VESTIBULAR ONTOGENY: MEASURING THE INFLUENCE OF THE DYNAMIC ENVIRONMENT

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In comparison to other special senses, we are only meagerly informed about the development of vestibular function and the mechanisms that may operate to control or influence the course of vestibular ontogeny.[1] Perhaps one contributing factor to this disparity is the difficulty of evaluating vestibular sense organs directly and noninvasively. The present report describes a recently developed direct noninvasive vestibular function test that can be used to address many basic questions about the developing vestibular system. More particularly, the test can be used to examine the effects of the dynamic environment (*e.g.* gravitational field and vibration) on vestibular ontogeny.

Vestibular compound action potentials: The new test involves the measurement of compound action potentials generated by the vestibular nerve and its central relay neurons. Transient linear acceleration of the cranium elicits a cohesive volley of action potentials in the vestibular nerve. These action potentials can be resolved from the surface of the skull using traditional signal averaging techniques.[6,8] Vestibular response thresholds can be determined by systematically reducing or increasing stimulus intensity and thus provide a measure of the overall sensitivity of the vestibular end organs. The sensitivity of gravity receptors changes as embryos grow and develop and the profile of maturation can be documented using vestibular response thresholds.

Stimuli: The adequate stimulus for vestibular responses is a linear acceleration pulse. Acceleration stimuli presented to the cranium are produced by an electromechanical shaker which is driven by a precisely defined voltage waveform.[10] The onset rise time of the stimulus is a critical parameter. Rise time values between 0.5 and 8.0 msec to peak acceleration have been used successfully. However, threshold is inversely proportional to the stimulus rise time as discussed below. The best stimuli have onset rise-times that are less than 2msec .

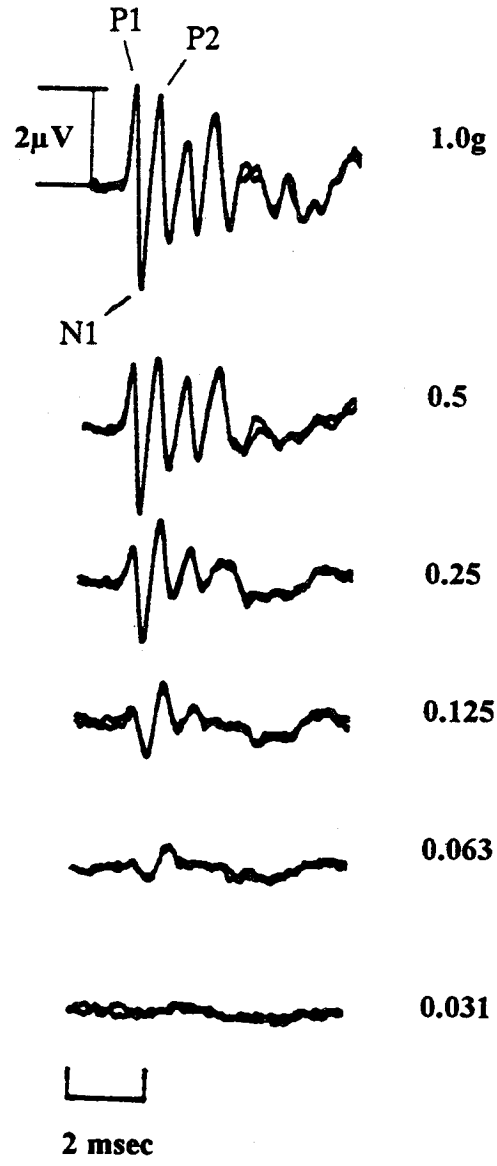

Figure 1: Vestibular responses to pulsed linear acceleration. Normal and inverted stimulus polarities are summed for each trace. First 3 major peaks are labeled (P1, N1, P2). 6dB intensity steps were used. Animal wa anesthetized.

Response Features and Origins: Figure 1 illustrates many of the general features of vestibular responses to pulsed linear acceleration as described originally by Jones and Pedersen.[10] Responses have short latencies (onset 1.0-2.0 msec) and small amplitudes (usually less than 20μV peak-to-peak). They are sensitive to core temperature change, they do not invert with stimulus inversion and they disappear immediately upon death. When stimulus intensity is increased, response onset latency decreases whereas amplitudes increase. Responses are dependent upon the labyrinths bilaterally and resemble compound action potentials of the auditory system but unlike auditory responses, they are highly resistant to intense acoustic masking. Moreover, response thresholds are unaffected by bilateral cochlear extirpation which spares vestibular structures.[6,20] These findings have shown therefore, that responses are neural and depend on the vestibular component of the eighth nerve bilaterally.

More recent studies confirm the hypothesis that the earliest components of vestibular short latency responses (*i.e.* P1, N1, P2) reflect activity within the eighth nerve whereas later response components are generated by higher order elements of the vestibular pathways.[18,19] It may be possible therefore to distinguish peripheral versus central changes in vestibular function.

In other studies we have found that the threshold of the compound vestibular response is inversely proportional to the rate of change in linear acceleration (*i.e.* da/dt or jerk[6,7,14,15]). Thus responses appear to be most sensitive to the first derivative of acceleration rather than the peak level of acceleration attained during stimulation.

Vestibular neurons that respond to this component of the acceleration stimulus waveform have been described in the frog.[16,17] These neurons are very likely among those neurons described as "irregular" units of the vestibular system[5] and those afferents that respond to dynamic components of stimuli in the bird.[2,3]

To further examine this issue we recently evaluated threshold as a function of jerk magnitude and found that when expressed in units of jerk (g/msec) thresholds are virtually constant[7] regardless of the onset rise-time of the stimulus at 1.0*g*. We have advanced the hypothesis therefore that vestibular responses to pulsed linear acceleration are produced in large part by vestibular jerk detectors. It will be important to rigorously test this hypothesis in future research.

Reporting and resolution of vestibular response threshold measurements: The sensitivity of the vestibular system is not constant throughout the life of a growing animal. Recent preliminary measurements suggest that thresholds fall sharply as the embryo develops and hatches (*e.g.* E19 embryo: 0.157*g* /msec, coefficient of variation (CV)= 52%; 21- day post-hatch chick: 0.073*g* /msec, CV= 55%). It is against the background of normal development and maturation that we must evaluate the effects of altered dynamic environments. It may be informative, therefore, to estimate the apparent resolution of the vestibular test under these circumstances.

Thresholds are determined by increasing or decreasing stimulus intensity in discrete steps (*i.e.* 6 or 3dB). Threshold is defined as the stimulus intensity midway between the minimum intensity producing a response and the maximum intensity failing to produce a response. Figure 1 illustrates a vestibular threshold test series using 6dB steps where threshold in this case is scored as 0.045*g* (0.09 *g*/msec or ≅ $-27dB_{re:1.0g}$).

Presumably, vestibular threshold is a continuous, normally distributed physiological variable. Threshold measurements, as described above, convert data into discrete levels or steps. If measurements employ large intensity steps relative to the true underlying data dispersion (*e.g.* variance), then sample distributions may be dis-

torted. This may in turn result in inaccurate estimates of the true mean and variance. Data that are reported in discrete steps or that are not normally distributed are best evaluated using non-parametric tests (*e.g.* Mann-Whitney U) since these tests depend on ranks and make no assumptions regarding the underlying data distribution. In any case threshold measurements described above provide an objective basis to rank the thresholds of individual animals.

In addition to quantizing data, the measurements impose a logarithmic transformation on threshold data (*i.e.* $y\ dB_{re:1.0g} = 20\log(x\ g/1.0g)$). Estimates of mean threshold and variance therefore should be calculated in $dB_{re:1.0g}$ and reported in $dB_{re:1.0g}$ or equivalent geometric means (in *g* or *g*/msec) for descriptive information. Descriptive information about data dispersion can be expressed as the CV in %. Statistical hypothesis testing should be based on mean and variance in $dB_{re:1.0g}$.

Given a normally distributed sample and reasonable estimate of population variance, the power of analysis of variance (ANOVA) tests for vestibular thresholds can be estimated. To this end, two questions can be entertained. What is the statistical power of the test given a change in threshold of 6dB? And what is the minimum threshold change that can be detected given an acceptable power of 0.8? We will assume a 'worst case' scenario where the test sample size will be n=5 and use the conventional α=0.05 as the criterion for statistical significance. We will estimate the population mean using threshold data from 27 normal vivarium control animals, Measurements were made using 6dB intensity steps and single polarity stimuli.

The value of the independent variable for analysis of variance power functions (Φ: "..a measure of the degree of falseness of the null hypothesis" [4]) is given by:[13]

$$\Phi=\{(\eta(\mu_2-\mu_1)^2)/\lambda\sigma^2\}^{1/2} \cong 2.06 \qquad \text{(eq 1)}$$

where η= the test sample size = 5, μ_1 = estimate of population mean = $-29.56dB_{re:1.0g}$, μ_2 = test mean which is set equal to 6dB above estimated population mean = $-23.56dB_{re:1.0g}$, λ= number of treatment factors (*e.g.* flight vs control) = 2, and σ = estimated population standard deviation = 4.6dB. The degrees of freedom for the numerator (df_{num}) and the denominator (df_{denom}) of the F-Ratio are given as $df_{num} = \lambda-1 = 1$, and $df_{denom} = \lambda(\eta-1) = 8$. The power corresponding to $\Phi \cong 2.06$ is approximately 0.72 (see Table A-2, pg 549 [13]).

Using the estimates of population mean and standard deviation (above) we can estimate the minimum threshold change that can be detected given an acceptable power of 0.8 and an α=0.05 as follows:

$$\mu_2-\mu_1=\{\lambda\Phi^2\sigma^2/\eta\}^{1/2} \cong 6.6dB \qquad \text{(eq 2)}$$

where Φ = 2.26 (from Table A-2 [13]), $df_{num} = 1$, $df_{denom} = 8$, and $\lambda = 2$.

These calculations imply that our resolution is close to that of the size of the protocol intensity step of 6dB. We can discern with some confidence threshold changes between 6 and 7dB. We would expect an improvement upon this resolution if smaller intensity steps were used (*i.e.* 3dB or less).

When nonparametric statistical tests are used one cannot estimate statistical power easily. However, using the same data employed in power calculations above, a simulated 6dB change in threshold was readily detected by a Mann-Whitney U test thus suggesting comparable power for changes near the size of the measurement step.

Recent Applications: Vestibular responses have been used recently to examine the effects of ototoxic drugs, space flight and vibration on vestibular function. Streptomycin at a dose of

600mg/Kg/day for 8 days was shown to produce a profound vestibular deficit evidenced by significant elevations of threshold.[8]

In a study of birds flown as embryos aboard the shuttle Discovery (STS-29), Jones and coworkers [11,12] reported that vestibular thresholds of flight animals were significantly greater than synchronous ground controls [12] but not vivarium controls.[11] Remarkably, measurements were not made until four weeks after the animals were returned to earth. In this case major putative effects of space flight were likely reduced to a minimum since adaptation to earth's gravity likely occurred upon return to 1.0G. Indeed if changes actually were present, then they were most probably less than 6dB and hence at the limit of test resolution.

Summary and acknowledgment: A new direct noninvasive method of testing peripheral vestibular function is available for use in the study of vestibular ontogeny and factors that potentially alter normal development such as the unusual dynamic environment of space flight. For small sample sizes, statistical power estimates for the measurements exceed 0.80 when treatment effects exceed 6.6dB. Smaller threshold shifts may be resolved by simply adjusting protocol step size. This work was supported by NASA Space Biology Program: NASA NAGW 1275.

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