HHS Public Access

Author manuscript

Exp Brain Res. Author manuscript; available in PMC 2016 June 01.

Published in final edited form as:

Exp Brain Res. 2015 June ; 233(6): 1825–1835. doi:10.1007/s00221-015-4254-4.

Preserved otolith organ function in caspase-3 deficient mice with impaired horizontal semicircular canal function

Patrick A Armstrong[1,2], **Scott J Wood**[1,3], **Naoki Shimizu**[1], **Kael Kuster**[2], **Adrian Perachio**[1], and **Tomoko Makishima**[1]

[1]Department of Otolaryngology, University of Texas Medical Branch, 301 University Blvd., Galveston, Texas, USA

[2]School of Medicine, University of Texas Medical Branch, Galveston, Texas, USA

[3]Azusa Pacific University, Azusa, California, USA

Abstract

Genetically engineered mice are valuable models for elucidation of auditory and vestibular pathology. Our goal was to establish a comprehensive vestibular function testing system in mice using: 1) horizontal angular vestibular-ocular reflex (hVOR) to evaluate semicircular canal function, and 2) otolith-ocular reflex (OOR) to evaluate otolith organ function, and to validate the system by characterizing mice with vestibular dysfunction. We used pseudo-off vertical axis rotation (pOVAR) to induce an otolith-only stimulus using a custom-made centrifuge. For the OOR, horizontal slow phase eye velocity (HEV) and vertical eye position (VEP) was evaluated as a function of acceleration.

Using this system, we characterized hVOR and OOR in the caspase-3 (*Casp3*) mutant mice. *Casp3* $^{-/-}$ mice had severely impaired hVOR gain, while *Casp3* $^{+/-}$ mice had an intermediate response compared to WT mice. Evaluation of OOR revealed that at low to mid frequencies and stimulus intensity, *Casp3* mutants and WT mice had similar responses. At higher frequencies and stimulus intensity, the *Casp3* mutants displayed mildly reduced otolith organ related responses. These findings suggest that the *Casp3* gene is important for the proper function of the semicircular canals but less important for the otolith organ function.

Keywords

Vestibular ocular reflex; counter rotation; pseudo off-vertical axis rotation; otolith ocular response; mice; caspase-3

Corresponding author: Tomoko Makishima, University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555-0521, Tel: 409-772-9946, Fax: 409-772-1715, tomakish@utmb.edu.

Conflict of interest:
The authors declare that they have no conflict of interest.

Introduction

Dizziness and balance problems are a significant problem in the United States, and ranked among the most common presenting symptoms in the health care system (Burt & Schappert, 2004; Kerber et al, 2008). The vestibular system is crucial in maintenance of balance, and vestibular dysfunction can significantly impact quality of life (Mira, 2008). The vestibular system is comprised of the semicircular canals (superior, horizontal and posterior), which detect head angular acceleration and the otolith organs (utricle and saccule), which detect linear head acceleration. In response to linear or angular acceleration stimuli, compensatory ocular reflexes are produced to stabilize images on the retina. These reflexes have been utilized to assess functions related to the semicircular canals or the otolith organs. Angular vestibular-ocular reflexes have been used routinely in humans to assess semicircular canal function (Fetter, 2007; Wuyts et al, 2007) and have been established in different animal models, including mice (Beraneck & Cullen, 2007; Faulstich et al, 2004; Kimpo & Raymond, 2007; Migliaccio et al, 2011; Stahl, 2002).

However, assessment of otolith organ function using ocular responses is performed less frequently. Centrifugation is one of the methods used to target stimulation to the otolith organs. In humans, induction of otolith-ocular reflex (OOR) has been established with off vertical-axis rotation (OVAR) (Furman et al, 1993; Guedry, 1965; Sugita-Kitajima et al, 2007), as well as eccentric counter-rotation (CR) (Benson & Barnes, 1973; Niven et al, 1966). OVAR typically entails constant rotational velocity of a subject's head about an axis that is tilted in respect to the earth vertical axis. After achieving steady state rotation, the semicircular canal response decays, and the resulting ocular response is otolithic in origin (Cohen et al, 1983; Guedry, 1965; Maruta et al, 2001). Similarly, CR provides rotating linear acceleration stimulus, but without relative initial angular acceleration. In animals, OVAR has been tested in several lateral-eyed mammals, including rabbits (Janeke et al, 1970; Maruta et al, 2001) and rats (Brettler et al, 2000; Hess & Dieringer, 1990; Rabbath et al, 2001), and CR has been tested in gerbils (Kaufman, 2002).

Mouse models have become prevalent for inner ear studies, since many human ortholog genes necessary for auditory and vestibular development and function have been identified (Anagnostopoulos, 2002; Fekete, 1999). In these genetically engineered mice, in contrast to the well-studied auditory system, the vestibular system remains relatively uncharacterized, due to the technical difficulties of manipulating mice for vestibular function analysis. Evaluation of the otolith organ function in mice has been reported using several methods: vestibular evoked potientials (VsEP), vestibular evoked myogenic potentials (VEMP) and maculo-ocular response (MOR). VsEPs are measurable electric potentials in the brainstem that are evoked by using angular or linear acceleration stimuli (Elidan et al, 1991; Jones & Jones, 1999; Plotnik et al, 1997). Linear VsEP is known to be a response to otolith stimulus, and has been a popular otolith function analysis method in mice (Jones et al, 2004; Jones et al, 2005; Jones et al, 2006; Jones et al, 2011a; Jones et al, 2002; Jones & Jones, 1999; Jones et al, 2011b; Lee et al, 2013; Mock et al, 2011; Robertson et al, 2008; Zhao et al, 2008). However, this method is used less frequently in humans, and is found in studies such as exploring angular VsEP (Elidan et al, 1991; Rodionov et al, 1996).

VEMP measures saccule-evoked potentials in head and neck muscles in response to sound stimulus, and utricular-evoked potentials related to otolith-ocular function. Due to technical difficulty performing this method in rodents, there are a limited number of studies in guinea pigs (Yang & Young, 2005) and only one report in mice (Sheykholeslami et al, 2009). MOR in response to maintained tilt created by off vertical axis rotation (OVAR) has also been used to assess otolith organ function in mice (Beraneck & Cullen, 2007; Oommen & Stahl, 2008; Romand et al, 2013; Stahl & Oommen, 2008; Stahl et al, 2012).

Our objective was to establish a comprehensive vestibular function analysis system in mice using ocular reflexes in hopes of obtaining results that is transferable between humans and mouse models. We used caspase-3 deficient mice (Morishita et al, 2001; Takahashi et al, 2001) which has unique vestibular properties: abnormal semicircular canal function and morphology (Makishima et al, 2011), with preserved otolith function and histologically intact otolith organs.

Methods

Mice

Male and female wild type (WT) mice (C57BL/6), *Casp3* mutant mice and *Apaf1* mutant mice at ages 2 – 6 months old were utilized in this study. The generation of the CPP32$^{ex3-/-}$ (*Casp3*−/−) and *Apaf1* mutant mice (Yoshida et al, 1998) has been previously described (Woo et al, 1998). Briefly, the *Casp3* mutant mice were generated by replacing a region including exon 3 of the *Casp3* gene with a neomycin cassette, thus creating a deletion of the *Casp3* gene downstream of the insertion. The genotype of the *Casp3* mutant mice was confirmed by PCR amplification of the region including the junction of the *Casp3* gene and the neomycin cassette as described (Woo et al, 1998). Protocol for animal use was approved by the Institutional Animal Care and Use Committee of the University of Texas Medical Branch. All animal studies were in compliance with the guidelines of the National Institutes of Health, United States.

Centrifuge

All vestibular function recordings were performed with a custom made centrifuge modified for use with mice (Fig 1 A, B). Details of the centrifuge have been previously described (Kaufman, 2002). In our rotational experiments, we employed rotation of the main and eccentric axes of the centrifuge capable of independent or simultaneous rotation in clockwise and counter-clockwise directions.

Horizontal Vestibulo-Ocular Reflex (hVOR)

WT mice (males n=15, females n=12), *Casp3* heterozygous mice (*Casp3*$^{+/-}$) (males n=6, females=4), *Casp3* homozygous mice (*Casp3* $^{-/-}$) (males n=5, females n=5) and *Apaf1* heterozygous mice (*Apaf1*$^{+/-}$) (male n=7, female n=5) were utilized in the evaluation of horizontal angular vestibular ocular response (hVOR). Mice aged 2 – 6 months were briefly anesthetized with isoflurane inhalation and were placed in a custom plastic conical jacket and placed on a support bed. The head was stabilized using a custom bite-block and head restraint and the head was positioned 30° nose-down. The bite-block and bed were attached

to a mini-platform designed to dock and secure the mouse to the animal centrifuge in a light tight drum. After being mounted to the centrifuge, mice were given a 10–15 minute rest interval to allow for full awakening and acclimation before testing. Pilocarpine otic drops (1%) were administered to the eyes to constrict the pupils allowing for optimal tracking of eye movements with video-oculography in darkness.

Horizontal canal vestibulo-ocular reflex function was evaluated using sinusoidal rotation of the eccentric axis between 0.05 Hz and 0.80 Hz at a peak velocity of 60 deg/s (horizontal VOR). Linearity of the VOR slow phase velocity was tested using 0.20 Hz stimuli at increasing peak velocities from 60° – 120°/sec.

Otolith-ocular reflex (OOR) Paradigms

WT (males n=4, females n=1), *Casp3*$^{+/-}$ (males n=3, females n=2), and *Casp3*$^{-/-}$ (males n=2, females n=3) mice at ages 2 – 6 months old were evaluated for the OOR tests with two paradigms: Counter Rotation (CR) and pseudo-Off Vertical Axis Rotation (pOVAR). Mice were prepared for stimuli administration and recording in the same manner as in hVOR protocol.

Counter Rotation (CR)

CR (Fig. 1C) was performed by slaving the eccentric axis to the velocity feedback signal of the main axis, allowing the eccentric axis to be rotated at an equal but opposite angular acceleration and constant velocity compared to the main axis. For example, when the main axis was rotated at 60 deg/s^2 to a constant velocity of 144 deg/s, the eccentric axis was rotated at -60 deg/s^2 and then reach a constant velocity of -144 deg/s. This rotational model resulted in no relative angular acceleration stimulus to the mice, producing a linear acceleration stimulus that constantly changed orientation relative to the head (Benson & Barnes, 1973). The rotational profiles used to test CR are listed in Table 1. For all profiles, the main axis was rotated at an acceleration of 60 deg/s^2 to constant velocity. The initial 60 seconds of recording were excluded to ensure steady state rotation.

Pseudo-Off Vertical Axis Rotation (pOVAR)

pOVAR (Kaufman, 2002) was performed using sustained angular rotation of the main axis independent of the eccentric axis at different velocities. Four separate sets of rotational profiles were tested for pOVAR (Table 2), with each set increasing in main axis rotational velocity, subsequently increasing equivalent linear acceleration and tilt angle vector. During pOVAR, the main axis was accelerated to peak velocity at 60 deg/s^2 and rotated for at least 60 seconds to allow the per-rotatory angular vestibular response to decay and reach steady-state. Main axis rotation was maintained at peak velocity throughout each set of eccentric axis rotation frequencies. To control for the initial angular acceleration stimulus, the initial 60 seconds of eccentric rotation recording were excluded from analysis to allow the canal and central signals to fully decay. After achieving steady-state rotation, the resultant vestibular stimulus was a continuously rotating linear acceleration similar to stimuli induced by OVAR. A schematic of eye position associated with acceleration stimulus orientation relative to the head is shown in Figure 2A.

Video-oculography

The right eye was used for monitoring ocular reflexes. A custom video-oculographic system was utilized using a near-infrared eye imaging camera. The maximum-likelihood based pupil tracking algorithm provides a robust best-fitting model that has been described elsewhere (Sung & Reschke, 1994). We derived a scale factor for gain calculations by estimating the average ocular globe diameter of mice at different ages relative to the number of pixels from our video images. Eye diameter was found to be lower in 1 month-old mice, resulting in a larger scale factor than older mice. Gain calculated by this method was verified by rotating a camera concentrically about the mouse eye at a fixed-radius, and therefore not dependent on visual following (Makishima, 2011). After applying the eye calibration, pupil position was differentiated, desaccaded using velocity thresholds, and then verified using a custom interactive script (MATLAB, The MathWorks). Response parameters for hVOR (gain and phase) were derived from least-squares sinusoidal fits of horizontal eye velocity. The response parameter of amplitude modulation for OOR paradigms were derived from sinusoidal fits of horizontal eye velocity and vertical eye position.

Gross behavioral analysis

Circling, tail hanging and air righting reflex was used to assess gross vestibular behavior (Al Deeb et al, 2000) in WT (n=8), heterozygous *Casp3*$^{+/-}$ (n=7) and homozygous *Casp3*$^{-/-}$ (n=6) mice at ages 5–6 months. For circling, the behavior was recorded on video for five minutes after placing the mouse in a clean cage with fresh bedding. Full 360° rotations, either in clockwise or counter-clockwise direction, were counted. For tail hanging, mice were lifted by the tail, and then lowered to the ground slowly. Normal response was considered when the mouse extended their forelimbs towards the ground, showing a ‘landing’ behavior. The mouse was considered abnormal if they bent ventrally. For air righting, mice were held supine and dropped from a height of 30 cm onto a foam cushion. The mice were considered normal if they landed on their feet. If they landed on their side or back, it was considered to be abnormal. Each test was repeated three times on three different days.

Statistical analysis

Ocular response parameters were assessed for statistical difference ($P<0.05$) using Student’s t test, as well as analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) using the Scheffe’s method. StatistiXL version 1.6 (available from http://www.statistixl.com/) was used with the Windows® version of Microsoft Excel®.

Results

Modulation of eye movements with linear acceleration stimuli in WT mice

We used the counter rotation (CR) and pseudo off-vertical axis rotation (pOVAR) paradigms to induce linear acceleration in mice. In WT mice (n=5), continuous unidirectional nystagmus in combination with a very slow vertical eye movement was observed in response to CR and pOVAR (Figure 2A, left panel). Horizontal slow phase eye

velocity (HEV) and amplitude of the vertical eye position (VEP) were used as parameters of otolith ocular response (OOR). Modulation of the amplitude of the sinusoidal component of OOR in response to increasing acceleration was apparent in both CR and pOVAR paradigms. Responses at 0.40 Hz showed similar linear stimulus intensity-dependent modulation of response parameters induced by pOVAR and CR (Figure 3). Given the equivalent response properties of the two stimulus paradigms, we chose pOVAR as the primary stimulus type to use in further studies due to higher ranges of stimulus intensities that could be provided with our centrifuge. With pOVAR, increasing acceleration increased the amplitude of HEV and VEP (ANOVA $P<0.05$). Increasing frequency increased the amplitude of HEV (ANOVA $P<0.05$), but did not modulate VEP in WT mice (Figure 4).

Impaired hVOR in *Casp3* mutant mice

We observed the hVOR at ages 2 – 6 months in heterozygous *Casp3*$^{+/-}$ mice (n=10), homozygous *Casp3* $^{-/-}$ mice (n=10) and heterozygous *Apaf1* $^{+/-}$ mice (n=12) and compared response parameters to WT mice (n=27) (Figure 5). The *Apaf1* mutant mice were included in the study as a random control heterozygous mutant strain. The *Apaf1* mice were nearly identical to WT in hVOR response (Figure 5A, B). Both *Casp3*$^{+/-}$ mice and *Casp3*$^{-/-}$ mice had significant differences in gain compared to WT mice at all frequencies tested (ANOVA, $P<0.05$), with *Casp*3 $^{-/-}$ mice exhibiting a greatly impaired gain and *Casp3*$^{+/-}$ mice having an intermediate response. Due to severely impaired hVOR gain, phase shift calculations were difficult to reliably quantify in *Casp3* $^{-/-}$ mice. *Casp3* $^{+/-}$ mice had elevated phase lead compared to WT at frequencies 0.20 Hz and higher (Figure 5B, Student's t test, $P<0.05$). *Casp3* $^{+/-}$ *and Casp3* $^{-/-}$ mice had linear VOR slow phase velocity which was significantly lower than the WT mice (Figure 5C). Phase shift decreased with increasing velocity, and was significantly higher in *Casp3*$^{+/-}$ than WT at 60 deg/s (Student's t test, $P<0.05$), but similar at other frequencies.

pOVAR response was mildly impaired in Caspase-3 mutant mice

pOVAR response in *Casp3*$^{+/-}$ (n=5) and *Casp3*$^{-/-}$ mice (n=5) were evaluated and compared to WT mice (n=5) (Figure 6). In response to pOVAR, *Casp3* $^{+/-}$ mice exhibited continuous unidirectional nystagmus comparable to WT. On the other hand, *Casp3* $^{-/-}$ mice exhibited similar slow phase eye movements, but typically required a higher stimulus intensity to reach threshold to produce robust continuous unidirectional nystagmus. Both *Casp3*$^{+/-}$ and *Casp3*$^{-/-}$ mice had a trend of exhibiting altered stimulus intensity-dependent modulation of HEV and VEP amplitudes in high frequencies compared to WT. Similar HEV responses were seen in WT and *Casp3* $^{-/-}$ mice across increasing stimulus intensities except for a significant difference at a single stimulus point of 0.1Hz at 8.2 m/s^2 (ANOVA $P<0.05$). Interestingly, *Casp3*$^{+/-}$ mice exhibited significantly elevated HEV amplitude compared to WT at higher intensities at 5.6 m/s^2 and 8.2 m/s^2 (ANOVA, $P<0.05$). *Casp*$^{-/-}$ mice had the lowest mean VEP amplitude with significantly lower responses at 3.6 m/s^2, 5.6 m/s^2 and 8.2 m/s^2 in higher frequencies (ANOVA, $P<0.05$). There was no difference in VEP responses between *Casp3* $^{+/-}$ and WT mice.

Abnormal gross vestibular behavior in *Casp3* deficient mice

We assessed gross vestibular behavior in WT, Casp3$^{+/-}$ and Casp3$^{-/-}$ mice by observing circling behavior, tail hanging test and air righting reflex. In tail hanging and air righting tests, four out of six Casp3$^{-/-}$ mice showed abnormal behavior, while all Casp3$^{+/-}$ mice (n=7) and WT mice (n=8) were all normal. In circling, the number of full 360° circling during a five-minute period was an average of 39 turns in Casp3$^{-/-}$ mice, while Casp3$^{+/-}$ and WT mice only circled less than two turns. Most Casp3$^{-/-}$ mice had a tendency to circle in the same direction on all three trials. There were more mice with a tendency to circle in the counter-clockwise direction (data not shown). The gross behavior trends were similar in older aged mice at >12 months (data not shown).

Discussion

Horizontal VOR and gross vestibular behavioral tests in mutant mice

We previously reported significantly impaired horizontal VOR in homozygous *Casp3* $^{-/-}$ mice and an intermediate response observed in heterozygous *Casp3* $^{+/-}$ mice (Makishima et al, 2011). Despite the lower gain observed in horizontal VOR, there were no apparent observable behavioral patterns such as circling, head bobbing or hyperactivity in the heterozygous Casp3$^{+/-}$ mice. In gross vestibular behavioral tests, circling, air-righting and tail hanging tasks did not evoke any abnormalities in the heterozygous Casp3 $^{+/-}$ mice. The discrepancy between the VOR results and behavioral test results suggest that: 1) gross behavioral tests are not as sensitive as VOR, 2) low horizontal VOR gain at approximately 50% of that of WT mice does not affect gross behavior in mice, and 3) our VOR video eye tracking system is suitable to detect subtle differences in vestibular performance not apparent in behavioral observations.

Comparison of CR and pOVAR paradigms

During CR in humans, the relationship of sinusoidal modulation HEV amplitude has been shown to be linear in response to increasing stimulus magnitude (Benson & Barnes, 1973). While we wanted to model our response parameters on a logarithmic frequency scale, it was technically difficult with the CR paradigm because the eccentric turntable could not rotate at velocities higher than 144 deg/s, limiting the range of linear stimuli intensity that could be provided. The lower intensity profiles of CR often did not reach the threshold for OOR response. On the other hand, our pOVAR paradigm was capable of delivering a wide range of linear stimuli intensities, making it an ideal test of otolith function. pOVAR is similar to OVAR, in that there is an initial angular stimulus until reaching steady state rotation, although the subject is not physically tilted. Analogous to CR stimuli, pOVAR equivalent tilt angle and magnitude of the linear acceleration vector are functions of the main axis rotational velocity, and the frequency rotation of the linear acceleration vector is a function of eccentric axis rotational velocity. Comparison between CR and pOVAR OOR demonstrated similar linear stimulus intensity dependent responses, providing evidence that our pOVAR response is also otolithic in origin.

Ocular response to pOVAR

Normative response—As shown in Figure 2A (WT data) in response to the pOVAR stimulus, we observed a constant horizontal nystagmus alongside a sinusoidal modulation of the horizontal eye velocity, and a very slow sinusoidal modulation of the vertical eye position. Due to the very slow movement and small amplitude in the vertical component of eye response compared to the horizontal component, the vertical eye velocity did not yield optimal values as a function of acceleration. Thus, we chose to evaluate the slow phase velocity of the horizontal eye movement and the vertical eye position for evaluation of otolith ocular response. We evaluated the sinusoidal modulation of the slow phase of HEV, which demonstrated a linear increasing modulation with stimulus magnitude. This is analogous to the changes in modulation with increasing tilt angle observed in mammalian OVAR studies (Darlot & Denise, 1988; Haslwanter et al, 2000). In OVAR, the linear stimulus intensity is modeled by $g \sin(\theta)$, where theta is the tilt angle and g is gravitational acceleration (Hess and Dieringer, 1990), thus the linear acceleration vector increases as tilt increases. HEV modulation amplitude also displayed an increase with increasing frequency of rotation, consistent with OVAR findings in other mammalian studies (Darlot & Denise, 1988; Jones et al, 2003). The modulation of HEV in mice appear to be consistent with gaze stabilization “translational” otolith-ocular reflexes; while the modulation of vertical eye position appears consistent with “tilt” otolith-ocular reflexes (Angelaki & Hess, 1996). The amplitudes of HEV and VEP modulation for pOVAR in WT mice were comparable to findings from OVAR in rats when compared at similar stimulus magnitude and frequency (Hess & Dieringer, 1990; Rabbath et al, 2001). These findings provide evidence for pOVAR as an otolithic test, and capable of differentiating both tilt and translational type otolith ocular responses.

Otolithic response in *Casp3* mutant mice is mildly impaired—Although *Casp3* $^{+/-}$ and *Casp3* $^{-/-}$ mice had severely impaired VOR response to angular stimuli compared to WT mice at all stimuli tested, there was a relatively small separation between genotypes with pOVAR OOR response. At the low and mid ranges of frequency and stimulus magnitudes there were no significant differences observed between *Casp3* mutant mice and WT. While *Casp3* $^{-/-}$ mice did not differ greatly from WT in HEV amplitude, surprisingly, *Casp3* $^{+/-}$ mice had multiple elevated HEV amplitude responses compared to WT at frequencies ≥0.20 Hz. Decreased modulation of OVAR HEV amplitude has been demonstrated to occur with gentamicin-induced lesions to the otolith organs in guinea pigs, likely due to the loss of afferent input from type I hair cells (Jones et al, 2003). Conversely, increases in modulation of OVAR HEV amplitude have been observed in humans with advanced age (Furman & Redfern, 2001), as well as cerebellar dysfunction (Anastasopoulos et al, 1998). It could be postulated that *Casp3* $^{+/-}$ mice have defects in the central processing of otolith signals, or a lack of inhibition of otolithic responses, however it is unclear why this is not observed in *Casp3* $^{-/-}$ mice. Alternatively, the enhanced HEV amplitude may be due to elevated peripheral input from the otoliths. *Casp3* $^{+/-}$ mice exhibit the trend of having increased number of hair cells compared to WT in the organs of the vestibule, whereas *Casp3* $^{-/-}$ mice have significantly fewer hair cells (Makishima et al, 2011). Additionally, the reduced modulation of amplitude in VEP for Casp3 $^{-/-}$ mice might reflect decrease in

peripheral vestibular function, which is suggested by the finding of Casp3 $^{-/-}$ mice having significantly fewer hair cells in the vestibule (Makishima et al, 2011).

Acknowledgments

We thank J. Stahl (Case Western Reserve University) for discussions and review of the manuscript, B. Lavery and J. Anderson for technical assistance and discussions of the project. *Casp3* mutant mice were a gift from Dr. Minna Woo (University of Toronto). *Apaf1* mutant mice were a gift from Dr. Tak Mak (University of Toronto). PA was supported by the Jeane B. Kempner fellowship. Research reported in this publication was supported by the National Institute on Deafness and Other Communication Disorders of the National Institutes of Health under Award Number K08DC011540 to TM.

References

Al Deeb S, Al Moutaery K, Khan HA, Tariq M. Exacerbation of iminodipropionitrile-induced behavioral toxicity, oxidative stress, and vestibular hair cell degeneration by gentamicin in rats. Neurotoxicology and teratology. 2000; 22:213–220. [PubMed: 10758350]

Anagnostopoulos AV. A compendium of mouse knockouts with inner ear defects. Trends in genetics: TIG. 2002; 18:499. [PubMed: 12350347]

Anastasopoulos D, Haslwanter T, Fetter M, Dichgans J. Smooth pursuit eye movements and otolith-ocular responses are differently impaired in cerebellar ataxia. Brain. 1998; 121:1497–1505. [PubMed: 9712011]

Angelaki DE, Hess BJ. Three-dimensional organization of otolith-ocular reflexes in rhesus monkeys. I. Linear acceleration responses during off-vertical axis rotation. J Neurophysiol. 1996; 75:2405–2424. [PubMed: 8793753]

Benson, AJ.; Barnes, GR. Responses to rotating linear acceleration vectors considered in relation to a model of the otolith organs. Fifth Symposium on the Role of the Vestibular Organs in Space Exploration; JAN 1, 1973; Washington D.C: NASA; 1973.

Beraneck M, Cullen KE. Activity of vestibular nuclei neurons during vestibular and optokinetic stimulation in the alert mouse. J Neurophysiol. 2007; 98:1549–1565. [PubMed: 17625061]

Brettler SC, Rude SA, Quinn KJ, Killian JE, Schweitzer EC, Baker JF. The effect of gravity on the horizontal and vertical vestibulo-ocular reflex in the rat. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale. 2000; 132:434–444.

Burt CW, Schappert SM. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 1999--2000. Vital Health Stat. 2004; 13:1–70.

Cohen B, Suzuki JI, Raphan T. Role of the otolith organs in generation of horizontal nystagmus: effects of selective labyrinthine lesions. Brain research. 1983; 276:159–164. [PubMed: 6626994]

Darlot C, Denise P. Nystagmus induced by off-vertical rotation axis in the cat. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale. 1988; 73:78–90.

Elidan J, Sela M, Liebner E, Sohmer H. Short latency vestibular evoked response to angular acceleration impulse in human beings. Otolaryngology--head and neck surgery: official journal of American Academy of Otolaryngology-Head and Neck Surgery. 1991; 105:353–359. [PubMed: 1945419]

Faulstich BM, Onori KA, du Lac S. Comparison of plasticity and development of mouse optokinetic and vestibulo-ocular reflexes suggests differential gain control mechanisms. Vision research. 2004; 44:3419–3427. [PubMed: 15536010]

Fekete DM. Development of the vertebrate ear: insights from knockouts and mutants. Trends in neurosciences. 1999; 22:263–269. [PubMed: 10354604]

Fetter M. Vestibulo-ocular reflex. Developments in ophthalmology. 2007; 40:35–51. [PubMed: 17314478]

Furman JM, Redfern MS. Effect of aging on the otolith-ocular reflex. Journal of vestibular research: equilibrium & orientation. 2001; 11:91–103. [PubMed: 11847453]

Furman JM, Schor RH, Kamerer DB. Off-vertical axis rotational responses in patients with unilateral peripheral vestibular lesions. The Annals of otology, rhinology, and laryngology. 1993; 102:137–143.

Guedry FE Jr. ORIENTATION OF THE ROTATION-AXIS RELATIVE TO GRAVITY: ITS INFLUENCE ON NYSTAGMUS AND THE SENSATION OF ROTATION. Acta oto-laryngologica. 1965; 60:30–48. [PubMed: 14337956]

Haslwanter T, Jaeger R, Mayr S, Fetter M. Three-dimensional eye-movement responses to off-vertical axis rotations in humans. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale. 2000; 134:96–106.

Hess BJ, Dieringer N. Spatial Organization of the Maculo-Ocular Reflex of the Rat: Responses During Off-Vertical Axis Rotation. The European journal of neuroscience. 1990; 2:909–919. [PubMed: 12106078]

Janeke JB, Jongkees LB, Oosterveld WJ. Relationship between otoliths and nystagmus. Acta oto-laryngologica. 1970; 69:1–6. [PubMed: 5310026]

Jones GE, Balaban CD, Jackson RL, Wood KA, Kopke RD. Effect of trans-bullar gentamicin treatment on guinea pig angular and linear vestibulo-ocular reflexes. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale. 2003; 152:293–306.

Jones SM, Erway LC, Johnson KR, Yu H, Jones TA. Gravity receptor function in mice with graded otoconial deficiencies. Hearing research. 2004; 191:34–40. [PubMed: 15109702]

Jones SM, Johnson KR, Yu H, Erway LC, Alagramam KN, Pollak N, Jones TA. A quantitative survey of gravity receptor function in mutant mouse strains. Journal of the Association for Research in Otolaryngology: JARO. 2005; 6:297–310. [PubMed: 16235133]

Jones SM, Jones TA, Johnson KR, Yu H, Erway LC, Zheng QY. A comparison of vestibular and auditory phenotypes in inbred mouse strains. Brain research. 2006; 1091:40–46. [PubMed: 16499890]

Jones SM, Robertson NG, Given S, Giersch AB, Liberman MC, Morton CC. Hearing and vestibular deficits in the Coch(−/−) null mouse model: comparison to the Coch(G88E/G88E) mouse and to DFNA9 hearing and balance disorder. Hearing research. 2011a; 272:42–48. [PubMed: 21073934]

Jones SM, Subramanian G, Avniel W, Guo Y, Burkard RF, Jones TA. Stimulus and recording variables and their effects on mammalian vestibular evoked potentials. Journal of neuroscience methods. 2002; 118:23–31. [PubMed: 12191754]

Jones TA, Jones SM. Short latency compound action potentials from mammalian gravity receptor organs. Hearing research. 1999; 136:75–85. [PubMed: 10511626]

Jones TA, Jones SM, Vijayakumar S, Brugeaud A, Bothwell M, Chabbert C. The adequate stimulus for mammalian linear vestibular evoked potentials (VsEPs). Hearing research. 2011b; 280:133–140. [PubMed: 21664446]

Kaufman GD. Video-oculography in the gerbil. Brain research. 2002; 958:472–487. [PubMed: 12470888]

Kerber KA, Meurer WJ, West BT, Fendrick AM. Dizziness presentations in U.S. emergency departments, 1995–2004. Academic emergency medicine: official journal of the Society for Academic Emergency Medicine. 2008; 15:744–750. [PubMed: 18638027]

Kimpo RR, Raymond JL. Impaired motor learning in the vestibulo-ocular reflex in mice with multiple climbing fiber input to cerebellar Purkinje cells. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2007; 27:5672–5682. [PubMed: 17522312]

Lee SI, Conrad T, Jones SM, Lagziel A, Starost MF, Belyantseva IA, Friedman TB, Morell RJ. A null mutation of mouse Kcna10 causes significant vestibular and mild hearing dysfunction. Hearing research. 2013; 300:1–9. [PubMed: 23528307]

Makishima T, Hochman L, Armstrong P, Rosenberger E, Ridley R, Woo M, Perachio A, Wood SJ. Inner ear dysfunction in caspase-3 deficient mice. BMC Neurosci. 2011; 12:102. [PubMed: 21988729]

Maruta J, Simpson JI, Raphan T, Cohen B. Orienting otolith-ocular reflexes in the rabbit during static and dynamic tilts and off-vertical axis rotation. Vision research. 2001; 41:3255–3270. [PubMed: 11718771]

Migliaccio AA, Meierhofer R, Della Santina CC. Characterization of the 3D angular vestibulo-ocular reflex in C57BL6 mice. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale. 2011; 210:489–501.

Mira E. Improving the quality of life in patients with vestibular disorders: the role of medical treatments and physical rehabilitation. International journal of clinical practice. 2008; 62:109–114. [PubMed: 17537195]

Mock B, Jones TA, Jones SM. Gravity receptor aging in the CBA/CaJ strain: a comparison to auditory aging. Journal of the Association for Research in Otolaryngology: JARO. 2011; 12:173–183. [PubMed: 21052761]

Morishita H, Makishima T, Kaneko C, Lee YS, Segil N, Takahashi K, Kuraoka A, Nakagawa T, Nabekura J, Nakayama K, Nakayama KI. Deafness due to degeneration of cochlear neurons in caspase-3-deficient mice. Biochemical and biophysical research communications. 2001; 284:142–149. [PubMed: 11374883]

Niven JI, Hixson WC, Correia MJ. Elicitation of horizontal nystagmus by periodic linear acceleration. Acta oto-laryngologica. 1966; 62:429–441. [PubMed: 5956521]

Oommen BS, Stahl JS. Eye orientation during static tilts and its relationship to spontaneous head pitch in the laboratory mouse. Brain research. 2008; 1193:57–66. [PubMed: 18178173]

Plotnik M, Elidan J, Mager M, Sohmer H. Short latency vestibular evoked potentials (VsEPs) to linear acceleration impulses in rats. Electroencephalography and clinical neurophysiology. 1997; 104:522–530. [PubMed: 9402894]

Rabbath G, Necchi D, de Waele C, Gasc JP, Josset P, Vidal PP. Abnormal vestibular control of gaze and posture in a strain of a waltzing rat. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale. 2001; 136:211–223.

Robertson NG, Jones SM, Sivakumaran TA, Giersch AB, Jurado SA, Call LM, Miller CE, Maison SF, Liberman MC, Morton CC. A targeted Coch missense mutation: a knock-in mouse model for DFNA9 late-onset hearing loss and vestibular dysfunction. Human molecular genetics. 2008; 17:3426–3434. [PubMed: 18697796]

Rodionov V, Elidan J, Sela M, Nitzan M, Sohmer H. Vertical plane short and middle latency vestibular evoked potentials in humans. The Annals of otology, rhinology, and laryngology. 1996; 105:43–48.

Romand R, Krezel W, Beraneck M, Cammas L, Fraulob V, Messaddeq N, Kessler P, Hashino E, Dolle P. Retinoic acid deficiency impairs the vestibular function. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2013; 33:5856–5866. [PubMed: 23536097]

Sheykholeslami K, Megerian CA, Zheng QY. Vestibular evoked myogenic potentials in normal mice and Phex mice with spontaneous endolymphatic hydrops. Otology & neurotology: official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology. 2009; 30:535–544.

Stahl JS. Calcium channelopathy mutants and their role in ocular motor research. Annals of the New York Academy of Sciences. 2002; 956:64–74. [PubMed: 11960794]

Stahl JS, Oommen BS. Eye hyperdeviation in mouse cerebellar mutants is comparable to the gravity-dependent component of human downbeat nystagmus. Progress in brain research. 2008; 171:503–508. [PubMed: 18718346]

Stahl JS, Thumser ZC, Oommen BS. The ataxic mouse as a model for studying downbeat nystagmus. Journal of vestibular research: equilibrium & orientation. 2012; 22:221–241. [PubMed: 23302704]

Sugita-Kitajima A, Azuma M, Hattori K, Koizuka I. Evaluation of the otolith function using sinusoidal off-vertical axis rotation in patients with benign paroxysmal positional vertigo. Neuroscience letters. 2007; 422:81–86. [PubMed: 17597299]

Sung K, Reschke MF. A four dimensional video eye movement tracking system. Aviat Space Environ Med. 1994; 65:464.

Takahashi K, Kamiya K, Urase K, Suga M, Takizawa T, Mori H, Yoshikawa Y, Ichimura K, Kuida K, Momoi T. Caspase-3-deficiency induces hyperplasia of supporting cells and degeneration of sensory cells resulting in the hearing loss. Brain research. 2001; 894:359–367. [PubMed: 11251216]

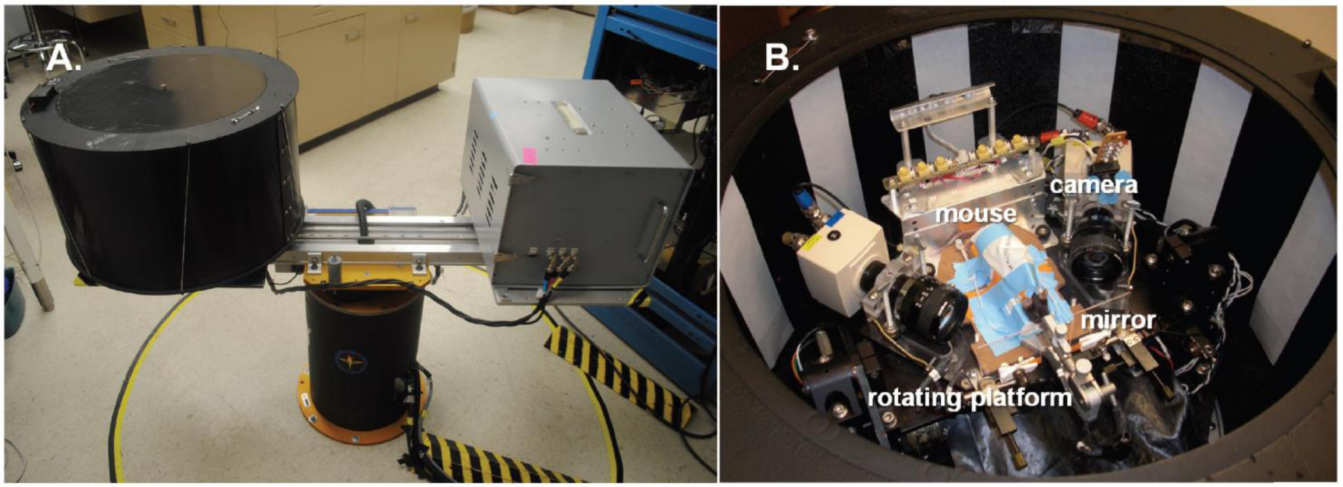
Woo M, Hakem R, Soengas MS, Duncan GS, Shahinian A, Kagi D, Hakem A, McCurrach M, Khoo W, Kaufman SA, Senaldi G, Howard T, Lowe SW, Mak TW. Essential contribution of caspase 3/CPP32 to apoptosis and its associated nuclear changes. Genes & development. 1998; 12:806–819. [PubMed: 9512515]

Wuyts FL, Furman J, Vanspauwen R, Van de Heyning P. Vestibular function testing. Current opinion in neurology. 2007; 20:19–24. [PubMed: 17215684]

Yang TH, Young YH. Click-evoked myogenic potentials recorded on alert guinea pigs. Hearing research. 2005; 205:277–283. [PubMed: 15953537]

Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R, Penninger JM, Mak TW. Apaf1 is required for mitochondrial pathways of apoptosis and brain development. Cell. 1998; 94:739–750. [PubMed: 9753321]

Zhao X, Jones SM, Yamoah EN, Lundberg YW. Otoconin-90 deletion leads to imbalance but normal hearing: a comparison with other otoconia mutants. Neuroscience. 2008; 153:289–299. [PubMed: 18355969]



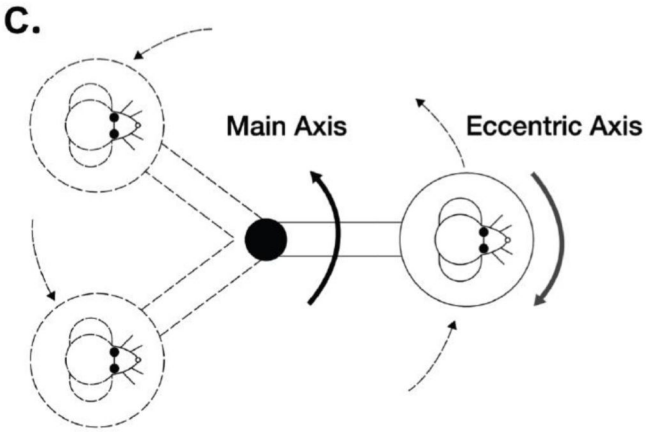


Figure 1.

Rodent centrifuge set-up used in the studies. (A) Main axis at the center and eccentric axis covered by light-tight drum. (B) The mouse is placed in the center of the drum for eye recordings in the dark. (C) Counter rotation paradigm. During counter rotation, the eccentric axis rotates with equal and opposite acceleration and velocity, resulting in no net angular stimulus.

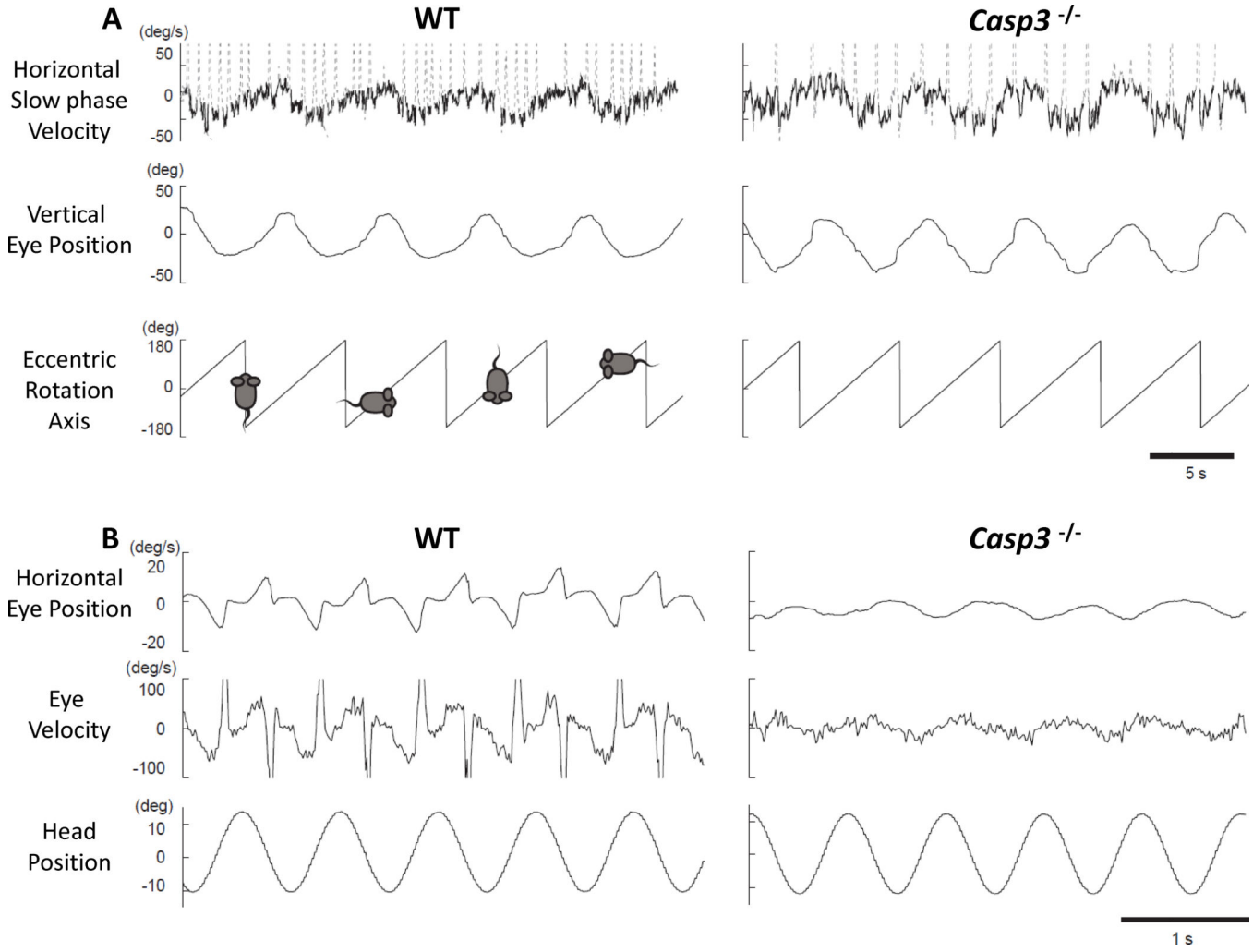


Figure 2.

Otolith ocular response and horizontal angular VOR: raw eye tracings and schematic. Representative data of WT and *Casp3* deficient (*Casp3*$^{-/-}$) mice on pOVAR (A) and horizontal VOR (B). (A) pOVAR stimulus (eccentric rotation at 72 deg/s, main rotation at 245 deg/s) induced similar otolith ocular response in WT (left panel) and *Casp3* deficient mice (right panel). Lower panel shows a schematic of the direction of acceleration relative to head orientation and associated eye movement. (B) angular VOR of WT mice (left panel) and *Casp3* deficient mice (right panel) were induced by sinusoidal rotation of 0.80Hz, peak velocity 60 deg/s. *Casp3* deficient mice had significantly reduced response (right panel) compared to WT mice (left panel).

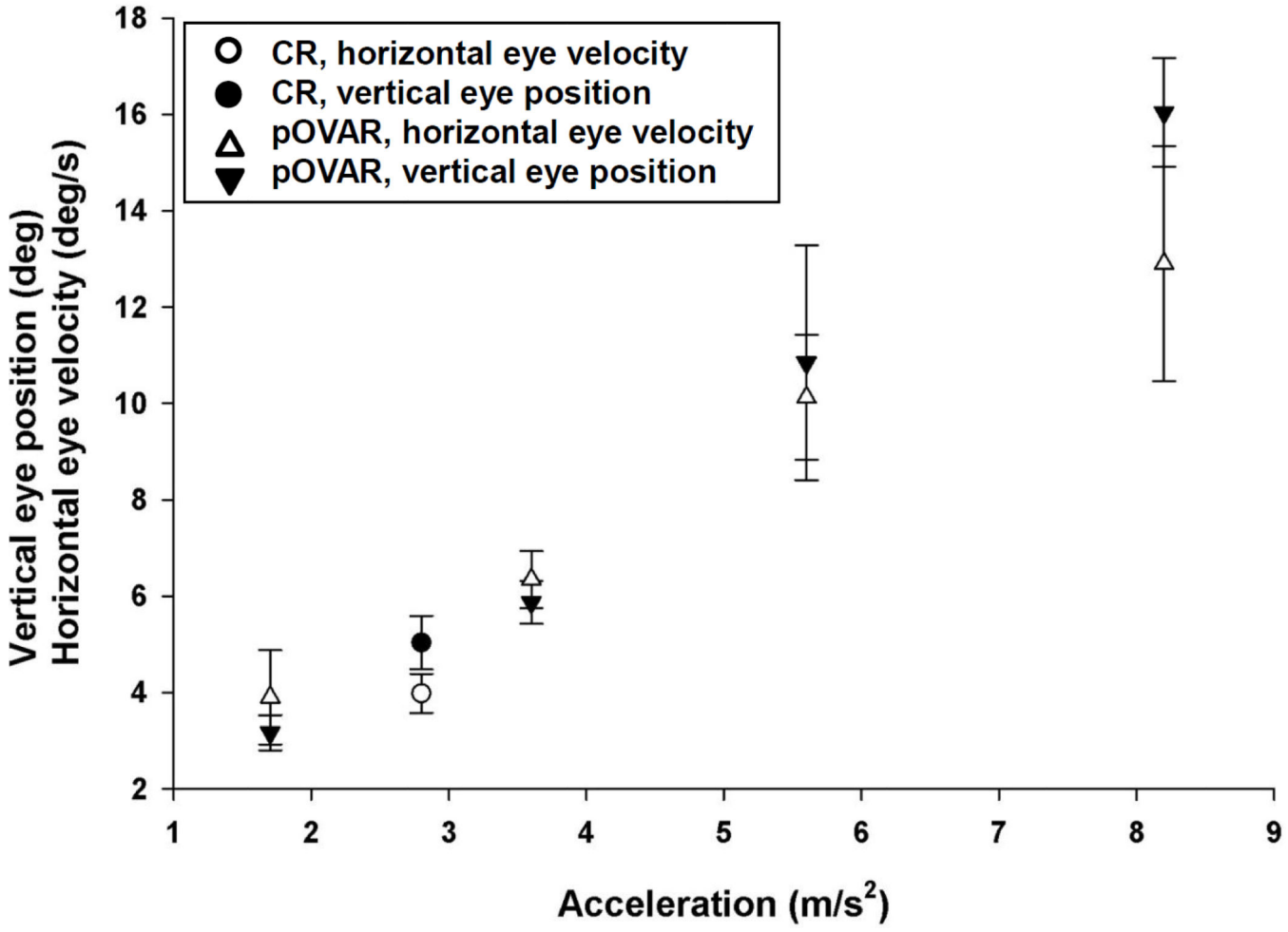


Figure 3.

Similar responses to counter rotation (CR) and pseudo off vertical axis rotation (pOVAR) during equivalent linear acceleration. Horizontal eye movement and verticle eye position response between pOVAR and CR are linear and comparable. Error bars, ± SEM.

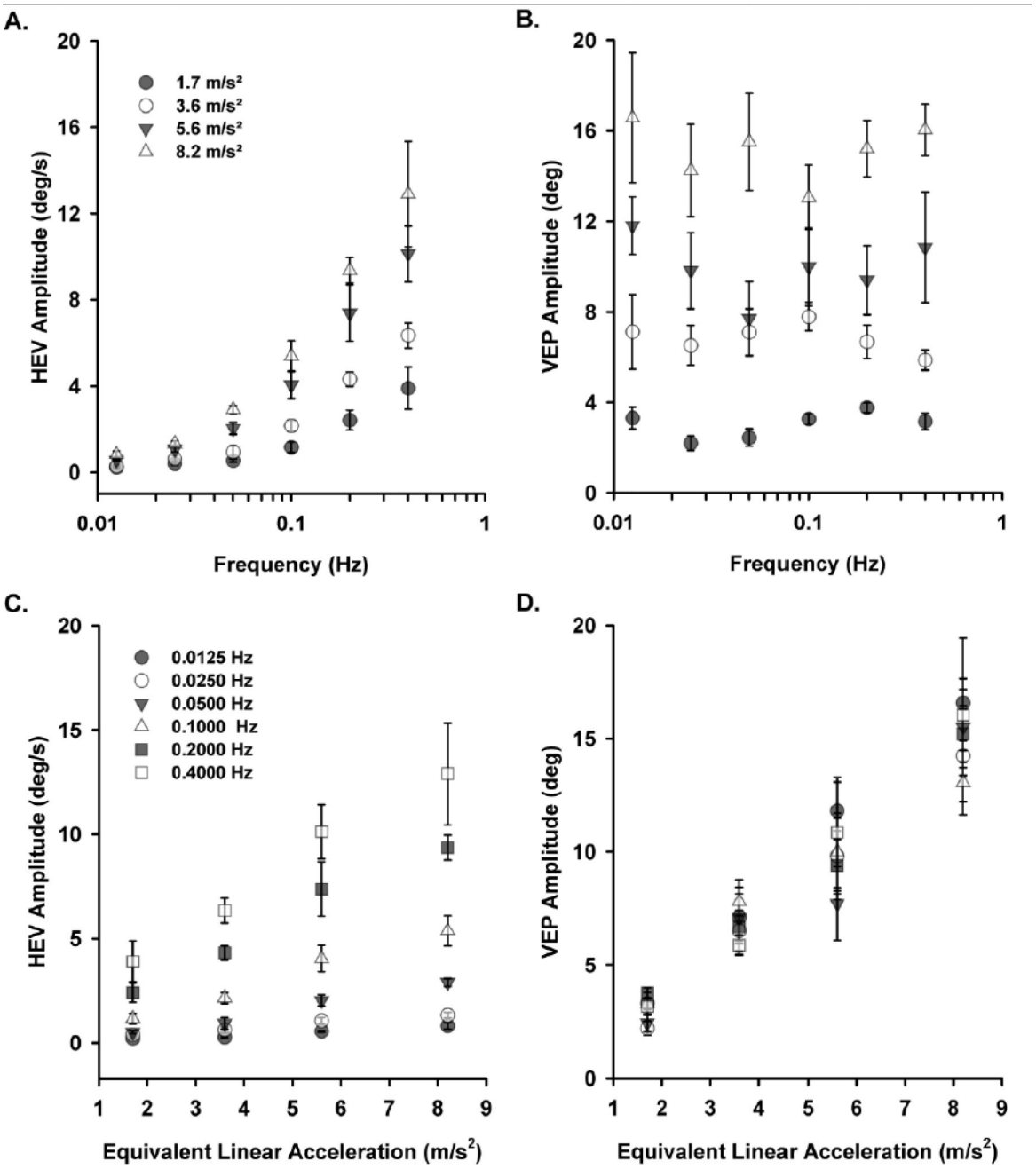


Figure 4.

pOVAR produces robust OOR in WT mice. Bode plots of sinusoidal modulation of HEV (***A***) and VEP (***B***) at stimulus intensities of 1.7 (●), 3.6 (○), 5.6 (▼), and 8.2 (△) m/s^2. Sinusoidal modulation of HEV (***C***) and VEP (***D***) at frequencies of 0.0125 Hz (●), 0.025 Hz (○), 0.05 Hz (▼), 0.10 Hz (△), 0.20 Hz (■), and 0.40 Hz (□). Error bars, ± SEM.

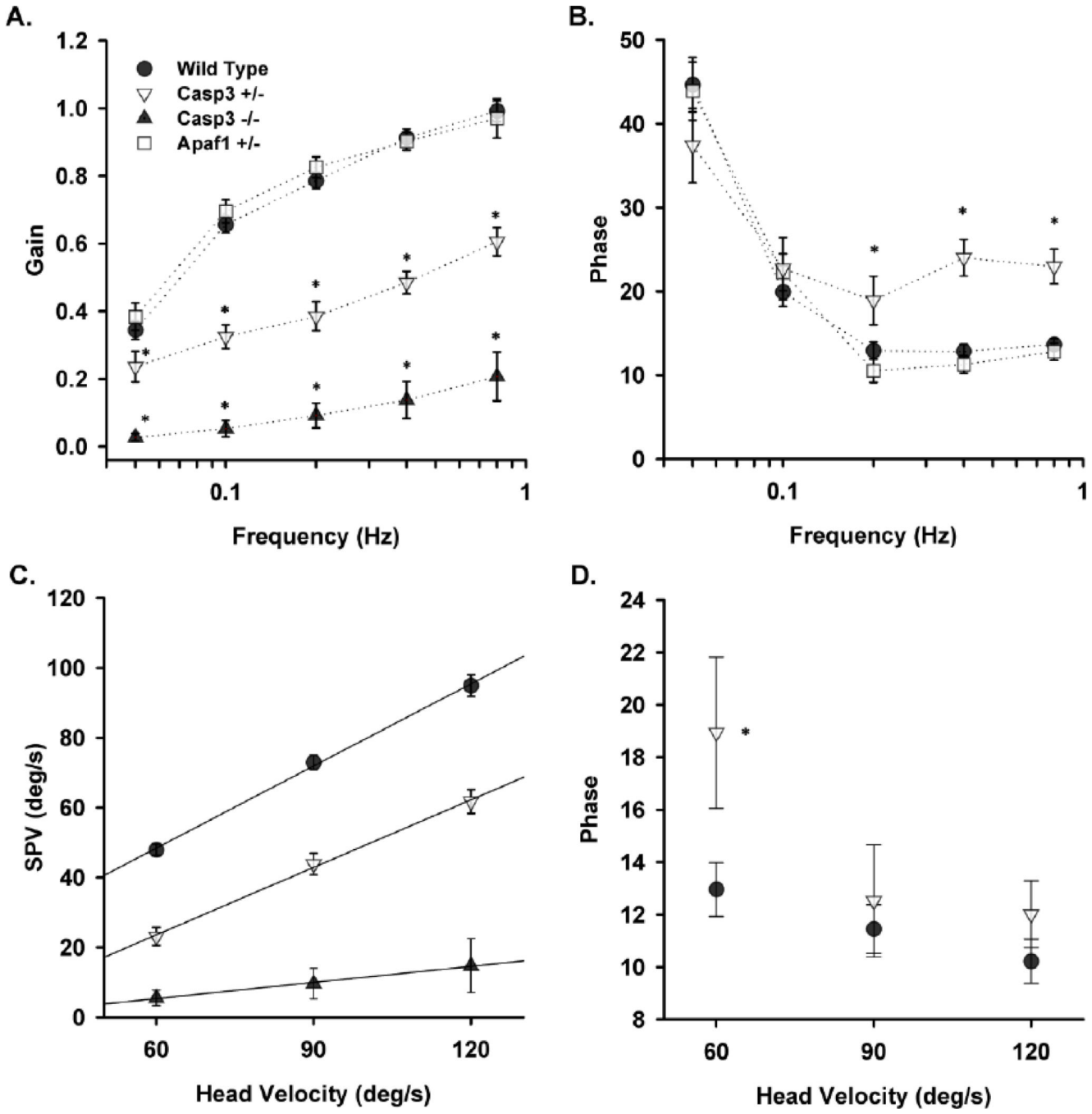


Figure 5.

Impaired horizontal VOR in *Casp3* mutant mice. Gain (A) and phase (B) of horizontal VOR in WT (n=27), *Casp3* heterozygous ($Casp3^{+/-}$) mice (n=10), *Casp3* deficient ($Casp3^{-/-}$) mice (n=10), and *Apaf1* heterozygous ($Apaf1^{+/-}$) mice (n=12). Slow phase velocity (SPV) (C) and phase (D) in different genotypes. WT (●), $Apaf1^{+/-}$ (■), $Casp3^{+/-}$ (▽) and $Casp3^{-/-}$ (▲). Error bars, ± SEM. *, $p<0.05$, Student's unpaired two-tailed *t* test.

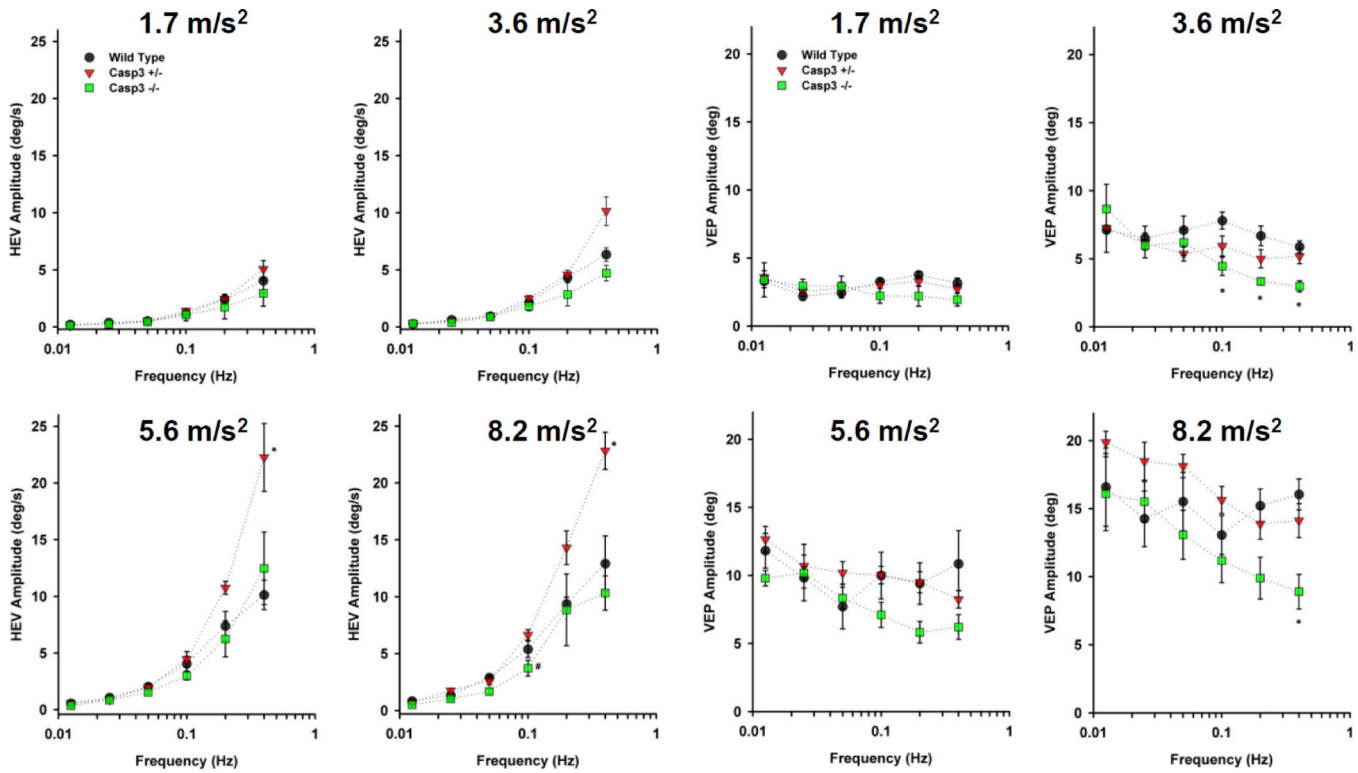


Figure 6.

pOVAR horizontal eye velocity response is mildly impaired in *Casp3* mutant mice. Bode plots comparing WT (black circle), *Casp3*$^{+/-}$ (red triangle), and *Casp3*$^{-/-}$ (green square) pOVAR response of sinusoidal modulation of HEV and VEP at stimulus intensity of 1.7 m/s,^{2} 3.6 m/s^2, 5.6 m/s^2, and 8.2 m/s^2. Error bars, ± SEM. *, $p<0.05$, ANOVA. $^{\#}$, $p<0.05$, ANOVA.

Table 1

Counter Rotation (CR) profiles

Profile type	Eccentric Axis Rotation Frequency (Hz)	Eccentric Axis Velocity (deg/s)	Main Axis Velocity (deg/s)	Radius (cm)	Equivalent Acceleration (m/s/s)	Equivalent Tilt (deg)
Counter-Rotation	0.05	-18	18	45	0	0.3
	0.1	-36	36	45	0.2	1
	0.2	-72	72	45	0.7	4.1
	0.4	-144	144	45	2.8	16.2

Table 2

Pseudo-off vertical axis rotation (pOVAR) profiles

Profile set	Eccentric Axis Rotation Frequency (Hz)	Eccentric Axis Velocity (deg/s)	Main Axis Velocity (deg/s)	Radius (cm)	Equivalent Acceleration (m/s/s)	Equivalent tilt (deg)
Set #1	0.0125	-4.5	112	45	1.7	10
	0.025	-9	112	45	1.7	10
	0.05	-18	112	45	1.7	10
	0.1	-36	112	45	1.7	10
	0.2	-72	112	45	1.7	10
	0.4	-144	112	45	1.7	10
Set #2	0.0125	-4.5	161	45	3.6	19.9
	0.025	-9	161	45	3.6	19.9
	0.05	-18	161	45	3.6	19.9
	0.1	-36	161	45	3.6	19.9
	0.2	-72	161	45	3.6	19.9
	0.4	-144	161	45	3.6	19.9
Set #3	0.0125	-4.5	203	45	5.6	30
	0.025	-9	203	45	5.6	30
	0.05	-18	203	45	5.6	30
	0.1	-36	203	45	5.6	30
	0.2	-72	203	45	5.6	30
	0.4	-144	203	45	5.6	30
Set #4	0.0125	-4.5	245	45	8.2	40
	0.05	-18	245	45	8.2	40
	0.1	-36	245	45	8.2	40
	0.2	-72	245	45	8.2	40
	0.4	-144	245	45	8.2	40