Peripheral generators of the vestibular evoked potentials (VsEPs) in the chick 1

Pedro Weisleder a, Timothy A. Jones b and Edwin W Rubel a

a University of Washington, Seattle, WA (U.S.A.), and b University of Nebraska Medical Center, Lincoln, NE (U.S.A.)

(Accepted for publication: 29 December 1989)

Summary Electrophysiological activity in response to linear acceleration stimuli was recorded from young chickens by means of subcutaneous electrodes. This investigation had 2 purposes: (1) to establish the vestibular origin of the potentials; and (2) to investigate the contribution of each vestibular labyrinth to the response. The stimuli consisted of pulses of linear acceleration delivered by a mechanical vibrator (shaker). In the first set of experiments vestibular evoked potentials (VsEPs) were recorded prior to and 24 h after bilateral cochlea removal. In the second set of experiments responses were recorded before and after unilateral or bilateral intralabyrinthine injections of tetrodotoxin (TTX). Different groups of subjects were used for each experimental condition. The general morphology of the VsEPs was maintained after bilateral cochlea removal. Absolute latency of wave P2, the most prominent component of the response, was not significantly affected by the manipulation. Unilateral intralabyrinthine TTX injections consistently prolonged the latency and reduced the amplitude of wave P2. Following binaural TTX injections we were unable to elicit responses at the acceleration levels used in this study. The results from these experiments suggest that: (1) the activity recorded in response to linear acceleration stimuli is vestibular in origin; (2) when recorded from intact animals the evoked response is composed of activity from both vestibular systems; and (3) TTX consistently blocks the activity of the vestibular portion of the VIIIth cranial nerve.

Key words: Vestibular physiology; Avian

Tests that evaluate the electrophysiological activity in response to acceleration stimuli in a non-invasive fashion have the potential of becoming routine tools for objectively assessing the vestibular system's function both in the laboratory and the clinical setting. Vestibular evoked potentials (VsEPs) have been recorded from rats (Elidan et al. 1982; Hoffman and Horowitz 1984a,b), cats (Elidan et al. 1984a,b, 1986, 1987a,b), and chickens (Jones 1988, 1989a,b; Jones and Pedersen 1989; Weisleder et al. 1989). VsEPs in rats and cats were recorded in response to angular acceleration stimuli while pulses of linear acceleration were used for the experiments on chickens.

The avian VsEPs consist of a series of well defined waves which appear 1.5 msec after the onset of the stimulus. The responses can be replicated and have little variability from subject to subject. Latency/ and amplitude/acceleration functions for the most prominent components can be constructed and used as normative data. Threshold responses are recorded, on the average, at 0.0935 g (Weisleder et al. 1989). These values compare favorably with those previously reported by Jones and Pedersen (1989).

Researchers have used several methods to rule out somatosensory and auditory contributions to the VsEPs. Elidan et al. (1982) demonstrated that the responses do not change when recorded from paralyzed animals. Jones and Pedersen (1989) re-

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1 This study was conducted at the University of Washington, Seattle.

Correspondence to: Edwin W Rubel, Ph.D., Department of Otolaryngology, RL-30, University of Washington, Seattle, WA 98195 (U.S.A.).

0013-4649/90/$03.50 © 1990 Elsevier Scientific Publishers Ireland, Ltd.
ported that acceleration of the trunk, leaving the head stable, did not yield recordable potentials. Elidan et al. (1984a), Hoffman and Horowitz (1984b), Jones (1988a,b), Jones and Pedersen (1989), and Weisleder et al. (1989) showed that high levels of acoustic white noise do not affect the VsEP. Conversely, these authors established that the responses disappear after VIIIth cranial nerve sectioning, labyrinthectomy, inner ear activity blockade, or death. Unfortunately, both auditory and vestibular components are affected by the latter series of manipulations. Masking experiments are the only ones that selectively alter the auditory system’s function.

Removing the cochlea (basilar papilla) of a chicken is a simple procedure. By excising both cochleae without permanently damaging the vestibular labyrinth it should be possible to rule out all auditory contributions to the VsEP. The advantage of this manipulation over the use of auditory masking is that the peripheral generators of electrophysiological activity from the auditory system can be completely eliminated (Born and Rubel 1984).

Born and Rubel (1988) established that tetrodotoxin (TTX), injected into the perilymph, reversibly blocks the activity of the auditory component of the VIIIth cranial nerve. This effect is localized to the treated ear and thus allows assessment of the non-injected ear. Binaural applications eradicate all potentials generated by either inner ear.

The chicken’s cochlear duct is a slightly curved, finger-like epithelial tube that runs almost perpendicular to the base of the skull. Its distal tip is directed medially. The duct is composed of 3 different structures: the basilar papilla (sensory organ); the tegmentum vasculosum; and, an otolithic macula, peculiar to lower vertebrates, at the distal tip (Tanaka and Smith 1978). The vestibular system of birds is composed of 3 semicircular canals with their corresponding ampullas, and 2 otolithic organs: the utricle and the saccule. The organization of these structures is similar to that found in the mammalian vestibular labyrinth (Ramprashad et al. 1986).

The purposes of this investigation were 2-fold: (1) to definitively establish the vestibular origin of the VsEP; and (2) to investigate the contribution of each ear to the VsEP. In order to achieve our goals the following experiments were undertaken: (1) VsEPs were recorded before and after bilateral cochlea removal. This manipulation would eliminate any possible auditory components of the response. (2) VsEPs were recorded after unilateral intralabyrinthine injection of TTX. This manipulation allows assessing the contributions of each ear to the VsEP. (3) The electrophysiological activity in response to linear acceleration stimuli was recorded following binaural TTX injections. This manipulation was used to confirm the effect of the unilateral injection. It also provided information regarding any somatosensory contributions to the VsEP.

Materials and methods

Fifteen, 2-week-old White Leghorn chicks (Gal- lus domesticus) were tested. The animals were anesthetized (ketamine 0.8 mg/10 g and Equithesin 0.015 ml/10 g) and subcutaneous stainless steel electrodes were positioned in a bipolar configuration: vertex of the skull (non-inverting input), 2–4 mm behind the opening of the external auditory meatus (inverting input), and skin of the neck (ground). Animals were placed prone, with the beak facing down, on a heating pad, their temperature was monitored and kept constant at 39°C. Different groups of subjects were used for each experimental condition.

A sigmoid-shaped voltage function generated by a digital-to-analog converter (12-bit), was applied to the transducer to produce pulses of linear acceleration (Fig. 1A). The signal from the digital-to-analog converter was amplified (RAMSA WP-9055) and attenuated. The output of the attenuator was routed to a 4-pound shaker (Ling 203B; solenoid-based linear mechanical vibrator; maximum displacement 5 mm), which delivered the stimuli. The initial movement of the shaker, in the vertical plane, was an upward displacement. Subjects were tightly coupled to the shaker by embedding the beak, facing down, in a fast-setting plaster pedestal attached to a small Plexiglas platform. This platform was in turn linked to the shaft of
A small piece of surgical gel (Gelfoam, Upjohn) was placed inside the oval window to prevent perilymph leakage. Twenty-four hours later, after assessing that the subjects were able to walk around their cage, their heads were not tilted and were able to feed themselves, responses were recorded again.

Unilateral inner ear activity blockade (5 subjects) was achieved through intralabyrinthine injection of TTX, a voltage-gated sodium channel blocker (Narahashi et al. 1967). TTX (Sigma) (2.5 \times 10^{-4} \text{ mg}) was administered in a vehicle of 12\% poly-vinyl-alcohol (DuPont) and 0.005 ml of the solution was injected through the oval window of the ear contralateral to the recording electrode. The oval window was accessed by dissecting the external auditory meatus, opening the tympanic membrane, and removing the columella. At the end of the recording session the test ear was also injected with TTX. Disappearance of all evoked activity confirmed the success of the first injection.

**Results**

Fig. 2 presents a representative intensity series from an intact animal displaying the nomenclature adopted by our laboratory. As can be seen, the avian VsEP is composed of a positive wave and a prominent negativity followed by 3–5 positive waves which occur within 10 msec after the onset of the stimulus. The latency between stimulus onset and the initiation of the response is about 1 msec. The first 3 elements (P1, N1, and P2) are the most consistent and prominent components of the response. At an acceleration of 2.00 g (1.00 g = 9.81 m/sec^2) the amplitude of these components ranges between 2 and 5 \mu V.

**Bilateral cochlea removal**

Cochlea removal experiments were carried out in 2 sessions. On the first day, baseline responses recorded from intact animals were followed by bilateral cochlea removal. Twenty-four hours later, after assessing that the subjects had no aberrant vestibular behavior, VsEPs were again recorded.

After recording baseline responses, bilateral cochlea removals were performed in 5 animals. Utilizing a lateral-to-medial approach and under direct observation (surgical microscope), the tympanic membrane was incised and the columella was removed. The oval window was visualized and fine-tip forceps were used to pull out the cochlear membranous labyrinth. The basilar papilla was examined to confirm removal in its entirety. A small piece of surgical gel (Gelfoam, Upjohn) was placed inside the oval window to prevent perilymph leakage. Twenty-four hours later, after assessing that the subjects were able to walk around their cage, their heads were not tilted and were able to feed themselves, responses were recorded again.

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PERIPHERAL ORIGIN OF CHICK'S VsEP

Fig. 4A presents averaged latency/acceleration functions of the P2 component before and after cochlea removal. Latency measurements were computed from the onset of the stimulus waveform. Two factor repeated measures (treatment × acceleration) analysis of variance (ANOVA), revealed no reliable effect of the treatment on the latency of P2 ($F (1, 4) = 0.4$, $P > 0.05$). As ex-
are reduced in amplitude and the latter most components, P5 and P6, become difficult to identify.

Data analysis was based on the properties of wave P2. This is the most prominent component of the response and it is the last element to disappear as acceleration is reduced.

Fig. 3A presents an intact animal's response to 1.00 g stimulation. Fig. 3B presents a response obtained under the same stimulus conditions 24 h following bilateral cochlea removal. As can be seen, the general morphology of the VsEP is maintained after the manipulation. The first 3 components of the response are preserved without major observable changes in shape. Waves beyond P2

Fig. 2. Intensity series from an intact animal with the nomenclature adopted by our laboratory. Stimulus intensity appears to the right of each trace (1.00 g = 9.81 m/sec$^2$). Averages are replicated to show consistency.

Fig. 3. Avian vestibular evoked potentials to 1.00 g stimulation. A: responses from an intact bird. B: responses recorded from the same animal after bilateral cochlea removal (C.R.). C: responses from a different chicken following unilateral injection of TTX (u-TTX). D: slow, low amplitude activity recorded following binaural injections of TTX (b-TTX). E: recording obtained 30 min following the animal's death (DEATH). The last 2 traces were obtained from the same animal as in C. Averages are replicated to show consistency.
Input/output (I/O) functions for P2 are presented in Fig. 5A. For graphical purposes, the average value of P2 at 2.00 g for the intact population was normalized to 100%. Examination of Fig. 5A reveals fairly linear I/O functions both for the normal and bilateral cochlea removal subjects. A treatment × acceleration ANOVA revealed a reliable effect of the cochlea removal on the absolute amplitude of P2 (F (1, 4) = 7.99, P < 0.05). P2 amplitude increased with increasing acceleration (F (3, 12) = 75.74, P < 0.001). Treatment × acceleration interaction was reliable (F (3, 12) = 5.83, P < 0.05). Thus, bilateral cochlea removal has a significant effect on the amplitude of P2 at the acceleration levels used in this study.

Unilateral TTX injection

In the second set of experiments VsEPs were recorded before and after unilateral intralabyrinthine injection of TTX. Panel C of Fig. 3 presents a response to 1.00 g stimulation 30 min after expected, the latency of the component decreased with increasing acceleration (F (3, 12) = 92.53, P < 0.001). Treatment × acceleration interaction was not reliable (F (3, 12) = 1.06, P > 0.05). Thus, bilateral cochlea removal did not have a significant effect on the latency of wave P2 of the VsEP.

Visual detection threshold (VDT) was defined as the level in between the last acceleration at which response components were recognized and the acceleration at which no waves were identified. In this context VDT refers to the ability of the investigator to detect the response as it emerges from the background noise, not to the intensity of the stimulus needed to activate the sensory endings 50% of trials. Mean threshold for intact animals was 0.081 g, whereas the average response threshold for cochlea removal subjects was 0.1 g. Response thresholds were not significantly different between the 2 groups of subjects (paired t test (t (4) = 2.44, P > 0.05)).
unilateral injection of TTX. The overall morphology of this trace does not differ significantly from the normal response. The first 3 waves are easily identified, later components are reduced in amplitude. An acceleration series revealed reduction in the amplitude of the components at all acceleration levels. Early disappearance of P4, P5 and P6 was also observed.

Fig. 4B presents averaged latency/acceleration functions of P2 before and after unilateral TTX injection. As can be seen, this treatment prolonged the wave's latency. A treatment x acceleration ANOVA revealed a reliable effect of the TTX injection on the latency of P2 (F (1, 4) = 17.1, P < 0.01). The expected decrease in latency as a function of increased acceleration was confirmed by the statistical analysis (F (3, 12) = 77.67, P < 0.001). Treatment x acceleration interaction was not reliable (F (3, 12) = 0.46, P > 0.05). Thus, the statistical analysis confirms that a unilateral intralabyrinthine injection of TTX produces a significant increase in the latency of P2.

Threshold responses for this experimental group of animals were recorded at 0.11 g. This represents a significant threshold elevation when compared to the intact animals' data (paired t test (t (4) = 6, P < 0.05)). Unilateral intralabyrinthine injection of TTX resulted in prolonged latency and reduced amplitude of the components. Finally, all responses disappeared following binaural TTX injections.

Discussion

Electrophysiological activity in response to linear acceleration stimuli was recorded from 2-week-old chicks by means of subcutaneous electrodes. The objectives of this study were to demonstrate that such activity is of vestibular origin, and to investigate the contribution of each ear to the response. VsEPs were successfully recorded after bilateral cochlea removal, although morphological changes in the response were observed. Unilateral intralabyrinthine injection of TTX resulted in prolonged latency and reduced amplitude of the components. Finally, all responses disappeared following binaural TTX injections.

Vestibular origin

We consider cochlea removal to be the definitive experiment to establish that auditory responses are not responsible for the VsEP. Our ability to record potentials following bilateral cochlea removal indicates that the response is indeed not cochlear in origin. Bilateral cochlea removal did not impede our capability of identifying the most prominent components of the response, nor did it affect the latency of wave P2 in a significant way. The amplitude of P2 was, however, somewhat altered by the manipulation. Residual damage to the vestibular portions of the labyrinth, slight persistent perilymph leakage, and removal of the macula of the lagena may have contributed to the observed changes. In addition, perilymph may have leaked out during removal of
the cochleae. It is possible that surgical trauma to some of the presumed generators of the response may have resulted in reduced amplitude of the components.

The alternative procedure to rule out auditory contributions to the VsEP has been the use of auditory maskers (Elidan et al. 1984a; Hoffman and Horowitz 1984b; Jones and Pedersen 1989; Weisleder et al. 1989). The disadvantage is that the cochlea is still in place; thus, it could presumably still be stimulated by high levels of acceleration. Removal of both cochleae eliminated the known peripheral generators of auditory potentials; therefore, the remaining activity generated within the inner ear is suggested to be of vestibular origin.

**Bilaterality**

The results from these experiments also indicate that when recording from intact animals, where both inner ears are being stimulated, the evoked response is composed of activity from both vestibular systems. Pharmacological blockade of the activity generated by one vestibular system had the effect of consistently increasing the latency of the components and reducing their amplitude. These results compare favorably with previous observations by Jones and Pedersen (1989). These authors reported that unilateral labyrinthectomy results in elimination or reduction of a portion of the response. A similar trend was observed in mammals by Elidan et al. (1987a) after unilateral section of the VIIIth cranial nerve. According to these investigators, surgical interruption of one afferent vestibular pathway yields prolonged and less prominent responses.

Bilateral intralabyrinthine injections of TTX abolished all VsEP components. The importance of this finding is 3-fold. First, disappearance of the activity in response to linear acceleration stimuli indicates that the generators of the VsEP are located in the inner ear. Second, absence of the characteristic wave forms following TTX injections rules out motion artifacts as the source of these potentials. Third, TTX has been proven to be a reliable blocker of the vestibular portion of the VIIIth cranial nerve. Similar results were described in rats and cats by Elidan et al. (1982, 1984a,b), and in chickens by Jones and Pedersen (1989). These investigators reported that, subsequent to bilateral VIIIth cranial nerve sectioning or aspiration of inner ear contents, they were unable to record activity in response to acceleration stimuli.

A slow, low amplitude potential could be recorded in all subjects following bilateral TTX injections. This wave could only be detected at the 2 highest acceleration levels (2.00 and 1.00 g). Elidan et al. (1984a,b) attributed an analogous finding to myogenic reaction in response to the stimulus. Jones (personal communication) commented on a similar wave that did not disappear even after death. Our experiments also suggest that this 'response' is an artifact due to electrode and skin motion. We do not believe that the potential is of microphonic origin as described by Wit et al. (1986), since the wave is of very low frequency (170 Hz) and can still be identified, without amplitude changes, in recordings obtained after the animal's death.

In conclusion, the results from this investigation show that the electrophysiological activity recorded in response to linear acceleration stimuli has its origin in the vestibular system. The close relationship that exists between the stimulus and the VsEP provides further evidence that the recorded potentials are indeed in response to acceleration. Vestibular evoked potentials have the advantage over traditional vestibular testing methods in that the responses originate from the primary vestibular pathways and do not depend on the integrity of other systems. The method can be used to study the normal physiology of the vestibular system, the physiology of the pathologic vestibular system, and to evaluate the effects of medications on the vestibular system. In addition, once technological difficulties dealing with stimulus delivery are overcome, this test has the potential of becoming a tool in clinical electrophysiology.

This study was supported by NIH Grant DC 00395 (EWR). Additional funds were provided by a Grant-in-aid of research from Sigma-Xi Scientific Research Society and Cora M. Poncin Foundation (PW), and NASA NAGW-1275 (TAJ).
The authors wish to thank Michael J. Wilson and Richard L. Hyson for their helpful comments on earlier versions of this manuscript.

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