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Research report

Bone conducted vibration activates the vestibulo-ocular reflex in the guinea pig

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ABSTRACT

The aim of the study was: (a) to test whether short duration (6 ms) 500 Hz bone-conducted vibration (BCV) of the skull in alert head free guinea pigs would elicit eye movements; (b) to test whether these eye movements were vestibular in origin; and (c) to determine whether they corresponded to human eye movements to such stimuli. In this way we sought to establish the guinea pig as an acceptable model for testing the mechanism of the effect BCV on the vestibulo-ocular reflex. Consistent short-latency stimulus-locked responses to BCV were observed. The magnitude of eye displacement was directly related to stimulus intensity as recorded by accelerometers cemented onto the animal's skull. The strongest and most consistent response component was intorsion of both eyes. In lateral-eyed animals intorsion is produced by the combined contraction of the inferior rectus and superior oblique muscles. In humans the same pair of muscles acts to cause depression of the eye. To test whether the movements were vestibular we selectively ablated the vestibular endorgans: 3 of the 8 animals underwent a bilateral intratympanic injection of gentamicin, an ototoxic aminoglycoside antibiotic, to ablate their vestibular receptors. After ablation there was an overall reduction in the magnitude of eye displacement, as well as a reduction in the effectiveness of the BCV stimulus to elicit eye movements. The animals' hearing, as measured by the threshold for auditory brainstem responses, remained unchanged after gentamicin, confirming that the cochlea was not affected. The reduced magnitude of responses after vestibular receptor ablation demonstrates that the eye-movement responses to BCV are probably caused by the stimulation of vestibular receptors, which in turn activate the vestibulo-ocular reflex.

1. Introduction

For the last 15 years bone-conducted vibration (BCV) has been used to assess vestibular function. The original study [8] found that a light tap on the forehead caused a bilateral myogenic response recorded by electrodes on a tensed sternomastoid muscle. This response has been referred to as the cVEMP (cervical vestibular-evoked myogenic potential). More recently a similar myogenic potential has been recorded beneath the eyes and has been named the ocular vestibular-evoked myogenic potential or the oVEMP [14], with the assumption that these potentials originate from extraocular muscles. Evidence from guinea pig primary vestibular afferents [2] showed that 500 Hz BCV selectively activates vestibular otolithic receptors. On the basis of such evidence cVEMP and oVEMP tests are used to assess vestibular function via the vestibulo-collic and vestibulo-ocular reflexes (VCR and VOR), respectively. The growing body of animal and clinical research led Curthoys to argue that the oVEMP assesses primarily utricular and the cVEMP primarily saccular function [3]. The present study uses an animal model to test the relationship between BCV and VOR.

Curthoys and Vulovic [4] confirmed that in guinea pigs at low intensities BCV does activate primary vestibular afferents and confirmed that low-intensity 500 Hz BCV preferentially activates otolith irregular afferents in the superior vestibular nerve. The majority of otolith fibres running in the superior vestibular nerve are utricular [5], demonstrating that BCV is a very effective utricular stimulus. In the vestibular periphery such irregular afferents most often synapse on Type I receptors in the utricle [7]. These afferents respond to changes in acceleration or "jerk" stimuli. The 500 Hz BCV which was used in these studies provides 500 sudden changes in acceleration, making it an ideal stimulus for otolithic Type I receptors.

Given that BCV activates the vestibular system including utricular macula receptors, it is also important to substantiate the pathway which would lead from utricular stimulation to extraocular muscle potentials found in the oVEMP. Utriculo-ocular pathways have been demonstrated in previous research [19]. In a

Abbreviations: ABR, auditory brainstem response; ACS, air conducted sound; BCV, bone conducted vibration; cVEMP, cervical vestibular-evoked myogenic potential; IO, inferior oblique; ITG, intratympanic injection of gentamicin; oVEMP, ocular vestibular-evoked myogenic potential; VCR, vestibulo-collic reflex.

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classic study Suzuki et al. [17] used unilateral electrical stimulation of the utricular nerve in the cat to demonstrate the functional outcome of utriculo-ocular pathways by measuring the resulting eye movements. Utricular nerve stimulation caused conjugate torsion, with the superior pole of both eyes rolling away from the stimulated side, and with small vertical and horizontal components. Suzuki et al. also measured muscular tension of all the extraocular muscles, and showed that utricular nerve stimulation caused strong activation of ipsilateral superior oblique and superior rectus as well as contralateral inferior oblique and inferior rectus. While utriculo-ocular pathways are strong and oligosynaptic, sacculo-ocular pathways were found to be relatively weak and polysynaptic [10]. Therefore, even though BCV activates otolith irregular afferents from both the utricle and the saccule, oVEMPs are likely to be primarily caused by utricular stimulation. A more complete description of these pathways can be found in a review by Curthoys [3].

According to the above studies, applying BCV to the skull in human subjects would stimulate the otoliths and should result in measurable eye movements similar to those found by Suzuki et al. [17] acting via the same utriculo-ocular pathways. This prediction was confirmed by Cornell et al. [1]. BCV at 500 Hz applied at the mastoid resulted in consistent stimulus-locked eye movements as recorded by high-resolution video measures. Cornell et al. found that unilateral BCV stimulation of the mastoid with gaze directed towards the stimulated side caused a downward movement of both eyes. This suggested activation of the ipsilateral superior oblique and contralateral inferior rectus. When gaze was directed away from the stimulated side, BCV caused an upward movement of both eyes. This suggested activation of the ipsilateral superior rectus and contralateral inferior oblique. It can be concluded that with unilateral BCV stimulation ipsilateral superior oblique and superior rectus were probably activated, along with contralateral inferior oblique and inferior rectus. Cornell et al. therefore corroborated the findings of Suzuki et al. [17]. Bilateral stimulation at the mastoids produced predominantly vertical downward eye movements in both eyes irrespective of gaze direction. Comparing bilateral with unilateral stimulation suggests a relative dominance in superior oblique and inferior rectus activation in both eyes, compared with the activation of the inferior oblique and superior rectus.

Since it has been established that in guinea pigs 500 Hz BCV activates otolith irregular afferents, the missing step is to show that the same BCV causes a pattern of eye movements in guinea pigs consistent with utricular activation. We sought to test this by delivering BCV to alert guinea pigs with unrestrained heads and measuring their eye movements bilaterally using dual (3d) scleral search coils on each eye. This method allows for measurement of eye movements in all three dimensions [9]. The consistency of the BCV stimulus was assessed with triaxial accelerometers chronically implanted on the dorsal surface of the skull, adjacent to where the stimulus was delivered. The head was not restrained since that manoeuvre can substantially alter the characteristics of the BCV by changing the resonance of the skull.

One obstacle in comparing results from guinea pigs directly with those of Cornell et al. [1] and Suzuki et al. [17] is the fact that humans and cats are frontal-eyed whereas guinea pigs are lateral-eyed. In the guinea pig, the axis of the eye is oriented 62° to the median plane, whereas in humans the axis of the eye is oriented 23° to the median plane. Simpson and Graf [15] studied the eye muscle geometry of species with different levels of eye lateralisation and concluded that "the requirements for producing the different appropriate compensatory eye movements are basically fulfilled in the orbit by the changes of the insertions and lines of action of the vertical extraocular muscles" (p. 28). Therefore, in frontal- and lateral-eyed animals, activation of the same extraocular muscles produces the same eye movements relative to head coordinates, but not to orbit coordinates. This would allow the same eye muscles to compensate for head movements via the VOR, thus preserving the vestibulo-ocular pathways regardless of eye lateralisation. For example the superior oblique and inferior rectus depress the eye in humans whereas they intort the eye in guinea pigs. In both species this pair of muscles causes eye rotation about the same interaural axis of the head and hence compensates for a pitch nose-up head rotation.

The animal model allowed us to perform a selective ablation of vestibular receptors using intratympanic injection of gentamicin (ITG) to test the vestibular origin of these eye movements. Obviously BCV activates more than just the otoliths: auditory and proprioceptive receptors would also be activated. Therefore it is possible that the stimulus-locked eye movements to BCV may be caused via other sensory modalities. Selective bilateral ablation of the vestibular system leading to a reduction in the eye movement magnitude would support the hypothesis that eye movements to BCV are vestibular in origin. Previous data shows that bilateral labyrinthectomies abolish the eye-movement response to BCV (unpublished observations). While this shows that the eye-movement response to BCV originates from the labyrinth, it does not exclude the possibility that there is an auditory contribution to the eye-movement response. Since the vestibular system and the cochlea are both anatomically close and physiologically similar, most ablative manoeuvres that affect one would affect the other. It was therefore imperative to ensure the auditory receptors remained intact during the experiment. To establish this we determined auditory brainstem response (ABR) thresholds to sound and vibration before and regularly after ITG to ensure the animal's hearing remained intact. It could thus be concluded that any reduction in the eye-movement intensity after ITG would be due to vestibular and not cochlear ablation.

The ototoxic properties of aminoglycoside antibiotics can be exploited to achieve a selective ablation. Gentamicin is ideal as it is more vestibulotoxic than cochleotoxic [16]. Previous researchers delivered gentamicin surgically into the guinea pig middle ear and placed it on the round window. Yang et al. [20] used this approach to show a complete absence or a reduction of the contralateral oVEMP after unilateral ITG. In that study electron microscopy further demonstrated a 68% reduction in the number of hair cells in all vestibular end organs.

The mechanism of gentamicin vestibulotoxicity is still not completely understood. Takumida et al. [18] found an increase in free-radical concentration in vestibular end organs of guinea pigs after ITG. It is known that gentamicin is taken up more vigorously by Type I vestibular receptors [12], and it is these receptor cells which are most sensitive to the toxicity, resulting in apoptosis [11]. The fact that Type I vestibular receptor cells are more susceptible to the toxicity of gentamicin is important, because they were also identified as probably being the receptors selectively activated by BCV [2].

In summary, the first aim of our study is to characterise eye movements caused by BCV of the head in the guinea pig, to determine whether they are repeatable from trial to trial and from day to day and how variable they are between animals. Demonstrating eye movements with the same species and equipment as used in Curthoys et al. [2] and Curthoys and Vulovic [4] who found the selective activation of irregular otolithic afferents by BCV would be an important outcome. Comparing our results with those found to BCV stimulation in humans [1] is also relevant. The second aim of our study is to establish the vestibular origin of the eye movements caused by BCV. This will be determined by observing whether bilateral IT gentamicin leads to a decrease in the magnitude of eye movements without a significant decrease in hearing.