

# Response of human jaw muscles to axial stimulation of the incisor

Russell S. A. Brinkworth, Kemal S. Türker and Andrew W. Savundra\*

Department of Physiology and \*Department of Dentistry, University of Adelaide, SA 5005, Australia

The role of periodontal mechanoreceptors (PMRs) in the reflex control of the jaw muscles has thus far been mainly derived from animal studies. To date, the work that has been done on humans has been limited and confined to orthogonal stimulation of the labial surface of the tooth. The purpose of this study was to investigate the response of the masseter and digastric muscles in humans to controlled axial stimulation of the upper left central incisor, both before and during a local anaesthetic block of the PMRs. Ten neurologically normal young adult females were tested, each on two separate occasions to confirm the reproducibility of the results. It was found that the reflex response in the masseter was modulated by the rate of rise of the stimulus used and, to a lesser degree, the level of background muscle activity. There was little detectable change in the activity of the digastric muscle under the tested conditions and what was found could be attributed to cross-talk with the masseter. The reflex responses obtained were significantly different between subjects; however retesting the same subject on a different occasion yielded similar results. The results indicate that the most common response of the masseter muscle to brisk axial stimulation of the incisor is a reflex inhibition at 20 ms, followed by a late excitation at 44 ms. However, it is possible that this late excitation could be due to delayed action potentials and hence be artefactual. As the application of a local anaesthetic block removed or significantly reduced both of these responses, it was concluded that they originated from the PMRs. Unlike during orthogonal stimulation, slowly rising stimuli did not produce any excitatory reflex activity. This indicated a difference in jaw reflexes to forces applied in different directions, possibly due to the activation of different receptor types when stimulating the tooth in either the orthogonal or axial directions.

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**Corresponding author** K. S. Türker: Department of Physiology, University of Adelaide, SA 5005, Australia.  
Email: kemal.turker@adelaide.edu.au

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

During mastication, the forces that are applied to the teeth displace them in their sockets, thus stimulating PMRs. The

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

The easiest way to mechanically stimulate teeth is by means of tapping the labial surface of incisor teeth with a probe (orthogonal stimulation). This approach has been tried by a number of researches and most studies have shown that tooth tapping in humans produces jaw reflexes that are multi-phasic and comprise a complex sequence of short- and long-latency inhibitory and excitatory reflexes (van der Glas *et al.* 1985), although many of the secondary

and later reflexes may be partially or fully artefactual (Türker & Cheng, 1994; Türker & Powers, 1999).

One of the problems with many of the experimental mechanical stimuli used is that the taps contain very high frequency components. It is known from both animal (Linden & Millar, 1988) and human (Trulsson & Johansson, 1994) studies that PMRs are sensitive to the total force as well as the rate of force application. To overcome the problem of high frequency components in the stimuli, the concept of preload was introduced (Brodin *et al.* 1993b). This involves holding the probe against the tooth at a low force level (0.5–1 N) in between stimuli. By using a preload, the high frequency components that were contained in the beginning of the stimuli, when the probe took up the slack in the stimulus delivery system, can be reduced. It was therefore put forward that an exact stimulus profile could only be applied to a tooth if a preload was used (Türker *et al.* 1997).

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

Since it is known that PMRs code the direction of force applied to the teeth (Trulsson *et al.* 1992; Dessem, 1995) it is likely that the direction of force application is a contributing factor in the reflex response of the human jaw muscles. Hence experiments that study human jaw reflexes due to axial stimulation are likely to provide a

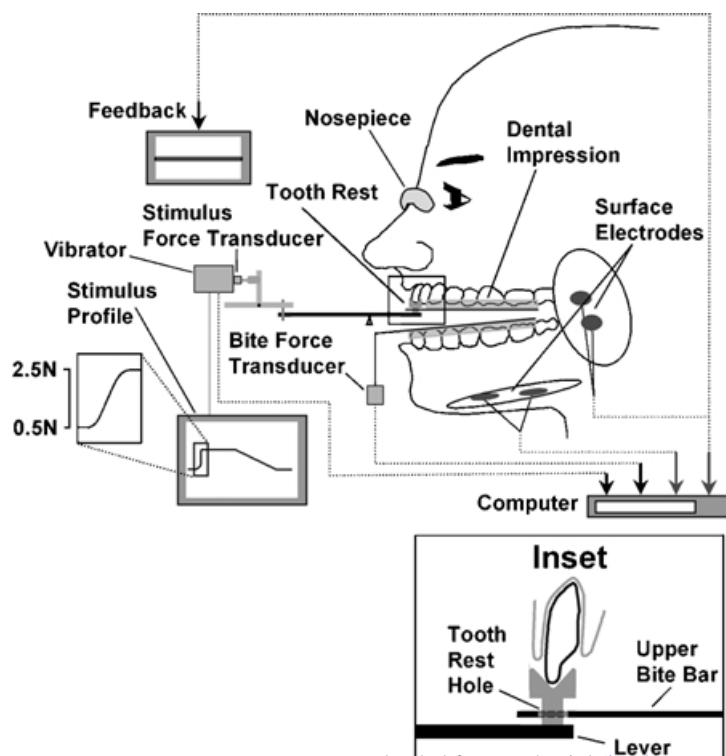
more accurate picture of what happens during normal incising activity than those utilizing orthogonal stimulation. However, this experiment was not intended to directly compare axial and orthogonal stimulation of teeth, but rather to provide the next logical step in experimental design, ensuring the direction of the mechanical stimulus is similar to that encountered during mastication. Preliminary results from this study have been published in abstract form (Brinkworth *et al.* 2002).

## METHODS

Written informed consent was obtained from 10 adult female volunteers with healthy teeth and gums, and no history of orthodontic treatment or dysfunction. The age range of the subjects was 18–25 years. Each subject was tested twice, no less than 1 week or more than 8 weeks apart. The experiments were approved by the Human Ethics Committee of The University of Adelaide and all procedures used conformed to the Declaration of Helsinki.

### Protocol

Subjects were instructed to bite into impression material (Formasil II, Heraeus Kulzer-Wehrheim) mounted on two bite bars with their upper left central incisor sitting on a tooth rest; the mean separation distance of the bars was set to 13 mm. A tooth stimulator, shown in Fig. 1, was used to stimulate the upper left central incisor. Movement of the subject's head was minimized by the use of a fixed nosepiece, which also counteracted the axial forces applied to the tooth. The dental impression was cut away from around the incisor so it could be contacted and stimulated by a small brass tooth rest (Fig. 1 inset). The impression of the subject's upper teeth was taken in such a way as to direct the force of stimulation along the long axis of the tooth. This involved slightly tilting the head forward while taking the impression of the upper teeth. During the experiment the subject was required to



**Figure 1. Experimental set up**

The subjects bit into impression material mounted on two bite bars with their upper left central incisor resting on a tooth rest that fitted into a hole in the upper bite bar (inset). Movement of the subject was further minimized by the use of a fixed nosepiece. The nosepiece also counteracted the axial forces applied to the tooth. A computer then produced the desired force profile randomly between 1.5 and 3 s. Two force transducers, one located in the motor arm and the other below the lower bite bar, picked up the stimulus force applied to the tooth and the total force generated by the jaw, respectively. Both the masseter and digastric EMG activity were recorded as well as the outputs of both force transducers; in addition, the SEMG of the masseter was fed into a filter box and used for on-line feedback.

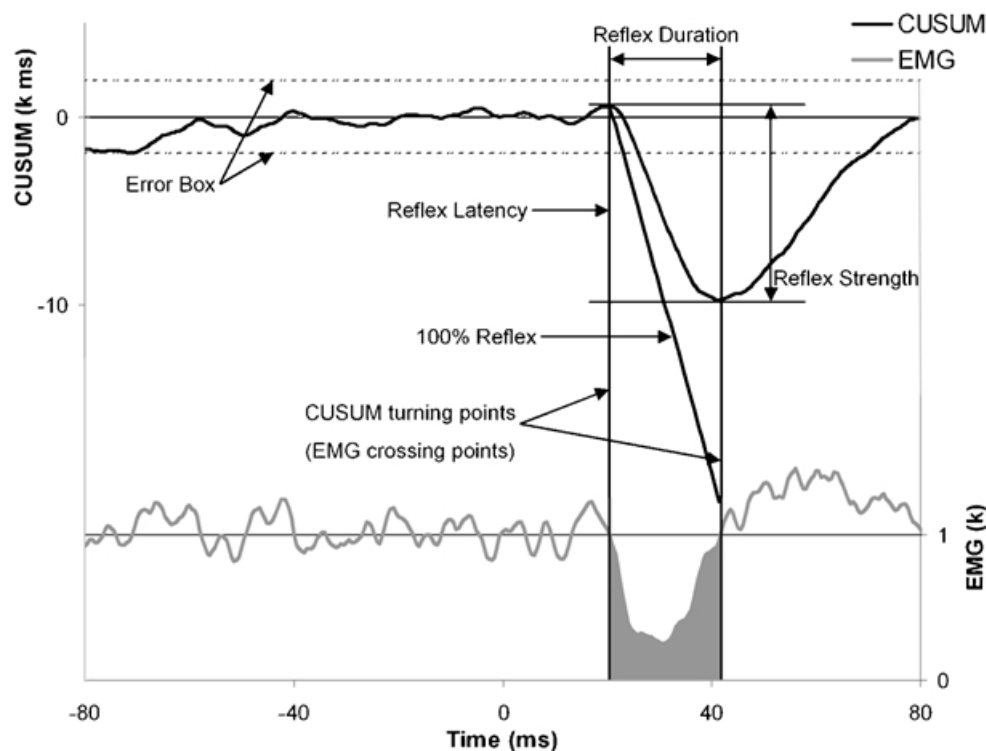
lean into the nosepiece, which reduced the possibility of the head tilting backwards. In addition, the tooth rest was not fixed to the lever or upper bite plate allowing for small amounts of subject movement while ensuring stimulation along the long axis of the tooth.

A computer generated force profile was used as the input signal to a small mechanical vibrator connected to the tooth via a lever system and tooth rest. Since the sound of a tap is sufficient to evoke an inhibitory reflex in the masseter similar to that resulting from periodontal receptors (Sato *et al.* 1994), the subjects wore headphones through which white noise was played during the experiment to mask any acoustic vibrations.

Surface electrodes were placed on the skin overlying the left masseter and digastric muscles in order to detect the SEMG activity. The EMG was amplified ( $\times 3000$ ) and band-pass filtered (cut-off frequencies of 5 and 1000 Hz) before recording. The subject was given on-line feedback showing their current level of muscle activity so it could be maintained at a specified level throughout the trial. For this purpose the masseter SEMG was full wave rectified and low-pass filtered (cut-off frequency 0.1 Hz) then presented to the subject on an oscilloscope screen. Grounding of the subject was achieved by the use of a lip-clip electrode (Türker *et al.* 1988). To ensure correct force application, the push force was recorded via a transducer located on the arm of

the vibrator. A bite force transducer was connected to the lower bite bar (see Fig. 1) to measure the forces generated by the subject's jaw musculature. This was done to find the overall response of the masticatory muscles to the stimulus as it has been shown that the EMG of one or two jaw muscles may not be indicative of the overall bite force, which is the net response of all jaw muscles (Yang & Türker, 1999). All data channels were sampled at 12-bits and 2 kHz using a specially designed LabView (National Instruments) computer program.

It was necessary to apply the stimuli at random intervals so the subject could not predict when they would occur. Hence there would be little, if any, EMG change due to anticipation of the stimulus (Ottenhoff *et al.* 1992b). An inter-stimulus interval between 1.5 and 3 s was used. Throughout the experiment three bite levels were used: 5, 10 and 20% of maximum voluntary contraction (MVC). These contraction levels were selected as most people can sustain them for the expected duration of the trials, and they represented muscle contraction levels likely to be used during mastication (Anderson, 1956). The stimulus profile used was a slow sinusoidal form as described in the literature (Türker *et al.* 1997). This, along with a preload of approximately 0.5 N, ensured that high frequency components in the stimuli were minimized. These parameters maximized the possibility of obtaining excitatory reflexes while still containing the required parameters for inhibitory reflexes as established for orthogonal



**Figure 2. EMG reflex parameters**

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

stimulation (Türker *et al.* 1997). Three different stimulus rise times were used: fast (12 ms), medium (20 ms) and slow (90 ms). A pre-load of 0.5 N and a push force of 2 N were selected as the non-varying stimulus parameters.

Once all conditions had been performed, approximately 4 ml of LA (Xylocaine – lignocaine hydrochloride with adrenaline 1:80 000) was administered to the gingival and palatal region in order to block the PMRs from all upper front teeth (canine to canine). Once the LA block was in place, the experimental procedure was repeated. The success of the LA was ensured when the subject could no longer feel the stimulus around the teeth; this took approximately 15 min to achieve. Some subjects reported feeling the fast stimuli as a faint vibration at the base of the skull. In total, nine different mechanical conditions were performed twice on a given day, once before LA and then once during LA block. The subjects then came back on a separate occasion to undertake the experiment a second time.

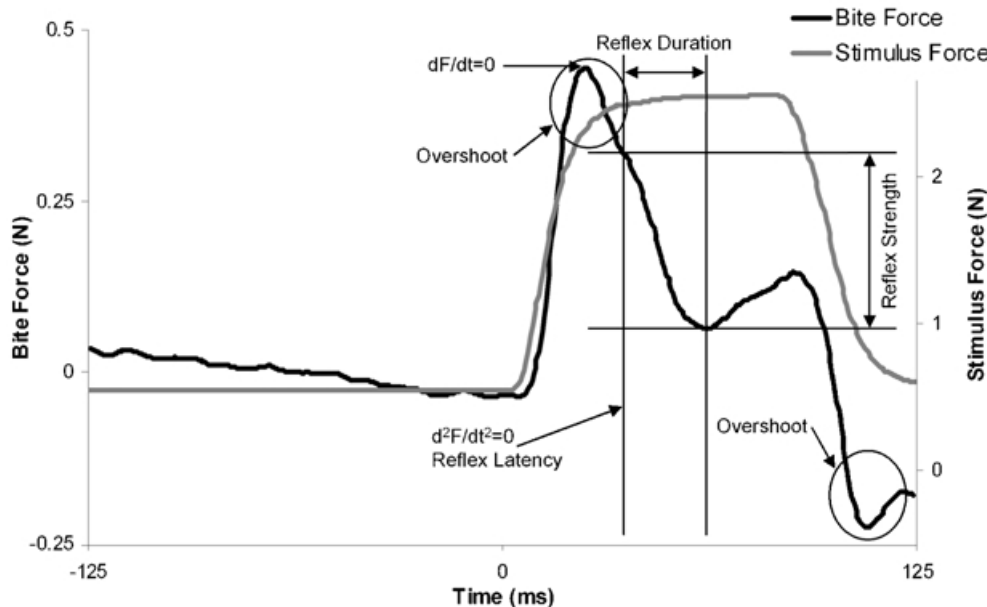
It is known that the teeth are displaced in their sockets following mechanical stimulation and successive taps do not permit them to return to their original position. In fact, the tooth is displaced during the first few taps by different amounts, only reaching a relatively stable position after a number of stimuli (Moxham & Berkovitz, 1995). In order to overcome this change, the first 10 taps of each trial were not included in the analysis so a steady state response could be found. Each trial lasted approximately 2.5 min, and subjects were asked to stay on the bite bars for three consecutive trials before coming off to rest.

### Analysis

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

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

Figure 2 illustrates the measured reflex characteristics. A symmetrical error box was constructed that took into account the largest pre-stimulus CUSUM deviation in either direction (Türker *et al.* 1997). If the post-stimulus CUSUM deviated by an amount greater than the error box, without a turning point (i.e. EMG crossing the pre-stimulus mean) in between, then a reflex was recorded. The latency and end of a reflex were defined as the turning points at the start and end of a reflex, respectively. The duration of a reflex was defined as the difference between the



**Figure 3. Bite force reflex parameters**

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



latency and the end. Since the EMG was normalized, the largest possible inhibition corresponded to a CUSUM slope of  $-1$  ( $EMG = 0$ ), hence all reflex strengths were calculated as a percentage of this theoretical maximum. Excitations were given a positive percentage while inhibitions were defined as negative.

Averaged bite force records were assembled in the same way as EMG records, but normalization consisted of subtracting the average pre-stimulus bite level from the trace to observe the change in bite level, hence making it possible to compare the changes between trials with different pre-stimulus averages. The bite force was averaged then passed through a 9-bin moving average filter centred on the current value (equivalent to zero-phase low-pass filtering at 111 Hz). Figure 3 illustrates the measured reflex characteristics from the bite force record.

A change in the bite force was only classified as a reflex if its strength was greater than twice the maximum pre-stimulus variation (error box). As with the EMG latency, the bite force latency was defined as the point where the bite force started to turn (derivative equals zero). The end of the reflex was defined as the next turning point and the duration as the difference between the latency and the end. The strength of the reflex was the vertical distance (amplitude) between the latency and the end. The main problem encountered in the bite force analysis was the overshoot that was present in a number of the records. When the stimulus was applied to the tooth, a small movement of the head was induced causing the bite force record to follow the shape of the stimulus superimposed upon it. However, in many of the fast stimulus conditions, an overshoot was produced at the end of the rising and falling phases of the stimulus. Therefore, the first turning point in the bite force following the stimulus corresponded to the peak of the overshoot, not the start of a reflex. Hence, if calculations showed that the latency of the reflex was the first turning point after the rising edge of the stimulus then the next time the slope changed (second derivative equals zero) was taken as the latency of the reflex. Only by using the second

derivative could the reflex latency be established when it occurred close to the end of the rising edge of the stimulus. To ensure that this analysis method yielded accurate results the following precaution was taken: if the latency of a reflex in the bite force recording did not occur between 7 and 25 ms after an associated inhibition in the EMG, then an exception was generated and the records closely scrutinized. Although this was rare, the most common reason for this was excessive variability in the bite force recordings. Under such circumstances, the bite force reflex was removed from further analysis.

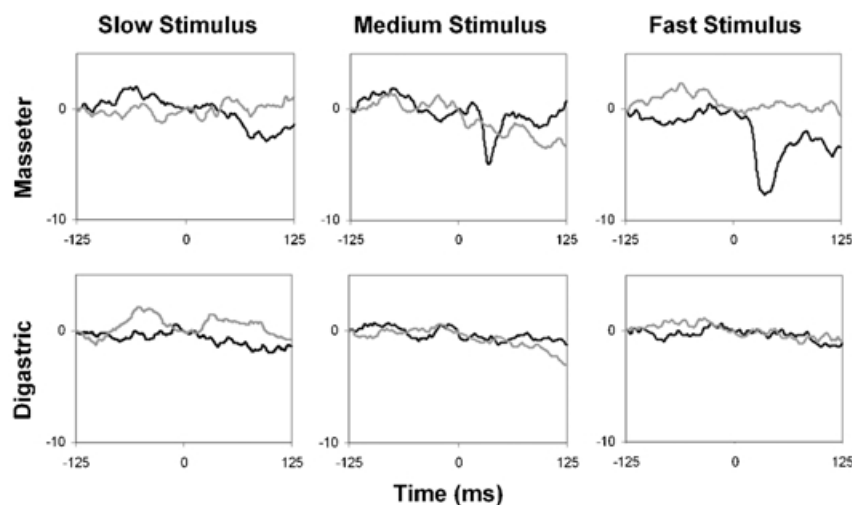
### Statistics

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

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

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

For all tests, the level of significance was set to 5%. Results are given in the form mean  $\pm$  1 S.D.; time calculations are given to the nearest 0.5 ms, force values are given to 0.01 N accuracy, while strength and frequency calculations are given to the closest per cent.



**Figure 4.** Jaw muscle response to various stimulus rise times

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

## RESULTS

The data were separated into the three reflex conditions as observed from the EMG records, early excitation (E1), inhibition (IN) and late excitation (E2); and one from the bite force record, change in bite force due to reflex (BR). The observed changes in the bite force record were only ever inhibitory. Although the rate of occurrence was low, E1 was elicited at least once in 5 of the 10 subjects before LA and all 10 subjects during LA. IN and E2 were found in all 10 subjects both before and during the application of LA; however the occurrence rate during LA was drastically reduced. BR was found in all subjects before LA and 7 out of the 10 during LA; however as with IN and E2, the occurrence rate during LA was reduced.

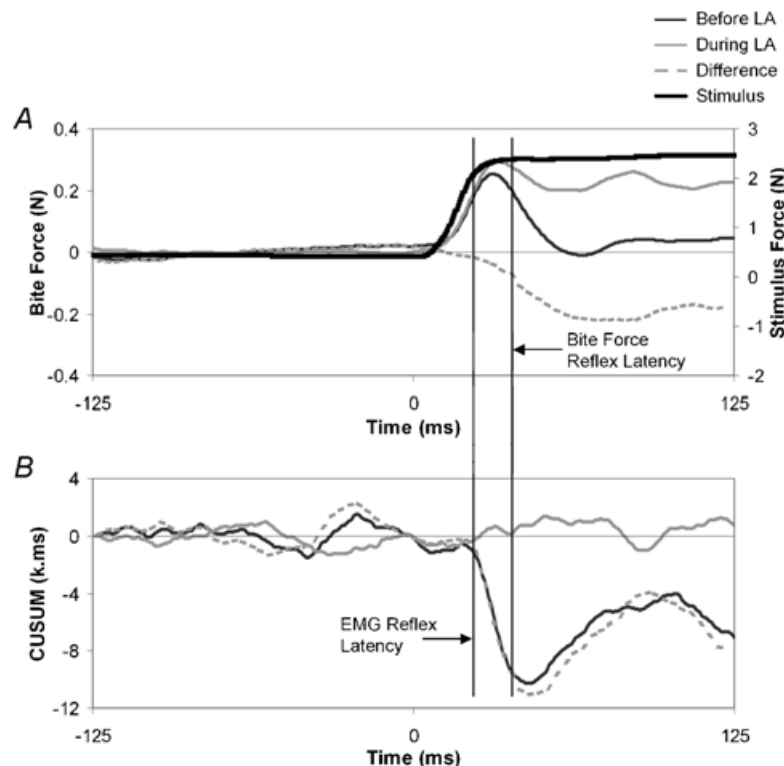
Figure 4 is an example of the results from one subject during trials conducted at 20% MVC. It illustrates the CUSUM of the masseter and digastric muscles to slow, medium and fast stimuli both before and during a LA block. The only time a masseteric response was elicited was during a medium or fast push before the LA block. There were no significant deflections in the CUSUMs associated with the digastric under any of the stimulus conditions.

Figure 5 shows the effect of LA on both the CUSUM of the masseter and the bite force recorded from one subject during a 10% MVC contraction of the masseter. As described above, the start of the stimulus profile was evident in the bite force records under both conditions. The large negative deflections in both the force and CUSUM traces were drastically diminished during LA indicating a reduction in the inhibitory reflex.

### Masseter muscle

The effect that both stimulus rate and LA block had on the latency, strength and duration of the three reflex events seen in the masseter (E1, IN and E2) is illustrated in Fig. 6 and described in detail in the following sections.

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



**Figure 5. Bite force and CUSUM of SEMG before and during application of LA**

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

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

There was no significant difference between day 1 and day 2 in the number of occurrences of E1. Both the application of LA ( $P < 0.001$ ) and the rate of stimulus application ( $P < 0.005$ ) affected the occurrence frequency of E1. No inter-subject differences were observed; however this might be due to the low number of E1 occurrences ( $n = 38$  from 360 trials). As illustrated in Fig. 7A, while the rate of E1 incidence was low before LA (regardless of the stimulus rate), during the application of LA increasing the push rate increased the number of E1 reflexes observed. The best stimulus condition to use in order to elicit E1 was a fast push during the application of LA block, although even then it occurred in only 28% of the trials.

**Inhibition.** Paired sample *t* tests on the results of IN showed that there was no difference between day 1 and

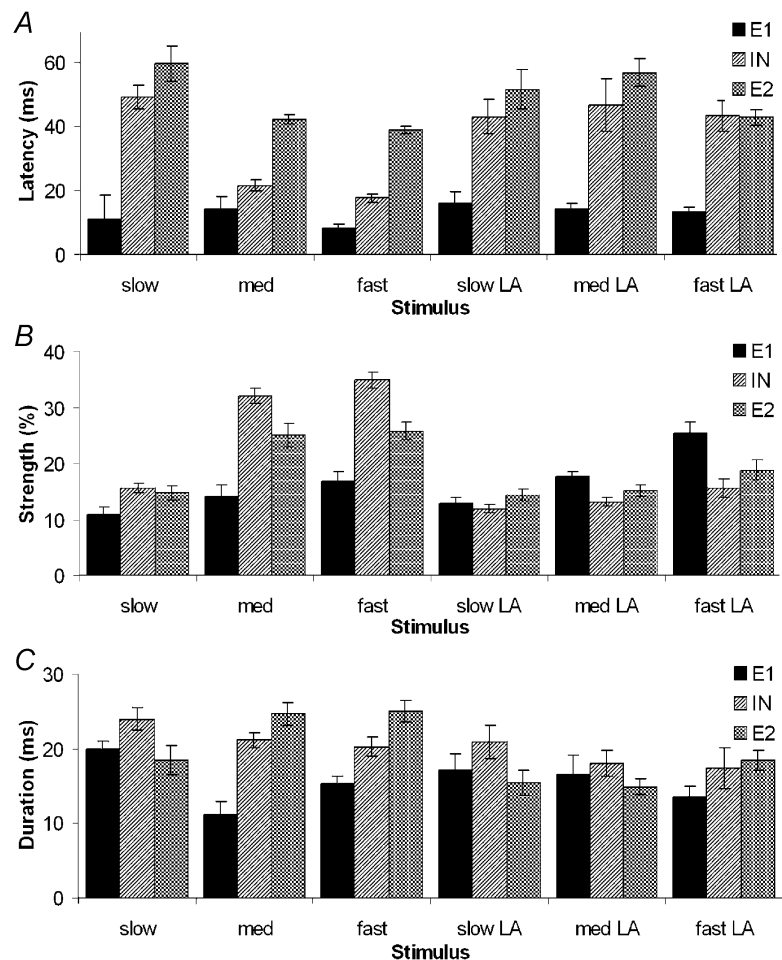
day 2 recordings for the latency or strength of IN, but there was a significant difference for the duration of the reflex ( $P < 0.005$ ). Hence the data from the 2 days was combined for further latency and strength analysis while separate reflex duration analysis was performed for the day 1 and day 2 recordings. Table 1 shows that, in addition to significant inter-subject differences for each of the measured statistics, all stimulus conditions had significant effects on at least one of the measured values.

The number of observed inhibitions was not significantly different between day 1 and day 2. As with E1, the stimulus rise time ( $P < 0.05$ ) and LA ( $P < 0.001$ ) had significant effects on the number of occurrences of IN. Figure 7A shows the increase in occurrence of IN with the increase in the speed of the stimulus before LA and a large reduction in the number of reflexes observed during LA. Hence, the best stimulus condition to elicit an inhibitory reflex was a fast push before LA. The bite level had no significant influence on the number of IN reflexes. Furthermore, there was a significant difference in the number of occurrences between subjects ( $P < 0.001$ ).

While the combination of push and LA had a significant impact on the IN latency ( $P < 0.001$ ) there were two main groups. The longest latencies (and largest variability) of IN

**Figure 6. Reflex characteristics of the masseter versus stimulus rise times**

The effect of altering stimulus rise time on the latency (A), strength (B) and duration (C) of reflexes seen in the masseter both before and during the local anaesthetic block. E1, IN, E2 and LA stand for early excitation, inhibition, late excitation and local anaesthetic, respectively. Columns indicate the average value while the error bars show the S.E.M. Strength is given as an absolute value so inhibition can be easily compared to the excitations. The effects that the stimulus conditions had on the measured reflex parameters are discussed in the relevant sections in the text and shown in Table 1.



**Table 1. Results of univariate analysis of reflexes**

Reflex	Statistic	Effected by	Significance
Inhibition	Latency	Push*LA	†††
		Subject	††
	Strength	Push*LA	†††
		Subject	†††
	Duration day 1	Bite	†
		Subject	†
Duration day 2	Bite	††	
	LA	††	
	Subject	†††	
Late excitation	Latency day 1	Push*LA	†
	Latency day 2	Bite*Push*LA	†
		Subject	††
	Strength	LA	†††
		Push	†
		Subject	†††
Duration	LA	††	
	Subject	†	
Bite reflex	Latency	Push	†††
	Strength	LA	†
		Subject	†††
	Duration	Push*LA	†
Subject		†††	

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

occurred during the application of LA or when a slow push was used, in which case the average latency was  $46.5 \pm 20$  ms (reflex observed 29 % of the time); on the other hand when a medium or fast push was used before the application of LA, the latency was  $19.5 \pm 11$  ms (incidence of reflex was 82 %). Similar to the latency, the strength of IN was weakest during LA or when a slow stimulus was used,  $-14 \pm 5$  %, compared to a medium or fast stimulus,  $-34 \pm 10$  % ( $P < 0.001$ ). Although the bite level had a statistically significant effect on the duration of IN on day 1 ( $P < 0.05$ ), the difference between the three levels was only 2.5 ms ( $19 \pm 6.5$  ms for 5 %,  $18.5 \pm 7.5$  ms for 10 % and  $21 \pm 9.5$  ms for 20 % MVC). However, for the data collected on the second experimental day, a bite level of 20 % MVC resulted in a significantly reduced duration when compared to the other levels ( $P < 0.01$ ),  $20 \pm 8$  ms for 20 % MVC versus  $23.5 \pm 8.5$  ms for 5 and 10 % MVC. The application of LA also decreased the duration ( $P < 0.005$ ) with an average before LA of  $24 \pm 8.5$  ms (74 % occurrence rate) and an average during LA of  $16.5 \pm 5.5$  ms (21 % occurrence rate).

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

There was no significant difference between the number of occurrences of E2 in day 1 compared to day 2. As with E1 and IN, the rate of stimulus delivery ( $P < 0.001$ ) and the application of LA ( $P < 0.001$ ) but not the bite level ( $P > 0.05$ ) affected the number of occurrences of the reflex. Figure 7A shows the increase in the number of occurrences of E2 with increasing speed of the stimulus. Just like IN, the best stimulus condition to elicit an E2 response was a fast stimulus before LA.

As shown in Table 1 the statistical analysis on the latency of E2 shows a complex interaction between all three stimulus parameters ( $P < 0.05$ ). In general, the slower the stimulus rise time the longer the latency of the reflex while no real trend with LA and bite level was evident. The largest E2 reflexes were observed before LA ( $P < 0.001$ ), with an average strength of  $24 \pm 12$  % (57 % incidence rate) or when the fast or medium push stimulus was used ( $P < 0.05$ ), strength of  $23 \pm 12$  % (69 % incidence rate). The smallest average reflex strength occurred during LA,  $17 \pm 7$  % (24 % occurrence rate) and as a result of a slow stimulus,  $14 \pm 4$  % (observed 17 % of the time). The E2 duration was longer before LA,  $24.5 \pm 9.5$  ms compared to during,  $17 \pm 5.5$  ms ( $P < 0.005$ ).

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

**Reflex change in bite force (BR).** BR was only ever inhibitory, with a latency that was significantly affected by the rate of stimulus application ( $P < 0.001$ ). The average latency of BR to a slow stimulus was  $86.5 \pm 6$  ms while the latencies evoked by the medium and fast stimuli were  $37.5 \pm 7.5$  ms and  $32.5 \pm 4.5$  ms, respectively. The duration of BR was affected by a combination of stimulus rate and LA ( $P < 0.05$ ). The average duration due to the medium and fast stimuli before LA was  $17.5 \pm 6.5$  ms while the duration under LA, or to a slow stimulus was  $21.5 \pm 6.5$  ms. The average strength of BR underwent a significant decrease from  $0.27 \pm 0.20$  N before LA to  $0.09 \pm 0.05$  N during LA ( $P < 0.05$ ). As shown in Fig. 7A the application of LA had a significant effect on the occurrence rate of BR ( $P < 0.001$ ). Prior to the LA, BR was rarely elicited by the slow stimulus (7 %) but was observed 52 % of the time in response to a medium stimulus and



67 % of the time to a fast stimulus. During LA, BR was not found in response to a slow stimulus and was elicited 18 % of the time during the medium and fast stimuli.

### Digastric muscle

Figure 7B shows the reflex occurrence for each of the stimulation rates both before and during LA block. No large reflexes or significant trends were seen in the analysis of the EMG recordings from the digastric muscle. The recordings made from the digastric muscle were of low amplitude and contained few recognizable reflexes. Only 5 % of records from both day 1 and day 2 had a detectable E1 in the digastric before LA indicating little, if any, early excitation of the muscle. It was found that 78 % of the IN reflexes recorded before the application of LA were also detected in the masseter with no significant difference between the paired latencies (digastric  $26.5 \pm 19$  ms, masseter  $31 \pm 21$  ms,  $P > 0.05$ ). A similar result was obtained for E2 with 83 % of the detectable reflexes observed in the digastric EMG before LA also observed in the masseter. The application of LA increased the incidence of E1 ( $P < 0.005$ ), and decreased the incidence of IN ( $P < 0.001$ ) and E2 ( $P < 0.01$ ); however similar results were also seen in the masseter. Additionally, the reflex occurrence rates for all reflexes elicited during LA were 16 %.

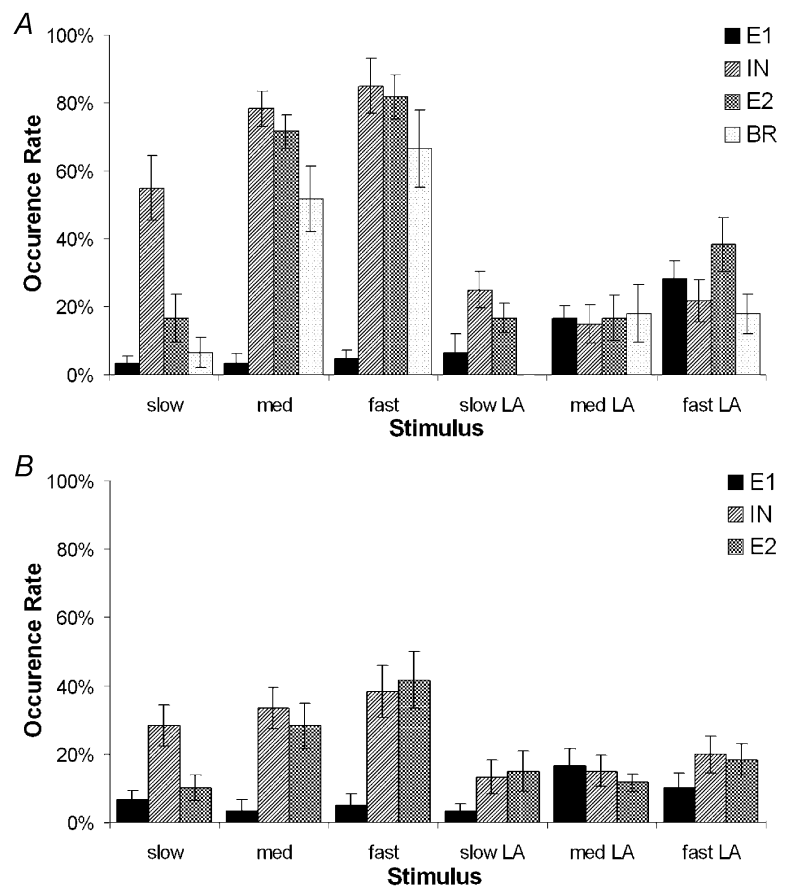
## DISCUSSION

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

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

### Figure 7. Reflex occurrence rate versus stimulus rise times

The effect of altering stimulus rise time on the occurrence of reflexes seen in the masseter (A) and digastric (B) both before and during the local anaesthetic block. E1, IN, E2, BR and LA stand for early excitation, inhibition, late excitation, reflex in bite force and local anaesthetic, respectively. Columns indicate the average occurrence rate over the 10 subjects while the error bars show the S.E.M. Bite level had no effect on the occurrence rate of the reflexes. The inhibition was the most often evoked reflex in the masseter before LA and increasing the stimulus rate increased the detection rate for all three reflexes. Early excitation was the only masseteric reflex to increase in frequency during the LA block. Many of the reflexes detected in the digastric, particularly before the application of LA, were simultaneously recorded in the masseter, indicating that they were the result of cross-talk.



### Reflex activity in the masseter muscle

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

In the current experiment, conditions that were likely to activate the muscle spindles were deliberately avoided. If a stimulus stretches (Matthews, 1975) or vibrates jaw muscles (Orchardson & Sime, 1981), then the muscle spindles will be activated and hence an E1 is likely to be generated. Stabilizing the head with tooth moulds and using a nosepiece to counter the axial force reduced both jaw movement and stretch in the current experiments. In addition, by using a stimulus with preload to reduce high frequency components in the stimulus, the possibility of stimulating the muscle spindles was further decreased. This was confirmed by the findings showing the occurrence of E1 was minimal and only increased when fast force rates (which have larger high frequency components) were used. The fact that E1 occurrences increased during LA application indicates that axial stimulation induces simultaneous activity in spindles and PMRs and that the latter part of the spindle response is obliterated by the simultaneously occurring stronger inhibitory response from the PMRs. During the LA block, PMR-related inhibition was dramatically reduced, hence exposing the early excitation. Therefore, during mastication these two pathways may be competing to regulate the activity in the jaw-closers.

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

By recording from the inferior alveolar nerve using tungsten electrodes, two different receptor types have been identified in humans (Trulsson *et al.* 1992; Trulsson & Johansson, 1994). The 'saturating group' are more active in response to static forces less than 1 N and lose their dynamic sensitivity above 0.5 N; the 'non-saturating' group display a linear response to forces up to 5 N and keep their dynamic sensitivity even during the application of static forces above this level. These 'saturating' and 'non-saturating' receptors are likely to correspond to the slow- and fast-adapting receptors as seen in animal studies (Linden, 1990).

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

Although the application of LA reduced the occurrence of IN, some inhibitory activity was still observed (approximately 20% of the time). While some of this activity may be due to the LA block wearing off, this effect would be minimal as any subjects who reported sensation to the tooth stimulation during the experiment had additional LA applied and the trial was repeated. During the LA block, subjects reported no feeling in their teeth, only a vibration in the base of the skull in response to the fast stimulus. Therefore, the expected cause of any persistent reflex activity may not be contained within the periodontal space. Although an attempt was made to remove the contribution of the receptors in the ears by playing white noise to the subject, these receptors, which are known to be inhibitory to jaw-closers (Sato *et al.* 1994), may still have been partially activated by the vibration conducted through the jawbone. During normal mastication, vibrations will only be significant during chewing of brittle food as soft foods will absorb much of the kinetic energy of the jaw. Therefore, this reflex may act as a constant 'break' on jaw-closer muscle activity during the chewing of brittle foods, thus possibly facilitating other factors such as the elasticity (Yemm, 1976) and the

length–tension (Mackenna & Türker, 1978) or velocity–tension relationship (van Willigen *et al.* 1997) of the jaw muscles in stopping the jaws from forcefully coming together if an object yields suddenly (reviewed in Türker, 2002).

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

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

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

The most likely explanation for the stimulus profile appearing in the bite force record was that the stimulus force on the incisor caused a slight upward movement of the head. Since the bite force is measured from the lower bite bar this upward movement of the jaw would cause a small increase in the observed force and since this increase is time locked with the stimulus it would not be averaged out to zero. This would also explain the existence of early excitation; spindles would be activated (due to jaw muscle stretch) as the upper jaw moved up while the lower jaw was stationary on the bite bar.

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

### Reflex activity in the digastric muscle

In humans it has been shown that while a reflex response in the masseter, and other jaw-closing muscles, can be easily identified, the digastric, and other jaw-opening muscles, have little or no reflex activity (Goldberg, 1976). Even painful electrical stimuli to skin and mucosal receptors, which are known to generate a jaw-opening reflex in animals, do not initiate activation of the digastric in humans (Yemm, 1972). While sudden downward movement of the mandible causes a strong contraction of the masseter and temporal muscles, it does not induce a reflex response in the digastric muscle (Lund & Olsson, 1983; De Laat, 1987). It has also been reported that there is no response in the digastric muscle to the unloading of incisors (Türker & Jenkins, 2000). The suggested reason for the discrepancy between the jaw-closer and opener muscles is that, unlike the masseter, the digastric does not contain muscle spindles (Taylor *et al.* 1976). This anatomical finding illustrates that there are fundamental differences in the wiring of the sensory feedback between the jaw-closing and jaw-opening muscles (Abbink *et al.* 1999). The current study indicates that, when observed, ‘reflexes’ in the digastric are more likely the result of cross-talk from the masseter rather than altered activity of the motoneurons controlling the digastric. Cross-talk results when potentials from adjacent muscles reach the recording site through volume conduction, thus contributing to the EMG signal (van Vugt & van Dijk, 2000). Experiments on cross-talk have shown that when using surface electrodes up to 16.8% of a signal detected above a muscle may be due to cross-talk rather than to activation of the muscle below the electrode (De Luca & Merletti, 1988).



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

### Experimental considerations

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

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

In line with the 'frequency principle' of inhibition established for motor units (Miles & Türker, 1986), the present study indicates that increasing bite force slightly decreases the duration of the inhibition in the range of bite levels used. The range of bite levels (5–20% MVC) was chosen due to their close relation to what is expected during mastication (Anderson, 1956). Thus, during regularly encountered muscle contraction levels there is only a small decrease in the inhibitory reflex duration due to an increase in muscle activity, assuming that the reflexes operate in the same way under dynamic conditions.

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

were unavoidable.

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

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