Semicircular canal occlusion causes permanent VOR changes

Darrin P. Gilchrist,[CA] Ian S. Curthoys, Ann M. Burgess, Andrew D. Cartwright, Ken Jinnouchi[2] Hamish G. MacDougall and G. Michael Halmagyi[1]

Vestibular Research Laboratory, Psychology Department, University of Sydney, NSW 2006; [1]Eye and Ear Research Unit, Institute of Clinical Neurosciences, Royal Prince Alfred Hospital, Sydney, NSW, Australia; [2] Department of Otolaryngology, Nippon Medical School, Tokyo, Japan

[CA]Corresponding Author

Received 3 May 2000; accepted 30 May 2000

We measured the guinea pig horizontal vestibulo-ocular reflex (hVOR) to high acceleration impulsive head rotations following a unilateral lateral semicircular canal (LSCC) occlusion. We found a significant hVOR deficit for rotations toward the side of the occluded LSCC and this deficit did not show systematic changes over 3 months. We considered the LSCC nerve was still functional as shown by the normal appearance of the crista of the LSCC ampulla and also electrical stimulation of the LSCC. We conclude that the VOR during angular acceleration in response to high acceleration shows no adaptive plasticity following a unilateral LSCC occlusion. *NeuroReport* 11:2527–2531 © 2000 Lippincott Williams & Wilkins.

Key words: Canal occlusion; guinea pig; Vestibular, vestibulo-ocular reflex

INTRODUCTION

Occlusion of a semicircular canal (SCC) preserves spontaneous primary vestibular afferent activity originating from the ampulla of that SCC, but dramatically modifies the response of that canal to dynamic rotational stimulation. This procedure is useful as an experimental tool to alter dynamic response from a specific canal without producing a lesion, and also as a less radical means of treating SCC dysfunction in humans than a unilateral neurectomy or labyrinthectomy. However, it is unclear from the literature whether there is a functionally effective recovery of the vestibulo-ocular reflex (VOR) following SCC occlusion. The present study sought to test in guinea pig whether lateral SCC (LSCC) occlusion does result in any horizontal VOR (hVOR) deficit and whether any observed deficit changes over time.

Following a unilateral SCC occlusion some authors have reported a recovery of VOR function in the monkey [1] and cat [2], whereas others reported no such recovery in the cat [3], rabbit [4] and human [5]. If all canals on one side are occluded, a recovery of VOR function has been reported for monkey in response to low frequency sinusoids, but not for high acceleration impulses [6]. In response to bilateral occlusion of a coplanar pair of SCCs, recovery of VOR function has also been observed in monkey [7,8]. Although a recovery of VOR function following bilateral occlusion of the LSCC has been reported in cat [9], Baker *et al*. [10] reported that there was little VOR recovery over several months in the same species. There is some debate as to whether any recovery following canal occlusion can be attributed to spatial adaptation from the remaining SCCs [7] or not [11,12].

Comparing results from one study to another is difficult given the species differences, the type of test stimulus used, exactly how the measure of hVOR gain is defined, and the fact that there is variation as to which, and how many of the SCCs are actually occluded. The aim of the present study was to help resolve some of these complications.

For the present study we have assessed hVOR function following occlusion of a single lateral canal in guinea pig. The hVOR response after unilateral labyrinthectomy or neurectomy in this species is similar to the hVOR response post-neurectomy in humans [13]. The guinea pig hVOR was tested in response to brief high acceleration impulsive yaw rotations, a stimulus in the range of angular acceleration encountered during natural head movements [14,15]. The use of low frequency sinusoidal stimulation to measure the asymmetry of VOR function post-lesion may be spurious when trying to assess the functional status of the VOR [13,16,17].

When assessing function after canal occlusion or vestibular lesion it is crucial to define exactly how gain is measured. In the present study the gain of the hVOR was measured during the step of acceleration, that is, when velocity was increasing. This type of measure has recently been referred to as an acceleration gain [6]. Broussard *et al*. [2], instead, measured an improved plateau gain following canal occlusion in cat, a gain measure of the portion of the impulse stimulus after the angular acceleration during which the angular velocity was constant (see inset to Fig. 1). Lasker *et al.* [6] have demonstrated that the plateau

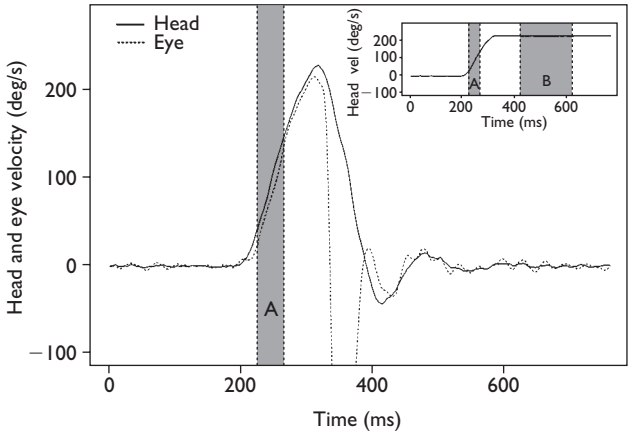

Fig. 1. A time series of head and eye velocity showing an example of the head rotation stimulus and the eye movement response during a single leftward impulse in a guinea pig prior to canal occlusion. For ease of comparison the eye velocity record has been inverted. The shaded part of the main trace during the portion of the stimulus when velocity is increasing (A) represents the segment of the impulse that is used to calculate the hVOR gain (acceleration gain). The inset is for comparison of the gain measure used in the present study, to the plateau gain measure that has been used in some other studies [2,6]. The shaded portion of the trace in the inset labeled B represents the portion of the stimulus during which a plateau gain would be calculated if rotation had been maintained.

gain measure is analogous to gain for a low frequency stimulus.

We found that following unilateral occlusion of the lateral semicircular canal in guinea pig there was a permanent asymmetry of the hVOR acceleration gain to high acceleration impulsive rotations. The acceleration gain of the hVOR for rotations toward the ipsilateral occluded side was reduced by 70% to 30% of normal, whereas gain for rotations to the contralateral side was reduced by 10% compared to normal.

MATERIALS AND METHODS

Five normal healthy pigmented guinea pigs weighing between 600 and 1000 g underwent surgery for a unilateral lateral semicircular canal occlusion (four on the right side and one on the left). The Animal Care and Ethics Committee of the University of Sydney, NSW, Australia approved all procedures used. The methods and conventions have been described in detail previously [13]. Briefly, each guinea pig was anaesthetized with Ketamil (ketamine hydrochloride, 100 mg/kg, i.m.; Troy Laboratories) and Xylase Injection (xylazine, 4 mg/kg, i.m.; Parnell Laboratories). A midline incision was made to expose the dorsal skull. A head holder consisting of a square plastic rod embedded in dental cement and anchored with small stainless steel screws (0-80 UNF $\times$ 1/8") was surgically implanted oriented parallel to the animal's interaural axis whilst the animal was positioned in a guinea pig nose bar (40° pitched nose down), to bring the lateral canals close to the plane of rotation [18].

At least one week later the guinea pig was re-anaesthetized as described above, the skin and tissue over the temporal bone was retracted by blunt dissection and a small hole was drilled into the temporal bone to expose the junction of the lateral and anterior SCCs using a fine dental burr. A small opening along the bony wall of the LSCC was made ~1 mm caudal from the entrance to the ampulla, leaving the membranous duct of the SCC intact. Sterile bone wax was gently inserted into the opening of the bony canal with a fine probe to compress the membranous duct. Upon completion of the LSCC occlusion the hole was sealed with dental acrylic and the wound sutured. Animals recovered from the anaesthesia under a heat lamp in a fully lit room. Recovery of each animal was closely monitored post-operatively to observe any nystagmus or postural symptoms indicative of a vestibular lesion rather than LSCC occlusion [19].

To measure the hVOR, three-dimensional head and eye position were obtained using the search coil technique, with the guinea pig's eye positioned at the centre of a 40 cm^3 transmitter field, having a linear region of around $\pm 30°$ (for detail of the calibration procedure and recording setup see [13]). Briefly, a small combination coil consisting of two small detector coils was placed on the locally anaesthetised cornea of the guinea pig with a drop of cyanoacrylate adhesive. A second similar combination detector coil was fixed to the head holder rod to measure head displacement. An *in vitro* calibration was performed at the beginning of each recording day. Guinea pigs were firmly restrained in a canvas bag with Velcro straps and placed in a Perspex guinea pig holding box. An IBM compatible PC running LabVIEW (National Instruments) was used to drive a velocity servo-motor (ASR Servotron) that delivered unpredictable, high acceleration head rotations in light. Each test impulse consisted of an angular displacement of 18° with a peak head velocity of 200°/s and a peak head acceleration of 3100°/s^2.

The methods for analysis of eye movement have been described previously [13]. To measure and define hVOR gain a method similar to that used by Lasker *et al*. [6] was employed. For each animal, eye velocity was plotted as a function of head velocity for each trial. To obtain the average hVOR performance for a testing session for a single animal, the data points for all impulses in the same direction were combined and a single lowess curve fitted to the data points. A linear regression fit to the averaged eye velocity/head velocity trace was calculated separately for rotations toward the occluded canal and away from the occluded canal. The line was fitted to the data during the period of the impulse when the head velocity was increasing from 50 to 150°/s, and corresponds to the time 30–70 ms after the onset of the stimulus. The slope of the line of best fit represents the gain measure (see Fig. 1). Unlike our previous gain measures, it does not reduce the gain value to a single arbitrary high head velocity [20] and the line of best fit to the data is not confounded by artifacts that may occur at very low velocities during the onset of the stimulus [13].

At least 4 months after behavioural testing each animal was anaesthetized with Nembutal (pentobarbitone sodium, 30 mg/kg, Rhone Merieux) and hypnorm (Fentanyl citrate, 2.5 mg/kg, Janssen). The ampulla of the LSCC was exposed and a fine stainless steel bipolar electrode, each wire 40 μm in diameter and Teflon insulated except at the tip, was carefully positioned near the ampulla. Brief trains of

electrical stimulation were delivered to the LSCC ampulla (square wave pulses of 100 μs duration at the rate of 400 pulses/s) [21] and the characteristics of the eye movement recorded as described above. Each animal was euthanased upon completion of the recording session.

RESULTS

Immediately after unilateral LSCC occlusion all animals had a transitory spontaneous ocular nystagmus (SN). In three of the animals the SN had disappeared within the first 10 h after surgery. In the remaining two animals a low frequency SN (4–8 beats per minute) was still apparent 14–18 h after surgery. At all times after surgery the frequency of SN was substantially less than that which occurs following UVD or LSCC nerve damage [22]. None of the five guinea pigs had postural asymmetry after surgery. These results contrast strongly with the behaviour following unilateral labyrinthectomy or unilateral damage to the nerve for the LSCC and are behavioural indicators that there was minimal damage to the lateral canal [22,23].

Before LSCC occlusion all animals had a hVOR gain close to 1.0 in response to high acceleration rotations. There was no significant difference between rotations toward the right and rotations toward the left ($t(4) = 1.06$; $p = 0.348$; Fig. 2a). Four of the five animals were able to have VOR testing 1 day after surgery. All animals were tested at 1 month, 2 months and 3 months afterwards. On the first test session 1 day after the LSCC occlusion, hVOR gain for rotation toward the occluded side was drastically reduced from preoperative values close to 1 to 0.25 ± 0.1 (mean ± 1 s.d of the mean). At this time hVOR gain for rotation toward the side contralateral to the occlusion was reduced from close to 1 to 0.9 ± 0.1. Over the next 3 months there was always a significant difference between gain values obtained when rotated toward the occluded side compared to rotations away from the occluded side ($F(1,3) = 227$, $p = 0.001$; Fig. 2a,c). The hVOR gain for rotations in either direction did not change over time ($F(3,9) = 1.17$, $p = 0.374$), that is, rotations toward the occluded side remained depressed by 70% compared to normal and rotations toward the side contralateral to the occlusion were always reduced by 10% compared to normal. Even at three months after the occlusion the small difference in gain obtained for rotation toward the side contralateral to the occlusion was significantly different from normal ($t(4) = 3.09$; $p = 0.037$).

To determine the degree of asymmetry over time for each animal, a directional deficit score was calculated as (hVOR gain for rotation toward contralateral side−hVOR gain for rotation toward occluded side)/(hVOR gain for rotation toward contralateral side + hVOR gain for rotation toward occluded side). The hVOR asymmetry did not improve during the first 3 months after LSCC occlusion ($F(3,9) = 1.364$, $p = 0.315$; Fig. 2b).

To determine whether the LSCC was still functional, we showed that high frequency electrical stimulation of the LSCC ampulla produced predominantly horizontal eye rotations (rotation around the z-axis; Fig. 3). The eye rotations were of LSCC origin, since stimulation of the nearby anterior SCC produced a different pattern of eye rotation. For each canal the direction of eye movement was approximately in the plane of the canal being electrically stimulated [18]. Our direct stimulation showed that the occluded LSCC was still functional in three of the five animals (for two of the animals the ampulla was damaged during the exposure to remove the dental acrylic). Furthermore, the procedure for high frequency electrical stimulation requires that the canal ampulla is exposed and viewed under high magnification. For the three animals in which stimulation was successful the crista of the LSCC ampulla appeared normal without any obvious signs of disease or damage. The standard histological control for SCC occlusion experiments (serial sections) does not unambiguously show the functional status of the ampulla of the SCC.

DISCUSSION

Following a unilateral LSCC occlusion all guinea pigs tested exhibited a severe deficit in hVOR gain for high acceleration impulsive rotations toward the occluded side. Although the gain for rotations to the intact side was only slightly impaired, the gain values were significantly lower compared to preoperative values. The impaired response for contralateral rotation was evident on the first testing session 1 day after the occlusion. The higher hVOR gain for contralateral *vs* ipsilateral rotations does not occur as a result of compensatory mechanisms that enhance contralateral gain after occlusion, but are evidence that, at least for high acceleration rotations, excitation is more important than commissural inhibition for generating the hVOR response. The gain of the hVOR for rotations in either direction did not recover in the 3 months after occlusion. This hVOR asymmetry and lack of recovery for high acceleration rotations in either direction is similar to that observed following unilateral vestibular deafferentation (UVD) in guinea pig [13].

We are confident that the canal occlusion procedure was successful in all animals tested. None of the five guinea pigs had postural asymmetry after surgery that indicated a unilateral labyrinthectomy had been performed [22]. Even a selective lesion of the lateral canal in guinea pig produces a marked head deviation in the horizontal plane for up to 24 h post-lesion [23]. Although we did observe a transient spontaneous nystagmus following the LSCC occlusion, transient symptoms are common after any opening of the inner ear, and have even been observed following opening of the middle ear [22]. A transient spontaneous nystagmus can last at least a week following unilateral canal occlusion in squirrel monkey [1,6], and human patients report a transient unsteadiness for up to 4 weeks following posterior canal occlusion [24].

High frequency electrical stimulation of the LSCC provided evidence that the canal was still functional. It is unlikely that our electrical stimulation activated anterior canal neurons since the direction of the eye movements elicited was in the plane of the lateral canal and not the vertical canal. Current spread would therefore be expected to produce a composite eye movement and not purely horizontal eye movement.

Many of the studies which report a recovery of VOR function post-canal occlusion use low frequency, low acceleration, sinusoidal stimulation [1,2,6–8] and in most cases recovery is modest [7,8]. With higher acceleration, high frequency stimuli both Lasker *et al*. [6] (in squirrel monkey) and Broussard *et al* [2] (in cat), report an increase in asymmetry between ipsilateral and contralateral rotations

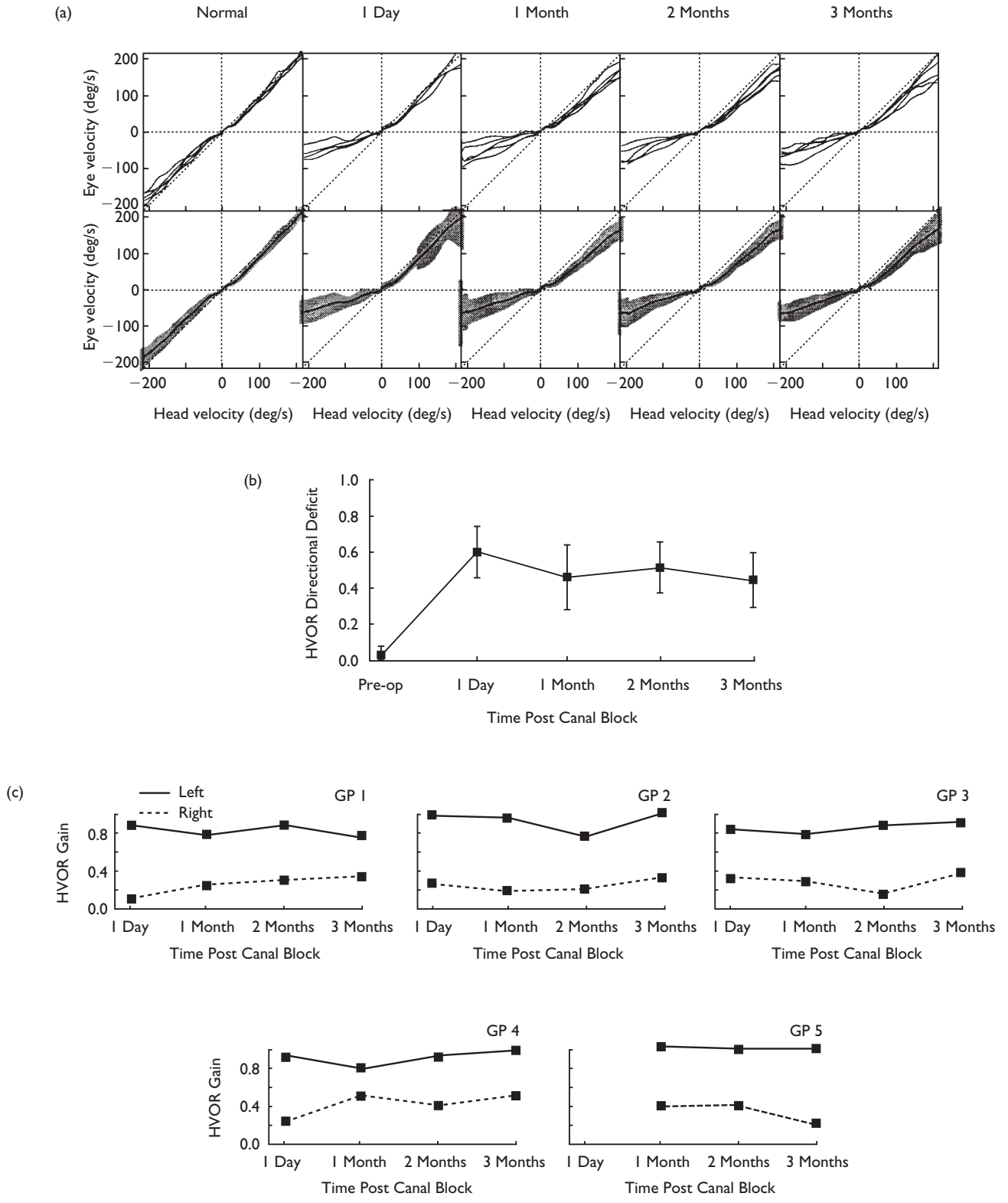

Fig. 2. Change in the hVOR over time after unilateral lateral semicircular canal (LSCC) occlusion. (**a**) The single best-fitting lowess line for each animal at each measurement time is presented in the upper panels and the corresponding two-tailed 95% confidence intervals in the lower panels. (**b**) Change in the asymmetry of the hVOR over time as defined by directional deficit. Data points represent mean ± 1 s.d. (**c**) Each plot represents the actual hVOR gain values (as defined in Materials and Methods) during high acceleration impulsive rotations, either toward the left (normal) side or right (occluded) side for one animal. For the one animal that had a left sided LSCC occlusion the data have been inverted for comparison to the remaining four animals.

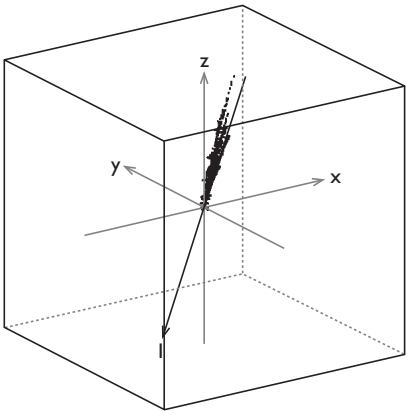

Fig. 3. Three-dimensional representation in rotation vectors of the eye movement response during repeated electrical stimulation of the lateral canal on the occluded side for one animal at least 3 months post occlusion. For lateral canal stimulation the rotation was predominantly around the z-axis (yaw eye movement), providing evidence that the canal was still functional and had not been lesioned as a result of the occlusion procedure. The line marked with the letter l, represents a vector perpendicular to the plane of the lateral canal. Details of the canal planes were obtained from [18].

following unilateral SCC occlusion. That asymmetry is marked and permanent when acceleration gain is used [6], but may be short-lived when plateau gain is studied [2,6]. There is also no recovery of the VOR gain to high acceleration head impulses towards the occluded side following occlusion of the posterior SCC in humans [5]. In the present study measures of VOR function have been made at regular times post-occlusion, a task difficult in human patient studies. Like Lasker *et al.* [6], we also find a permanent VOR deficit for high acceleration stimuli post-canal occlusion, with a large asymmetry between rotations toward the occluded side compared to the intact side. However, Lasker's results were obtained following occlusion of all the SCC's on one side, in the present study only the lateral SCC was occluded on one side. This would suggest that for these high acceleration stimuli, and following unilateral LSCC canal occlusion, there is no adaptive plasticity to account for the loss of input from that canal.

Yakushin *et al.* [12] have recently argued that the canal occlusion alters the dynamic response of the occluded canal to different frequencies. Similarly, Lasker *et al.* [6] have demonstrated that a change in gain of the linear part of the angular VOR pathway allows for a recovery of response to low velocity, low frequency stimuli. Lasker *et al.* [6] contend that the non-linear part of the angular VOR gain does not contribute to ipsilateral VOR responses after canal occlusion. It may be that for higher velocity rotations toward the occluded side and in the absence of excitation from the canal nerve on the occluded side, inhibitory cut-off on the intact side prevents it from modulating the ipsilateral vestibular nucleus resting discharge rate. This inhibitory cutoff permanently impairs responses for high frequency, high velocity rotations to the ipsilateral side [6]. For low frequency, or low acceleration stimuli there may well be some recovery of the VOR, but we question whether it is of functional value during natural head movement [14,15].

CONCLUSION

It is not clear in the literature as to whether there is a recovery of VOR function following canal occlusion. We have demonstrated in guinea pig, using time domain analysis, that for high acceleration impulses there is no recovery of the hVOR gain during the angular acceleration for rotations toward the occluded side following a selective unilateral occlusion of the LSCC.

REFERENCES

1. Paige GD. *J Neurophysiol* **49**, 152–168 (1983).
2. Broussard DM, Bhatia J and Jones GEG. *Exp Brain Res* **125**, 353–364 (1999).
3. Zuckerman H. *McGill Med J* **36**, 8–13 (1967).
4. Barmack NH and Pettorossi VE. *J Neurosci* **8**, 2863–2843 (1986).
5. Aw ST, Halmagyi GM, Pohl DV *et al. Exp Brain Res* **103**, 471–475 (1995).
6. Lasker DM, Backous DD, Lysakowski A *et al. J Neurophysiol* **82**, 1271–1285 (1999).
7. Angelaki DA, Hess BJM, Arai Y *et al. J Neurophysiol* **76**, 2941–2953 (1996).
8. Newlands SD, Ling L, Phillips JO *et al. J Neurophysiol* **81**, 2119–2130 (1999).
9. Böhmer A, Henn V and Suzuki J-I. Compensatory eye movements in the monkey during high frequency sinusoidal rotations. In: Roucoux A and Crommelinck M, eds. *Physiological and Pathological Aspects of Eye Movements*. The Hague: Dr W Junk Publishers; 1982, pp. 127–130.
10. Baker J, Goldberg J, Peterson B *et al. Brain Res* **252**, 151–155 (1982).
11. Yakushin S, Dai M, Suzuki J-I *et al. J Neurophysiol* **74**, 2722–2738 (1995).
12. Yakushin S, Raphan T, Suzuki J-I *et al. J Neurophysiol* **80**, 3077–3099 (1998).
13. Gilchrist DPD, Curthoys IS, Cartwright AD *et al. Exp Brain Res* **123**, 242–254 (1998).
14. Black RA, Halmagyi GM, Curthoys IS *et al. Exp Brain Res* **122**, 362–366 (1998).
15. Grossman GE, Leigh RJ, Abel LA *et al. Exp Brain Res* **70**, 470–476 (1988).
16. Foster CA, Demer JL, Morrow MJ *et al. Exp. Brain Res* **116**, 501–509 (1997).
17. Tabak S, Collewijn H, Boumans LJJM *et al. Acta Otolaryngol* **117**, 785–795 (1997).
18. Curthoys IS, Blanks RHI, Markham CH Acta Otolaryngol 83, 258-265 (1977).
19. Smith PF, Darlington CL and Curthoys IS. *Brain Res* **513**, 149–151 (1986).
20. Halmagyi GM, Curthoys IS, Cremer PD *et al. Exp Brain Res* **81**, 479–490 (1990).
21. Cohen B, Suzuki J-I and Bender M. *Ann Otol* **73**, 153–169 (1964).
22. Curthoys IS, Smith PF and Darlington CL. *Prog Brain Res* **76**, 375–384 (1988).
23. de Waele C, Graf W, Josset P *et al. Exp Brain Res* **77**,166–182 (1989).
24. Walsh RM, Bath AP, Cullen JR *et al. Clin Otolaryngol Allied Sci* **24**, 316–323 (1999).

Acknowledgements: This research was supported by a project grant from the National Health and Medical Research Council of Australia. The authors wish to thank Mr Warren Davies for his excellent technical assistance. K.J. was a visiting scholar from Japan, supported by a grant from the Foundation of Science for Long Life.