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Vestibulo masseteric reflex and acoustic masseteric Reflex. Normative data and effects of age and gender

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HIGHLIGHTS

- Normative data for the Vestibulo and Acoustic Masseteric Reflex were collected in a large sample.
- The effects of two different electrode configurations, of age and gender were analyzed.
- Normative data are useful for a clinical application of these reflexes in neurological conditions.

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.

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 and Colebatch, 2001; Rosengren et al., 2011, Sandhu et al., 2013; Rosengren, 2015; Govender et al., 2016). These VEMPs have found

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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 originally vestibulo-masseteric reflex (VMR) (Deriu et al., 2003) and more recently masseteric VEMP (mVEMP; De Natale et al., 2015a). The VMR was 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). Patients with cochlear damage showed a p11/n15 wave only while patients with vestibular lesion showed a preserved p16/n21 potential only (Deriu et al., 2007). This finding clarified the vestibular origin of the p11/15 wave (VMR) and the cochlear origin of the p16/n21 wave, that was termed acoustic-masseteric reflex (AMR). Anatomical studies conducted in rats revealed that, besides a multisynaptic vestibulo-trigeminal pathway (Giaconi 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 medial 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). The anatomical basis of the AMR are not precisely known. Functional studies suggested that besides the ventral cochlear nucleus, the superior olivary complex and the nucleus of lemniscus lateralis, the interneurons of the pontine reticular formation may constitute the connection trough the auditory system and the premotor area for the masseter muscle (Kiziltan 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 individually, 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 mVEMP, was employed as part of a comprehensive battery of VEMPs for the functional assessment of the brainstem in patients with Parkinson's disease (De Natale et al., 2015a, 2015b), idiopathic REM-Sleep Behaviour Disorder (de Natale et al., 2018) and amyotrophic lateral sclerosis (Liu et al., 2019). A mVEMP score was provided to assess the severity of brainstem dysfunction in neurological conditions (de Natale et al., 2015a, 2015b, 2018). These studies suggest the utility of the VMR and AMR as 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 participants underwent tonal audiometric (Amplifon audiometer 300E815) examination performed following international standard procedures ISO 6189-1983. All participants had normal audiograms.

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, 2007).

Rectified and unrectified EMG activity were bilaterally recorded (1902 Quad System Amplifier, Cambridge Electronic LTD, Cambridge, UK), amplified (x5000), 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 (Fig. 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 and Colebatch, 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 on the characteristics of the two reflexes, a subset of participants was stratified into six

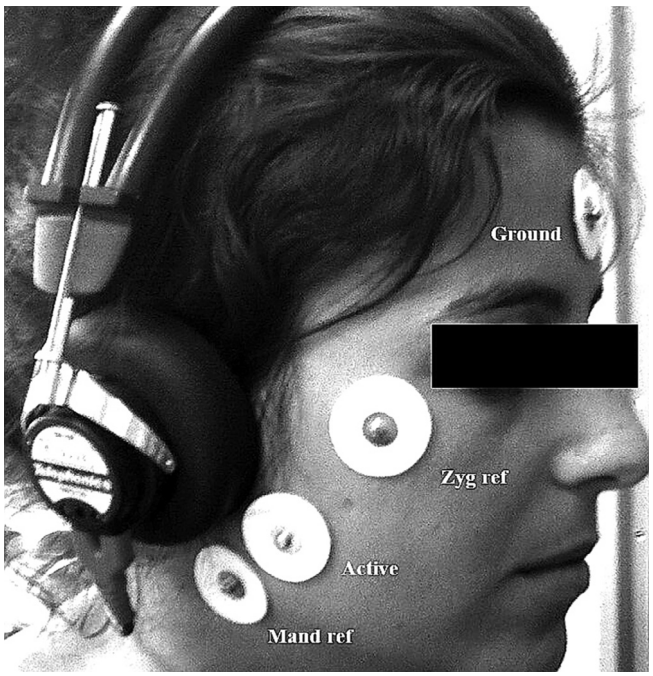

Fig. 1. Position of the electrodes for the recording of the acoustic-masseter reflex and of the vestibular-masseteric reflex in two different belly-to-tendon montages. The active electrode (Active) is positioned in the lower third of the masseter muscle and two reference electrodes are placed one at 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.

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. For each subject, 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, measured in the unrectified traces, namely, when they were >2SD of the pre-stimulus unrectified mean EMG (group average: 10.426 ± 5.122 μV in the zygomatic montage and 7.355 ± 3.779 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 rectified EMG activity in the 50 ms before the stimulus, since the response scales with the level of tonic activity (Deriu et al 2005). 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 and Colebatch, 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 Fig. 2.

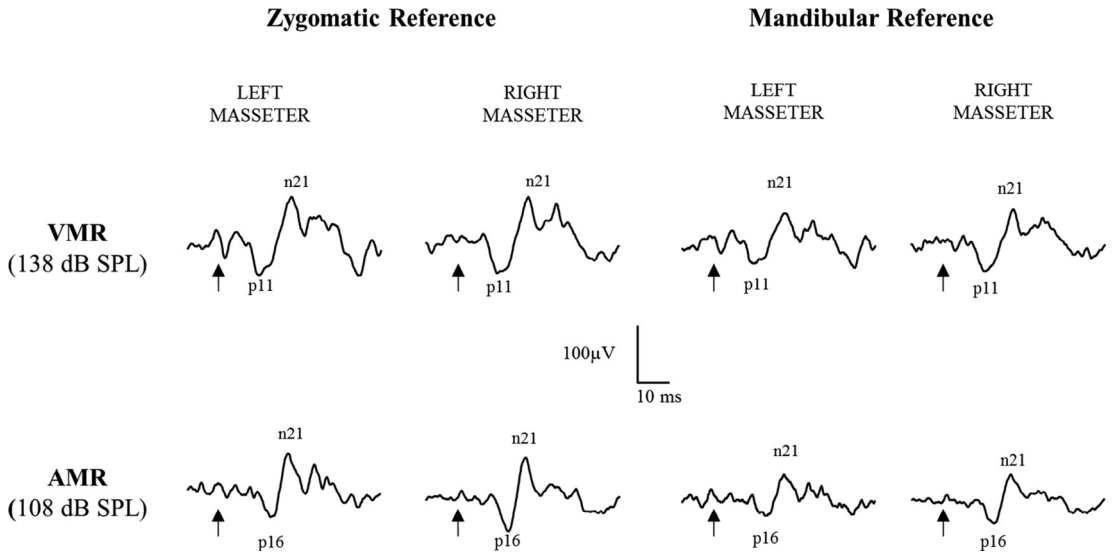

Fig. 2. VMR and AMR recorded in a representative subject. Averaged (n = 300 sweeps) unrectified EMG responses to the stimulation of the right ear (arrow) were recorded from active masseter muscles bilaterally. The VMR appears as a bilateral and symmetric p11 wave followed by an acoustic n21 wave. The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

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

Fig. 3 describes masseter responses to different click intensities. Recordings from a representative subject are shown in Fig. 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

Table 1
Characteristics of masseter muscle responses to clicks of 138 dB SPL intensity (VMR), recorded in 62 subjects (aged ≤55 years) in the mandibular and zygomatic electrode configurations.

MM parameters	Mandibular Montage					Zygomatic Montage					*Mand vs Zyg (Unilateral) p*	*Mand Vs Zyg (Bilateral) p*
	Unilateral		*Ipsi vs Contra p*	Bilateral	*Uni* vs Bil p*	Unilateral		*Ipsi vs Contra p*	Bilateral	*Uni vs Bil p*		
	Ipsilateral MM	Contralateral MM		Ipsi and Contra MM		Ipsilateral muscle	Contralateral muscle		Ipsi and Contra MM			
Frequency (ears)	98/124 (79.0%)	100/124 (80.6%)	*ns*	100/124 (80.6%)	*ns*	120/124(96.8%)	120/124 (96.8%)	*ns*	124/124 (100%)	*ns*	*<0.0001*	*<0.0001*
Onset (ms)	8.68 ± 1.15	8.86 ± 1.04	*ns*	8.55 ± 1.0	*ns*	8.52 ± 1.52	8.42 ± 1.14	*ns*	8.1 ± 1.01	*0.008*	*0.009*	*0.001*
p11 latency (ms)	11.37 ± 0.91	11.5 ± 0.87	*ns*	11.38 ± 0.91	*ns*	11.17 ± 0.98	11.38 ± 0.9	*ns*	11.19 ± 0.87	*ns*	*ns*	*ns*
n21 latency (ms)	19.75 ± 1.61	19.5 ± 1.84	*ns*	20.09 ± 2.06	*0.04*	19.68 ± 1.81	19.53 ± 1.9	*ns*	20.1 ± 2.07	*0.02*	*ns*	*ns*
Interpeak latency (ms)	8.38 ± 1.65	7.99 ± 1.73	*ns*	8.7 ± 1.97	*0.02*	8.52 ± 1.78	8.15 ± 1.87	*ns*	8.91 ± 1.97	*ns*	*ns*	*ns*
p11 raw amplitude (μV)	36.03 ± 24.9	36.53 ± 24.51	*ns*	48.71 ± 32.15	*0.001*	43.36 ± 26.26	47.54 ± 27.41	*ns*	66.7 ± 42.78	*<0.0001*	*<0.0001*	*<0.0001*
Mean rectified EMG (μV)	80.32 ± 39.76	79.48 ± 37.32	*ns*	80.08 ± 32.71	*ns*	104.31 ± 41.66	102.00 ± 40.96	*ns*	105.09 ± 39.66	*ns*	*<0.0001*	*<0.0001*
p11 corrected amplitude	0.46 ± 0.25	0.48 ± 0.28	*ns*	0.62 ± 0.33	*<0.0001*	0.46 ± 0.18	0.49 ± 0.2	*ns*	0.68 ± 0.28	*<0.0001*	*ns*	*ns*
p11/n21 raw amplitude (μV)	52.47 ± 33.5	50.69 ± 30.47	*ns*	70.57 ± 41.33	*<0.0001*	66.8 ± 41.98	72.23 ± 46.37	*ns*	99.05 ± 64.0	*<0.0001*	*<0.0001*	*<0.0001*
p11-n21 corrected amplitude	0.68 ± 0.33	0.68 ± 0.35	*ns*	0.92 ± 0.42	*<0.0001*	0.72 ± 0.31	0.74 ± 0.31	*ns*	1.0 ± 0.38	*<0.0001*	*ns*	*ns*
p11 latency interside difference (ms)	0.57 ± 0.39			0.49 ± 0.32	*ns*	0.59 ± 0.36			0.53 ± 0.36	*ns*	*ns*	*ns*
p11 latency asymmetry	2.5 ± 1.6			2.4 ± 1.6	*ns*	3.2 ± 2.4			2.6 ± 2.1	*ns*	*ns*	*ns*
p11 corrected amplitude asymmetry	17.91 ± 13.55			16.96 ± 14.06	*ns*	14.56 ± 11.8			13.27 ± 11.65	*ns*	*ns*	*ns*

VMR = Vestibular Masseteric Reflex; MM = masseter muscle; Unilateral and Uni = unilateral stimulation; Bilateral and Bil = bilateral stimulation; Ipsi = Ipsilateral; Contra = contralateral; Zygomatic and Zyg = zygomatic electrode montage; Mandibular and Mand = mandibular electrode montage; *ipsi- and contralateral responses pooled; *p = p* value, *ns* = non-significant; Statistics: chi-squared test for comparisons between frequencies; Mann-Whitney U-test for comparison between means.

Table 2
Characteristics of masseter muscle responses to clicks of 108 dB SPL intensity (AMR), recorded in 62 subjects (aged ≤ 55 years) in the mandibular and zygomatic electrode configurations.

MM Parameters	Mandibular Montage					Zygomatic Montage					*Mand vs Zig (Unilateral)**	*Mand vs Zig (Bilateral) p*
	Unilateral		*Ipsi vs Contra p*	Bilateral	*Uni* vs Bil p*	Unilateral		*Ipsi vs Contra p*	Bilateral	*Uni vs Bil p*		
	Ipsilateral MM	Contralateral MM		Ipsi and contra MM		Ipsilateral MM	Contralateral MM		Ipsi and contra MM			
Frequency (ears)	75/124 (60.5%)	69/124 (55.6%)	*ns*	92/124 (75.8%)	*0.035*	106/124 (85.5%)	106/124 (85.5%)	*ns*	112/124 (90.3%)	*ns*	*<0.0001*	*0.003*
Onset (ms)	12.34 ± 1.58	12.27 ± 1.83	*ns*	11.95 ± 1.48	*ns*	12.63 ± 1.69	12.5 ± 1.66	*ns*	12.05 ± 1.62	*0.008*	*ns*	*ns*
p16 latency (ms)	15.47 ± 1.04	15.63 ± 1.23	*ns*	15.24 ± 0.99	*0.04*	15.62 ± 0.91	15.55 ± 1.12	*ns*	15.3 ± 1.02	*0.02*	*ns*	*ns*
n21 latency (ms)	20.3 ± 1.69	20.59 ± 1.72	*ns*	20.23 ± 1.62	*ns*	20.5 ± 1.7	20.46 ± 1.8	*ns*	20.22 ± 1.67	*ns*	*ns*	*ns*
Interpeak latency (ms)	4.8 ± 1.5	5.0 ± 1.5	*ns*	4.99 ± 1.39	*ns*	4.9 ± 1.5	4.97 ± 1.56	*ns*	4.91 ± 1.31	*ns*	*ns*	*ns*
p16 raw amplitude (μV)	20.44 ± 13.18	21.4 ± 11.31	*ns*	26.46 ± 18.82	*0.015*	23.04 ± 15.98	24.91 ± 19.34	*ns*	32.68 ± 23.07	*<0.0001*	*ns*	*0.04*
Mean rectified EMG (μV)	74.48 ± 44.66	75.79 ± 40.87	*ns*	76.50 ± 42.15	*ns*	91.54 ± 59.53	94.25 ± 65.05	*ns*	95.37 ± 58.03	*ns*	*<0.0001*	*<0.0001*
p16 corrected amplitude	0.29 ± 0.13	0.29 ± 0.1	*ns*	0.36 ± 0.2	*0.005*	0.26 ± 0.14	0.27 ± 0.15	*ns*	0.35 ± 0.2	*<0.0001*	*ns*	*ns*
p16/n21 raw amplitude (μV)	29.24 ± 18.67	30.41 ± 16.04	*ns*	37.44 ± 22.3	*0.007*	37.79 ± 23.4	41.13 ± 28.9	*ns*	51.97 ± 34.56	*<0.0001*	*<0.0001*	*0.01*
p16-n21 corrected amplitude	0.45 ± 0.24	0.43 ± 0.22	*ns*	0.51 ± 0.23	*0.03*	0.43 ± 0.21	0.45 ± 0.22	*ns*	0.56 ± 0.28	*<0.0001*	*ns*	*ns*
p16 interside latency difference (ms)	0.6 ± 0.59			0.57 ± 0.42	*ns*	0.71 ± 0.61			0.53 ± 0.5	*ns*	*ns*	*ns*
p16 latency asymmetry	1.9 ± 1.4			2.1 ± 1.3	*ns*	2.5 ± 2.0			2.6 ± 2.1	*ns*	*ns*	*ns*
p16-n21 corrected amplitude asymmetry	17.9 ± 13.25			21.10 ± 12.76	*ns*	16.84 ± 12.62			16.06 ± 10.59	*ns*	*ns*	*ns*

AMR = Acoustic Masseter Reflex; MM = masseter muscle; Unilateral and Uni = unilateral stimulation; Bilateral and Bil = bilateral stimulation; Ipsi = Ipsilateral; Contra = contralateral; Zygomatic and Zyg = zygomatic electrode montage; Mandibular and Mand = mandibular electrode montage; *ipsi- and contralateral responses pooled; *p* = *p* value, *ns* = non-significant; Statistics: chi-squared test for comparisons between frequencies; Mann-Whitney U-test for comparison between means.

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 stimulation. 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 of mono- o binaural stimulations. Although statistically significant, the gender difference found was quite small in terms of absolute values (average difference: 0.4 ms for the p11, 0.5 ms for the p16 and 1.0 ms for the n21).

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

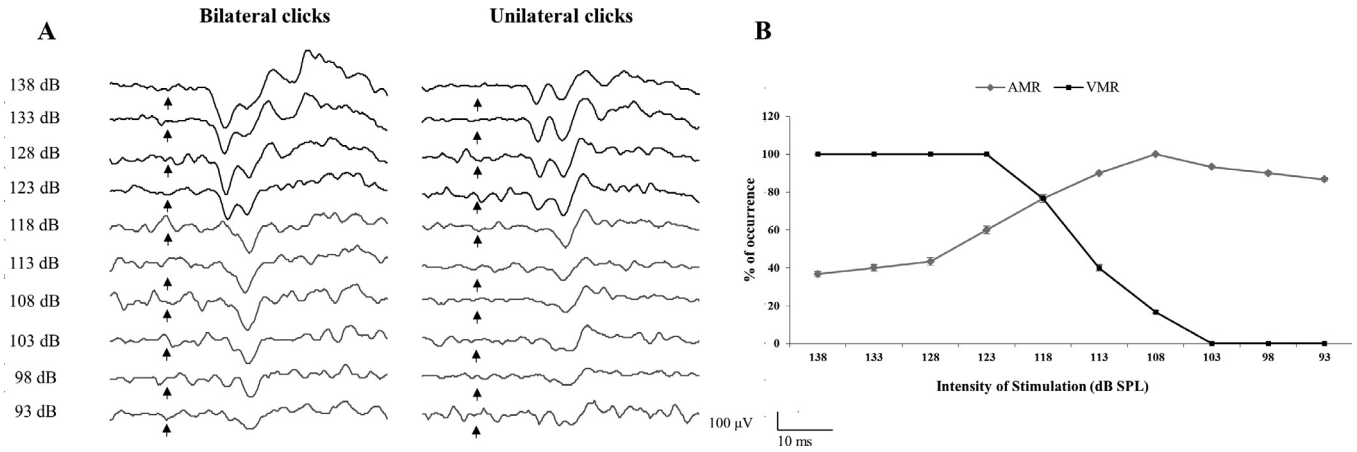

Fig. 3. VMR and AMR elicited by different intensities, in steps of 5 dB. **A.** Unrectified EMG recordings from a representative subject. **B.** Mean ± standard error of the percentage of reflex elicitation rate in 10 subjects. No differences in the percentage of elicitation of either reflex for unilateral 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).

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 result from the larger volume of muscle recorded in the zygomatic montage and, possibly, in the detection of a higher number of motor units involved in the reflex, with a consequent larger raw amplitude and a parallel higher pre-stimulus EMG activity. 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.

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).

For both VMR and AMR the detection rate was significantly higher in the zygomatic than mandibular montage. This may be due to the larger IED in the former configuration. 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, while 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, 2007, 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, 2015b, 2018; Magnano et al., 2016) 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. $\leq$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.

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, 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.

Table 3
Comparison of VMR and AMR parameters between age groups (n = 10 subjects) following unilateral and bilateral click stimulation.

		Unilateral Click Stimulation				Bilateral Click Stimulation			
Click intensity	Age category (years old)	Detection rate of the p11 wave (ears)	p11peak latency (ms)	n21peak latency (ms)	p11 corrected amplitude	Detection rate of the p11 wave (ears)	p11peak latency (ms)	n21peak latency (ms)	p11 corrected amplitude
138 dB SPL	<25	20/20 (100%)	11.37 ± 0.74	19.82 ± 1.13	0.60 ± 0.15	20/20 (100%)	11.22 ± 0.44	20.66 ± 2.06	0.87 ± 0.21
	26–35	20/20 (100%)	11.42 ± 0.76	19.88 ± 2.31	0.49 ± 0.21	20/20 (100%)	11.10 ± 0.53	20.98 ± 1.96	0.82 ± 0.38
	36–45	20/20 (100%)	11.38 ± 0.82	19.78 ± 2.02	0.50 ± 0.19	20/20 (100%)	11.41 ± 0.84	20.72 ± 2.82	0.7 ± 0.26
	46–55	18/20 (90%)	11.42 ± 0.98	20.03 ± 1.76	0.48 ± 0.25	18/20 (90%)	11.33 ± 1.17	19.72 ± 1.49	0.66 ± 0.34
	56–65	18/20 (90%)	11.97 ± 0.97*	20.76 ± 2.24	0.40 ± 0.14***	16/20 (80%)	11.55 ± 1.05	20.09 ± 1.56	0.56 ± 0.29*
	>65	16/20 (80%)	12.16 ± 0.93**	19.19 ± 2.36	0.39 ± 0.18***	16/20 (80%)	11.46 ± 0.76	20.12 ± 1.85	0.56 ± 0.23*
p		χ^2 = 10.714	F = 5.628	F = 2.189	F = 5.389	χ^2 = 12.655	F = 0.758	F = 1.142	F = 4.056
		p = 0.057	p < 0.0001	p = 0.06	p < 0.0001	p = 0.03	p = 0.582	p = 0.343	p = 0.02
Click intensity	**Age category (years old)**	**Detection rate of the p16/n21 wave (ears)**	**p16 peak latency (ms)**	**n21peak latency (ms)**	**p16/n21 corrected amplitude**	**Detection rate of the p16/n21 wave (ears)**	**p16 peak latency (ms)**	**n21peak latency (ms)**	**p16-n21 corrected amplitude**
108 dB SPL	<25	20/20 (100%)	15.25 ± 0.81	19.81 ± 1.26	0.48 ± 0.20	20/20 (100%)	14.79 ± 0.62	19.67 ± 0.51	0.74 ± 0.19
	26–35	20/20 (100%)	15.61 ± 0.85	20.55 ± 1.58	0.44 ± 0.21	20/20 (100%)	15.33 ± 0.90	20.60 ± 1.45	0.61 ± 0.21
	36–45	20/20 (100%)	15.80 ± 0.65	20.67 ± 1.09	0.45 ± 0.27	20/20 (100%)	15.6 ± 1.07	20.77 ± 1.45	0.56 ± 0.29
	46–55	16/20 (80%)	15.78 ± 0.90	20.66 ± 1.16	0.42 ± 0.15	18/20 (90%)	15.53 ± 0.70	20.55 ± 1.23	0.55 ± 0.22
	56–65	16/20 (80%)	16.50 ± 0.81***	21.25 ± 1.04**	0.35 ± 0.08**	16/20 (80%)	15.89 ± 1.2**	21.22 ± 1.06**	0.47 ± 0.17***
	>65	16/20 (80%)	16.59 ± 1.22***	21.34 ± 1.99**	0.33 ± 0.10**	16/20 (80%)	15.83 ± 0.91**	21.30 ± 1.30**	0.47 ± 0.25***
p		χ^2 = 13.333	F = 12.588	F = 6.199	F = 3.325	χ^2 = 12.655	F = 3.811	F = 4.547	F = 4.018
		p = 0.02	*p < 0.0001*	*p < 0.0001*	*p = 0.007*	p = 0.03	*p = 0.003*	*p = 0.001*	*p = 0.002*

Differences between frequencies are calculated with $\chi 2$ test. Difference between means are calculated with to-way ANOVA. Post-hoc Tukey test: * = <0.05; ** = <0.01; *** = <0.001.

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 and Colebatch, 2001; Basta et al., 2007; Brantberg et al., 2007; Janky and Shepard, 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 and Hawkins, 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 and Sheldrake, 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, this difference was questioned by a more recent anatomical work (Miller, 2007). In disagreement with our findings, studies on cVEMP (Ochi and Ohashi, 2003) and oVEMP (Sung et al., 2011; Versino et al., 2015) failed to demonstrate a gender difference. Additionally, caloric response is not different between genders, suggesting that no difference between males and females exists in the peripheral vestibular pathway. It should be considered that although the gender difference found in our study was statistically significant, the absolute difference was quite small, which makes difficult its biological interpretation. 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, 2015b, 2018; Magnano et al., 2016; de Natale et al. 2016). 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

Table 4
Comparison of VMR and AMR parameters according to gender.

Clicks intensity	Reflex parameters	Unilateral Stimulation			Bilateral Stimulation		
		Men	Women	*p*	Men	Women	*p*
138 dB SPL	onset (ms)	8.67 ± 1.3	8.56 ± 1.4	*n.s.*	8.14 ± 1.09	8.12 ± 0.91	*n.s.*
	p11 peak latency (ms)	11.65 ± 1.0	11.25 ± 0.9	*<0.0001*	11.36 ± 1.0	11.09 ± 0.7	*0.045*
	n21 peak latency (ms)	20.24 ± 2.2	19.17 ± 1.7	*<0.0001*	20.54 ± 1.9	19.65 ± 1.9	*0.004*
	p11 corrected amplitude	0.5 ± 0.2	0.5 ± 0.2	*n.s.*	0.67 ± 0.27	0.65 ± 0.29	*n.s.*
	p11/n21 corrected amplitude	0.7 ± 0.3	0.7 ± 0.3	*n.s.*	0.99 ± 0.37	0.99 ± 0.42	*n.s.*
108 dB SPL	onset (ms)	12.91 ± 2.0	12.73 ± 1.5	*n.s.*	12.25 ± 1.7	12.06 ± 1.47	*n.s.*
	p16 peak latency (ms)	15.95 ± 1.1	15.64 ± 1.0	*0.024*	15.81 ± 1.1	15.14 ± 0.90	*0.0001*
	n21 peak latency (ms)	21.20 ± 1.5	20.21 ± 1.8	*0.0001*	21.00 ± 1.4	19.96 ± 1.5	*0.0001*
	p16 corrected amplitude	0.26 ± 0.1	0.26 ± 0.1	*n.s.*	0.37 ± 0.20	0.37 ± 0.24	*n.s.*
	p16/n21 corrected amplitude	0.44 ± 0.2	0.43 ± 0.2	*n.s.*	0.60 ± 0.32	0.60 ± 0.40	*n.s.*

*p values refer to Mann-Whitney U-test.

counterbalanced by the preservation of the VMR response on the corresponding 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 standard electromyographic techniques, with sound stimuli intensity in the range for evoking cochlear as well as vestibular responses.

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Conflict of interest

The authors declare no conflicts of interest.

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