

Title

Vestibulo Masseteric Reflex and Acoustic Masseteric Reflex. Normative data and effects of age and gender.

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Abstract

Objective. To provide normative data for the Vestibulo-Masseteric Reflex (VMR) and Acoustic-Masseteric Reflex (AMR) in healthy subjects, stratified for age and gender.

Methods. A total of 82 healthy subjects (M:F 43:39, mean age 39.3 ± 18.4 years, range 13-79 years) underwent recording of click-evoked VMR and AMR (0.1 ms duration, 5 Hz frequency) from active masseter muscles. Masseter responses to uni- and bilateral stimulation were recorded in a zygomatic and a mandibular configuration, according to the position of the reference electrode. Stimulation intensity curves were recorded for each reflex in ten subjects (mean age 20.7 ± 8.1 years). Gender effect was investigated in 62 subjects and age effect was analyzed in six 10-subject groups aged from <25 to >65 years. Onset and peak latencies, interpeak intervals, raw and corrected amplitudes, latency and amplitude asymmetries were analyzed.

Results. VMR had a higher elicitation rate than AMR. For both reflexes, rates of elicitation, and corrected amplitudes were higher in the zygomatic configuration, and bilateral stimulation elicited larger responses. Best acoustic ranges of elicitation were 98-113 dB for AMR and 128-138 dB for VMR. Reflex latencies were shorter in females than males. Frequency and amplitude of VMR and AMR decreased substantially over 55 year olds.

Conclusions. VMR and AMR can be easily performed in any clinical neurophysiology laboratory.

Significance. These reflexes can find application in the investigation of brainstem function in central neurological disorders.

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1. Introduction

There is a long history of reflex responses to sound recorded in a number of cranial muscles that may be produced by peripheral cochlear (Meier-Ewert et al., 1974) or vestibular (Hickenbottom et al., 1985) stimulation. In this context, loud sound stimuli have been used to elicit vestibular evoked myogenic potentials (VEMPs) in active sternocleidomastoid muscles (cervical VEMP, cVEMP) (Colebatch et al., 1994) and inferior oblique muscles (ocular VEMP, oVEMP) (Rosengren et al., 2005). For cVEMPs and oVEMPs, standard values in healthy subjects are available (Welgampola et al., 2001, Rosengren et al., 2011, Sandhu et al., 2013, Rosengren, 2015, Govender et al., 2016). These VEMPs have found a wide application in the study of both vestibular and neurological disorders (Venhovens et al., 2016).

Vestibular stimulation at the end-organ level may also evoke a short-latency inhibitory EMG response in active masseter muscles. This response was first demonstrated following unilateral or bilateral transmastoid electrical stimulation as a bilateral and symmetric p11/n15 biphasic wave, termed vestibulo-masseteric reflex (VMR), (Deriu et al., 2003). The VMR was later shown to be also evoked by high-intensity acoustic stimulation, but in this case the n15 wave was inconsistently visible as a small deflection in a simple p11/n21 potential or not detectable at all (Deriu et al., 2005). This study demonstrated that the p11/n21 potential was the result of two overlapping components: a short-latency, high-threshold p11/n15 wave, not detectable in the rectified EMG, and a longer-latency, low-threshold p16/n21 wave, clearly visible in the rectified EMG as a transitory short period of EMG suppression (Deriu et al., 2005). A further study, performed in patients with selective vestibular or cochlear lesion (Deriu et al., 2007) clarified the vestibular origin of the p11/15 wave and the cochlear origin of the p16/n21 wave, and termed acoustic-masseteric reflex (AMR). Anatomical studies conducted in rats revealed that, besides a multisynaptic vestibulo-trigeminal pathway (Giacconi et al., 2006), possibly mediating excitatory long-latency trigeminal responses to vestibular stimulation (Tolu et al., 1996, Deriu et al., 1999, Deriu et al., 2000, Deriu et al., 2010), a monosynaptic connection between the vestibular nuclei and the trigeminal motor nucleus exists (Cuccurazzu et al., 2007). Although not yet confirmed in humans, this crossed and bilateral vestibulo-trigeminal pathway could be the anatomical substrate of the VMR (Deriu et al., 2010).

Both the VMR and AMR have been recently employed in pathological settings. For instance, they were used in patients with multiple sclerosis singularly, or in a battery along with other myogenic potentials, to improve the ability of clinical and neuroimaging examinations to detect brainstem dysfunctions (Magnano et al., 2014, Magnano et al., 2016). More recently, the VMR, also termed masseteric VEMP (mVEMP), was employed as part of a comprehensive battery of VEMPs for the functional assessment of the brainstem in patients with Parkinson's disease and idiopathic REM-Sleep Behaviour Disorder. A VEMP score was provided to assess the severity of brainstem dysfunction in this neurological condition (de Natale et al., 2015a, de Natale et al., 2015b; de Natale et al., 2018). These studies suggest the utility of the VMR and AMR as complementary tools in the assessment of brainstem function. However, unlike cVEMPs and oVEMPs, normative data for VMR (or mVEMP) and AMR are lacking, and this limits their potential use in clinical settings.

Consequently this study proposed to: a) test the click-evoked VMR and AMR in a large population of healthy subjects to establish normative parameter values; b) determine the optimal sound intensity for the elicitation of VMR and AMR; and c) investigate whether age and gender may affect these reflexes.

2. Methods

2.1 Subjects

A total of 82 healthy subjects (43 females and 39 males; mean age 39.3 ± 18.4 years, range 13-79 years) participated in this study, after giving their written informed consent. For underage subjects, written consent was provided by both parents. The study was approved by the local ethics authority (ASL1 Sassari, Prot. 693/L/08) and conducted in accordance with the Helsinki declaration.

Detailed personal history was collected for all participants to exclude previous or current medical conditions such as neuro-otological and stomatognathic disorders, cervical spine disturbances and migraine. In particular, to rule out conductive and/or neurosensorial hearing loss, all individuals underwent audiometric examination prior to enrollment.

Subjects were seated in a dim and quiet room and were asked to contract masseters at 30-50% of their maximal voluntary contraction, with visual feedback to help them to monitor their muscle contraction level.

2.2. Reflex recordings

During masseter contraction at the prescribed level, VMR and AMR were elicited through air-conducted clicks ($n = 300-500$ stimuli, 0.1 ms duration, 5 Hz frequency), generated by a 3505 HP attenuator driven by a Signal 5.0 script for VEMP (Cambridge Electronic Design, LTD, Cambridge, UK) and delivered through TDH-49P calibrated earphones (Telephonics, Huntington, NY) mono- and binaurally. VMR was elicited at an intensity of 138 dB SPL and AMR at 108 dB SPL; these intensities have been previously found to elicit distinct VMR and AMR responses (Deriu et al., 2005, Deriu et al., 2007).

Rectified and unrectified EMG activity were bilaterally recorded (1902 Quad System Amplifier, Cambridge Electronic LTD, Cambridge, UK), amplified ($\times 5000$), filtered (bandwidth 5-5000 Hz) and sampled (10 KHz) within a 200 ms window (50 ms before and 150 ms after stimulus delivery), using an analog/digital converter (1401 power, Cambridge Electronic Design LTD, Cambridge, UK) and Signal 5.0 software for PC.

2.3. *Electrodes montage*

In all subjects, masseter muscle EMG was recorded through surface bipolar silver/silver chloride electrodes placed in a double belly-to-tendon configuration, with the active electrode positioned in the lower third of the masseter muscle, two reference electrodes placed at the mandible angle (*mandibular montage*) and in the middle of the zygomatic arch (*zygomatic montage*) respectively, and the ground electrode over the forehead (Figure 1).

The differences in responses to unilateral and bilateral stimulation recorded with either mandibular or zygomatic montage, were analyzed in those subjects who were ≤ 55 years old (62 subjects, 30 males and 32 females; mean age 30.9 ± 11.1 years, range 13-54 years), to exclude any potential age effects (Welgampola et al., 2001).

2.4 *Intensity of stimulation*

The effect of stimulation intensity was analyzed in 10 subjects (4 males and 6 females; mean age 20.7 ± 8.1 years, range 20-50 years), who underwent unilateral and bilateral click stimulation at increasing intensities (steps of 5 dB SPL), within a range from 98 dB SPL to 138 dB SPL. Rates from left and right stimulations were pooled for the calculation of unilateral responses. Responses from both montage configurations were measured.

2.5 *Effects of age*

In order to analyze the effects of age in the characteristics of the two reflexes, a subset of participants was stratified into six age categories (<25 years, 26-35, 36-45, 46-55, 56-65, >65 years) each comprising 10 subjects, for a total of 60 subjects (33 males and 27 females, mean age 45.7±17.4 years, range 13-79 years).

2.6 Effects of gender

Gender differences in VMR and AMR were analyzed in subjects aged <55 years (62 subjects). As stated above, responses from both montage configurations were measured.

2.7. Data analysis

For each reflex, the rate of detection was first examined. The VMR and the AMR were considered present when a p11 or p16/n21 wave, respectively, was clearly discernible from the averaged background EMG activity, namely when they were larger than 2SD of the mean noise (>20.7 μV in the zygomatic montage and >14.9 μV in the mandibular montage). The averaged unrectified EMG was then used to measure the following parameters: onset and peak latency of the first positive wave or p1 (p11 for VMR and p16 for AMR); peak latency of the first negative wave or n1 (i.e. n21 for AMR); p1-n1 interpeak intervals (i.e. p11-n21 and p16-n21 intervals), peak (p11, p16 and n21 waves) and peak-to-peak (p11-n21 and p16-n21) raw and corrected amplitudes (expressed as ratio between the raw amplitude and mean EMG activity in the 50 ms before the stimulus). The asymmetries in both p1 latencies and corrected amplitudes were calculated with the following formula $[(Lx-Rx/Lx+Rx)*100\%]$ where Lx and Rx represent the latency and the amplitudes of the left and right responses (Welgampola et al., 2001). Inter-side differences in peak latencies were also measured.

2.8. Statistical analysis

All statistics were made with PASW Statistics (SPSS version 18 for Windows, Chicago, Illinois), with significance set at $\alpha < 0.05$.

Different montages were compared for all the parameters considered as well as for unilateral and bilateral responses, through paired t-tests. Comparison between VMR and AMR frequencies according to different intensities of stimulation was performed through the Chi-square test. The effect of age on the reflex morphology was tested with a one-way ANOVA with Tukey's post-hoc test and Greenhouse-Geisser correction in case of non-spherical data, as assessed by Mauchly's test.

3. Results

3.1. Reflex detection rate in the general population enrolled

A representation of a VMR and AMR recorded in the mandibular and zygomatic electrode montage following click stimulation is provided in Figure 2.

VMR. Within the whole cohort of 82 subjects studied, the VMR was detected in 93.9% of cases following unilateral stimulation (154/164 ears) and in 95.1% following bilateral stimulation (156/164 ears) in the zygomatic montage. In the mandibular montage, the rates of detection of the VMR were of 73.2% (120/164 ears) and 82.3% (135/164 ears) following unilateral and bilateral clicks, respectively. A significantly higher detection rate was found in the zygomatic compared with the mandibular montage, at both unilateral ($p < 0.0001$) and bilateral ($p < 0.0001$) stimulations. By contrast, within each montage no significant differences in the rate of elicitation were observed between unilateral and bilateral stimulations ($p < 0.05$). Three out of 82 subjects did not show any clear VMR in either electrode configurations (6/164 ears, 3.6%). Notably, 3 ears out of 164 (1.8%) showed the VMR in the mandibular recording only and 13 (7.9%) in the zygomatic configuration only.

AMR. In the zygomatic montage, the AMR was clearly detectable in 84.1% (138/164 ears) and 89.2% (146/164 ears) of subjects, following unilateral and bilateral stimulation, respectively. In the mandibular montage, rates of AMR detection were 62.2% (102/164 ears) following unilateral clicks and 71.9% (118/164 ears) following bilateral clicks. A significant difference was observed between the two montages for both unilateral ($p < 0.0001$) and bilateral stimulations ($p = 0.0002$). Of the 164 ears tested, 1 (0.6%) had the AMR in the mandibular configuration only, 26 (15.8%) in the zygomatic configuration only and 13 (7.9%) had no evocable AMR.

3.2. VMR and AMR parameters according to the electrode montage

At the standard intensities used, the frequency rate exhibited by the two reflexes was significantly different ($p < 0.01$ for all) according to the electrode montage and the side of stimulation. Data relative to VMR and AMR parameters recorded from subjects ≤ 55 years old at the time of enrollment ($n = 62$ subjects; 124 ears) are shown in Tables 1 and 2 respectively.

VMR. The VMR elicited by bilateral stimulation showed a significantly larger amplitude ($p \leq 0.001$) than that induced by unilateral stimuli. In the latter case, ipsi- and contralateral responses

did not differ as for latency and amplitude. Compared with the mandibular montage, in the zygomatic montage the VMR detection rate was significantly higher ($p < 0.0001$), the onset earlier ($p \leq 0.009$) and the amplitude larger ($p < 0.0001$) following both unilateral and bilateral stimulation. See Table 1 for details.

AMR. No significant differences between ipsilateral and contralateral responses to unilateral clicks were found in any of the parameters measured and between montages. By contrast, in both configurations, responses to bilateral stimulation exhibited significantly earlier onset and peak latencies and larger amplitudes in comparison with responses to unilateral stimulation. As to the montage, AMR showed a significantly higher detection rate and a larger amplitude in the zygomatic than mandibular montage following both unilateral and bilateral stimulation ($p \leq 0.01$). Additionally, the rate of elicitation of AMR in the mandibular configuration was higher after bilateral than unilateral stimulation ($p = 0.035$). See Table 2 for details.

3.3. Standardization of stimulation intensity

Figure 3 describes masseter responses to different click intensities. Recordings from a representative subject are shown in Figure 3A and mean responses from the subset of the 10 subjects investigated are shown in fig 3B. The AMR (p16/n21 wave) was clearly detectable in the 98-113 dB range, with no sign of the VMR (p11 wave) at these stimulation intensities. By contrast, the p11 wave of the VMR was clearly detectable at intensities ranging from 128 to 138 dB in all subjects. Due to the overlapping between the VMR and the AMR at these intensities, the n15 wave of the VMR was not detectable or appeared as a small deflection in the body of a p11/n21 bipolar mixed (vestibular/cochlear) potential. Within the intensity range of 113-123 dB, it was not possible to distinguish reliably clear potentials belonging to any of the two reflexes.

3.4. Effects of age on VMR and AMR

The differences in the main parameters of VMR (p11 wave) and AMR (p16/n21 wave) are displayed by age groups (Table 3).

VMR. The frequency of elicitation of the reflex tended to decrease with age. The p11 peak latency showed a trend to increase with age, with a sharp significant rise from 56-year-olds onwards, following unilateral ($p < 0.0001$) but not bilateral stimulations. A significant decline in the amplitude of the onset-peak p11 of the VMR was detected for both unilateral ($F_{2,59}: 5.389$, $p < 0.0001$) and bilateral stimulation ($F_{2,59}: 4.056$, $p = 0.02$).

AMR. The frequency of elicitation of AMR decreased with age, both for unilateral and bilateral stimulation. Mean peak latencies of the p16 and n21 waves showed a trend to increase with age for both unilateral and bilateral stimulations. This effect was significant from the 56-65 age group onwards ($p < 0.0001$). Moreover, p16/n21 corrected amplitudes decreased with age in a similar manner, with no differences in the trend according to the side of stimulation and with a significant drop in the two eldest age categories (Table 3).

3.5. *Effects of gender on VMR and AMR*

Gender differences between the two reflexes are displayed in Table 4. In women, the p1 and n1 peak latencies were significantly shorter in comparison with male subjects, regardless mono- or binaural stimulations. By contrast, corrected amplitudes did not differ significantly between genders in both reflexes.

4. Discussion

This study provides normative data on the characteristics of click-evoked VMR and AMR in a population of healthy subjects and describes methods to elicit and record vestibular and cochlear masseteric responses to loud sound.

4.1. *Electrode positioning.*

In line with previous studies on VEMPs (Vanspauwen et al., 2016, Leyssens et al., 2017) we found that the electrode configuration affected the characteristics of the VMR and AMR. In particular, when the reference electrode was positioned in the zygomatic arch rather than in the mandible angle, both reflexes exhibited significantly higher elicitation rates and raw amplitudes, but no differences in corrected amplitudes. The zygomatic montage, compared to the mandibular montage, has a higher inter-electrode distance (IED) which, employing a broader area of recording, prevents “reference contamination” (Piker et al., 2011). Surface EMG recording of the masseter muscle is highly influenced by IED, since even small changes in it may result in significant differences of both amplitude and variability of the recording (Castroflorio et al., 2006). In this regard, surface EMG recording during isometric sub-maximal contractions of the jaw-elevator muscles provides best results when a 30 mm IED is employed (Castroflorio et al., 2005).

The differences in the raw, but not in the corrected amplitude, according to the electrode configuration may be the result of the higher number of motor units involved in the zygomatic

configuration, for which a higher mean pre-stimulus EMG activity is expected. In addition, the raw amplitude of VEMPs is influenced by the pre-stimulus EMG activity (Deriu et al., 2003, Rosengren, 2015) and may be subject to wider swings according to this latter parameter. Applying the ratio of these two parameters smooths these differences and may explain the lack of significant difference between the corrected amplitudes, according to the electrode montage.

Moreover, the possible contribution of other masticatory muscles innervated by the trigeminal nerve (such as the pterigoidei muscles) to the amplitude obtained in the zygomatic configuration cannot be totally excluded in our sample, although this phenomenon on face muscles is more likely to occur at very high rates of muscle contraction (Rosengren, 2015).

The electrode montage affected significantly also the reflex rate of elicitation, as it was significantly higher in the zygomatic than in the mandibular montage, for both VMR and AMR. According to Piker et al. (2011), there is a considerable risk of obtaining equal EMG responses in the active and reference electrodes if they are too close. This may lead to a far-field contamination as a result of volume conduction (Rutkove, 2007). When this happens, the net effect of the synchronized EMG on both the active and reference electrodes is a subtraction of signal, which may result in a reduction of the reflex amplitude up to an absent response (Sandhu et al., 2013). In line with these observations, in our population of healthy subjects, we observed that in 7.9% of cases the VMR was detectable in the zygomatic montage only and in 1.8% of cases it was present in the mandibular montage only. For the AMR the difference in sensitivity between the two montages was more evident, being detectable in the zygomatic but not the mandibular montage in 15.8% of cases and in the mandibular but not the zygomatic montage in 0.6% of cases. Based on these findings, we suggest that, to ensure the highest detection rate, both electrode configurations be used when recording the VMR and the AMR.

4.2. Intensities of stimulation

The difference in activation threshold of cochlear and vestibular receptors to sound may explain the different characteristics of the low-threshold, longer-latency AMR (p16/n21 potential), which is cochlear in origin, and of the high-threshold, short-latency VMR (p11/n15 potential), which is of vestibular origin (Deriu et al., 2003, 2005, Deriu et al., 2007, Deriu et al., 2010). The overlap between these masseter responses makes it important to define which range of click intensity allows a clear detection and distinction between them. In line with previous studies (Magnano et al., 2014, de Natale et al., 2015a, de Natale et al., 2015b, Magnano et al., 2016, de Natale et al., 2018) we found that the best intensities to induce a clear VMR are in the range between 123-138 dB SPL,

with the optimal intensity at 138 dB SPL. At these intensities the p11 vestibular wave was clearly detectable. By contrast the n15 wave appeared as a deflection in a p11/n21 mixed vestibular-cochlear potential or not visible at all. The AMR was clearly detected at intensities sub-threshold for the VMR, i.e. ≤ 123 dB, with the best intensity at 108 dB, which in most of the subjects was unable to elicit even a small VMR.

In a previous work, the VMR was found to have the same elicitation intensity threshold of the cVEMP (Deriu et al., 2005). However, some differences between these VEMPs need to be acknowledged. Provided the stimulation intensity is the same, the amplitude of the mVEMP is around 30% smaller than the cVEMP (Deriu et al., 2005). In line with this finding, compared to the mVEMP, the cVEMP and oVEMP can be elicited with the proportion of 91% and 84% at 135 dB SPL respectively as well as with higher amplitudes (Rosengren et al., 2011). These data indicate that the vestibular projection to the sternocleidomastoid and ocular muscles is more powerful than the projection to the masseters. This may be a consequence of the predominant role played by neck and ocular muscles in postural control compared with that played by jaw-closing muscles. Another aspect that should be pointed out is that the level of contraction of the masseter used in our experiments (30-50% of the maximum voluntary contraction) is lower than the 80% usually employed for sternocleidomastoid muscle contraction in cVEMP recordings.

No comparison is possible at the moment between masseter responses to click versus tone stimulation, which is another type of stimulus commonly used to elicit cVEMPs and oVEMPs, with different degrees of sensitivity. The papers (Deriu et al., 2005, 2007) which first described VMR and AMR in healthy subjects as well as in clinical settings (Magnano et al., 2014, Magnano et al., 2016, De Natale et al., 2015a, 2015b, De Natale et al. 2018) have all used air-conducted click stimulation. For this reason, normative data collected here have been obtained using this mean of stimulation only. Further works may be warranted to investigate whether differences in mVEMP features and elicitation rate depending on different types of stimulation exist.

4.3. Effects of age and gender

In line with a considerable number of studies on cVEMP and oVEMP published in the last fifteen years (Welgampola et al., 2001, Basta et al., 2007, Brantberg et al., 2007, Janky et al., 2009, Piker et al., 2011, Rosengren et al., 2011), we found that age significantly affects the morphology of the VMR and AMR responses. For VMR, frequency of elicitation decreases in the category of over 65-year-olds. This can be explained by the progressive degeneration of the hearing and vestibular

systems that involves all its components, from a regular loss of the hairy cells (Rosenhall, 1973) and the cochlear system (Makary et al., 2011) to the Scarpa ganglion (Richter, 1980), up to the brainstem vestibular nuclei (Alvarez et al., 1998). Furthermore, it is acknowledged that masticatory muscles exhibit a decrease in strength and function with age, as revealed by the reduction in muscle thickness and maximal voluntary contraction after age 60 (Palinkas et al., 2010) as well as in EMG activity of masticatory muscles in elderly people (Cecilio et al., 2010). This would also have affected the outcomes of VMR and AMR recordings in elderly people. In addition, we have found a slight decrease in the rate of reflex elicitation also in younger age groups. It is known that a small but progressive loss of otoconia occurs in healthy subjects from the age of 30 (Johnsson et al., 1972) and this could at least in part explain this phenomenon. Moreover, age-related changes on the healthy hearing system are well described in both sensory neurons (Sergeyenko et al., 2013) and neurotransmitters (Lee, 2013) in a similar way to vestibular degeneration.

In this study, females exhibited significantly shorter peak latencies, for both unilateral and bilateral AMR and VMR recordings. A similar effect of gender has been described for Brainstem Auditory Evoked Potentials recordings (Beagley et al. , 1978, Trune et al. , 1988). It has been hypothesized that the difference of the cochlear average length, which is lower in females (Sato et al., 1991), may play a role. However, caloric response is not different between genders, suggesting that no difference between males and females exists in the peripheral vestibular pathway. Additionally, studies on cVEMP (Ochi et al., 2003) and oVEMP (Sung et al., 2011, Versino et al., 2015) failed to demonstrate a gender difference. Comparative studies between different VEMPs according to this parameter may better clarify the presence and the causes for this difference.

4.4. Clinical implications.

VEMPs are increasingly employed for research and clinical purposes in a wide number of neurological and neurotological disorders, with a diagnostic/differential diagnostic purpose. The reflexes here tested are able to indirectly study a significant portion of the brainstem and have been proven a useful complement to cervical and ocular VEMPs in the assessment of brainstem function (Magnano et al., 2014, de Natale et al., 2015a, de Natale et al., 2015b, Magnano et al., 2016; de Natale et al. 2016; de Natale et al., 2018). VMR has the advantage of investigating the trigeminal brainstem pathways and is more tolerated than the Trigeminal Cervical Reflex (which implies a stimulation which, although not nociceptive, can be distressing for the subject). VMR also provides a crossed and bilateral response to mono or bilateral stimulations; this feature may be useful when differentiating central neurological and peripheral vestibular disorders. In the latter case,

impairments in the stimulation of the affected side (peripheral vestibular damage) can be counterbalanced by the preservation of the VMR response on the correspondent target muscle from contralateral side stimulation (preservation of central pathways). We suggest the VMR and AMR as an additional useful tool in current clinical practice since they are easily performed, mono and binaurally, using common electromyographers, with sound stimuli intensity in the range for evoking cochlear as well as vestibular responses.

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FIGURE LEGENDS

Figure 1. Position of the electrodes for the recording of the acoustic-masseter reflex and of the vestibular-masseteric reflex in two different belly-to-tendon montages. The active electrode (Active) is positioned in the lower third of the masseter muscle and two reference electrodes are placed one at the mandible angle (mandibular montage, “Mand ref”) and the other in the middle of the zygomatic arch (zygomatic montage, “Zyg ref”). The ground electrode (Ground) is placed over the forehead.

Figure 2. VMR and AMR recorded in a representative subject. Averaged ($n = 300$ sweeps) unrectified EMG responses to the stimulation of the right ear (arrow) were recorded from active masseter muscles bilaterally. The VMR appears as a bilateral and symmetric p11 wave followed by an acoustic n21 wave. The AMR, appears as a p16/n21 wave.

Figure 3. VMR and AMR elicited by different intensities, in steps of 5 dB.

A. Unrectified EMG recordings from a representative subject. **B.** Mean \pm standard error of the percentage of reflex elicitation rate in 10 subjects. No differences in the percentage of elicitation of either reflex for unilateral or bilateral stimulations were detected, thus pooled data from both types of stimulation are reported in the graph. The p11 wave of the VMR (black line) was clearly detectable at stimulation intensities between 138 to and 128 dB SPL. The AMR (p16/n21 wave) was clearly detectable in the 98-118 dB range (grey line).