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Vestibular-evoked myogenic potentials in miniature pigs

Xi Shi [a], Yan Zhang [b], Ya Li [a], Shiwei Qiu [a], Shili Zhang [a], Yaohan Li [a], Na Yuan [a], Yuehua Qiao [a,d,*], Shiming Yang [c,**]

[a] *Institute of Audiology and Balance Science, Xuzhou Medical University, China*
[b] *Department of Otorhinolaryngology Head and Neck Surgery, First Hospital of Jilin University, Changchun, Jilin 130021, China*
[c] *Department of Otolaryngology—Head & Neck Surgery, Institute of Otolaryngology of PLA, Chinese PLA General Hospital, Beijing 100853, China*
[d] *Clinical Hearing Center, Affiliated Hospital of Xuzhou Medical University, China*

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Abstract

Objective: To report detection of vestibular-evoked myogenic potentials (VEMPs) in the miniature pig.
Methods: Potentials evoked by 1000 Hz tone bursts were recorded from neck extensor muscles and the masseter muscles in normal adult Bama miniature pigs anesthetized with 3% pentobarbital sodium and Carbachol II.
Results: The latency of the first positive wave P from neck extensor muscles was 7.65 ± 0.64 ms, with an amplitude of 1.66 ± 0.34 uv and a rate of successful induction of 75% at 80 dB SPL. The latency of potentials evoked from the masseter muscles was 7.60 ± 0.78 ms, with an amplitude of 1.31 ± 0.28 uv and a rate successful induction of 66% at 80 dB SPL.
Conclusion: The latencies and thresholds of VEMPs recorded from the neck extensor muscle and the masseter muscle appear to be comparable in normal adult Bama miniature pigs, although the amplitude recorded from the neck extensor muscle seems to be higher than that from the masseter muscle. However, because of their usually relatively superficial and easily accessible location, as well as their large volume and strong contractions, masseter muscles may be better target muscles for recording myogenic potentials.

Keywords: Vestibular evoked myogenic potentials; Miniature pig; Masseter myogenic potential

1. Introduction

Vestibular-evoked myogenic potentials (VEMPs) is an objective and accurate method to assess vestibular functions via recording the myogenic potential from the sternocleidomastoid muscle evoked by high intensity acoustic stimulation of the ipsilateral saccule (Shimizu et al., 2000). VEMPs may be used for diagnoses and prognoses purposes with high specificity in a number of diseases including sudden hearing loss, Meniere's disease and vestibular neuritis. VEMPs are divided into ocular vestibular-evoked myogenic potentials (oVEMPs) and cervicalves vestibular-evoked myogenic potentials (cVEMPs), depending on the specific neural transmission pathway.

Miniature pigs are comparable to humans in both anatomy and physiology. Advantages of using miniature pigs include their relatively light bodyweight, slow rate of growth and overall convenience in experimental operations. They have been used in studies involving tumors, cardiovascular diseases, diabetes, oral surgeries, plastic surgeries, hematologic diseases, genetic and metabolic diseases, and drug safety

* Corresponding author. Clinical Hearing Center, Affiliated Hospital of Xuzhou Medical University, China.
** Corresponding author. Tel.: +86 1 6821 1696.
E-mail addresses: oto8558@163.com (Y. Qiao), yangsm301@263.net (S. Yang).

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evaluation (Guo et al., 2015). The miniature pig is now beginning to be applied to inner ear studies. Compared to other rodent animals, the miniature pig has unique advantages in terms of ear anatomy: (1) The miniature pig has a complete middle ear and mastoid air cells system. (2) Its ear morphology, ossicular chain size and cochlear microstructure are similar to those of humans. (3) The morphology and structures of its vestibular system are also similar to those of humans. These make the miniature pig the best animal model for VEMP testing. While a few studies have reported VEMPs in guinea pigs and mice, none have applied the test to miniature pigs. This study examines VEMPs in the miniature pig.

2. Materials and methods

2.1. Experimental animals

Six months old female Bama pigs (n = 12, weight 25 kg) with completely normal hearing and balance functions were used for VEMP tests.

2.2. Anesthesia

Pentobarbital sodium, which has no effect on muscular resistance, was combined with Carbachol II for anesthesia: Carbachol II was injected into the neck muscle (0.1 ml/kg) and 3% pentobarbital sodium was administered through intravenous injection (1 ml/kg) at 2 ml/min. Anesthesia took effect within 10 min, marked by loss of corneal reflex. Because anesthesia would induce muscle relaxation, which affects the contraction of skeletal muscles, VEMPs were recorded from the same muscle under both relaxation and tension conditions for comparison. ABR tests were performed to ensure that there were no significant contaminations by hearing responses (Weiwei et al., 2015).

2.3. VEMP and ABR tests

VEMPs recorded from the sternocleidomastoid muscle are commonly used in the clinic to evaluate vestibular functions, especially saccular functions. This method excludes elderly patients and patients with cervical diseases because it requires extensive contraction of the sternocleidomastoid muscle. The miniature pig neck extensor muscle is similar with the human sternocleidomastoid muscle in anatomy and function. The neck extensor muscle is the thickest muscle on both sides of the neck. It originates from the humerus crest and terminates at the occipital bone and petrous. Due to thick adipose tissue found around the miniature pig neck, the neck extensor muscle is difficult to access. Recently, the masseter muscle has been used to record VEMPs in elderly patients and those with cervical spondylosis for vestibular reflex evaluation. Xie (Sujiang, 2007) recorded masseter myogenic potentials evoked in awake guinea pigs. The miniature pig masseter muscle starts in the cheekbone and ends in the lateral surface of the mandible, covered by facial muscle and facial nerve. It is a short and thick muscle in an approximately square shape. The deep surface of the masseter muscle is lateral to the mandibular ramus (Zhu et al., 2004). The masseter muscle is reasonably easy to access. In this study, we recorded VEMPs from both the neck extensor and masseter muscles in the miniature pig.

The SmartEP (Intelligent Hearing Systems, USA) was used to record the muscle potential in an open sound field with a 30-3 kHz band-pass filter setting and 30 k EMG signal amplification. The analysis window was 50 ms and electrode resistance <3 kΩ, with superposition of 128 responses to short sound stimulation at 1000 Hz. Stimulus intensity started at 120 dB SPL and was decreased in 5 dB increments until the reproducible waveform disappeared. Two consecutive records were acquired to ensure repeatability. A custom-made silver needle electrode (silver nickel alloy of 90% purity, 0.9 × 50 mm) covered with Teflon was used for recording. A current meter was used to test the electrode.

2.3.1. Neck extensor muscle VEMPs

VEMPs were recorded from the neck extensor muscle first in a relaxed state. The miniature pig was then positioned on the side on the test bench with the head turned to the other side at a 45° angle. A special device was used to maintain tension in the neck extensor muscles, prior to measurement of myogenic potentials. To prevent airway blockage, the tongue was pulled out of the mouth before the test. The recording electrode was placed at the bifurcation of the neck extensor muscle. The reference electrode was inserted into the top of the head at the surface of the ridge crest periosteum. The grounding electrode is inserted into the proboscis (2 cm in depth, <3 kΩ in resistance). VEMP waveforms under various sound stimulus intensities were visually examined and the threshold was determined as the stimulus intensity associated with the disappearance of the first positive P wave.

2.3.2. Masseter muscle VEMPs

Similar with neck extensor muscle VEMP recording, masseter muscle VEMPs were also first recorded in a relaxed condition. A roll of gauze wrapped around a stick was then placed in the mouth to create tension in the masseter muscle before recording myogenic potentials was repeated. The recording electrodes was inserted roughly a third of the way into the masseter muscle on the test side. Reference and ground electrodes placement was similar to neck extensor muscle VEMP recording. VEMP waveform examination and threshold determination were also similar to those for neck extensor muscle VEMPs.

2.3.3. Auditory brainstem response

The recording electrode was inserted into the top of the skull. The reference electrode was placed on the recording side earlobe. The ground electrode was connected to the nose tip. Impedance between electrodes was less than 3 kΩ. Clicks stimulation was started at 100 dB SPL and decreased to 20 dB SPL by 10 dB steps. Filter setting was 100–3000 Hz. ABR threshold was the stimulus intensity associated with disappearance of wave V.

3. Results

Evoked potential could be recorded neither in neck muscle nor in masseter muscle (Fig. 1), which may result from a relaxed state. After we changed the head position and managed to make the animal bite a stick to increase muscle tension (Fig. 3), the VEMP was successfully recorded from the neck (Fig. 2A) and masseter extensor muscles (Fig. 2B). Meanwhile, the detected ABRs showed that the miniature pigs with normal hearing were usded in this study (Fig. 4).

4. Discussion

VEMP testing is commonly used in the evaluation of neurotologic diseases including acoustic neuroma, Meniere's disease, vestibular neuritis, superior canal dehiscence syndrome, multiple sclerosis, benign paroxysmal positional vertigo, vestibular migraine, and other peripheral or central vestibular disorders. The amplitude of the VEMP from human skeletal muscles is positively correlated with the level of muscle tension (Deriu et al., 2003, 2005). Variation of the absolute amplitude appears to only be affected by muscle tension and not related to vestibular function (Sun et al., 2005). Due to limitations on recording conditions, we were unable to adequately determine the intensity of background electric activities in the muscle, and therefore elected not to elaborate on VEMP amplitudes in this study. Measures of VEMPs include threshold, latency and P13-N23 amplitude. A number of factors can affect VEMP peak amplitude and latency, including gender, age, muscle characteristics, subcutaneous fat thickness, skin electrical impedance, stimulation intensity, electrode placement, testing equipment, etc.

In our study, when stimulation intensity was decreased, only the amplitude decreased while the latency remained unchanged. P13 latencies from the two muscles were between 7 and 9 ms and N23 latency was 10 ms. The resulting P wave showed good repeatability and stability. The N wave (negative phase wave) was weak, possibly due to low muscle tension.

To rule out contamination by auditory responses, ABR was tested in addition to VEMPs. Under high intensity sound stimulation, the latency of wave V was 5–6 ms and the threshold was about 25 dB SPL, significantly different than VEMP latencies (7–9 ms) and thresholds (about 80 dB SPL) and effectively excluding the possibility of contamination by auditory potentials on VEMPs. The anesthetic drugs used in this study can induce muscle relaxation, which affects contraction of skeletal muscles in experimental animals. When we first recorded VEMPs under the condition muscle relaxation, obtained responses showed good repeatability with relatively consistent baselines, although with no obvious P waves (Fig. 1). By increasing muscle tension using a custom-made device, a distinct positive wave P were recorded with a latency of 7–9 ms, although the amplitude was relatively small. These results indicate that muscle tension in the recording position is very important in VEMP tests (Yang and Young, 2005).

The effect of anesthetics on VEMPs in miniature pig needs to be further studied, as no suitable VEMP methods are available for testing in unanesthetized pigs. The effect of anesthesia on VEMP tests plays a crucial role. While under anesthesia, miniature pigs display decreased EEG activities and reduced muscle movement in the head, snout and limbs. Muscle movement results in inaccurate VEMP measurements and should be minimized. Such muscle movements are observed when 3% sodium pentobarbital is used as the anesthetic agent regardless of administration techniques. Carbachol II, a muscle relaxant and weak analgesic, is used in studies to minimize disturbance to EMG measurements. It does have an inhibitory effect on respiration and the heart, and increases salivary glands and trachea glands secretion (Shang,

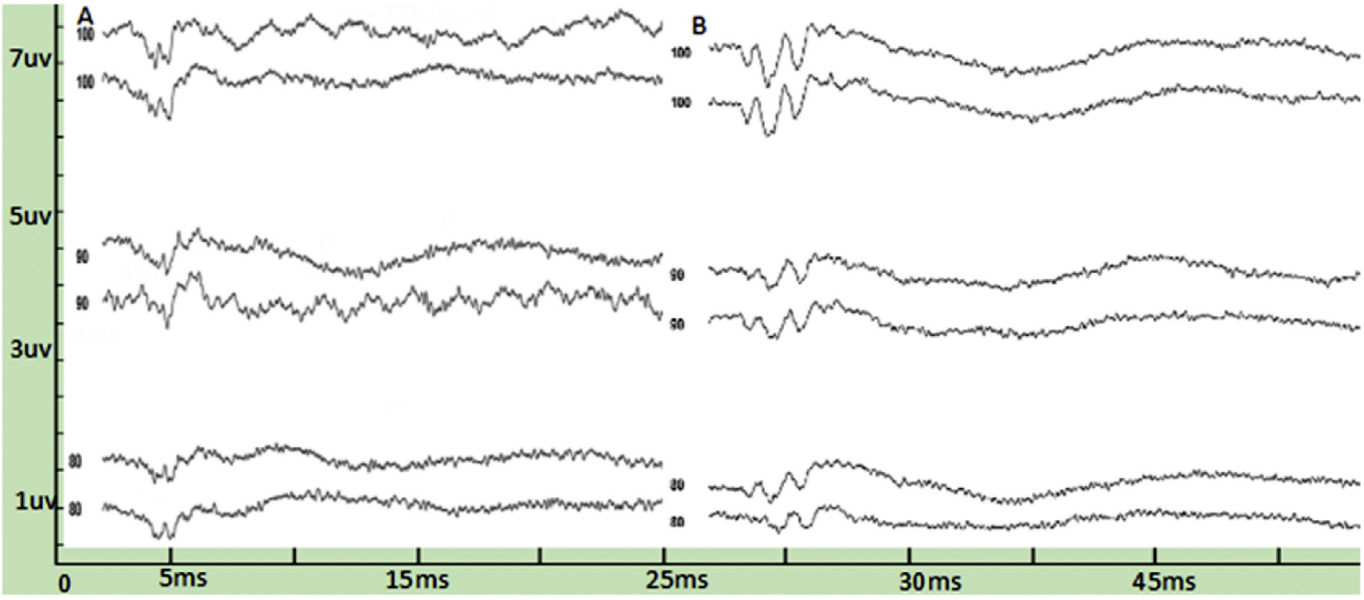

Fig. 1. Responses when neck extensor (A) and masseter (B) muscles are relaxed, showing no obvious P waves.

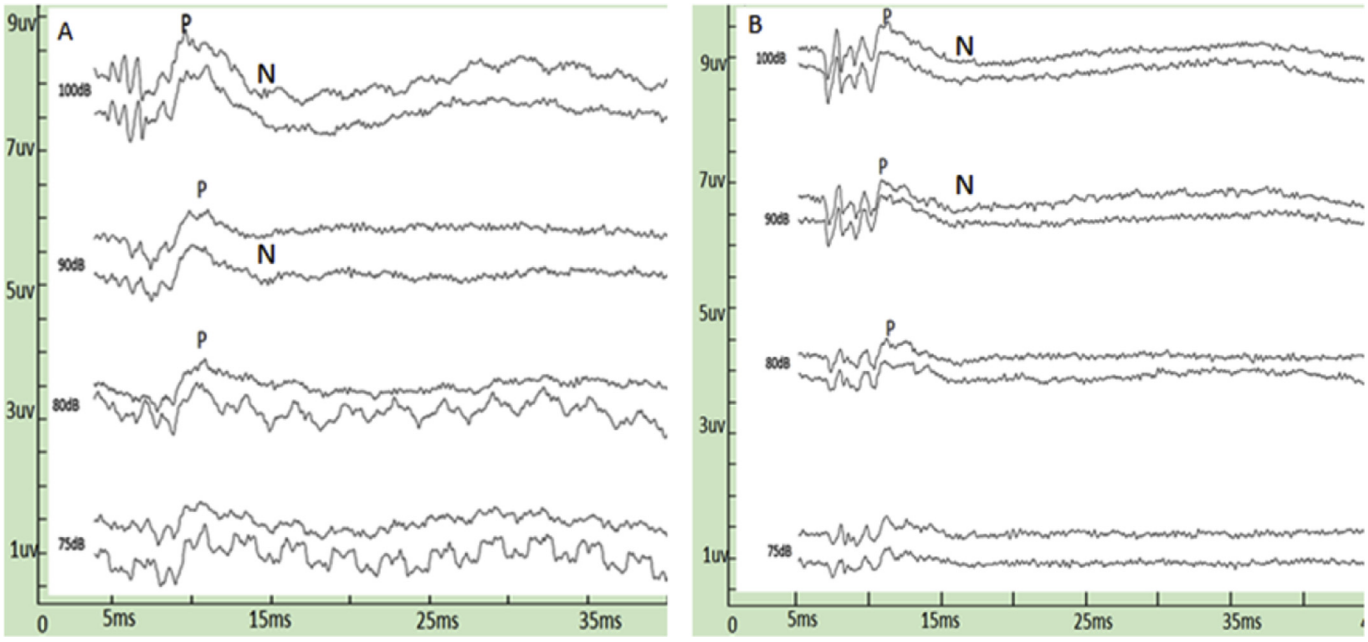

Fig. 2. A: Neck extensor muscle VEMPs in a 6 months old female miniature pig. The latency of the first positive wave is 7.52 ms, with an amplitude of 1.50 uv and a threshold of 75 dB SPL. The latency of the first negative wave is 9.87 ms. B: Masseter muscle VEMPs in a 6 months old female miniature pig. The latency of the first positive wave is 7.63 ms, with an amplitude of 1.60 uv and a threshold of 75 dB SPL. The latency of the first negative wave is 11.63 ms.

Fig. 3. Positioning of miniature pigs during testing.

1992). Airway must therefore be carefully maintained throughout the experiment. The inhibitory effect of sodium pentobarbital on the respiratory system is related to dosage and injection speed. Faster rates result in higher incidence of respiratory depression (Colebatch and Halmagyi, 2000). Therefore, body temperature, respiratory rate, heart rate and skin color of the experimental animals must be observed closely throughout testing. In case of an abnormal situation, it is necessary to intervene in a timely manner. The duration of anesthesia is related to the location and method of

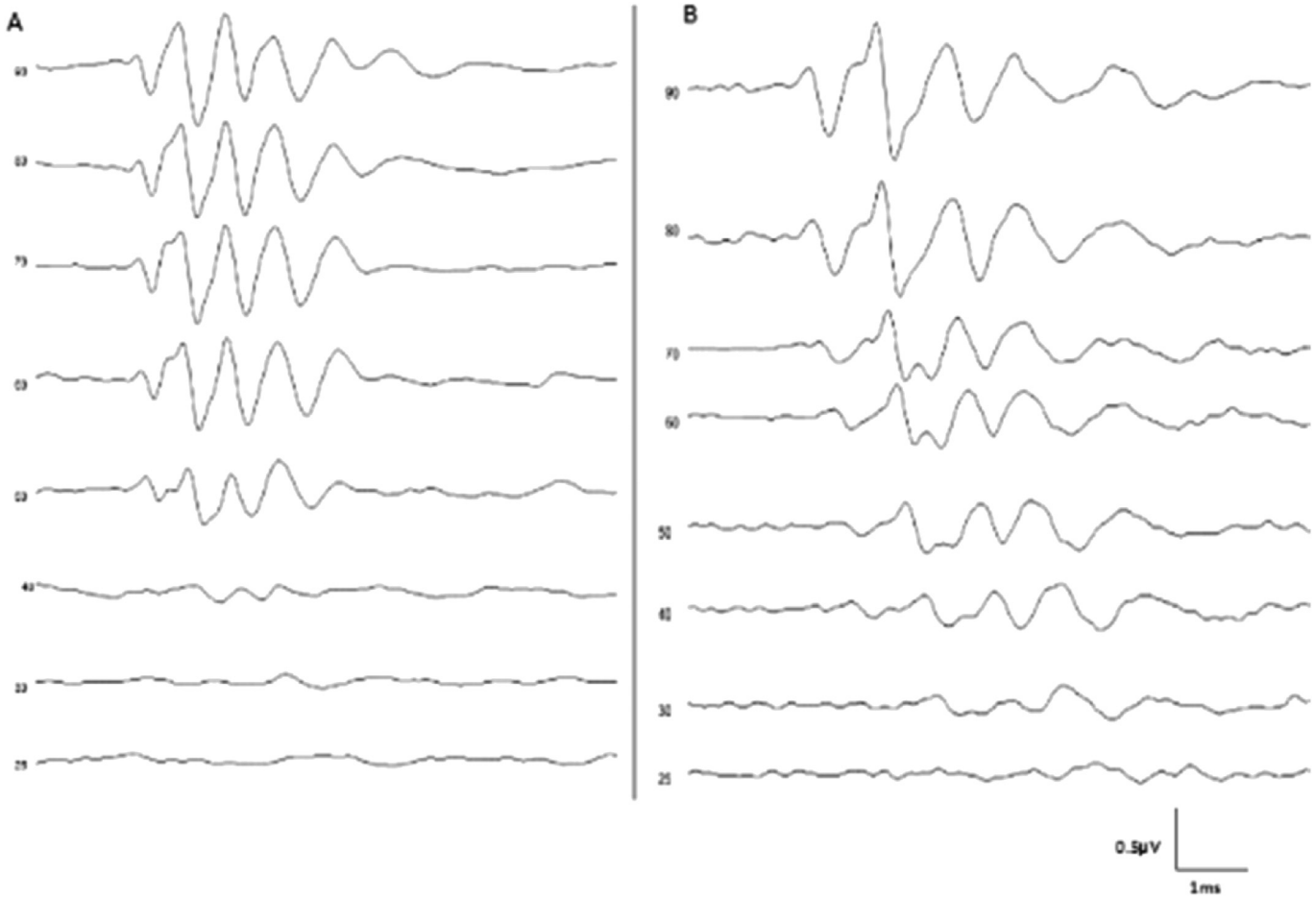

Fig. 4. Normal ABRs in miniature pigs. Threshold is approximately 25 dB SPL for both A and B.

administration. Generally, administration to the neck muscles can be maintained for 3–4 h. In the process of anesthesia, dosage should be strictly controlled and should err on the side of caution (Ye et al., 2000).

Under the same intensity of sound stimulation, neuromuscular reflex excitability is closely related to the degree of muscle tension. In order to maintain muscle strain, a specific device was used to provide muscle tension in the recording area during the experiment. For intense sound evoked myogenic potentials, latency may be related to the speed of nerve conduction. The latency of clicks evoked masseter muscle VEMPs is 6–8 ms in miniature pigs, similar with that of neck extensor muscle VEMPs. This is shorter than masseter muscle response latency in humans (Deriu et al., 2005; Xie et al., 2006) but slightly longer than neck extensor muscle response latency in rats (Xie et al., 2006; Sakakura et al., 2003).

This study shows that the latencies of VEMPs recorded from the neck extensor muscle and the masseter muscle are similar, while the amplitude of VEMPs from the neck extensors is slightly higher than that of VEMPs from the masseter muscle. This discrepancy is likely related to differing degrees of muscle tension. Neck extensor muscles are not easy to locate because of overlying thick adipose tissue, making it difficult to consistently induce muscle tension. In the recording process, experimental animals are prone to unexpected shortness of breath, producing more difficulty in recording VEMPs. The superficial masseter muscle is easy to locate and to consistently induce muscle tension, providing more precise and accurate VEMP measurements. In addition to the sternocleidomastoid muscle and masseter muscle, similar testing may also be done from the trapezius muscle (Sujiang, 2007). There have also been previous studies which recorded VEMPs in the arm or leg (Ferber-Viart et al., 1997). There are several techniques of recording VEMPs in miniature pigs. Further research is needed to determine the optimal experimental method for recording VEMPs.

Conflict of interest

There is no any conflict of interest.

Acknowledgments

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