction was used for the DOWNHOLD task (Fig. 6.1B) i.e. the target declined continuously from 20-10N over 1 minute then remained constant at 10N for a further minute. A further 2 minutes of static biting (Fig. 6.1C) (STATIC) at 10N was recorded. These tasks are illustrated by the force recordings in Figure 6.1.
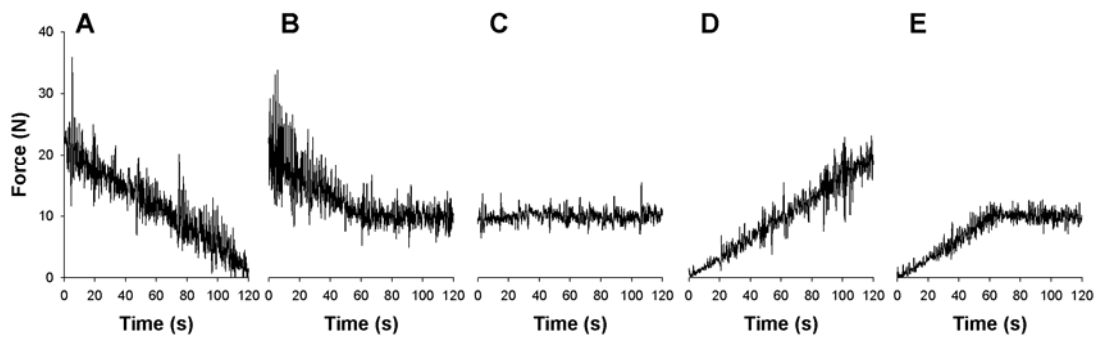
Figure 6.1. Force recorded from a single subject during biting tasks. A) DOWN task. B) DOWNHOLD task. C) STATIC task. D) UP task. E) UPHOLD task. Raw force records were downsampled to 50Hz prior to plotting.

Biting tasks were randomised and interspersed with 30-second rest periods. The first segment henceforth referred to as "Control 1", consisted of 5 biting tasks (UP, DOWN, UPHOLD, DOWNHOLD, STATIC). Once the biting tasks for Control 1 had been completed, the subjects rested for 20 minutes. The protocol was then repeated ("Control 2") with a different randomisation of biting tasks so as the effect of time could be assessed. After completion of Control 2, subjects had their upper and lower peri-incisal periodontium anaesthetised by local anaesthetic (LA) infiltration (4ml, Xylocaine 2%®, Astra Pharmaceuticals Pty. Ltd. NSW, Australia). Adequate anaesthesia was determined to have been achieved when the subject no longer had tactile or vibratory sensation in either labial or buccal aspects of the upper or lower incisor teeth canine to canine (Türker and Jenkins, 2000; Brinkworth *et al.*, 2003). The above biting tasks were then repeated with a different randomisation 5 minutes after completion of the anaesthetisation procedure (about 15 minutes after the initial dose of the LA). This third segment will be henceforth referred to as "LA".

6.2.3 Data Analysis

Cross-spectral analysis was performed in accordance with the methods of Rosenberg *et al.* (1989) and Halliday *et al.* (1995) to determine if any correlation existed in the frequency domain between the simultaneously recorded EMG and force signals. Coherence spectra like those calculated here, give an estimate of the frequency domain coupling between two time series, from which significant coherence at any given frequency infers rhythmicity at that frequency in both signals (Hansen and Nielsen, 2004). Coherence analysis has been implemented here using a custom designed LabVIEW® (National Instruments, Texas) based computer program.

Four variates were automatically extracted for statistical testing from the spectral analysis results by an Excel macro. We chose to compare the effect the factors of Direction (UP, DOWN, STATIC) and Condition (CONTROL 1, CONTROL 2 and LA) had on the following three variates: 1) the size of physiological tremor in the force record which we defined as the area under the force power spectrum between 6 and 12Hz, 2) the mean firing frequency of the motor unit pool, defined as the frequency at which the sub 30Hz maximum occurred in the multi-unit power spectrum, and 3) the area of 6-12Hz coherence between the rectified multi-unit and force. These variates were tested using a within subjects design i.e. a multi-variate 2 factor repeated measures ANOVA (SPSS®). In order to compare the means of the main effects we used a priori defined contrast matrix that compared each of the Direction means, and for Condition, compared the means of CONTROL 1 and CONTROL 2 and then the pooled mean of the 2 controls against LA. Mauchly's test was used to assess the sphericity of data prior to further analysis of results and significance was set at the 5% level. To assess the relationship between the mean firing frequency of MUs and the area of physiological tremor measured in force, we plotted these variables against one another and fitted an S-curve (Gompertz Model) to the relationship as described by the following equation: $y = \exp(a + b/x)$ where y = the force tremor amplitude and x the mean MU firing rate (SPSS®). Pearson's R^2 was used to determine the strength of this relationship. For the ramp and hold tasks, only the data from the hold period contiguous to the ramp was analysed.

6.3 Results

Clearly identifiable, sub 30Hz peaks in the EMG power spectra were evident in all trials and, in 7 of the 10 subjects these peaks were at higher frequencies, clearly independent of the physiological tremor frequency peak in the force spectrum. Typically, this EMG peak occurred in the 15-25Hz frequency range. For three of the subjects, during the DOWN task, the maximal sub 30Hz EMG peak occurred at a frequency that overlapped the frequency at which physiological tremor occurred. The 2 patterns of EMG spectra are illustrated in Figure 6.2. Examples of the EMG and force spectra seen during the HOLD phase of the ramp-and-hold tasks are presented in Figure 6.3.
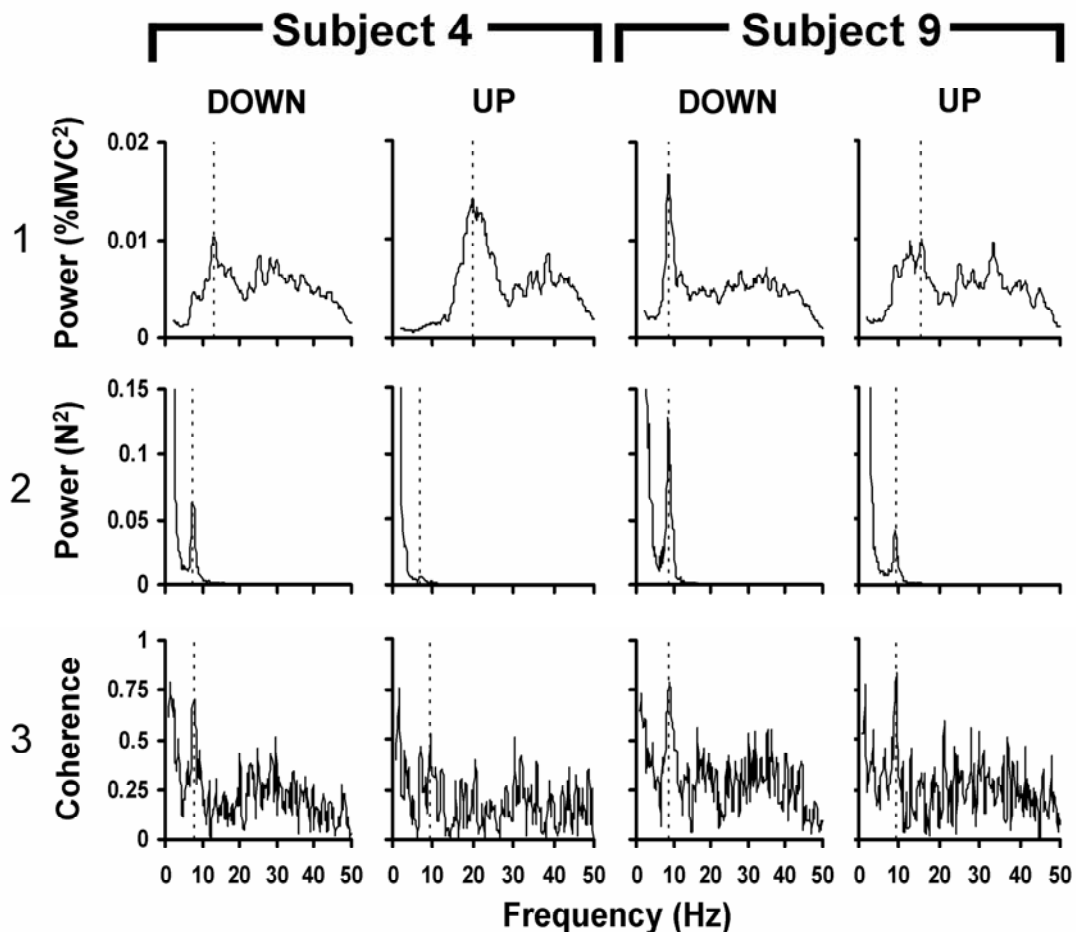
Figure 6.2. Physiological tremor spectra; both UP task and DOWN task under force feedback. This figure shows: (1) the EMG power spectra, (2) the mandibular force power spectra and (3) the coherence spectra between EMG and force for the

2 minutes of biting for the respective biting task. Subject 4 is an example of the type of EMG power spectrum that was typical of most (7 out of 10) subjects i.e. a sub 30Hz maximum at a frequency higher than that at which the dominant physiological tremor frequency occurred in the force spectrum. A smaller peak in the power spectrum is also evident at ~7.5Hz where a peak in the force spectrum also occurs. Peak coherence above 2Hz occurs at this frequency also. Subject 9 is an example of the other type of spectrum that was seen in 3 of the subjects. During the DOWN task the peak in the EMG spectrum occurs at the same frequency as that in the force power spectrum. In both subjects shown here, the peak frequency in the EMG power spectrum moves to the right during the UP task, indicating a higher mean MU firing rate. This rightward shift in the EMG spectral peak occurs concurrently with a decrease in the amplitude of force physiological tremor.
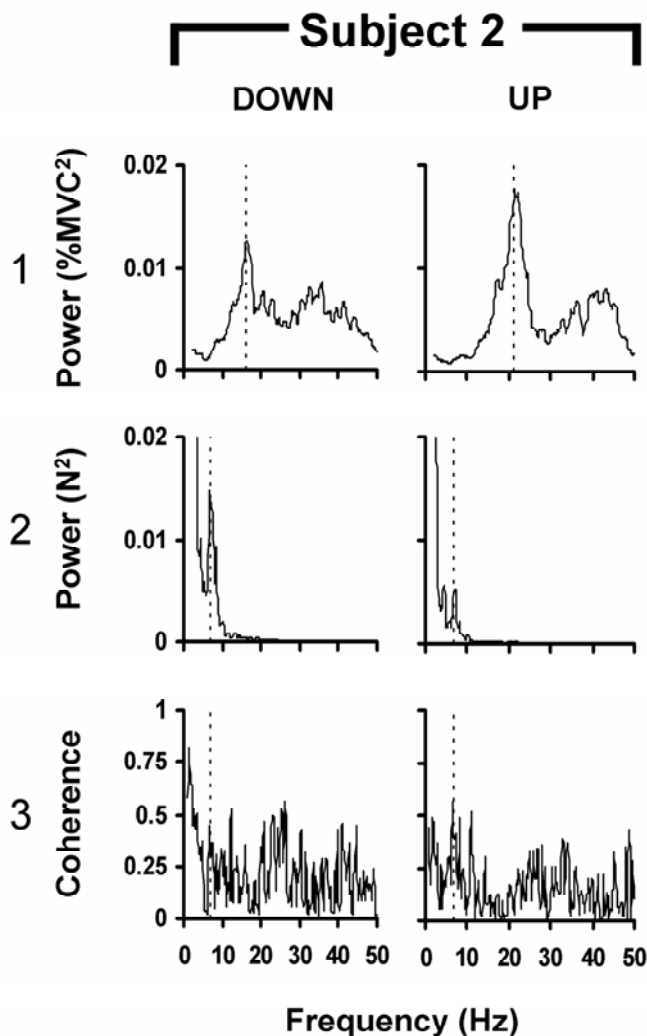
Figure 6.3. Physiological tremor spectra during static holding following either an UPHOLD or DOWNHOLD task. This figure shows: (1) the EMG power spectra, (2) the mandibular force power spectra and (3) the coherence spectra between EMG and force for 1 minute of static biting at 10N following 1 minute of the contiguous respective biting task. The subject presented here shows that a similar pattern of EMG peak rightward shift during the HOLD phase of the UPHOLD task compared to the HOLD phase of the DOWNHOLD task as is seen in Fig.2 where the UP task is compared to the DOWN task. As with the dynamic tasks,

this rightward shift in the EMG is accompanied by a decrease in the magnitude of

the physiological tremor peak in the force spectrum.

6.4 Group Results

ANOVA showed that there was no significant interaction between condition and direction for any of the variates tested, therefore only the main effects of direction and condition are presented.

6.4.1 Direction

There was a significant effect on the average dominant EMG frequency. During the UP task the dominant frequency (19.64 ±0.90Hz) was significantly higher than both the DOWN (15.48 ±1.11Hz) and STATIC (17.133 ±0.85Hz) tasks but there was no significant difference between DOWN and STATIC see Figure 6.4. The frequency of peak coherence was not changed by the direction of bite task, remaining constant at a mean ±SEM frequency of 7.25 ±0.5Hz, clearly a frequency independent of the overall mean dominant EMG frequency of 17.5 ±0.75Hz. As shown in Figure 6.4 the area of physiological tremor was almost four times larger during DOWN (0.067 ±0.022) than UP (0.018 ±0.006) and also larger than STATIC (0.048 ±0.023). Likewise, coherence areas were significantly larger during DOWN (1.9 ±0.20) than during UP (1.41 ±0.15) or STATIC (1.55 ±0.22), indicating that there was more physiological tremor during the DOWN task.
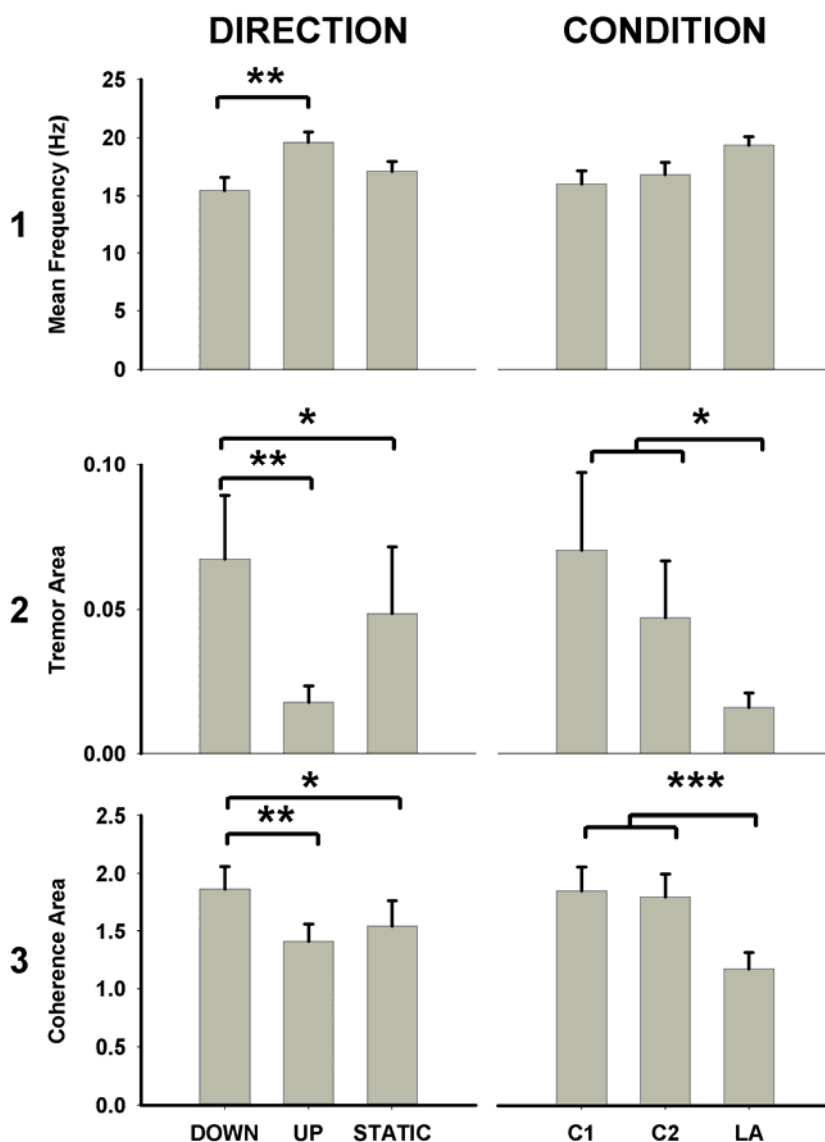
Figure 6.4. Average data across all subjects (n=10, mean ± SEM) during the continuous force ramps and STATIC task. 1) the frequency of the sub 30Hz maximum in the EMG power spectrum for each of the direction tasks and the control tasks (C1 and C2) vs. the tasks conducted during PMR anaesthetisation (LA). There was a significantly higher mean firing frequency during the UP task compared to the DOWN task p<0.01. There was an increase in mean frequency during LA compared to the controls that was almost significant (p=0.07). 2) Effect

on 6-12Hz physiological tremor area in the force record. Physiological tremor was significantly greater during the DOWN task compared to the UP task (p<0.01) and significantly larger than during the STATIC task (p<0.05). During the controls the area of 6-12Hz physiological tremor was significantly greater than during LA (p<0.05) and there was no statistically significant difference between the physiological tremor size during the 2 controls. 3) Effect on the area of coherence between EMG and force. Coherence was significantly greater during the DOWN task compared to the UP task (p<0.01) and significantly larger than during the STATIC task (p<0.05). During the controls the area of 6-12Hz coherence was significantly greater than during LA (p<0.001) and there was no significant difference between coherence areas during the 2 controls.

6.4.2 Condition

Anaesthetisation of the PMRs caused a significant main effect on all measured parameters. A priori defined contrasts revealed that for all measures except the dominant frequency of EMG, the significant difference seen was due to the LA condition being significantly different from the pooled controls, whereas no significant difference between controls was evident. The application of LA increased the dominant frequency of the EMG from 16.03 ±1.16Hz during CONTROL 1 and 16.83 ±1.05Hz during CONTROL 2 to 19.4 ±0.73Hz during LA. However this difference was not large enough to cause a significant difference between the pooled controls and the LA condition (p=0.07). The application of LA significantly decreased the area of physiological tremor from 0.070 ±0.027 during CONTROL 1 and 0.047 ±0.019 during CONTROL 2 to 0.016 ±0.005 during LA. The coherent area (CONTROL 1 =1.85 ±0.21, CONTROL 2 =1.80 ±0.20, LA =1.17 ±0.14) was similarly altered (Figure 6.4).

6.4.3 Correlation between Firing Rate and physiological tremor

There was a significant (p<0.001) negative curvilinear relationship [S-Curve: Force Tremor Area = exp(-6.054 + 34.175)/Firing Frequency] between the physiological tremor area and the dominant EMG frequency during both control periods. No relationship was evident during LA (Figure 6.5).
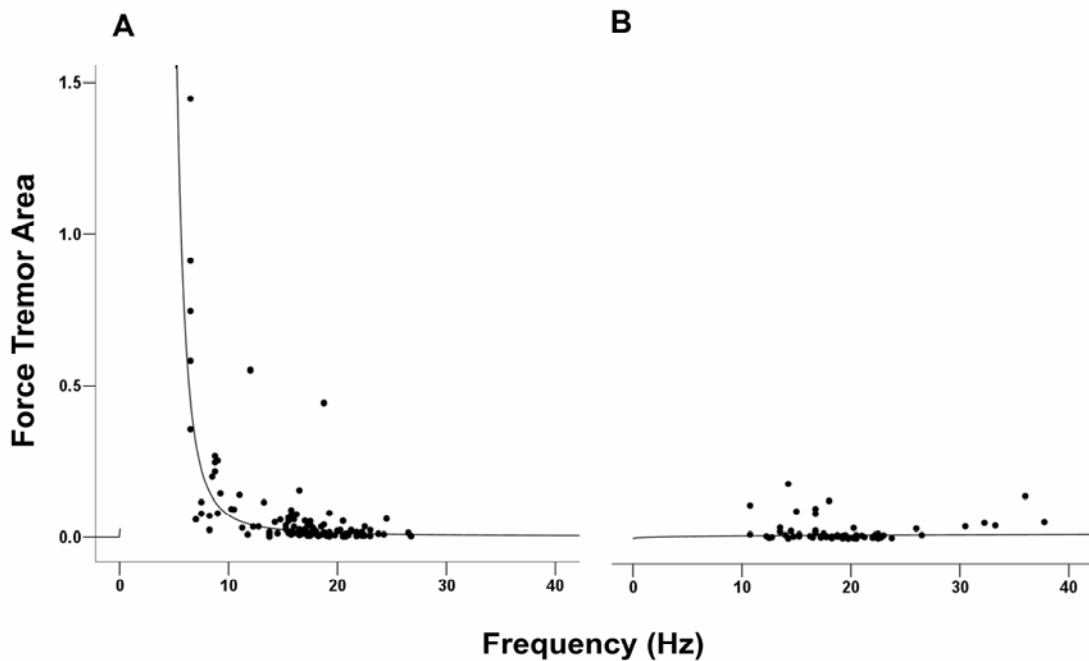
Figure 6.5. Relationship between mean firing rate of motor units and magnitude of 6-12Hz physiological tremor in force. (A) includes both control conditions and all directions. There is a significant (R^2 =0.477, p<0.001) negative curvilinear relationship (S-Curve) between the mean firing frequency of the sampled MU pools and the concurrent 6-12Hz physiological tremor measured in jaw force. (B) includes all directions during LA (R^2 =0.004).

6.5 Hold Experiment

6.5.1 Direction

During the hold phase of the UPHOLD task, the dominant EMG frequency (18.8 ±1.36Hz) was significantly higher than the hold phase of the DOWNHOLD task (16.68 ±1.20Hz) but there was no difference between the dominant EMG frequency during the hold phase of the DOWNHOLD and STATICHOLD tasks (17.66 ±1.06Hz) see Figure 6.6. The physiological tremor area (DOWNHOLD =0.057 ±0.021, UPHOLD =0.012 ±0.003) (Figure 6.6) and coherence area (DOWNHOLD =1.71 ±0.20, UPHOLD =1.35 ±0.14) were both significantly larger during the hold phase of the DOWNHOLD task than during hold phase of the UPHOLD and the coherence area was significantly larger during the hold phase of DOWNHOLD than STATIC (DOWNHOLD =1.71 ±0.20, STATIC =1.67 ±0.18), indicating that there was increased physiological tremor during the hold phase of the DOWNHOLD task Figure 6.6. The tremor during the hold phase of the DOWNHOLD task was almost five times larger on average than that seen during the hold phase of the UPHOLD task.

6.5.2 Condition

Anaesthetisation of the PMRs caused significant changes in both physiological tremor area (Figure 6.6) and coherence area (CONTROL 1 =1.67 ±0.20, CONTROL 2 =1.86 ±0.19, LA =1.19 ±0.13). The frequency of coherence and EMG remained unchanged. A priori defined contrasts revealed that for both physiological tremor area and coherence area, the significant difference was due to the LA condition being significantly different from the pooled controls, whereas there was no significant difference between controls, see Figure 6.6.
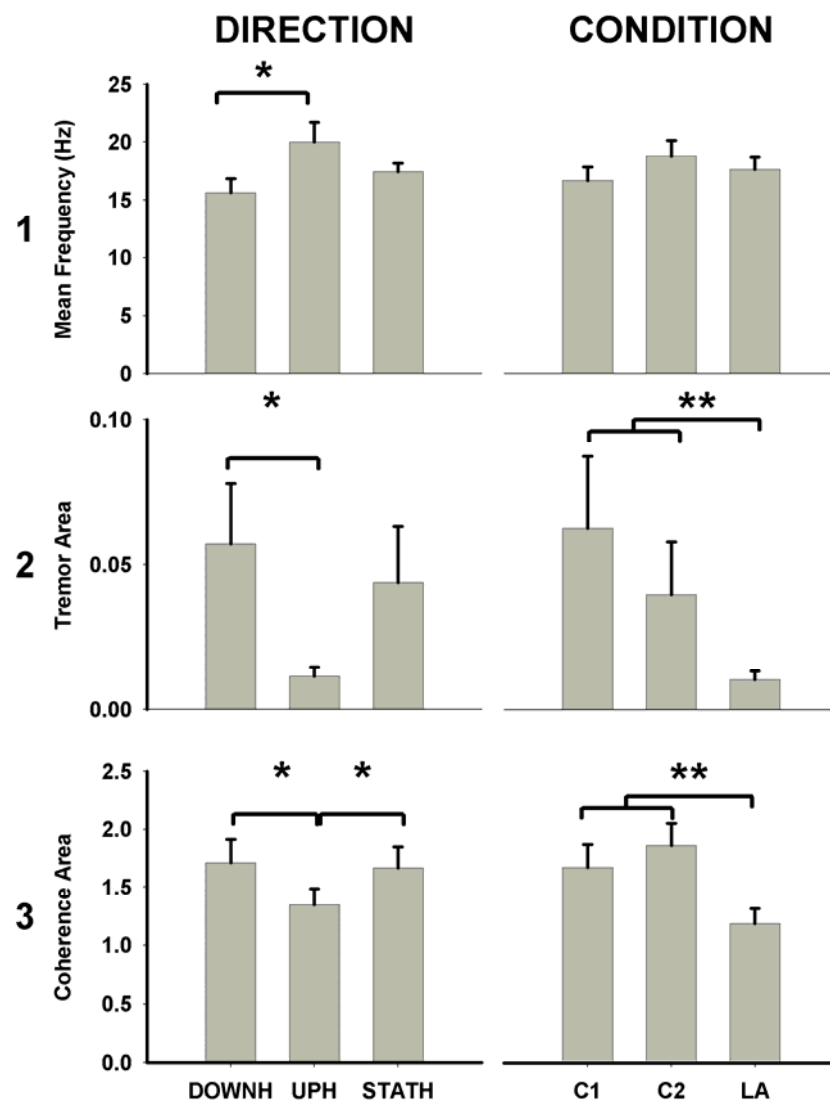
Figure 6.6. Average data across all subjects (n=10, mean ± SEM) during the hold phases of the ramp and hold experiments. 1) Frequency of the sub 30Hz maximum in the EMG power spectrum for each of the direction tasks and the control trials (C1 and C2) and the trials conducted during PMR anaesthetisation (LA). There was a significantly higher mean firing frequency during the UPHOLD (UPH) task compared to the DOWNHOLD (DOWNH) task p<0.05. 2) 2) The effect on 6-12Hz physiological tremor area in the force record.

physiological tremor was significantly greater during the DOWNH task compared to the UPH task (p<0.01) but not significantly different from the STATIC (STATH) task. During the controls the area of 6-12Hz physiological tremor was significantly greater than during LA (p<0.05) and there was no significant difference between the physiological tremor size during the 2 controls. (3) Effect on the area of 6-12Hz coherence between EMG and force. Coherence was significantly greater during the DOWNH task compared to the UP task (p<0.05) Coherence was also significantly greater during the STATH task compared to the UPH task (p<0.05). During the controls the coherence was significantly greater than during LA (p<0.01) and there was no significant difference between coherence areas during the 2 controls.

6.6 Discussion

There are four main findings in this chapter. The first is that the type of contraction performed under isometric conditions can influence the expression of mandibular physiological tremor. During a ramped isometric relaxation, the magnitude of 6-12Hz physiological tremor as measured in mandibular force is significantly greater than that seen when the subject performs the contraction in the opposite direction even though the mean force exerted over the time of task is identical. Furthermore, the magnitude of physiological tremor during the hold phase of a ramp-and-hold task is similarly altered depending on the direction of the contiguous ramp phase, the amount of physiological tremor being almost five times greater during the hold phase of a DOWNHOLD task than and UPHOLD task. The randomisation of the order these tasks were performed in, combined with the fact that we did not use the conventional triangular force profile where the DOWN phase contiguously follows the UP phase, negates the likelihood that fatigue would have influenced these results. Moreover, the maximum force of 20N used here is within the very low range of a system that can sustain loads above 100N for several minutes (Sforza *et al.*, 2007).

Given that 6-12Hz physiological tremor is the result of synchronisation of MU firings at this frequency (Erimaki and Christakos, 1999; Halliday *et al.*, 1999; Christakos *et al.*, 2006; Sowman *et al.*, 2007), we propose that the direction of the force ramp dictates the degree of MU synchronisation. Specifically, a declining force ramp causes an increase

in MU synchronisation compared to an increasing force ramp. This is evidenced here by the significant increase in 6-12Hz physiological tremor in the mandibular force record and the significant increase in the area of coherence between EMG and force seen during the DOWN contraction and also during the HOLD phase of the DOWNHOLD task.

The second finding is that the type of contraction performed under isometric conditions can influence the firing statistics of a population of MUs within the masseter muscle. During a declining force ramp, the overall average firing rate of the sampled population is significantly lower than during an increasing force ramp of identical magnitude. Furthermore, the overall average firing rate of the sampled population is significantly lower during the hold phase of a ramp-and-hold task when direction of the contiguous ramp phase was declining as opposed to increasing.

The third finding in this chapter was that PMR anaesthetisation decreased physiological tremor under all contraction conditions. This result is in good agreement with our previous findings (Sowman *et al.*, 2006; Sowman *et al.*, 2007) and extends those findings to incorporate time varying isometric contractions.

The fourth finding presented here is that the mean firing rate of the MU pool was negatively correlated with the size of mandibular tremor. This finding is in agreement

with previous studies that have shown that the modulation of single MU firing (Ellaway and Murthy, 1985; Matthews, 1997) or multiple MU synchronisation (Türker and Powers, 2002; Lowery and Erim, 2005) in response to an input is negatively related to firing rate.

6.6.1 Physiological Tremor and the EMG Power Spectrum

The 6-12Hz synchronised firing of MUs underlying physiological tremor has been shown to occur at frequencies independent of the actual firing rates of the MUs (Elble and Randall, 1976; Wessberg and Kakuda, 1999) i.e. the rhythmic component of tremor is not due to synchronous firings of MUs at their intrinsic firing rate, but rather, rhythmic modulations of this intrinsic firing rate.

MU firing statistics cause distinctive patterning of the EMG power spectra [described in detail by (Blinowska *et al.*, 1979, 1980)] whereby distinctive peaks in the power spectra, when present, can be interpreted to give an indication of the firing statistics of the underlying population. Such frequency peaks are especially pertinent to the muscles of the face and the jaw elevators as these have a narrower spread of firing frequencies than larger limb muscles (Van Boxtel and Schomaker, 1983). Furthermore, the validity of this spectral characteristic is likely to be especially relevant in this chapter where we have, by using multiunit EMG recorded from fine-wire electrodes,

sampled a small, homogenous population of masseteric MUs. By doing this we have also ensured that our recordings were not contaminated by EMG arising from overlying mimic muscles (Warwick and Williams, 1995).

Blinowska suggests that the EMG power spectrum can be categorised into 3 main types: The A-type spectra, consists of a single well defined first maximum, the frequency of which represents the uncorrelated mean firing rate of the group. This type of spectra also gives rise to a second maximum at a harmonic frequency of the first. This is particularly well illustrated in the left hand panel of Figure 6.3. The B-type spectrum occurs with increasing correlated discharge between units. As synchronisation increases, so too does effective dispersion of interspike intervals (ISI). A sharp peak at the lower, synchronisation frequency is evident, but the increased dispersion causes the size of the peak at the firing rate frequency to lessen but not necessarily disappear (Weytjens and Van Steenberghe, 1984b). This type of spectral appearance is particularly well illustrated by the right hand panel of Figure 6.3. Weytjens and van Steenberghe (1984b) suggest that both information about ISI statistics and the level of synchronisation are obtainable from the EMG spectra.

6.6.2 Decrement in Physiological Tremor Related to Increase in Firing Rate

In both of the control conditions there was a significant negative sigmoidal relationship demonstrated between the dominant firing rate of the sampled MUs and the magnitude of the physiological tremor in the force record. This is strikingly similar to that relationship presented by Harrison *et al*. (1991) between firing rate and synchronisation. The left hand side of the graph (Figure 6.5) describing the relationship consists mostly of those results obtained from the DOWN task and the STATIC task where the mean firing rate was lower and the physiological tremor higher. This relationship was abolished during LA. During LA there was a significant increase in the dominant firing rate of the sampled MUs contingent with a decrease in the magnitude of physiological tremor in the force record.

A possible way in which the gain of the response to a rhythmic input may be modulated, is by changes in the ratio between the carrier frequency and the input frequency (Matthews, 1997). In a motoneurone pool, the carrier frequency is dictated by the mean firing rate of the motoneurones in that pool and therefore, for a given input frequency, changes in the mean firing rate that bring the carrier frequency closer to the frequency of a common input will cause an increase in gain. When considering multiple MUs, increasing the gain to a rhythmic input across multiple units by manipulating the carrier frequency also increases the likelihood of MU synchronisation at that frequency (Lowery and Erim, 2005). Such a relationship between firing rate and motor unit synchronisation that agrees with our interpretation of data presented herein has also previously been described empirically (Kirkwood and Sears, 1978, 1982;

Kirkwood *et al.*, 1982; Ellaway and Murthy, 1985; Harrison *et al.*, 1991) and is known to follow a negative linear (Türker and Powers, 2002) or curvilinear relationship (Ellaway and Murthy, 1985) i.e. as MU firing rate increases, the level of synchronisation (Türker and Powers, 2002), and associated force tremor (Yao *et al.*, 2000), decreases.

6.6.3 The Influence of Afferent Input on MU Firing Rate and Physiological Tremor

While MUs are known to follow an orderly recruitment and derecruitment strategy (Henneman, 1981), several factors including afferent inputs (Garnett *et al.*, 1976; Garnett and Stephens, 1978, 1980, 1981; Kimura *et al.*, 2003a), are known to alter recruitment patterns and mean firing rates. The experiments of Garnet and colleagues showed that afferent input may bias the recruitment of high threshold MUs which are capable of developing higher forces at lower firing frequencies, than lower threshold MUs. For a given level of force output, the presence of afferent input may dictate a lower mean firing rate in the MU pool. This biasing toward high threshold units has also been shown in the human jaw when PMRs are stimulated (Yang and Türker, 2001) and may therefore be an alternative explanation for the diminution of mandibular physiological tremor seen when PMRs are blocked by LA (Van Steenberghe and De Vries, 1980; Sowman *et al.*, 2006; Sowman *et al.*, 2007). This thesis is not supported by the data presented here however. Whilst there was a trend toward increasing firing rates with LA in the dynamic experiment, this trend was not significant. Furthermore,

in the HOLD experiments, LA caused a marked reduction in tremor whilst there was no coincident increase in firing rate present.

6.6.4 The Influence of Contraction Type on MU Firing Rate and Physiological Tremor

Increased tremulous activity (Christou and Carlton, 2002) along with increased MU synchronisation is known to occur during lengthening contractions (Semmler *et al.*, 2002a). This has been attributed to the increased involvement of the stretch reflex during lengthening, however there is the coincident fact that MU firing rates are significantly lower during lengthening contractions compared to shortening contractions carrying the same load (Christova and Kossev, 2001; Semmler *et al.*, 2002a; Semmler *et al.*, 2002b). It has further been proposed that during eccentric contraction there is a bias toward the recruitment of high threshold units (Nardone and Schieppati, 1988; Nardone *et al.*, 1989). Therefore, it remains a possibility that, during lengthening contractions, the larger tremor-causing synchronisation might be due to a decrease in the carrier frequency for an extrinsic oscillatory input. Furthermore, it has been proposed that both cortical input (Bigland and Lippold, 1954; Howell *et al.*, 1995; Kimura *et al.*, 2003b) and stretch reflex sensitivity (Kimura *et al.*, 2003a) are actually decreased during lengthening contractions, suggesting that an increase in oscillatory input from either the cortex or stretch reflex may not be the factor that increases oscillatory output at physiological tremor frequency during lengthening contractions.

Whilst the contractions performed here were isometric, similarities exist between MU behaviour during lengthening contractions and isometric relaxations (Tax *et al.*, 1989;

Tax *et al.*, 1990a; Tax *et al.*, 1990b); moreover, it is known that a significant amount of shortening and lengthening of muscle fibres occurs during isometric ramp contractions (Ito *et al.*, 1998).

6.7 Limitations of this Chapter

The main limitation of this chapter is that with an increase in the synchronisation of MUs the dominant frequency peak in the power spectrum comes to represent the frequency of synchronisation rather than the dominant firing rate. Under circumstances where there is a high degree of MU synchronisation (and large physiological tremor), the effect of synchronisation is to cause increasing non-stationality in the signal. Therefore, while a large maximum at the frequency of synchronisation is present, the shortening or elongation of intervals preceding the spike that is time-locked to the synchronising input causes ISI dispersion and loss of firing rate information. While the occurrence of this type of spectrum was only evident in three subjects, the large tremor associated with a low frequency maximum may be indicative of a large level of synchronisation that obscures the firing information. The possibility remains however that in these cases that there was a true overlap between the dominant firing frequency and the frequency of the synchronising input (Christakos *et al.*, 2006).

The application of LA in this chapter always followed the control condition. This fixed ordering may have introduced a time effect into the results. For this reason we used

two control periods so as to examine the effect of repeated trials. Chapter 3 used this technique to analyse the effect of time showed no difference to occur (Sowman *et al.*, 2006) but the current data shows that physiological tremor size is reduced in the second control compared to the first, albeit statistically insignificantly. It is unlikely that this was a result of cumulative fatigue as this has been shown repeatedly to increase rather than decrease physiological tremor. Given the known effect of PMR anaesthesia on physiological tremor, we suggest that repeated biting tasks may have produced a partial pressure-anaesthesia (Dahlin *et al.*, 1989) of the PMR afferents that caused reduced physiological tremor during the second control period.

Whilst we designed this experiment so that the amount of PMR stimulation was to be identical on average between conditions, the fact that the DOWN ramp was performed without a preceding UP ramp necessitated the subject making a rapid increase in bite force prior to the DOWN ramp proceeding. It is possible that, due to the viscoelastic properties of the tooth/periodontal interface, less tooth intrusion occurred during the DOWN ramp. It is known that tooth intrusion is inversely correlated with the rate of force application (Schoo *et al.*, 1983), and hence, during the up phase where force application was always slow in rate, more tooth intrusion may have occurred, saturating the rapidly-adapting receptors and lowering the overall PMR outflow during the UP ramp compared to the DOWN ramp.

6.8 Conclusions

This chapter shows that manipulating the firing rate of the MU pool during isometric contractions can effect the expression of physiological tremor in the human mandible. A lower mean firing rate during the DOWN phase of an isometric ramp contraction is associated with an increase in the 6-12Hz physiological tremor in the force record. Furthermore, the relationship between the magnitude of physiological tremor and the firing rate of the MU pool follows the negative curvilinear relationship described previously between firing rate and synchronisation. Whilst these data suggests that the difference in tremor that occurs when an increasing-force ramp contraction compared to a decreasing-force ramp contraction is performed can be explained by changes in the overall firing statistics of the MU pool, they do not support such a mechanism accounting for the decrement in tremor seen during PMR LA. Similar tremor reduction can be seen following an increasing-force contraction as can be observed when the PMRs are anaesthetised. However, the associated increase in MU firing rate observable with the contraction-type manipulation is not consistently a feature of PMR LA. Furthermore, whilst PMRs play a significant role in the occurrence of physiological tremor in the mandible, we have shown here that the expression of physiological tremor can be significantly altered in the presence of comparable levels of PMR activation. We therefore posit that, while the reduction in physiological tremor seen during PMR anaesthesia may in part be due to effects on MU firing rate statistics, there remains a further contribution of these receptors yet to be elucidated. This contribution may be an

amplification of a pre-existent supraspinal rhythm or oscillation of their dependent reflex loop.

6.9 Clinical Relevance

This chapter has stressed the importance of PMR activation on normal jaw tremor. When PMR activity is normal, it keeps the jaw in a responsive, underdamped state where it can more rapidly respond to sudden, potentially damaging force changes between the teeth.

Chapter 7
Concluding Remarks

7.1 Publication Acknowledgement

The following is a modification of "Mandibular tremor during isometric contractions"

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7.2 Introduction

The experimental work within this thesis has shown that the human jaw, like other body parts, exhibits small oscillations in torque during isometric contractions. These small oscillations, referred to as 'physiological tremor', occupy a frequency band in the power spectrum between 6 and 10Hz. While such oscillatory activity has been recognised and discussed for many years, the mechanism that gives rise to this fundamental phenomenon is still debated. There have been a number of hypotheses put forward in the literature to explain the appearance of physiological tremor and these can be broadly incorporated into 2 main categories (Stein *et al.*, 1978): 1) mechanical resonance phenomena; and 2) oscillatory activity that is described to be of 'neurogenic origin'. The contribution of intrinsic mechanical properties of the body part under study is unarguable however the assertion by many that this constitutes the entirety of

physiological tremor has now been comprehensively disproved. The existence of a frequency invariant component of tremor that arises from a neural source is well accepted, although the neural source remains debated. These proposed neural sources generally fit within two broad categories: 1) reflex feedback loops; and 2) rhythmic supraspinal drives. While it seems that these theories will remain debated for much time to come, it is certain that any input that causes movement or the exertion of force will be subject to reflex feedback. Consequently, any source of tremor, be it a purely mechanical phenomenon, or as a consequence of a pulsatile supraspinal input, will be subject to complex interaction with reflex feedback loops. The focus of this thesis has been the neurogenic component of physiological tremor that can be observed in the mandible during isometric biting. This thesis has attempted to identify the extent to which exteroceptive reflex activity arising from the PMRs contributes to this physiological tremor and in doing so has considered the above hypotheses for physiological tremor development.

7.2.1 Mechanical Resonance

The mechanical resonance hypothesis is based on the premise that body parts can be thought of as second order mechanical systems, that when perturbed tend to exhibit damped oscillation at their natural frequencies. The frequency of this oscillation is determined by the system's moment of inertia and its stiffness. Such an explanation for physiological tremor would require that there be ongoing perturbation of the system for it to continuously oscillate and it has accordingly been proposed that the unfused

twitches of newly recruited motor units or the pressure pulse associated with blood circulation could provide such an impetus.

While mechanical properties of they masticatory apparatus undoubtedly confer a degree of instability upon the jaw that results in tremulous movements, such movements are confined to an area of the spectrum below 3Hz. The mechanical component of mandibular tremor is especially evident at rest and has been shown to be driven predominantly by the cardioballistic effect of blood ejection (Mccarroll and De Vries, 1988). Evidence presented in this thesis argues that a distinct component of physiological tremor exists in the jaw that is not the result of its mechanical properties. The first item of evidence is that physiological tremor has been observed to occur in a frequency range that is relatively invariant across a number of body parts that have vastly different natural frequencies. Physiological tremor is seen in the finger, arm and in the jaw at 8Hz (Raethjen *et al.*, 2002; Sowman and Türker, 2005) whilst the natural frequency of the finger has been calculated to be as high as 25Hz, the jaw 3Hz (Cooker *et al.*, 1980) and the arm as low as 2Hz (Stiles and Randall, 1967; Joyce and Rack, 1974).

Junge *et al* (1998) provide further specific evidence against a purely mechanical explanation for this component of mandibular physiological tremor. They showed that increasing the load on the mandible, which would increase its moment of inertia, did not change the frequency of physiological tremor, nor could they show any evidence of damped oscillation following a sharp perturbation of the mandible. Furthermore, chapter 2 of this thesis showed, that over a range of different isometric bite forces,

where the stiffness of the joint would be expected to vary significantly, the frequency of the 8Hz component of physiological tremor remains unchanged see figure 2.3.

7.2.2 Reflex Feedback

It has been expounded by many authors that the stretch reflex loop is primary responsible for normal physiological tremor (Lippold, 1970; Matthews and Muir, 1980; Durbaba *et al.*, 2005) but the possible involvement of exteroreceptors has only been considered briefly (Van Steenberghe and De Vries, 1980; Fisher *et al.*, 2002). Based on the findings of van Steenberghe and de Vries (1980) , who showed that the frequency of mandibular tremor shifted to lower frequencies during local anaesthetic (LA) block of the peri-incisal periodontium, this chapter 2 examined experimentally the possibility that exteroceptive input, rather than proprioceptive input, could be responsible for the genesis of isometric mandibular physiological tremor. For the PMR reflex pathway to explain the mandibular oscillation, several criteria should be evident:

1. The amplitude of the tremor should be proportional to the gain of the reflex and therefore blocking the receptors responsible for initiating the reflex, i.e. reducing the gain in a given reflex, should reduce the amplitude of tremor produced by that reflex component.

2. The reflex should exhibit damped oscillation at the frequency of tremor in response to an impulse stimulus.

3. Delay in the reflex loop should be equal to half the period of oscillation.

In addition to these criteria, there needs to some evidence that the proposed reflex tremor is generated as a consequence of PMR activation and not because of muscle spindle reflexes activated in concert with PMRs.

Evidence that suggests the periodontal reflex could satisfy each of these criteria is presented below:

7.2.3 Manipulating the Gain of the Reflex

If a reflex causes physiological tremor then any alteration in the gain of this reflex will alter that amplitude of physiological tremor. This line of reasoning has been used to support the contribution of the stretch reflex to physiological tremor (Young and Hagbarth, 1980). With regard to the periodontal reflex, factors influencing the gain of the reflex should also affect the amplitude of physiological tremor. While experiments to increase the gain of periodontal reflexes have not been performed, Mcfarland and colleagues (Mcfarland *et al.*, 1986) showed across a cohort of subjects that the amplitude of mandibular tremor was proportional to the size of mechanically evoked intraoral reflexes i.e. those subjects with large amplitude tremor also had large amplitude reflexes.

Reducing the periodontal reflex gain with anaesthesia is a straightforward experimental proposition in the jaw and has the added bonus of not directly influencing the muscle spindles. Using this approach and following up on the work by van Steenberghe and de Vries (1980), chapter 3 shows conclusively, in a cohort of 10 subjects, that the 8Hz component of mandibular physiological tremor can be abolished during low-load (2-10% MVC) isometric biting by anaesthetising the periodontium. Furthermore, chapter 3 has shown this to occur under conditions of force feedback and EMG feedback, lessening the likelihood that this phenomenon is due to a change in the mean size of active motor units only because of increased bite force during LA.

7.2.4 Impulse Response

Lippold (1970) suggests that oscillatory characteristics of a reflex feedback loop can be investigated by introducing a step-function into the loop. Provided that the loop is underdamped, the response should be characterised by oscillations that are at a frequency indicative of the loop time of the reflex. Whilst such experiments have not been performed with the express ambition of examining the periodontal contribution to mandibular physiological tremor, many reflex experiments that include force measurements have been performed. Close examination of such records appears to show the requisite damped oscillation at a period consistent with the frequency of mandibular physiological tremor [e.g. see figure 6. Yang and Türker (1995)].

7.2.5 Reflex Loop Time

Any body part subject to reflex feedback is susceptible to oscillation at a frequency determined by the sum of the delays in the reflex response pathway. These delays include the activation time of the receptors initiating the reflex, the following delay in the afferent and efferent pathways, including synaptic time, and the delay in the subsequent force output of the effector muscles that subserve the reflex response. For reflex feedback to account for the frequency of oscillation, the reflex delay should be equivalent to half the oscillation period –approximately 60ms for a tremor frequency of 8Hz. While it is often argued that this is a reason against the reflex hypothesis, loops being longer in the arm than in the jaw for example, the contribution to the loop time made by the length of the nerves is very small relative to the time taken up by electromechanical delay and the synaptic transit. Therefore, the total reflex loop time can be similar across all systems as it is mostly dependent on electromechanical delays.

By recording ongoing tremor and the EMG associated with it, an estimate of the time delay between the onset of EMG and the onset of force can be attained by means of the phase argument associated with the coherence, or the cumulant density function estimated between the two signals. Accordingly, this Chapter 2 has found this time delay to be approximately half the period of oscillation of physiological tremor (see Chapter 2, figure 2.3). Whilst this fits well with the requirements for a reflex origin for physiological tremor, it is considerably longer than that time measured for the electromechanical delay by reflex experiments. This discrepancy is interpreted to be due to the fact that most reflex experiments utilise large step-like stimuli that cause a very synchronous recruitment of motor units, as opposed to the small amplitude

approximately sinusoidal stimulation of periodontal receptors caused by physiological tremor.

7.2.6 Muscle Spindle Feedback

The reflex hypothesis for physiological tremor is most often applied to the model where the myotactic muscle spindle loop provides the reflex feedback. For mandibular physiological tremor to be of periodontal origin there needs to be some evidence that the link is not a spurious one due to the misattribution of effects that arise from coincidental masseteric muscle spindle activation. The first indirect evidence for this comes from the contention of several authors that the stretch reflex should not operate, and therefore tremor would not be present under true isometric conditions. Durbaba, Taylor *et al*. (2005) , restated the position put forward by Matthews and Muir (1980) that, under true isometric conditions with the body part fixed against a rigid transducer, the ~8 Hz component of tremor is abolished. Furthermore, Durbaba and colleagues showed this to be the case in seven of nine subjects tested in their experiment, arguing that the use of acceleration, velocity or displacement to measure tremor inevitably includes stretch-reflex mechanisms, as these measures require the body part to be free in its movement at the end of the lever opposite the hinge, a requirement that in turn allows the body part oscillate in space and increases the gain in the stretch-reflex loop.

This thesis shown that physiological tremor can be recorded from the jaw under true isometric conditions (Chapters 2, 3 and 4). Under such conditions, even though it is

expected that motor unit firing would cause changes in internal muscle length, enough to modulate spindle discharge (Ito *et al.*, 1998), activity in the stretch reflex loop should be small enough to be considered negligible, yet unlike the aforementioned studies, physiological tremor can be seen to persist. In this case then, mechanoreceptors in the periodontium are likely to be implicated more heavily than the muscle spindles.

Additionally, the LA in our experiments should not have affected physiological tremor if it was primarily of muscle spindle origin, as the infiltration of LA around the incisors could not have directly reduced the efficacy of the muscle spindle response. Furthermore, there is evidence of no change to the jaw jerk reflex when the periodontium is anaesthetised (Hoogmartens and Caubergh, 1988; Poliakov and Miles, 1994)

7.3 Evidence against a Purely PMR-Reflex Origin for Mandibular Physiological Tremor.

Whilst the distribution of tremor amplitude seems unaffected by the range of forces tested in Chapters 1 and 2, it is known that this is well within the saturating force range of the PMRs. It would therefore be expected that if physiological tremor were due to PMR reflexes, a diminishing amplitude of tremor would be evident; whereas the opposite phenomenon occurs i.e. as force increases, the amplitude of tremor increases such that the distribution of power in the force spectrum remains unchanged (see Chapters 1 and 2). For this reason chapter 5 sought to characterise the saturating response of this reflex to see if it paralleled the response seen in single receptors. As

Chapter 5 shows, the reflex responses from a single tooth saturates in a similar way to the single receptor output. Even if a progressive increase in recruitment of distant receptors via mechanical coupling occurs with increasing force, as the data in Chapter 3 seems to indicate (physiological tremor reappeared with increased bit force after during LA) this compensation is negligible in reflex terms (dynamic reflex sensitivity is greatly reduced with 2N preloading). There remains then a conflict of evidence whereby it has been repeatedly demonstrated that blocking afferent outflow from the PMRs profoundly diminishes mandibular physiological tremor, yet progressive physiological loading of the PMRs to levels that would be expected to result in a progressive decline in the dynamic sensitivity required to maintain an ongoing reflexive oscillation, does not affect tremor amplitude. However, it is possible that the experimental paradigm used in Chapter 5 to test the saturation characteristic of the PMR reflex (orthogonal stimulation of an isolated incisor) negates the extent of biomechanical coupling that would occur when these forces are applied in a the more physiologically relevant axial direction. Furthermore, with physiological loading, the level of background motoneuronal excitation is always proportional to the tooth load, whereas in Chapter 5, tooth load was varied independently of the level of background excitation. Increasing background excitation would normally be expected to reduce the response of the motoneuronal pool to a common input however, so this source of confounding seems an unlikely explanation. A further difference that occurs with physiological loading is the increase in reaction force that opposes jaw closing. If the increased loading reduces the reflex size by a saturation effect, the actual amplitude of mechanical excursion in response to that reflex could be maintained by virtue of the parallel increase in reaction force that

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occurs. This last reason may explain the lack of change in tremor amplitude as bite force increased beyond that level at which the PMR reflex was seen to be greatly reduced.

7.4 An Alternative Explanation for the Effect of PMRs on Mandibular Physiological Tremor

For the reasons discussed in 7.2, Chapter 6 attempted to investigate the possibility that, rather than providing a rhythmic input, the activation of PMRs during isometric biting may provide a tonic input to the motoneuronal pool such that conditions for the expression of physiological tremor are enhanced, i.e. PMR activation allows for the enhanced expression of another rhythmic input. Given that the PMR reflex is primarily inhibitory in its action, and that inhibition of motoneuronal output is a frequency based phenomenon (Türker and Powers, 2001), this thesis tested the hypothesis that tonic input from the PMRs could work to slow the overall firing rate of the motoneuronal pool in submaximal contractions. Such an effect would increase the probability that any excitatory input would recruit a 'motoneurone in waiting' by the fact that the overall proportion of neuronal waiting time is greater when motoneurones fire slowly (Matthews, 1996; Türker and Powers, 2001). The recruitment of more than one motoneurone simultaneously would then cause the requisite synchronicity of MUs that underlies physiological tremor as demonstrated in Chapter 4. While there seemed to be an effect on the firing rates in the right direction generally, the increase in firing rate seen during PMR LA did not always parallel the extent of the reduction in physiological tremor. So, whilst Chapter 6 added to the body of evidence that suggests population

firing rates influence the extent of synchronous MU activity and hence tremulous behaviour, it was unable to conclusively link the two. Nevertheless, it seems likely that this mechanism plays some part in a multifactorial process that links the activation of PMRs to the expression of jaw tremor.

7.5 Supraspinal Oscillators?

While the theory that cortical oscillatory activity could be the source of oscillatory motor output has been around for a long time, recent advances in the ability to enhance signal to noise ratios and the advent of MEG have allowed such theories to gain currency. A number of recent studies have shown evidence of cortico-muscular coupling at frequencies associated with physiological tremor [e.g. see Raethjen *et al* (2002)] While such evidence continues to build, the likelihood that central oscillators are solely responsible for physiological tremor is small. Rather, such experimental evidence would suggest that central rhythmicities and peripheral reflex mechanisms act like coupled oscillators (Cathers *et al.*, 2006) contributing more or less, depending on experimental conditions, to produce physiological tremor. With specific regard to the jaw system, there have been no published examinations of the existence or not, of coherent oscillatory activity linking the cortex and the masticatory muscles at the frequency of physiological tremor. Furthermore, there are significant challenges involved in finding such coupling if field potentials are to be used. The superior attachment of the temporalis muscle is high enough on the skull to mean that and any attempt to record EEG [Sowman and Murphy (2005) unpublished] or MEG during contraction of the jaw elevators will encounter significant crosstalk problems.

7.6 Conclusion

The contribution of reflex feedback from the periodontium to mandibular physiological tremor is significant under isometric conditions. Blocking the outflow of the PMRs causes a significant diminution of this tremor and a significant diminution of a common input to masseteric motoneurones at the frequency of this input. However, the existence of this tremor solely as a consequence of this reflex feedback loop seems unlikely given the lack of a tremor saturation phenomenon that parallels the reflex saturation that occurs with increased tooth loading. Given the strong evidence supporting the existence of central oscillators that are coherent with peripheral physiological tremor in other body parts, it seems likely that physiological tremor in the mandible may be due to the interaction of a supraspinal oscillator and the PMR reflex loop. While the exact mechanism by which this occurs was not determined by this thesis, evidence presented herein shows that exteroceptive reflexes play a significant role in what is most likely a phenomenon that results from the complex interaction of multiple reflex pathways and a putative supraspinal oscillator. Further studies of physiological tremor should consider the role of exteroceptive reflexes carefully, especially if those studies are using the jaw as their model system.

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