



Vestibular-evoked myogenic potentials recorded from miniature pigs and rats

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Abstract

Objective: To report vestibular evoked myogenic potentials from different recording sites (neck extensor or masseter muscles) in miniature pigs and rats.

Methods: Potentials were recorded using 1000 Hz tone bursts from the neck extensor muscle or masseter muscle in normal adult Bama miniature pigs and rats anesthetized with 3% pentobarbital sodium and Sumianxin II.

Results: At 80 dB SPL, the first positive wave (P wave) of VEMPs was recognizable in 58% of rats with a latency of 6.45 ± 0.23 ms and an amplitude of 1.45 ± 0.49 μ V when recorded from the neck extensor muscle, and in 50% of rats with a latency of 6.38 ± 0.34 ms and an amplitude of 1.57 ± 0.35 μ V when recorded from the masseter muscle. In miniature pigs, at the same stimulus intensity, P wave was recognizable in 58% of the animals with a latency of 7.65 ± 0.64 ms and an amplitude of 1.66 ± 0.34 μ V when recorded from the neck extensor muscle, and in 50% of the animals with a latency of 7.65 ± 0.64 ms and an amplitude of 0.31 ± 0.28 μ V when recorded from the masseter muscle.

Conclusion: VEMP can be induced from both neck extensor and masseter muscles in the miniature pig and rat. For a given species, the site of recording affects P wave induction rate and amplitude but not latency. Consistency and repeatability analysis suggests that the masseter muscle is a better recording site in miniature pigs while the cervical extensor is a better recording site in rats. For a given recording site, both latency and amplitude of the P wave are slightly greater in miniature pigs than in rats.

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Keywords: Vestibular evoked myogenic potentials; Miniature pigs; Rats; Masseter muscle; Cervical extensor muscle

1. Introduction

Vestibular evoked myogenic potentials (VEMPs) are produced in a tensioned skeletal muscle by a loud sound

stimulating the saccule. VEMPs reflect the integrity of the vestibulothalamic pathways in humans and animals. VEMP is an objective and noninvasive electrophysiological method with clinical practicality (Shimizu et al., 2000; Taylor et al., 2012) in the diagnosis of vestibular diseases. Routine VEMP testing requires maintenance of tension in the sternocleidomastoid muscle, making it a challenge for elderly patients and those with cervical vertebral diseases. Deriu et al. (Deriu et al., 1999; Murofushi, 2014) have recorded VEMPs from the masseter muscle, and Xie et al. (Sujiang, 2007) have established a masseter muscle myogenic potentials model with the rat.

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Miniature pigs have been widely used in biomedical research. With respect to otological studies, miniature pigs are very similar to humans in anatomy and physiology. Similar structures and positions of the vestibular system (Lili, 2013) make miniature pigs a useful experimental model in the study of human otological diseases (Hsu, 1982; Guo et al., 2015). The masseter muscle in miniature pigs is a relatively thick, roughly square-shaped muscle, running from the cheekbone across the jaw and covering the mandible (Weiwei et al., 2015). Its superficial location and bulky size make approaching the muscle relatively easy. The masseter muscle in rats is covered with subcutaneous fat and positioned relatively deep. In the clinic, the cervical extensor (sternocleidomastoid) muscle has been widely used for VEMP recording as a method of evaluating vestibular functions. This study selected the neck extensor and masseter muscles for VEMP recording in miniature pigs and rats.

2. Materials and methods

2.1. Experimental animals and grouping

Normal 6 months old female Guangxi Bama miniature pigs ($n = 12$, weighing about 25 kg), provided by the North Large Animals Research Base of Chinese Academy of Sciences, were used in the study. These animals were examined for signs of ear infection or vestibular/balance disorders (abnormal gaits, head swings, trunk twisting, circling, etc). Twelve adult female rats (Academy of Military Sciences Laboratory Animal Center), weighing about 300 g were also chosen and examined for auricle reflex sensitivity, limb strength and positions, as well as balance functions. Normal hearing was verified using ABRs (Fig. 1).

2.2. Anesthesia

To minimize interference by brain electrical activities and extremity movement, animals were anesthetized before experiment, after fasting for 12 h. Miniature pigs were injected intramuscularly with 3% pentobarbital sodium (1 ml/kg, Beijing Daniel Spulber Biotech) + Sumianxin II (0.1 ml/kg, Jilin Huamu Animal Care Products, Ltd) which led to slumped head, decreased activities, gait instability and lying down in 3–5 min. Rats were given intraperitoneal 1% pentobarbital sodium (Beijing Daniel Spulber Biotech) at 0.4 ml/100 g. Successful anesthesia was verified by loss of corneal reflex and pain reflex.

2.3. VEMP testing

In rats, the recording electrode was inserted into the lower third of the masseter muscle, the reference electrode in the tip of the nose, and the ground electrode in the scalp at the vertex. A plastic ball was placed between the upper and lower incisors to open the upper and lower jaws as wide as possible and maintain tension in the masseter muscle. To record VEMPs from the neck extensor muscle, the recording electrode was

placed at the midpoint of the muscle, the reference electrode placed at the vertex, and the ground electrode at the nasal tip. While the body was held in a bottle, the head was turned to one side, to maintain tension in the neck extensor muscle, and held in place via a custom made band (Fig. 3). A Smart EP evoked potentials system (Intelligent Hearing Systems, USA) was used to record VEMPs evoked with 1000 Hz tone burst stimulation presented at 5 Hz via the ER3A earphone inserted into the external auditory canal (model number MO15300). The myogenic signals were amplified by 100 k and band passed between 30 and 1000 Hz. The scan time was 50 ms, and 128 sweeps were averaged. Starting stimulus intensity was 100 dB SPL, decreasing at 10 dB steps until the waveform disappeared. Two consecutive recordings were collected at threshold levels to verify repeatability and stability.

The masseter muscle of miniature pigs is approximately square in shape and located superficially underneath the skin. It contains thick muscle fibers that run from the cheekbone to the mandible. Facial muscles and facial nerves run on top of the muscle and the muscle covers the ramus (Weiwei et al., 2015). The neck anatomy of the miniature pig is different from that of humans, with thicker fatty tissue layers and narrower inter-vertebral spaces, which limit head motions. The extensor muscle in miniature pigs, however, shares some similarities with the sternocleidomastoid muscle in humans, both in anatomy and physiology. The muscle is located on the side of the neck, originates from the humerus crest and terminates in the occipital and petrosal temporal bones. It is covered by thick adipose tissues and difficult to palpate. It is an important muscle in the neck and controls head rotation and elevation. The same Smart EP evoked potentials system and test protocol described above were used to record VEMPs from the masseter and neck extensor muscles in miniature pigs (Fig. 3), except testing was conducted in an open sound field.

3. Results

For VEMPs recorded from the neck extensor muscle in rats, the latency of the first positive wave P was 6.45 ± 0.23 ms, with an amplitude of 1.64 ± 0.49 μ V. The rate of VEMP induction at 100 dB SPL, 90 dB SPL and 80 dB SPL was 100%, 75% and 100%, respectively, with 80 dB SPL being the threshold. For VEMPs from the masseter muscle, the latency of wave P was 6.42 ± 0.34 ms, with an amplitude of 1.47 ± 0.35 μ V; and induction rate at 100 dB SPL, 90 dB SPL and 80 dB SPL was 100%, 66% and 100% respectively, again with 80 dB SPL being the threshold. In miniature pigs, the latency of wave P in VEMPs from the neck extensor muscle was 7.65 ± 0.64 ms, with an amplitude of 1.66 ± 0.34 μ V, and induction rate at 100 dB SPL, 90 dB SPL and 80 dB SPL was 100%, 83% and 100%, respectively, with the threshold being 80 dB SPL. For VEMPs from the masseter muscle in miniature pigs, the latency of wave P was 7.60 ± 0.78 ms, with an amplitude of 1.31 ± 0.28 μ V, and induction rate at 100 dB SPL, 90 dB SPL and 80 dB SPL was 100%, 75% and 100% respectively, with the threshold being 80 dB SPL. See Fig. 2 for examples of VEMPs from a miniature pig.

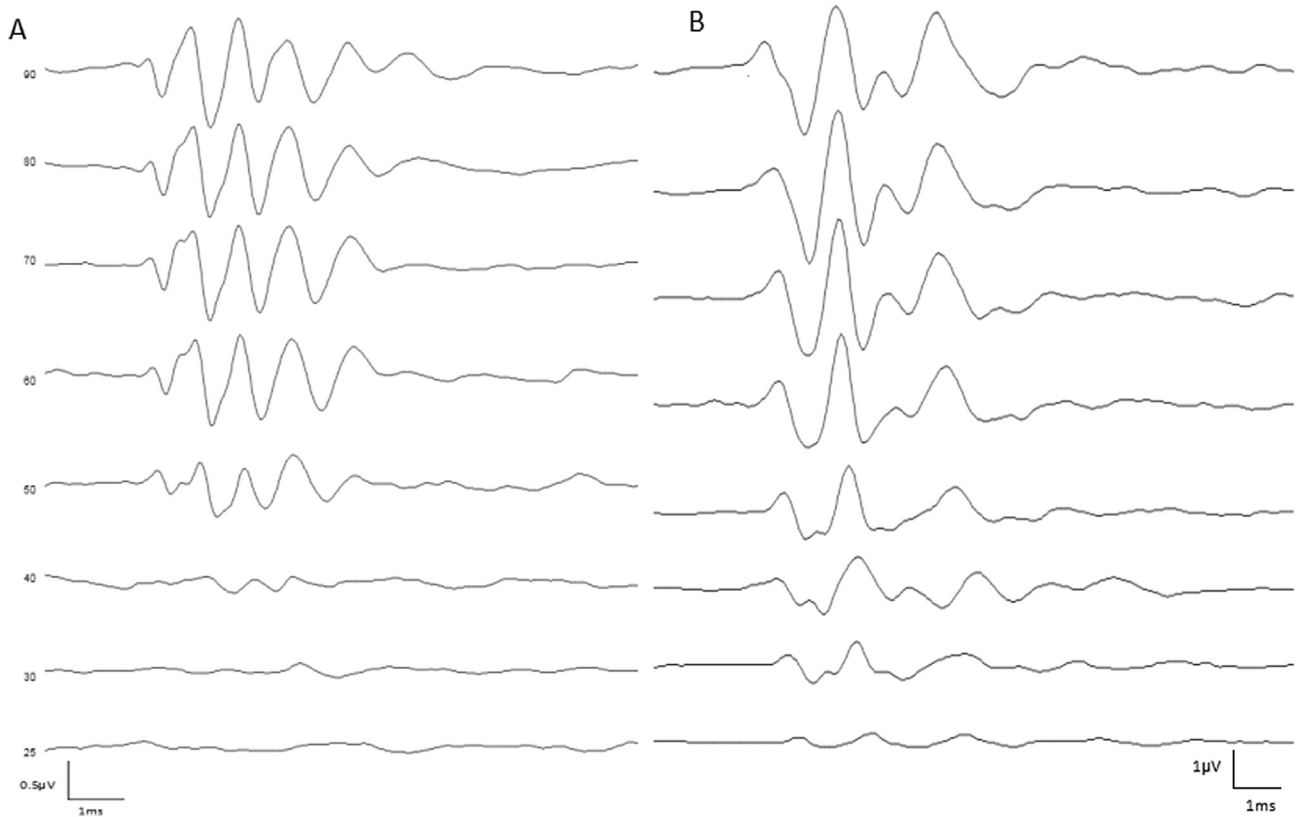


Fig. 1. Normal ABRs in a miniature pig (A) and a rat (B), showing thresholds at 25 dB SPL.

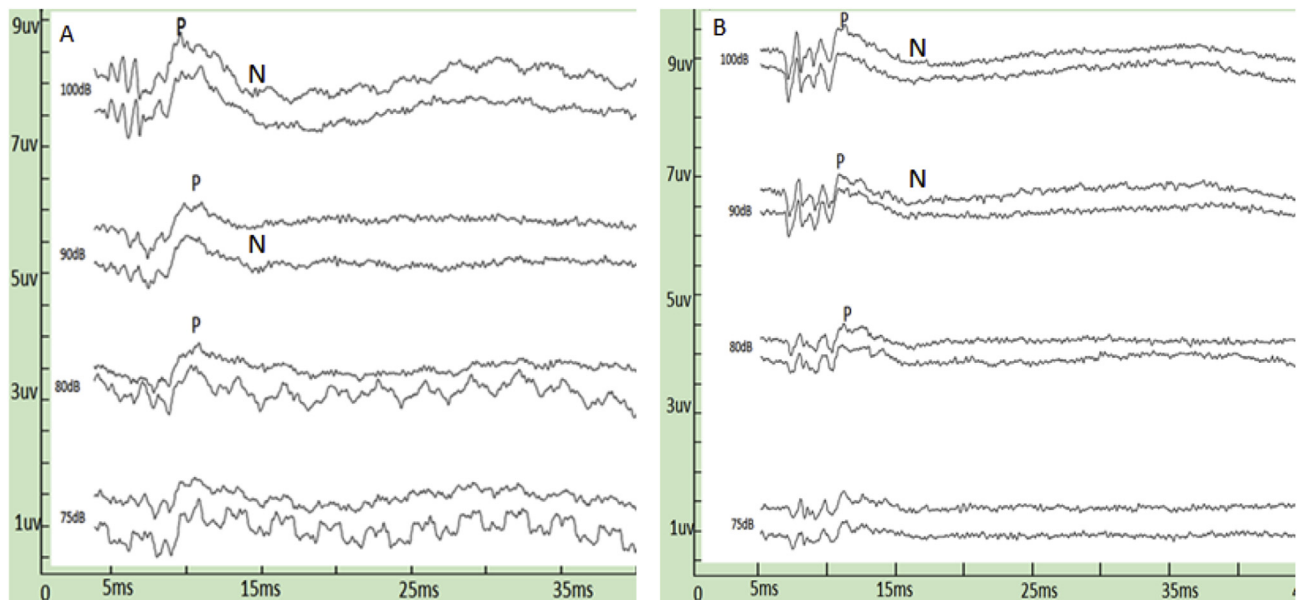


Fig. 2. A: VEMPs from neck extensor muscle in a 6 months old female miniature pig. Latency of the first positive wave P is 7.52 ms, with an amplitude of 1.50 μ V, at 75 dB SPL. Latency of the first negative wave N is 9.87 ms. B: VEMPs from masseter muscle in a 6 months old female miniature pig. Latency of wave P is 7.63 ms, with an amplitude of 1.60 μ V, at 75 dB SPL, and latency of wave N is 11.63 ms.

4. Discussion

Clinically, VEMP testing is used to diagnose neurotology diseases such as acoustic neuroma, Meniere's disease,

vestibular neuritis, superior semicircular canal dehiscence syndrome, multiple sclerosis, BPPV, vestibular migraine and other peripheral or central vestibular diseases. VEMP amplitude is an important index for the evaluation of vestibular

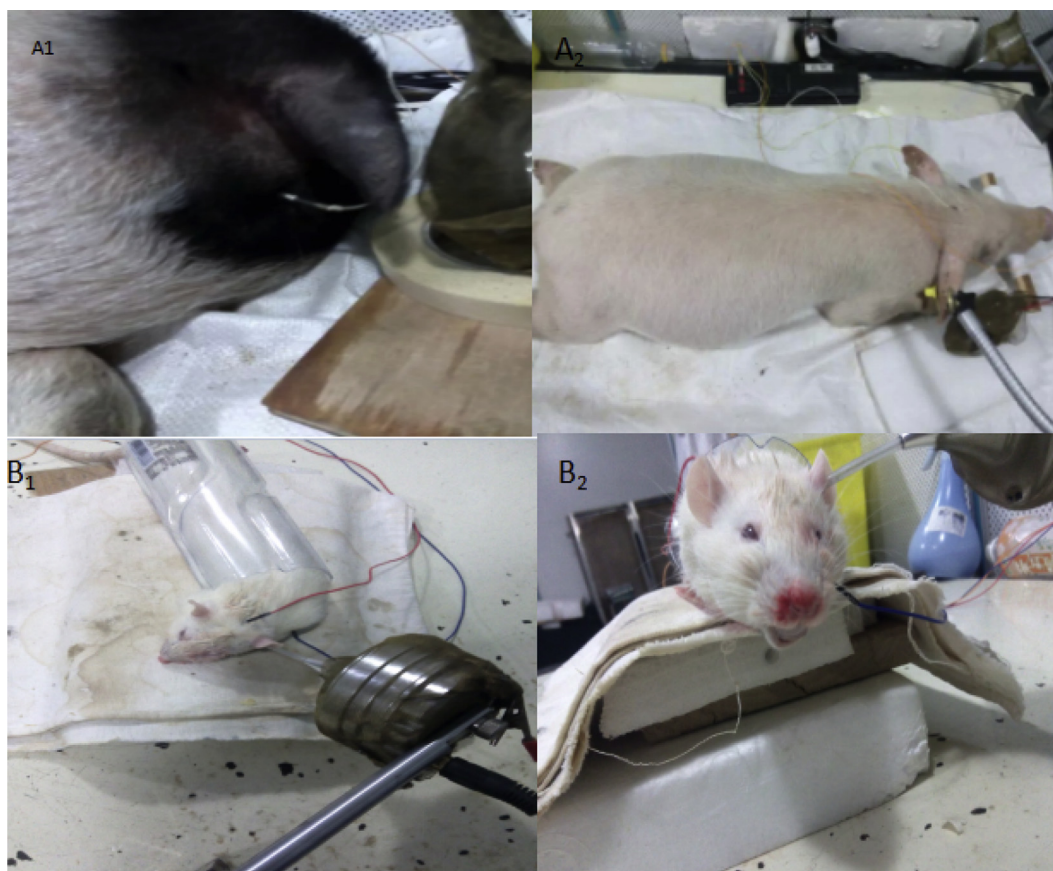


Fig. 3. Setups for VEMP recording from neck extensor and masseter muscles in a miniature pigs (A1 and A2) and in a rat (B1 and B2).

functions, but it is vulnerable to interference by factors such as age, muscle strength, skin thickness and gender (Lopez-Escamez et al., 2015). To minimize interference, this study used only female animals maintained at a certain weight. Zhou (Zhou et al., 2004) reported inter-individual differences in VEMP amplitude but not in latency. While surface electromyography has been used during VEMP testing as a measure of sternocleidomastoid muscle tension and an optimal level of 30–50 μV has been suggested (Sun et al., 2005), there is no standard method established yet to reduce the inter-individual differences (Akin et al., 2004; Westin and Brantberg, 2014). Masseter and neck extensor muscle tone is increased as much as possible in animal VEMP experiments to optimize VEMP waveforms, but this inevitably causes decreased sensitivity and objectivity of amplitude and other test parameters. VEMP amplitude is positively correlated to the level of surface electromyography activities of the test muscle. The tone of the muscle directly affects the amplitude of VEMPs (Taylor et al., 2012). In the masseter muscle, VEMP amplitudes in miniature pigs were lower than those in rats. This may be because 1) affected by physiological factors, there may be some atrophy changes in vestibular nerve fibers and the masseter muscle in miniature pigs, which can lead to reduced nerve conduction velocity and muscle tension; 2) testing in miniature pigs was longer than in rats, and extended time of tension in the masseter muscle may lead to muscle fatigue and therefore

reduced muscle contraction and potential amplitudes; 3) being an omnivorous rodent species, rat masseter muscle fibers show higher densities, greater numbers and larger fiber sizes compared to miniature pigs; 4) the custom made device to increase masseter muscle tension may introduce man-made factors that may artificially produce lower muscle tone in miniature pigs than in rats. From the current study, it is clear that high background muscle tension is needed for recording VEMPs from either the neck extensor or masseter muscle. Due to anatomical and functional differences of animal muscles from the human, there has not been a suitable method to selectively increase neck extensor muscle tension while keeping other neck muscles relaxed. As a result, the methods used in the current study are prone to be affected by animal conditions, causing baseline instability, interferences and amplitude variations. Efforts must be made to reduce such interferences during experiment to ensure reasonable waveform repeatability and stability. Because anatomy of miniature pig neck muscle is complex and the muscle is covered by thick adipose tissues, maintenance of neck extensor muscle tension can be a challenge and location of the recording electrode may shift, adding to signal variations. Extended period of neck extension can lead to airway and ventilation difficulties, snoring, increased airway secretions, and even suffocation. When recording VEMPs using the masseter muscle, the animal is in a prone position with its trunk and neck laid on the test bench

and relaxed, thus reducing the myoelectric activity interference, which contributes to improved waveform repeatability and baseline stability. This study argues that, although VEMPs from the masseter muscle may show lower amplitudes and induction rates at some stimulation intensities than those from the neck extensor, for animal safety and signals repeatability and stability reasons, as well as the ease of experimental operation, VEMP recording from the masseter muscle is superior over from the neck extensor muscle in miniature pigs in terms of waveform quality. However, judging from waveform repeatability, amplitude size and rate of successful induction, we recorded better VEMPs in rat cervical extensor muscle than from the masseter muscle, consistent with previous reports by some scholars. We feel that this may be related to the anatomical and physiological features of the muscle itself and/or the way of signal recording in this study, and needs to be further investigated in future studies. Regarding the VEMP amplitude in different animal models, due to the constraint of recording techniques, there is currently no effective way to simultaneously monitor electromyography activity levels in the background muscle. Information of background muscle tension levels is needed for reliable and objective VEMP testing. Accurate detection of background muscle tension can be the key to application of masseter myogenic potentials testing. Currently, variation in absolute amplitude values is considered to be mainly an effect of muscle tension, and has nothing to do with vestibular function. Focusing on variation of absolute VEMP amplitudes is therefore meaningless under current conditions (Murofushi, 2014).

VEMP latency is an important index for evaluating vestibular functions. In animal models of VEMP, the first positive wave has the highest rate of induction (100% at 100 dB SPL) (Lopez-Escamez et al., 2015; Taylor et al., 2012), and the highest application significance. It is therefore the focus of this study. Reported latencies for the first positive wave in high intensity tone bursts triggered masseter or neck extensor muscle myogenic potentials in miniature pigs are in the range of 7–9 ms, similar to the findings in this study. The same latency range in rats is 6–8 ms. There is no statistically significant difference in the latencies between the two kinds of experimental animals. At 80 dB SPL, the rate of successful induction of VEMPs from neck extensor muscle was higher than from the masseter muscle in both animals. By comparison, the latency of VEMPs from the masseter muscle in miniature pigs is close to that of humans (although shorter), but longer than that of rats, probably related to the length of neural pathways and the diameter of nerve fibers. The VEMP latency represents the time needed for activation of neuronal receptors along the peripheral sensory nerve-brainstem-masseter motor neuron/nerve pathway (Wang, 2010; Wang Boshen, 2010). The longer latencies in miniature pigs and humans may be related to longer time needed for saccular signals to travel to the central nervous system. In the clinic, increased VEMP latencies may be caused by vestibular function impairment and decreased conductivity from degenerative changes in myelinated nerve fibers following aging. According to Cazals and Didier (Wu et al., 2004; Cazals et al.,

1987; Zuo et al., 2002), response latency to acoustic stimulation in rats is 1 ms in vestibular nerve and 3 ms in vestibular nuclei. If the masseter muscle VEMP originates from vestibular activation, the latency of its first positive wave should be longer than 4 ms and probably less than the 11 ms in humans (Murofushi, 2014; Didier and Cazals, 1989; Papathanasiou et al., 2014). The 6–8 ms P wave latencies seen in this study from the neck extensor and masseter muscles in rats fall right in this range.

Although VEMPs can be used to objectively evaluate vestibular functions and have been widely used in clinical practice, many issues remain to be investigated. First of all, although it has been confirmed that VEMPs come from saccular activation, their relationship with the rest vestibular organs remains unclear (Zhang et al., 2012). Secondly, there lacks an animal model of specific saccular dysfunction, limiting relevant animal experiments. Again, there is currently no universally accepted standard for VEMP examination in animal experiments. Therefore, much remains to be done regarding VEMPs (especially VEMPs in animals).

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