RESEARCH NOTE

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Vestibulo-collic reflex (VCR) in mice

Abstract The vestibulo-collic reflex (VCR) attempts to stabilize head position in space during motion of the body. Similar to the better-studied vestibulo-ocular reflex, the VCR is subserved by relatively direct, as well as indirect pathways linking vestibular nerve activity to cervical motor neurons. We measured the VCR using an electromagnetic technique often employed to measure eye movements; we attached a loop of wire (head coil) to an animal's head using an adhesive; then the animal was gently restrained with its head free to move within an electromagnetic field, and was subjected to sinusoidal (0.5–3 Hz) or abrupt angular acceleration (peak velocity approximately 200°/s). Head rotation opposite in direction to body rotation was assumed to be driven by the VCR. To confirm that the compensatory head movements were in fact vestibular in origin, we plugged the horizontal canal unilaterally and then retested the animals 2, 8 and 15 days after the lesion. Two days after surgery, the putative VCR was almost absent in response to abrupt or sinusoidal rotations. Recovery commenced by day 8 and was nearly complete by day 15. We conclude that the compensatory head movements are vestibular in origin produced by the VCR. Similar to other species, there are robust compensatory mechanisms that restore the VCR following peripheral lesions.

Keywords VCR · Mice · Vestibular compensation · Head movement · Vestibular system · Semicircular canals

Abbreviations CCR: Cervico-collic reflex · VCR: Vestibulo-collic reflex · VOR: Vestibulo-ocular reflex

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Introduction

The vestibulo-collic reflex (VCR) attempts to stabilize head position in space during movements of the trunk (Peterson et al. 1981; Wilson and Schor 1999). Similar to the more frequently studied vestibulo-ocular reflex (VOR, for review see Wilson and Melvill Jones 1979; Leigh and Zee 1999), the VCR has access to direct as well as indirect pathways that link sensory neurons in the vestibular periphery to cervical motoneurons innervating the neck musculature (Wilson and Peterson 1978; Wilson et al. 1995; Wilson and Schor 1999). During coordinated eye and head movements, the VOR and VCR operate synergistically to control gaze position and stabilize retinal images (Cullen and Roy 2004). The VOR is often used as a model for studies of motor control and sensory motor integration in many species, but has not been well studied in mice, perhaps because of the difficulty in measuring eye movements in this species (Stahl et al. 2000; Iwashita et al. 2001; van Alphen et al. 2001; Stahl 2004b). However, the mouse has become an animal model of choice for many types of genetic, cellular and molecular studies of brain function (Lalonde and Strazzielle 2003; Stahl 2004a; Porras-Garcia et al. 2005). These studies provide valuable data, but the surgical preparations required to implant eye coils in mice or to stabilize their heads for video recording of eye movements limit their usage to research that involves small numbers of animals on whom recovery surgeries can be performed. To circumvent this difficulty, we recorded the VCR as an alternative to the VOR using a method that does not require surgical preparation. First, the VCR is measured with the head free to move, so it is not necessary to implant a device to fix or stabilize the head. Second, "head coils" can be affixed to animals' heads with surgical adhesive in order to measure head movements non-invasively. Thus, we believe that measurement of head stability in rodents is an efficient and effective way to assess vestibular function if surgical manipulation is not feasible or if large numbers of

animals are required. Furthermore, given the similarity of their function and anatomical organization, either the VCR or VOR may be considered model systems with which to study sensory-motor integration and central physiology (de Zeeuw et al. 1998).

We subjected mice to passive, whole body vestibular stimulation about a vertical axis (yaw rotation) and measured head movements about the same axis. As demonstrated in other species, the VCR partially stabilizes head position in space during trunk rotation (Goldberg and Peterson 1986; Guitton et al. 1986; Keshner and Peterson 1995). We quantified the VCR by computing the ratio of head angular velocity to body angular velocity (VCR gain). Plugging the lateral semicircular canal unilaterally led to decreased head stability and a smaller VCR gain.

Materials and methods

Six female CB6F1 mice weighing 20–30 g were used. The experimental protocol was reviewed and approved by the animal care and use committee at the University of Michigan and conformed to National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

During experiments, the mouse was immobilized with a restraint jacket (Lomar Biomedical Inc., modified by us to provide free head movement). Initially, data were collected in the dark or with light and VCR responses were found to be similar in both conditions. Based on this observation, all of the data reported in this paper were obtained in the dark. Head movements were measured using an electromagnetic sensor technique that has been employed to measure eye movements in other species (Robinson 1963; Judge et al. 1980) and, in a few labs, in the mouse (de Zeeuw et al. 1998; Stahl et al. 2000). The mouse was placed with its head centered within an alternating electromagnetic field (CNC Engineering, Seattle, WA, USA). A small search coil (constructed of twenty 12-mm diameter turns of multiple strand stainless steel wire) was attached to the mouse's scalp (after hair removal) using Skin-Bond (a removable adhesive manufactured by Smith + Nephew Inc.). During the experiment, head position was related to the search coil's position within a magnetic field and could be detected with sensitivity of 0.1°. Although the skin on the mouse's head could move relative to the scalp, we rarely recorded motion artifacts except when the mouse actively moved its whiskers so that its scalp moved. These artifacts were easily identified by their relatively high frequency rhythmic waveform and low amplitude as confirmed by visual observation of the mouse.

The field coils used to produce the magnetic field were centered and mounted on a servo-controlled rotating platform upon which the mouse was held. The platform could be rotated sinusoidally about the yaw (vertical) axis at frequencies up to 3 Hz or could generate velocity steps (approximately 200°/s) with abrupt onsets. Since the field coils rotated with the platform, the search coil system (CNC Engineering phase detection system) detected head movement with respect to the platform and the mouse's body. Head pitch and yaw position were recorded throughout an experiment. Pitch rotations of the head during yaw rotation were absent or negligible except when the mouse actively moved its head. Such intervals were relatively infrequent and were excluded from the data analysis. We were unable to detect head rotation in the roll plane with our system. At the end of an experiment, the coil was removed from the animal's head using a solvent (Uni-Solve, Smith + Nephew Inc.) and the mouse restored to its cage.

Voltages proportional to head and platform position were sampled at 1 KHz using a CED Power 1401 data acquisition system with 16-bit accuracy and stored on a PC hard drive for later analysis. Data were analyzed using Spike2 (CED) and an Excel spreadsheet. The head coil was calibrated prior to experiments by mounting it on the apparatus and rotating it through known angles. Over the range of head movements monitored in this study (less than 30°), the relationship between coil voltage and position was linear.

For surgical canal plugging, animals were anesthetized with a combination of intra-peritoneal ketamine (120 mg/kg) and xylazine (4 mg/kg). Under sterile conditions, a post-auricular incision on the left side was made and the underlying muscle was dissected to expose the posterior and lateral semicircular canals. A small hole in the lateral semicircular canal was made using a needle. A muscle fragment, taken from the surgical wound, was inserted into the hole, followed by cement to seal the hole. The surgical wound was then closed. The small muscle piece inserted into the lateral semicircular canal blocked the circulation of inner ear fluid. Surgically impaired animals were retested 2, 8, and 15 days after their surgery.

Results

Figure 1 shows a typical mouse's VCR responses to body rotation, before and 2 days after canal plugging. Each graph shows the mean and standard error of 10 or more trials. In response to abrupt steps of body angular velocity, the mouse consistently produced a small counter-rotation of its head shortly after the onset of body motion (panels A and C). The direction of the evoked head rotation tended to stabilize head position in space as would be expected for the VCR. The ratio of head to body velocity was measured 150 ms after the onset of body motion in order to compute reflex gain. This time interval was selected to maximize response amplitude whilst minimizing any effects of visual feedback. For all animals, the gain was 0.13 ± 0.04 for rightward rotations ($n = 3$) and 0.11 ± 0.03 for leftward rotations ($n = 6$, see Table 1). Two days after unilateral canal plugging, the VCR was nearly abolished bilaterally
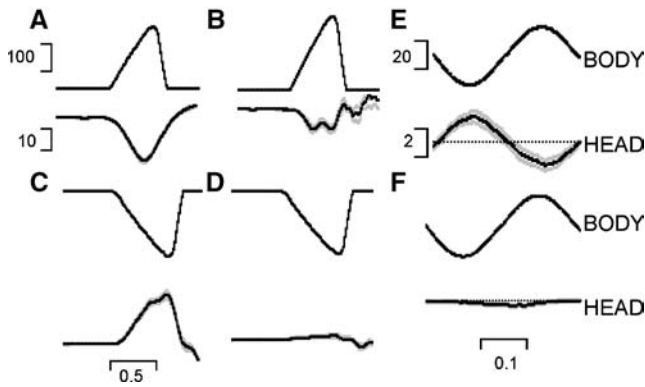
Fig. 1 Averaged head and body velocity (mean ± standard error) during abrupt steps (*A–D*) or sinusoidal (3 Hz) whole body passive rotation (*E, F*) about a yaw axis. Notice that the mouse's head rotates opposite to its body. *A, C* Intact mouse, abrupt velocity steps to the right (*A*) or left (*C*); *B, D* velocity steps 2 days after left-sided canal plug. *E* 3 Hz sinusoidal rotation; *F* 3 Hz rotation 2 days after canal plug. In all panels, the heavy *black line* is the mean velocity and the *gray shaded lines* indicate the standard error (in many traces, the standard error is within the thickness of the black trace and cannot be seen). Calibration bars are *degr*/s or s. *Dashed lines* indicate zero head velocity (*E, F*)

(gain 0.03 ± 0.01, *n* = 6, panels B and D), as would be expected if the head movement were initiated by the vestibular system. VCR gain partially recovered 15 days after surgery to 0.10 ± 0.01 on the lesioned side (*n* = 5) and 0.06 ± 0.01 (*n* = 5) on the intact side (see Table 1). In six mice, the latency between the onset of body rotation and the onset of the compensatory head movement was estimated by determining when head velocity first exceeded two standard deviations of the pre-stimulus baseline. Latencies ranged from as little as 13–74 ms with a mean of 44.7 ± 20.8 ms (*n* = 10, ipsi and contra

side rotations included; in two mice data were obtained only for one direction). These latencies were all less than 100 ms consistent with a vestibular reflex rather than visual or voluntary responses to the stimulus.

Figure 1 also shows VCR responses to sinusoidal body rotation at 3 Hz about the yaw axis. Prior to surgery (panel E), the head rotated 180° out of phase with respect to the body. For six mice, the mean gain averaged over all tested frequencies was 0.08 ± 0.01 and the mean phase lead (with respect to inverted head velocity) was 24.4 ± 4.4°. Data were averaged across frequencies since there was little variation over the tested range (0.5–3 Hz). After surgery, the response amplitude was reduced and the mean phase lead increased (gain = 0.02 ± 0.00; phase = 70.3 ± 16.7°, panel F). The enhanced phase lead is consistent with a peripheral lesion. These responses partially recovered to a mean gain of 0.05 ± 0.01 and phase lead of 37.8 ± 26.1 15 days after the surgery. These data are summarized in Table 2.

Discussion

The results show that consistent VCR responses to body motion can be measured in mice using a non-surgical technique. Although the amplitude of the compensatory head movements is small (circa 10% of body rotation), the responses are stereotypical and can consistently be demonstrated during abrupt, unpredictable steps of body angular rotation or steady state sinusoidal rotation up to 3 Hz. Qualitatively similar responses can be measured in light or darkness suggesting that visual inputs contribute minimally to the observed responses. It is also likely that attentional mechanisms and interactions with other reflexes (e.g., the cervico-collic reflex) influenced

Table 1 VCR responses to velocity steps

Mouse #	Side	M1	M12	M2	M24	M25	M4	Average	SEM
Before	L	0.05	0.15	0.23	0.08	0.07	0.09	0.11	0.03
	R	–	0.07	0.20	–	–	0.14	0.13	0.04
2 days post	L	0.02	0.04	0.04	0.01	0.05	0.04	0.03	0.01
	R	0.01	0.03	0.01	0.02	0.05	0.05	0.03	0.01
8 days post	L	0.12	0.14	0.08	0.07	–	0.09	0.10	0.01
	R	0.06	0.04	0.11	0.03	0.11	0.05	0.07	0.01
15 days post	L	–	0.12	0.09	0.13	0.09	0.08	0.10	0.01
	R	–	0.10	0.09	0.05	0.04	0.04	0.06	0.01

Table 2 VCR responses to sinusoidal rotation

Mouse #	Side	M1	M12	M2	M24	M25	M4	Average	SEM
Before	Gain	0.03	0.06	0.13	0.09	0.07	0.07	0.08	0.01
	Phase	35.2	33.0	10.5	12.1	31.0	24.9	24.4	4.4
2 days post	Gain	0.01	0.02	0.01	0.01	0.03	0.02	0.02	0.00
	Phase	96.4	38.4	36.0	82.3	134.0	34.9	70.3	16.7
8 days post	Gain	0.6	0.03	0.05	0.02	0.02	0.05	0.04	0.01
	Phase	71.0	135.7	17.0	74.7	135.4	38.1	78.7	20.0
15 days post	Gain	0.06	0.06	0.05	0.02	0.05	0.04	0.05	0.01
	Phase	160.7	32.2	16.8	−29.3	22.0	24.2	37.8	26.1

the magnitude of the responses we measured (Wilson and Schor 1999) and will need to be systematically studied in this species.

Unilateral canal plugs transiently reduced the VCR and confirmed a vestibular origin for the stereotypical head movements. The reduced gain and increased phase lead after canal plugging is consistent with a peripheral lesion. Although the canal plug was unilateral, the rotational stimulus bilaterally activates the system allowing the intact side to drive the VCR after recovery from the immediate injury. The effects of the surgery were not permanent and partial recovery was achieved within 15 days suggesting either central or peripheral compensation for the lesion as has been observed in other species (Baker et al. 1982; Ris et al. 1999; Hess et al. 2000).

We observed little or no systematic change in gain or phase of the VCR over a frequency range of 0.5–3 Hz. In comparison, Peterson et al. (1985) reported large gain and phase changes in cervical electromyographic recordings over the same frequency range in decerebrate cats. Differences in preparation might account for the observed differences. First, the cat data reflect electromyographic activity and do not include the dynamic contributions of the head and neck plant, which could be significant. Second, in the decerebrate cat, descending tract modulation of the VCR and cervico-collic reflex is likely to be very different from what it would be in an intact animal and could lead to differences in dynamics. Finally, the VCR electromyographic data were obtained under open loop conditions whilst our results were closed loop.

Our results demonstrate that the VCR may be used to assess vestibular function in mice. Similar to the VOR, the VCR is produced by direct and indirect pathways that link vestibular receptors to cervical motoneurons (Wilson and Maeda 1974; Wilson and Peterson 1978; Wilson et al. 1995; Wilson and Schor 1999). Previous studies have shown that indirect pathways, (e.g., reticulospinal, see Wilson and Schor (1999) for review) may play a significant role in generating or modulating reflex activity. Thus, the small gains recorded under our experimental conditions might reflect a lack of a behavioral context or motivation (e.g., a reinforcement for stabilizing gaze direction) that would be conveyed by indirect pathway modulation of VCR activity. Although speculative, this idea could be explored in future experiments. It is also likely that there are visual influences on the VCR that were not addressed in this study. For example, at low frequencies of head rotation (< 1 Hz), eye movements can be driven by retinal slip in addition to the VOR. It is likely that the VCR is similarly influenced, presumably via the indirect pathways described above. However, we were unable to detect any systematic difference in VCR measurements obtained in the light or dark, perhaps reflecting poor attention or the relatively low contrast visual environment that surrounded our subjects. The VCR and VOR probably share primary sensory neurons and perhaps some of the secondary neurons involved in the reflexes (Wilson et al. 1995). However, these pathways are less established in

the mouse. Unlike the VOR, however, the VCR is intrinsically more complex since the head, but not the eye, has significant inertia (Goldberg and Peterson 1986). Furthermore, mechanical properties such as elasticity and viscosity of the head-neck system may be related to neck-muscle activity levels generated by the VCR itself or other motor behaviors (Goldberg and Peterson 1986; Keshner et al. 1999). Unlike the VOR, the VCR is a closed loop reflex in the sense that VCR activity reduces the head acceleration that gives rise to the reflex. Furthermore, the VCR is opposed by the cervico-collic reflex (CCR), a stretch reflex that tends to stabilize head position with respect to the body (Goldberg and Peterson 1986). These considerations suggest that the VCR is a complex behavioral response that is likely to be dependent on attentional mechanisms, intention, and sensory inputs from the vestibular and visual systems. If it is to be used successfully as an assay of vestibular function, the relative influence of these factors on the strength of VCR responses to rotation should be established in future studies. Finally, these experiments were restricted to motion about the yaw axis that stimulated primarily the horizontal semicircular canals. Motion about other axes (pitch and roll), would stimulate the otolith organs as well as the semicircular canals, thus offering the possibility of studying canal-otolith interactions in this species.

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