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Normal side-to-side variation of the exteroceptive suppression of masseter muscle in young dentate adults

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SUMMARY **Previous studies have shown that various stimuli applied in the orofacial region, evoke bilateral inhibitory responses in the jaw elevator muscles. The exteroceptive supression (ES) of the masseter muscle after electrical stimulation of the mental nerve, often appears as a double phase of inhibition interrupting the voluntary sustained contraction of the muscle. The aim of the present study was to investigate the normal bilateral variation of the masseteric ES in a control group of 20 healthy dentate adults, and to determine the range of its boundaries. The reflex was elicited by electrical stimulation of the mental nerves, during maximum intercuspal clenching. Generally, the overall mean values for the latencies and durations were in line with those reported in previous investigations, while no significant differences were found between the left and the right masseter muscles, regardless of the side of stimulation. However, in the intra-individual analysis, significant bilateral variation was occasionally recorded, particularly in the latency and duration of the late exteroceptive suppression. It was concluded that the exteroceptive suppression of the masseter muscle is a repeatable and clear bilateral reflex response, although an increased number of observations is recommended in normal subjects, before its use could be extended for diagnostic purposes.**

Introduction

Among the various neurophysiological investigations of mandibular reflexes, the exteroceptive suppression (ES) (also reported as cutaneous suppression, masseter inhibitory reflex (MIR), masseter inhibitory period, or inhibitory antinociceptive brain-stem reflex) of the masseter or temporalis muscles after electrical stimulation of the orofacial region, provides important information on the anatomical and functional organization of the brain-stem motor systems involved in mastication and speech (Yemm, 1972; Godaux & Desmedt, 1975; Cruccu *et al.*, 1984; Göbel *et al.*, 1992). Evoked by electrical stimulation of the mental nerve, the exteroceptive suppression of the masseter or temporalis muscles, normally appears bilaterally as a double phase of inhibition interrupting the voluntary sustained contraction of the muscle. Together with the jaw jerk and blink reflex, exteroceptive suppressions have also been used to provide information on the integrity of mandibular, maxillary and ophthalmic divisions of the trigeminal nerve. Particularly, exteroceptive suppression of masseter and/or temporalis has been proposed as an aid in the neurophysiological assessment of trigeminal function after radiofrequency thermocoagulation and microcompression of gasserian gaglion when treating trigeminal neuralgia, in identifying abnormalities in patients with Huntington's chorea, Parkinson's disease, dystonia, multiple sclerosis and unilateral (hemi)masticatory spasm, in vascular or neoplastic brain-stem lesions, in differentiating between tension headache, migraine and other types of headaches, and even in temporomandibular joint disorders (Cruccu *et al.*, 1987; Cruccu *et al.*, 1991; Schoenen *et al.*, 1987; Ongerboer De Visser *et al.*, 1989). In a general neurophysiological context, exteroceptive suppressions evoked by electrical stimulation of sensory and mixed nerves, have been used for the detection of conduction

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abnormalities in small sensory fibres, not recorded by other routine nerve studies (Leis *et al*., 1991). Similar transient decreases in EMG tonic activity of the mandibular elevator muscles during sustained contraction (silent period (SP)), are evoked by mechanical tapping on the chin. In the dental literature the term silent period is usually applied to the specific cluster of inhibitory reflex responses evoked after chin tapping, tooth tapping or unloading of the muscle spindles, where, however, the involvement of several receptor sites, complicates the interpretation of the responses (Sharav, McGrath & Dubner, 1982; Hellsing & Klineberg, 1983; Van Willigen *et al*., 1993; Türker, Yang & Brodin, 1997).The silent period after chin tapping was extensively investigated and discussed, in younger and older dentate humans as well as in denture wearers. Different types of silent periods were recorded and analysed, and a range of normal values was reported for each particular group (Kossioni & Karkazis, 1995a,b). It was the aim of the present experiments to determine the normal boundaries of side-to-side differences in exteroceptive suppression of the masseter muscle, using electrical stimulation of the mental nerve, as an aid in a further assessment of its potential diagnostic value in the dental profession.

Material and methods

Material

Twenty young volunteers (10 males and 10 females) with a mean age of 25 years (range 20–24 years) were carefully selected for this study. All subjects were healthy, with 28–32 teeth present in an acceptable arch form, with no history suggesting neuromuscular disease or disease affecting neuromuscular performance (e.g. diabetes), not taking any prescription medications (e.g. psychotropic drugs) or over the counter medications (e.g. aspirin), without signs and symptoms of stomatognathic system dysfunction upon thorough clinical examination, and able to clench maximally and produce an interferential EMG pattern. All subjects had given their written consent for the procedures which were adequately described to them beforehand, and were free to withdraw at any stage of the experiment. The experiments were discussed in detail and approved by the head of the department.

EMG and stimulating techniques

A computerized recording and analysis system was used. A detailed description of the electromyographic technique has been previously described (Kossioni & Karkazis, 1993, 1995a,b). In order to diminish the systematic influence of various neuropsychological factors (e.g. stress) on our data, all recordings took place in a quiet room under controlled environmental conditions, and the volunteers got acquainted with the experimental environment beforehand. The mental nerve was stimulated with single electric square pulses of 0·2 ms from a constant current unit (Grass Square pulse stimulator S48K, constant current unit (CCU1), and stimulus isolation unit (SIU5))*, delivered by a bipolar stimulating electrode, to the skin of the lower lip directly over the corresponding mental foramina. The cathode was always positioned proximal to the anode and placed over the selected and marked skin area. The stimulus intensity was gradually increased from a subthreshold level up to 30 mA (150 V), and finally adjusted about two times the threshold of the reflex, a level that most subjects could bear without considerable discomfort. The threshold was identified as the stimulus intensity for which a clear dual suppression could be identified. The occasional mechanical twitch of the lower lip did not influence the recordings. The procedures were absolutely safe, and the electrical currents used were well accepted by all subjects without any discomfort. A total of 20 electrical stimulations (10 for each side) were delivered at 60 s intervals during maximum clenching with the aid of visual feedback (Oscilloscope, Hameg HM 205-3)†. Online observation of the recordings also enabled immediate replacement of the unmeasurable records with additional stimulations. An inter-stimulus interval of at least 60 s was considered necessary to prevent fatigue and habituation (Cruccu *et al*., 1991). Subjects were instructed to continue clenching at least 1–2 s after the stimulus delivery, in order to avoid interruption of the recording due to voluntary relaxation. No facilitating manoeuvres remote from the orofacial region (e.g. Jendrassic) were employed at any stage of the experiment.

EMG activity was recorded simultaneously from the superficial portion of the right and the left masseter

* Astro-Med Inc., W. Warwick, RI, U.S.A.

† Hameg Instruments, Frankfurt, Germany.

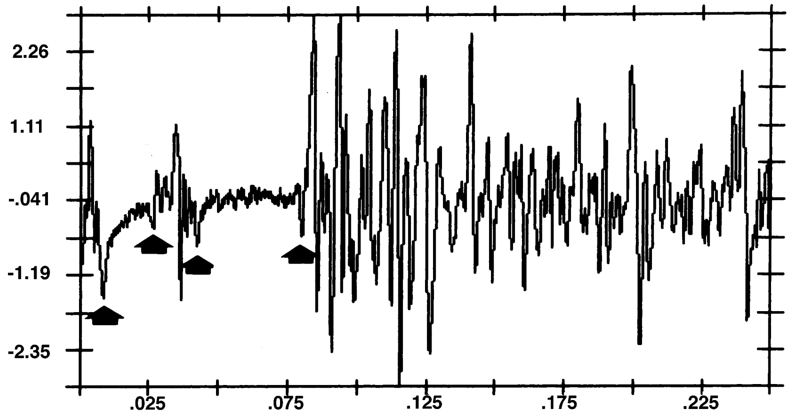

Fig. 1. Measurements of the exteroceptive suppressions induced by electrical stimulation of the mental nerve during maximum voluntary contraction. The arrows indicate the start and the end of the first and the second exteroceptive suppressions, respectively. Calibrations: *y*-axis, amplitude in volts at input to A/D converter, amplification gain (500–10 ×); *x*-axis, real time in seconds.

muscles by bipolar surface electrodes (Duo-Trode, silver/silver chloride)‡. Computer sweeps were triggered by a synchronous output pulse of 1 ms provided by the square pulse stimulator. All signals were differentially amplified (NL 850 isolated preamplifier and NL 104 A.C. Preamp. differential main amplifier; Neurolog system)§, integrated (RMS integrator NL 705)§, AD converted (DAS-1600 Keithley MetraByte)¶ and stored on a personal computer for off-line analysis. The sides of stimulation were randomly alternated.

Data analysis

The analysis was performed off-line by one experienced examiner from the raw EMG and its RMS expression, using a commercial software package (Easyest LX)** The parameters studied from the raw and RMS recordings were the latencies and durations of the early and the late exteroceptive suppressions (ES1 and ES2) identified as absolute or relative decreases in EMG activity. The latency of the ES1 was defined as the time interval from the electrical stimulation to the last significant peak in EMG activity before the first inhibition, while the latency of the ES2 as the time interval from the electrical stimulation to the last peak in EMG activity before the start of the second inhibition (Fig. 1). The duration of both inhibitory phases was defined as the time interval from the last significant peak before the inhibition to the first peak after the return to previous levels of background activity. The intra-observer variation in the accuracy of defining latencies and durations of reflex events has been previously tested and found to be satisfactory (Kossioni & Karkazis, 1993, 1995a,b).

‡ Myotronics Inc., Seattle, WA, U.S.A.

§ Digitimer Ltd, Hertfordshire, U.K.

¶ Keithley Data Acquisition, Taunton, MA, U.S.A.

** Keithley Asyst Software Technologies, Inc., U.S.A.

Statistical analyses

After the assessment of the ability to perform parametric tests (i.e. normality of distributions and equality of variances) overall mean values and standard deviations were calculated and compared with two-way ANOVA, to investigate the main effects of the recorded and the stimulated sides and their possible interaction (Hassard, 1991; Snedecor & Cohran, 1991). The normal variability of the difference between the left and the right masseter muscles was analysed by calculating the mean, the standard deviation and the range of the individual paired differences between the left and the right sides on data pooled together from right and left stimulations ($n = 40$). All calculations were based on the means of the 10 recordings captured for each subjects side, the latter acting as one statistical unit. Statistical analysis was conducted at the 0·05 level of significance.

Results

Less than 5% of the sweeps were unclear during online inspection, and were omitted and replaced during the experiment. For comparison purposes overall mean values and standard deviations for latencies and durations of ES1 and ES2 are presented in Table 1. Two-way ANOVA (recorded by stimulated side) revealed no significant main effects or interactions ($P > 0{\cdot}05$), which suggests: (a) the lack of statistically significant side-to-side asymmetry in all variables and (b) the negligible contribution of the side of stimulation to the side-to side-variation (Table 2). The normal variability of the differences between the left and the right masseter muscles, based on the individual paired differences, is presented in Table 3. The normal range of bilateral differences for ES1 latency and duration was 2·10 and 4·45 ms, respectively. This is in contrast with the significantly higher range, recorded in the ES2 parameters (ES2 latency, 8·05 ms; ES2 duration, 10·44 ms). Figures 2 and 3 illustrate the inter-individual normal side-to-side variation of the ES1 and ES2 latencies and durations.

Discussion

Reflex patterns and measuring techniques

Investigating reflexes mediated through the 5th cranial nerve is an important part of a routine neurological examination. However, because clinical observation cannot solely assess parameters such as reflex inhibition, excitability of the reflex pathways, latencies and durations of the reflex responses, or even real absence of the reflexes, electrophysiological studies offer additional useful information. The wealth of the literature about the much debated problem of trigeminal reflexes confirms among others that tapping on the chin during sustained contraction of mandibular elevator muscles, produces various patterns of inhibitory and excitatory reflex responses. This variability and the inherent difficulty in pattern recognition, have been attributed to various physiological factors such as the magnitude and direction of the tapping force, the clenching level and the activation of different receptor sites (muscle, joint, tendon, acoustic, cutaneous, bone, mucosal in denture wearers, and even remote somatic receptors) either directly or through transmission of the vibration from the chin tap (Bessette *et al*., 1973; Desmedt & Godaux, 1976; Palla *et al*., 1981; Kroon & Naeije, 1984; Olsson & Landgren, 1990). Moreover, these patterns require a laborious analysis before the technique could be further used for diagnostic purposes.

On the contrary, if performed under standardized conditions as in the present investigation, electrophysiological testing of the trigeminal nerve usually elicits a bilateral double phase of inhibition. This reflex complex is more easily identified and measured either on an averaged signal of 10 or more responses, a technique that increases the sensitivity of the method, or using conventional techniques such as the tedious one-by-one analysis. It should be noted, however, that due to its polysynaptic nature, the latency of the late exteroceptive suppression is particularly influenced by the strength of stimulation. An increase in the afferent volley leads to a decrease of the latency and vice versa (Godaux & Desmedt, 1975). Among the various methods described for the determination of the beginning and the end of exteroceptive supressions, the peak to peak measurement from the last EMG peak before, to the first peak after the suppression, was the criterion preferred in our experiments. Our previous experience has shown that the subjective evaluation based on the visual impression of many recordings, enables in most cases, clear marking of the beginning and the end of the events. Amplitude criteria such as millivolts or percentages of maximum amplitude (e.g. 80%) in order to determine the true suppressions should be employed with caution because the small fragments of activity before and between the exteroceptive suppressions could be incorporated in latency and duration measurements (Paulus *et al*., 1992).

Table 1. Normal bilateral variation of latency and duration of ES1 and ES2 ($n = 20$)

	Left stimulation		Right stimulation	
Variable (ms)	Left masseter ($\bar{X} \pm$ SD)	Right masseter ($\bar{X} \pm$ SD)	Left masseter ($\bar{X} \pm$ SD)	Right masseter ($\bar{X} \pm$ SD)
ES1 latency	9·68 ± 1·00	10·05 ± 1·23	9·62 ± 0·87	9·55 ± 1·21
ES2 latency	41·91 ± 4·53	41·97 ± 4·74	44·16 ± 4·56	43·57 ± 4·67
ES1 duration	19·46 ± 2·81	19·46 ± 2·93	20·48 ± 3·44	21·15 ± 3·99
ES2 duration	37·92 ± 7·20	38·47 ± 7·30	37·75 ± 9·64	39·58 ± 9·57

n, number of subjects in the group.

Table 2. Two-way ANOVA (recorded by stimulated side) of latency and duration of ES1 and ES2

	ES1 latency			ES2 latency			ES1 duration			ES2 duration		
Source of variation	DF	*F*-test	*P*	DF	*F*-test	*P*	DF	*F*-test	*P*	DF	*F*-test	*P*
Recorded side	1	0·55	0·45	1	0·06	0·80	1	0·20	0·64	1	0·39	0·53
Stimulated side	1	1·51	0·22	1	3·46	0·06	1	3·32	0·07	1	0·06	0·80
Record X Stimul	1	1·04	0·31	1	0·10	0·75	1	0·19	0·65	1	0·11	0·73

Underlying neurophysiological mechanisms of ES

Exteroceptive suppression appears to be a tri-phasic reflex response consisting of two inhibitory responses (ES1 and 2) separated by an excitatory and facilitatory phase (Göbel *et al.*, 1992). Although neither the precise neuronal circuitry nor the neurotransmitter systems are fully understood, the two phases of depression seem to be related to different underlying mechanisms. Human studies and results in cats (Goldberg & Nakamura, 1968; Kidokoro *et al.*, 1968; Nakamura, Mori & Nagashima, 1973), indicate that the two inhibitions may not share the same afferents as argued by others (Godaux & Desmedt, 1975), although they both belong to the Aβ intermediate myelinated fibre group mediating pain impulses (antinociceptive brain-stem reflex) (Göbel *et al.*, 1992). Studies on central delay recovery curves to paired stimuli and habituation during repetitive stimulation, which are thought to depend on the number of synapses involved, suggest that the two inhibitory phases are mediated by separate neural nets in the brain stem: a disynaptic post-synaptically inhibited net for ES1 and a polysynaptic pre-synaptically inhibited net for ES2 (Cruccu *et al.*, 1984; Cruccu & Bowsher 1986; Göbel *et al.*, 1992). The inhibitory interneuron(s) involved are located in the proximity of the trigeminal motor nucleus for ES1 and in the lateral reticular formation at the ponto-medullary junction for ES2. Because anatomical data obtained in animal studies indicated that these structures receive strong input from various limbic structures (Kuypers, 1958; Nazaki, Enomoto & Nakamura, 1983) it is hypothesized that the exteroceptive suppressions in humans are also under the control of the limbic system as well as the cortex, the cerebellum and other anatomical structures (periaquaductal gray, amygdala, hypothalamus, orbitofrontal gyrous) that similarly project onto the pontomedullary lateral reticular formation (Cruccu *et al.*, 1991). These anatomical connections emphasize the modulating role of higher centres on the involved interneurons and explain to some extent the intra and inter-individual normal biological variation recorded in the present experiments. It should be noted that the strong pyramidal activation that takes place during maximum clenching is a fundamental prerequisite for a clear reflex response, because this condition affects the responsiveness of interneurons and motor neurons to the afferent volleys (Lundberg & Voorhoeve, 1962; Desmedt & Godaux, 1976). The importance of the ascending volleys has been pointed out by Leis *et al.* (1991) who concluded that the ascending volley following electrical stimulation of a mixed peripheral nerve (orthodromic sensory and antidromic motor) produces the SP without the apparent contribution from the descending volley, and the changes in spindle discharges created by the superimposed direct muscular twitch, as it was generally accepted previously (Matthews, 1931; Higgins & Lieberman, 1968). Therefore, the state of the peripheral receptors is unrelated to the morphology of the exteroceptive suppression.

Bilateral variation in the ES of the masseter muscle

Most of the overall mean values of the parameters studied were within the range of those reported in previous investigations (Cruccu *et al.*, 1987; Ongerboer De Visser *et al.*, 1989; Cruccu *et al.*, 1991; Kossioni & Karkazis, 1993, 1995a,b). Slight differences, however, can be attributed to variations in the recording and

Table 3. Mean of individual paired differences between right and left masseter muscles

Variable (ms)	Mean	SD	Min	Max	Range
ES1 latency	0·50	0·48	0·01	2·11	2·10
ES2 latency	1·82	1·67	0·06	8·12	8·05
ES1 duration	1·44	1·19	0·05	4·5	4·45
ES2 duration	3·80	2·64	0·16	10·6	10·44

Data pooled together from the right and left stimulations ($n = 40$).

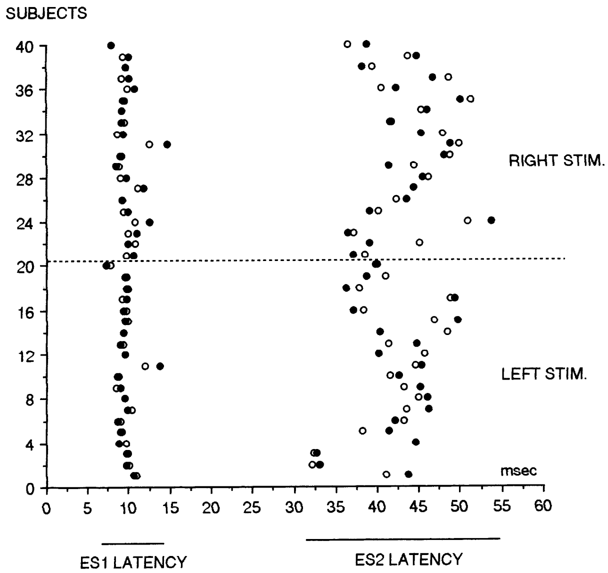

Fig. 2. Normal side-to-side variation of the latencies of the early and late exteroceptive supressions after single electrical shocks, in 20 subjects (10 averaged responses for each subjects side). The relatively symmetrical distribution of values above and under the horizontal dotted line reflects the equivalent side-to-side responses regardless of the side of stimulation (○, left masseter; ●, right masseter).

analysis techniques used. Within the limitations of the present experimental set-up which is used in the every day clinical neurophysiology practice, no overall significant differences in the latencies and durations of ES1 and ES2 were recorded between sides (Tables 1 and 2). However, significant differences were occasionally recorded in the intra-individual analysis, and were more prominent in the latency and duration of ES2 (Table 3). The upper limits of normal assymetry were 2·10 ms for the latency of ES1 and 8·05 ms for the latency of ES2, while higher differences were recorded for the durations (4·45 and 10·44 ms, respectively). The latency asymmetry values were slightly higher than the corresponding values reported by other authors using a similar experimental set-up. These authors reported side-to-side differences that varied between 1·5 and 2 ms for ES1 and between 5 and 6 ms for ES2 (Cruccu *et al.*, 1987; Ongerboer De Visser *et al.*, 1989). From the clinical point of view, the range of the individual mean differences provides a more reliable measure of normal side asymmetry and should be taken into consideration when analysing pathological situations in age- and sex-matched groups. For example, the intra-individual side-difference together with other criteria is regarded as an effective index for testing asymmetry in unilateral brain lesions, and in this respect its use could be extended in TMD patients (Cruccu *et al.*, 1987, 1991). Moreover, the significant consistency in the pattern of the reflex response and, especially, in the latencies of ES1 and ES2, provide additional evidence of its possible diagnostic significance, because smaller variability and narrower normal range increase sensitivity. Finally, the lack of considerable bilateral variation in relation to the side of stimulation suggests that unilateral stimulation may produce equal bilateral responses in normal subjects. If, however, only one side is recorded, contralateral stimulation is preferred to reduce possible stimulus artifact.

Conclusions

(1) When elicited and recorded in the proper manner, the exteroceptive suppression of the masseter muscle is a sufficiently repeatable and clear reflex response,

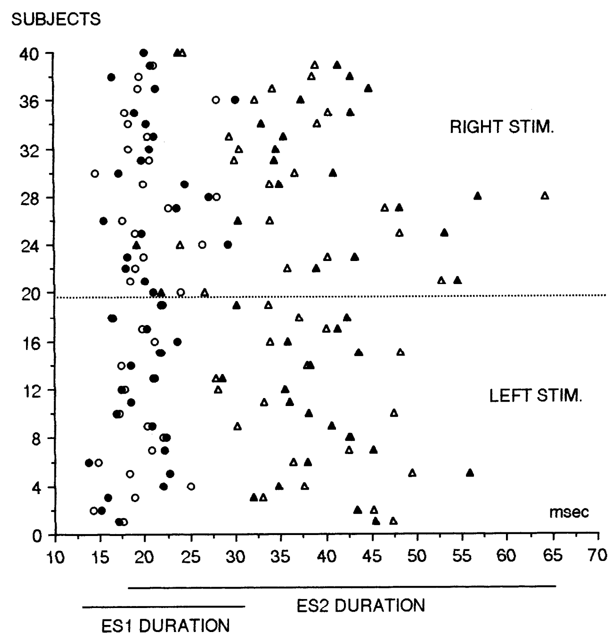

Fig. 3. Normal side-to-side variation of the durations of the early and late exteroceptive suppressions after single electrical shocks, in 20 subjects (10 averaged responses for each subjects side). The higher inter-individual variation seen in ES2 duration probably reflects the polysynaptic nature and the modulating role of higher centres on the neuronal net in the brain stem, mediating the above inhibitory response (○, ES1 duration, left masseter; ●, ES1 duration, right masseter; △, ES2 duration, left masseter; ▲, ES2 duration, right masseter).

comprising a double phase of inhibition (ES1 and ES2) interrupting the voluntary sustained contraction of the muscle.

(2) Although group analysis did not reveal significant variation between sides, significant differences were occasionally recorded in the individual analysis. Therefore, an increased number of observations should be averaged and rectified before analysis and normal ranges for age- and sex-matched groups should be established, before the masseter exteroceptive suppression could be further used as a possible diagnostic tool in pathological situations.

(3) In normal subjects, unilateral stimulation of the mental nerve may produce equivalent bilateral responses. If, however, only one side is recorded, contralateral stimulation is preferred to reduce possible stimulus artifacts. This facilitates fast recordings of normal values, in order to obtain the appropriate pool of data for comparisons with patients.

Acknowledgments

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