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Research report

Saccule contribution to immediate early gene induction in the gerbil brainstem with posterior canal galvanic or hypergravity stimulation

Thomas H. Marshburn [a], Galen D. Kaufman [b], Ian M. Purcell [b], Adrian A. Perachio [b,c,d,*]

[a] *University of Texas Medical Branch, Department of Internal Medicine, 7.102 MRB, Galveston, TX 77555-1063, USA*
[b] *University of Texas Medical Branch, Department of Otolaryngology, 7.102 MRB, Galveston, TX 77555-1063,USA*
[c] *University of Texas Medical Branch, Department of Physiology / Biophysics, 7.102 MRB, Galveston, TX 77555-1063, USA*
[d] *University of Texas Medical Branch, Department of Anatomy / Neuroscience, 7.102 MRB, Galveston, TX 77555-1063, USA*

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Abstract

Immunolabeling patterns of the immediate early gene-related protein Fos in the gerbil brainstem were studied following stimulation of the sacculus by both hypergravity and galvanic stimulation. Head-restrained, alert animals were exposed to a prolonged (1 h) inertial vector of 2 G (19.6 m/s^2) head acceleration directed in a dorso-ventral head axis to maximally stimulate the sacculus. Fos-defined immunoreactivity was quantified, and the results compared to a control group. The hypergravity stimulus produced Fos immunolabeling in the dorsomedial cell column (dmcc) of the inferior olive independently of other subnuclei. Similar dmcc labeling was induced by a 30 min galvanic stimulus of up to -100 μA applied through a stimulating electrode placed unilaterally on the bony labyrinth overlying the posterior canal (PC). The pattern of vestibular afferent firing activity induced by this galvanic stimulus was quantified in anesthetized gerbils by simultaneously recording from Scarpa's ganglion. Only saccular and PC afferent neurons exhibited increases in average firing rates of 200–300%, suggesting a pattern of current spread involving only PC and saccular afferent neurons at this level of stimulation. These results suggest that alteration in saccular afferent firing rates are sufficient to induce Fos-defined genomic activation of the dmcc, and lend further evidence to the existence of a functional vestibulo-olivary-cerebellar pathway of adaptation to novel gravito-inertial environments. © 1997 Elsevier Science B.V.

Keywords: Fos; Vestibular; Otolith; Inferior olive; Semicircular canal

1. Introduction

Much research has been directed towards a better understanding of central nervous system function in novel gravito-inertial environments. While vestibular primary and secondary afferent pathways have been well-described [1,9,10,13–15,23,24,27], immunohistochemical techniques have been used to better understand the function and plasticity of this anatomical framework [3,4,7,8,21,27,31]. In particular, the immediate early gene (IEG) c-*fos* has been used as a marker for cellular activation following various end-organ stimuli [3,4,6–8,17,19]. This proto-oncogene gives rise to the inducible transcription factor (ITF) Fos which, when dimerized with the ITF Jun, directs DNA transcription of proteins in response to specific second messenger events. The presence of labeled Fos in the nuclei of neurons indicates upregulated genomic activity, and therefore has been considered to be a marker for sites of cellular adaptation [14].

Previous work has used Fos immunolabeling (IL) to develop a functional description of the central vestibular pathways in response to various inertial and galvanic stimuli. Kaufman et al. [18] studied the patterns of Fos IL in the brainstem of the rat following hemilabyrinthectomy and exposure to a hypergravitational stimulus from centripetal acceleration, with the animals positioned so that the utricular maculae were coplanar with the plane of rotation. They found a strong recruitment of cells in the inferior olive after 1 h of exposure to the centripetal stimulus. In particular, cells within the olivary subnucleus dmcc showed strong Fos IL independently of other olivary subnuclei. The lack of dmcc labeling in hemilabyrinthectomized animals in some orientations relative to the vector

Abbreviations: μA, micro-amps; IEG, immediate early gene; ITF, inducible transcription factor; IL, immunolabeling; DC, direct current; dmcc, dorsomedial cell column of the inferior olive; MVN, medial vestibular nucleus; PrH, prepositus hypoglossi; IV, fourth ventricle; AC, anterior canal; PC, posterior canal; HC, horizontal canal; G, gravity unit (9.8 m/s/s).

* Corresponding author. Fax: +1 (409) 772-5893.

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of centripetal acceleration indicated a dependency of dmcc activation on an intact labyrinth. This work therefore suggested a strong contribution by the otolith organs, primarily the utricle, to dmcc genomic upregulation [18].

Galvanic stimulation of the labyrinth at the oval window in the gerbil has likewise induced ITF labeling in the inferior olive, including the dmcc [20]. The gerbil was used as the animal model because unlike the rat, the gerbil has a post-auricular thin-walled bulla that allows easy access to a large bony labyrinth, allowing precise positioning of a stimulating electrode.

This work in the gerbil suggests a means of further delineating the contribution of specific vestibular end-organs, in particular, the saccule, to genomic activation in the dmcc. While the saccular macula lies deep within the bony labyrinth, the afferent nerve from its posterior portion arises from the inferior division of Scarpa's ganglion along with the axons innervating the PC. Since the PC crista is a relatively accessible, superficial structure, galvanic stimulation through the PC ampulla could lead to increased firing rates in the PC afferent neurons. Spreading current should activate adjacent afferent neurons from the posterior saccular macula. Using this approach we reasoned that we might determine the contribution of novel saccular firing rates to observed brainstem Fos IL.

This work therefore attempts to ascertain whether activation of the saccule afferent neurons leads to genomic activation in the dmcc using two stimulus paradigms: (1) galvanic stimulation from an electrode placed at the PC ampullary wall, and (2) a natural hypergravity stimulus with animal orientation specific for saccular stimulation.

2. Materials and methods

Twenty-one female gerbils (*Meriones unguiculatus*) were divided into one of three experimental groups. All procedures used in these studies were approved by the Animal Care and Utilization Committee of the University of Texas Medical Branch. One group of eight animals were head restrained and exposed to either brief angular ($n = 3$) or sustained hypergravity ($n = 3$) head accelerations on a small animal centrifuge, or were statically head restrained in the supine position ($n = 2$). In another group of eleven animals, galvanic stimulation was applied through the perilymph within the ampullary wall of the PC. Of these eleven, an anesthetized subgroup ($n = 5$) was used for simultaneous recording from the vestibular nerve. In the other subgroup ($n = 6$), galvanic stimulation was applied at the PC for 30 min through chronically implanted electrodes in unanesthetized gerbils. This time course was chosen since Fos expression is known to occur in 15–30 min of novel stimulus application, and to last for 8 h following stimulus application [20]. The animals in this subgroup were sacrificed to obtain brainstem tissue for Fos IL. Finally, two animals underwent only handling or mock surgery.

2.1. Electrode implantation

Eleven animals, weighing between 55 and 70 g, were anesthetized with a combination of sodium pentobarbital (35 mg/kg i.p.) and ketamine hydrochloride (35 mg/kg i.m.). Supplementary ketamine at the same dose was administered as needed throughout the experiment. Body temperature was maintained by a sodium acetate heating pad (Prism Technologies). The heads of the animals were then secured in the standard position in a Kopf stereotaxic frame with the incisor bar secured 8 mm below the zero ear bars, to attain a coplanar alignment of the horizontal stereotaxic plane with the plane of the lateral canals [25].

The post-auricular bulla of the temporal bone was opened to expose the bony labyrinth. The bone overlying the ampulla of the PC was abraded with a 28 G needle, exposing the perilymph, allowing the placement of the 1 mm exposed tip of a 0.01 inch teflon-coated silver wire electrode. The tips of the electrodes were electroplated with gold and platinum. After placement, the electrodes were secured with bone wax at the bulla and at the parietal bone with a self-tapping, stainless steel screw. Dental cement was used to immobilize the electrode in position on the screw, but was not allowed to flow into the bulla. Previous work has shown that the monopolar configuration of the stimulating electrode drives primary afferent responses that best mimic natural stimuli [20]. Therefore a chlorided silver reference electrode with a 10 mm tip was placed in the neck musculature posterior to the stimulating electrode and on the side opposite the side of stimulation. Electrode resistance values across the labyrinth with this configuration were in the range of 30–500 KΩ.

2.2. Vestibular afferent recording with galvanic stimulation

While the animals were still under anesthesia, the occipital plate was removed in five of the eleven animals. The stereotaxic frame was mounted to a motor driven turntable which was attached to an airbearing linear track (Dover Corp. Model #8454) driven by a separate DC torque motor (Inland Corp.) to provide time-varying linear or angular head accelerations, respectively. Micropipettes filled with 2 M KCl were driven through the intact cerebellum at an angle of 25 degrees off the sagittal plane. Penetration of the vestibular nerve was achieved 5–6 mm from the insertion site [25]. The head was held in a position that oriented the major plane of the horizontal canal (HC) and the utricle coplanar to the plane of horizontal rotation or linear translation.

Vestibular afferent neurons were identified by their activity during angular or linear accelerations of the stereotaxic frame [25]. Anterior canal (AC) afferent neurons were identified by their decreased firing activity with angular acceleration toward the recording side during manual rotation of the turntable. Horizontal and PC afferent

neurons were identified by their increased firing activity with ipsilateral angular acceleration, or with additional ±10 degrees pitching movements.

Utricular afferent neurons showed increases in firing activity with horizontal linear accelerations only. The response gain and phase of these neurons was determined by measuring the rate of firing during linear acceleration at each of six polar angle positions, spaced 30 degrees apart, of the animal about its yaw axis. A response vector was assigned to each utricular afferent corresponding to the direction and angle of the highest sensitivity. The response vector angle was defined as the angle of the animal's naso-occipital axis to the linear acceleration stimulus vector, increasing clockwise from a nose-forward (0 degrees) position.

Saccular afferent neurons could be distinguished by a maximum response to the linear acceleration stimulus within 15 degrees of a coalignment of the vector of linear acceleration with the naso-occipital head axis. Response magnitude decreased as a cosine function of the stimulus polar angle relative to the vector of maximum sensitivity.

A DC galvanic stimulus was generated by a constant current generator driven by a function generator. The biphasic stimulus isolation unit and microammeter were placed in series with the pulse generator and electrode. Two stimulus levels, −25 and −50 μA were applied to the PC ampulla for most of the afferent neurons characterized. Each stimulus was maintained for 15–60 s. A similar stimulation has previously been shown to produce both behavioral postural changes in the alert animal and IEG activation [25].

Vestibular afferent firing activity was amplified 1000 times and band-pass filtered before digital conversion and storage on VHS magnetic tape. The action potentials were analyzed off-line after digital conversion and input to a peripheral interface device (Cambridge Electronic Design, 1401) and a computer. The mean firing rate, mean interspike interval (ISI), standard deviation of the ISI, and the coefficient of variation (CV = ISI standard deviation divided by ISI mean) of each vestibular afferent were calculated from data obtained under each test condition (see Table 1). The CV was used to classify the neurons as regular, intermediate, or irregular based on ISI variability [25,29].

2.3. Galvanic stimulation and ITF expression

Teflon-coated silver wire electrodes were implanted unilaterally in six other animals as described above. In this group, the stimulus and ground electrodes were positioned on the dorsum of the head and affixed with dental acrylic, and the animals were then given 24 h for post-surgical recovery. Just prior to the stimulus protocol, five of the six animals were given isoflurane gas anesthesia to allow connection of the stimulus and ground leads. Previous work [20] has shown no effect on Fos induction with this method of anesthesia. The animals were alert in 30 s. One animal received no isoflurane, the leads having been attached with manual restraint.

Monopolar DC cathodal current of −25 to −100 μA was delivered to five of the six animals by a digital function generator through a stimulus isolator/constant current generator (Bak Electronics, Model BSI-1). The

Table 1
Vestibular afferent responses to PC galvanic stimulation

Regularity	End-organ	Average baseline firing rate (spikes/s)	CV	% change of activity from baseline with −50 (−25 [a]) μA
I	SACC	11.95	1.05	352.6
IR	SACC	26.28	0.54	34.0
IR	SACC	116.04	0.74	4.8
I	PC	27.2	0.86	394.3
I	PC	18.2	1.18	196.3 [a]
I	PC	25.8	1.28	227.2 [a]
R	Utricle	40.1	0.05	32.9
R	Utricle	71.0	0.03	2.5
IR	Utricle	28.1	0.12	−10.6
IR	Utricle	21.9	0.14	0
IR	Utricle	79.6	0.12	−17
IR	Utricle	61.6	0.20	−10.6
IR	Utricle	28.3	0.11	59.7
I	Utricle	69.4	0.60	−25.2 [a]
I	Utricle	61.7	0.62	9.9
R	AC	37.0	0.05	−4.2
I	AC	29.7	1.02	32.7
I	AC	56.5	1.07	−5.3
IR	HC	39.6	0.12	−2.6

AC = anterior canal, PC = posterior canal; HC = horizontal canal; SACC = saccule. I-highly irregular (CV > 0.5); IR = intermediate regularity (CV > 0.1, < 0.5).; R = highly regular (CV < 0.1). [a] Percentage increase at application of −25 μA.

current was monitored by a microammeter in series with the electrodes and isolator. One animal, the surgical control, received no stimulus. The animals were allowed free movement within a circular enclosure 25 cm in diameter, and were closely monitored during the stimulation. No signs of discomfort, such as tetany of the vibrissae or facial twitching or vocalization were noted, but signs of vestibular asymmetries, such as head tilt and hindlimb extension, were evident. The stimulus was delivered for 30 min, after which the animals were allowed spontaneous activity for 30 min post-stimulus to give ample time for ITF expression to occur. No signs of ataxia or vestibular activation were noted following cessation of the stimulus.

All six animals were then given a lethal dose of anesthetic (urethane, 500 mg i.p.), and were then transcardially perfused with 100 ml 0.9% normal saline followed by 300 ml of 4% paraformaldehyde in PBS (0.05 M Na_2HPO_4, 0.137 M NaCl, pH 7.4). The brain was immediately removed intact and immersed in 4% paraformaldehyde for 8 h at 4°C. The tissue was then immersed in 30% sucrose in PBS for at least 24 h, also at 4°C.

Serial 40 μm sections of the brainstem were cut on a cryostat. Non-specific binding sites were blocked with normal goat serum (Vectastain™) for 30 min at room temperature (RT). Anti-Fos rabbit polyclonal antibody (Santa Cruz) in blocking solution was then applied overnight at 4°C, followed by incubation in secondary sheep anti-rabbit IgG in PBS for 1 h at RT, and finally ABC (avidin-biotin-complex) in PBS for 1 h, also at RT. The antibody recognizes c-*fos* and Fos-related antigens, i.e. other proteins that are variable forms of Fos which are also under transcriptional control [20].

2.4. Head restrained hypergravity group

For application of the head restraint, six female gerbils weighing between 55 and 70 g were anesthetized with the same protocol as noted above. After exposing the parietal bone bilaterally, a 5 mm × 2 mm slot was cut through the bone on each side with a 0.6 mm burr drill. The slots extended down to the dura, and were placed along the interaural line approximately 7 mm on either side of bregma. The head of a 0/80 stainless steel screw, after being milled into a 1/2 mm thick crescent, was inserted into each slot, leaving the posts of the two screws exposed. This allowed strong purchase of a 2 × 2 cm polypropylene head restraint cap, set onto the posts and cranium with dental acrylic. The animal was allowed to recover for 24–36 h post surgery.

The animal was wrapped in VetWrap™ and was rendered essentially immobile with the cap fit tightly against the inside surface of a polypropylene tube and secured with two 0/80 nylon screws in a restraint pod. The hypergravity group was rotated in the dark, counterclockwise at 375°/s at the end of a 50 cm arm for 1 h. The sagittal plane of the animals was positioned perpendicular to the centripetal acceleration vector, nose forward, and 30 degrees from the horizontal plane, so that the resultant 2 G force was perpendicular to the major plane of the utricular maculae.

2.5. Hypergravity control groups

Three animals were rotated at a constant velocity for 1 h left side down, with the interaural axis aligned with the axis of rotation, to provide a lateral 1 G stimulus to the otolith organs and only brief angular acceleration at the onset and termination of rotation. Two other animals were head restrained in a static supine position for 90 min. These animals were then sacrificed and perfused within 5 min of termination of the stimulus, and the brains fixed according to the procedure noted above. Two other animals were sacrificed, perfused, and their brains fixed for Fos immunohistochemical analysis after an hour of handling only. As noted above, one animal underwent placement of a stimulating electrode according to the above

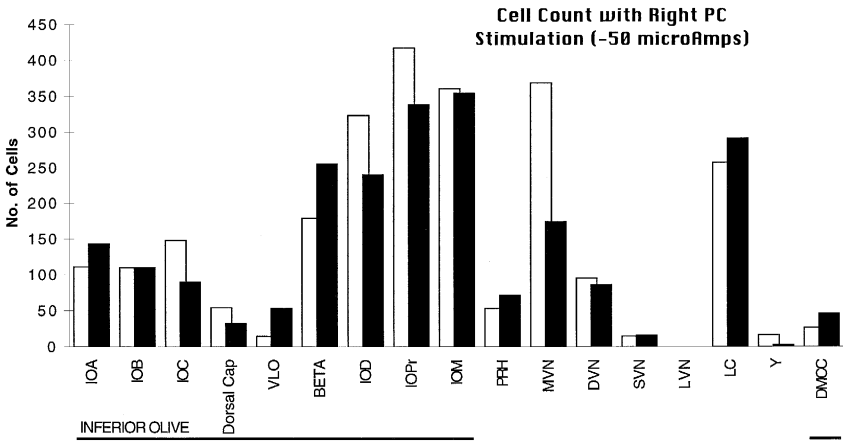

Fig. 1. Graph of Fos IL cell counts in one animal in the nuclei and subnuclei listed following 30 min of −50 μA galvanic DC stimulation to the PC ampulla. Black bars represent ipsilateral counts, white bars are contralateral to the side of stimulation (right).

protocol, and allowed to recover for 24 h post-surgery. No stimulation was applied prior to sacrifice and preparation for Fos immunohistochemical analysis.

2.6. Analysis

Tissue sections were examined for Fos IL. All magnification and illumination settings were the same for all sections during each counting session. Only those neurons with darkly labeled nuclei were counted in one focal plane, although only a few cells lay out of the focal plane in each section. However, in those nuclei that had no apparent Fos IL, all focal planes were scanned for any labeling. A total cell count for one nucleus was determined by adding the cell counts in each section for that nucleus. Right and left sides of each nucleus were counted separately to measure any asymmetry in Fos IL (e.g. Fig. 1).

3. Results

3.1. Afferent recording and galvanic stimulation results

The labyrinth end-organs further away from the PC ampulla (HC, AC, and utricle) revealed the smallest average change in spontaneous firing rate following application of -25 or -50 μA galvanic stimulation at the PC. A total of nine utricular afferent neurons were identified and characterized. Two were regularly firing, ($CV < 0.1$), two were highly irregular ($CV > 0.5$), and the rest were of intermediate regularity ($0.1 < 0.5$) (see Table 1). Each neuron displayed a maximum gain at a particular vector angle and a null response at the orthogonal vector in the horizontal head plane (data not shown). The average change in firing rate from spontaneous discharge seen in the utricular afferent neurons during PC galvanic stimulation was $18.7 \pm 18.5\%$ S.D. For AC and HC afferent neurons ($n = 4$), these values were $11.2 \pm 14.4\%$ S.D.

In contrast, saccular and PC afferent neurons on average showed a large increase in firing rate upon application of PC galvanic stimulation. Three saccular afferent neurons were identified, and all showed increases in average discharge rate with a galvanic stimulus at the PC ($130.5 \pm 192.9\%$ S.D.). Similarly, three PC afferent neurons showed an increase in firing activity of $272.6 \pm 106.5\%$ S.D.

3.2. Fos expression with galvanic stimulation

The ITF expression seen in specific brainstem nuclei following unilateral cathodal stimulation at the perilymph of the PC ampulla is here summarized. These results are compared with the ITF expression found in the same brainstem nuclei of the surgical controls. Fig. 1 shows the cell counts obtained from a representative animal from this group.

3.2.1. Caudal brainstem / inferior olive

Several similarities were identified in both the surgical control and galvanic stimulation groups. Occasional labeling was identified in the bilateral paratrigeminal, cuneate, and spinal trigeminal nuclei in both surgical and stimulus groups. Cells within the lateral reticular tract likewise showed bilateral labeling in both groups, with a predominance ipsilateral to the surgical site. Labeled neurons were also identified bilaterally in both groups in the tractus solitarus, mostly along the border with the area postrema. Only in those animals that had undergone galvanic stimulation at the PC was Fos expression identified in the dmcc of the inferior olive (Fig. 2). The A and beta nuclei, the ventrolateral outgrowth, and the ventral loop of the inferior olive showed sparse labeling in the stimulated group. The expression was symmetric except for the beta nucleus, which was labeled on the ipsilateral side.

3.2.2. Nucleus prepositus hypoglossi

Occasional symmetric labeling was identified in the surgical controls, similar to that seen in previously described handled-only animals [18]. In the stimulated group,

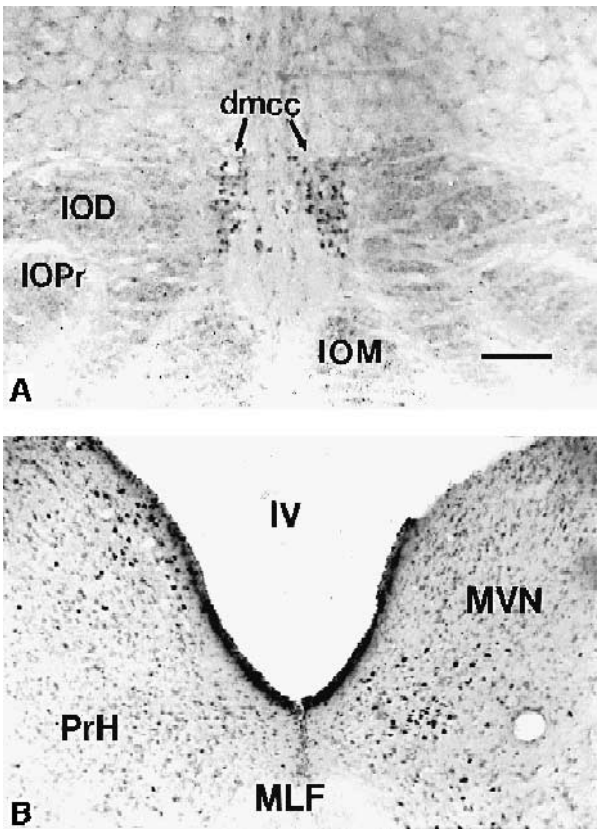

Fig. 2. (A) Fos labeling in the dmcc of the inferior olive after 30 min of galvanic stimulation at the posterior canal with -50 μA. IOD, dorsal nucleus of the inferior olive; IOPr, principal nucleus of the inferior olive; medial nucleus of the inferior olive. Scale bar = 100 μm (for all micrographs). (B) Asymmetric labeling in the contralateral MVN and ipsilateral PrH. IV, fourth ventricle; MLF, medial longitudinal fasciculus; PrH, nucleus prepositus hypoglossi; MVN, medial vestibular nucleus.

strong asymmetric labeling was easily identified within the ipsilateral nucleus prepositus hypoglossi (PrH), particularly along the border with the medial longitudinal fasciculus. Note that this is in contrast to an anodal galvanic or destructive labyrinth lesion, which produces contralateral PrH Fos IL [20].

3.2.3. Vestibular nuclei

No labeling was found in either the surgical controls or galvanically stimulated groups in the lateral or superior nuclei, and only a few scattered cells were found in the descending nucleus. Labeling was noted bilaterally but stronger contralaterally in the medial vestibular nucleus (MVN), with a higher concentration along the ventricular border (Fig. 2). Also, small concentrations of a few labeled neurons were identified dorsal to the genu of the seventh nerve bilaterally, where cell bodies of vestibular efferent neurons are known to be located [26]. Sparse labeling was also seen in the dorsal cochlear nuclei bilaterally.

3.2.4. Mesencephalon

As previously reported, [18] the bilateral locus coeruleus displayed strong ITF expression in both control and stimulated groups. Likewise, the dorsolateral and dorsal periaqueductal gray also showed bilateral labeling, as did the parabrachial nucleus and the inferior colliculus. A few cells were noted in the dorsal raphe nucleus, but no labeling was found in either the cerebellar or pontine nuclei.

3.3. Fos expression with hypergravity

The animals exposed to protracted linear head acceleration displayed Fos labeling which is described here and compared with the labeling seen in the on-axis control animals.

3.3.1. Caudal brainstem / inferior olive

Sparse bilateral Fos IL was seen in the periphery of the spinal trigeminal nucleus, next to the spinal trigeminal tract. The area postrema and raphe obscurus also displayed sparse Fos IL in a similar pattern seen in both groups. Likewise, bilateral Fos expression was found in a small number of neurons in the lateral reticular tract, tractus solitarus, and the x vestibular nucleus. Similar patterns were observed in the hypergravity and control groups, although the animals exposed to hypergravity showed more stained cells per nucleus than the control group. The greatest differences were seen in the dmcc. Strong Fos expression was seen in the dmcc independent of other olivary subnuclei (Fig. 3) following exposure to hypergravity, whereas the 1 G control group displayed no Fos IL in the inferior olive.

3.3.2. Vestibular nuclei

No Fos IL was observed in the superior or lateral vestibular nuclei for either the hypergravity or on-axis control group. Both groups displayed sparse, symmetric labeling of the MVN and the descending nucleus. The y nucleus, Roller's nucleus, and the raphe pallidus also showed sparse, symmetric labeling in the hypergravity group only.

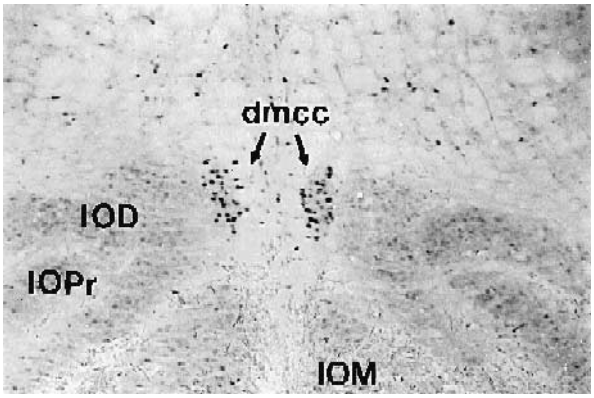

Fig. 3. Fos labeling in the dmcc after 60 min of a 2 G hypergravity stimulus. IOD, dorsal nucleus of the inferior olive; IOPr, principal nucleus of the inferior olive; medial nucleus of the inferior olive.

3.3.3. Other nuclei

Sparse labeling was identified in the PrH for the hypergravity and on-axis control animals. The locus coeruleus, gigantocellular tract, dorsomedial and dorsolateral tegmental nucleus, and the central gray alpha all displayed more widespread Fos IL in the hypergravity group as compared to on-axis controls.

3.4. Fos expression with sustained supine position

Symmetric, bilateral labeling was observed in several brainstem nuclei of gerbils restrained in the supine position. Occasional Fos expression seen in the spinal trigeminal tract, the nucleus tractus solitarus, and the area postrema was similar to that seen in the on-axis and hypergravity groups. There was no Fos IL seen in any of the subnuclei of the inferior olive. Likewise, sparse labeling was identified in the medial and descending vestibular nuclei and the cochlear nucleus. The dorsomedial and dorsolateral tegmental nuclei and the locus coeruleus also contained a symmetric distribution of Fos labeled neurons.

4. Discussion

The pattern of genomic activation in brainstem nuclei of the gerbil obtained in the hypergravity series of experiments are similar to those obtained by Kaufman et al. in the rat [18]. The upregulated genomic activity in the dmcc suggested by the intense Fos labeling seen in this subnucleus in all of the animals exposed to hypergravity and galvanic stimulation of the PC further reinforces the proposal that the dmcc is involved as a part of an otolith-

olivo-cerebellar system to regulate adaptation of the vestibular system to novel inertial stimuli.

Several changes in experimental design were made from previous work [18] to more clearly delineate the role of the saccule in dmcc activation. First, in this study the animal's head was rigidly restrained throughout the application of 2 G dorso-ventral acceleration, ruling out canal cross-coupling from spontaneous head movements.

Second, the animal was placed in the restraint pod so that the hypergravity stimulus vector was aligned with the animal's dorso-ventral head axis. The response magnitude of otolith afferent neurons to inertial acceleration stimuli is known to decrease as a sine function relative to the plane of the maculae, so that the afferent neurons show no response to inertial forces orthogonal to the macular plane [11,22]. The animal alignment should have therefore minimized activation of utricular afferent neurons, and maximized saccular activity. In the previous work, the animals were rotated with the major plane of the utricular macula nearly aligned with the radial vector of centripetal acceleration [18]. Since a portion of the anterior saccule in the gerbil curves toward the transverse head plane, afferent neurons associated with both otolith organs could have been activated with laterally directed acceleration.

The alignment of the interaural axis of the statically tilted control animals in this study essentially removed any inertial force at the labyrinth other than the earth's gravito-inertial force directed orthogonal to the major plane of the saccular maculae. The lack of dmcc labeling following this stimulus suggests that restraint itself, the stress associated with restraint, and auditory stimuli do not contribute to the dmcc labeling pattern. This same result was obtained in other control groups in previous work [18]. Furthermore, otolith organ damage is not a likely reason for the brainstem changes seen. Other work examining macular ultrastructure in squirrel monkeys exposed to greater than 10 G for up to 10 h showed no effect after histological examination [16].

The simultaneous afferent recordings from the vestibular nerve and galvanic stimulation at the PC ampulla further support the role of the saccule in stimulating genomic alteration of the dmcc, since current intensity sufficient to produce dmcc labeling in alert animals produced increases in saccular afferent neuron activity in anesthetized animals.

Previous work has demonstrated that galvanic stimulation at the labyrinth with a monopolar electrode induces current spread through the perilymph [12]. Due to the close proximity of the saccular macula and PC crista in the labyrinth, and to their proximity in the inferior division of the vestibular nerve, galvanic stimulation applied at the PC ampulla of appropriate magnitude could therefore induce afferent firing rate increases in PC and saccular afferent neurons. This expected pattern of current spread matches that observed with stimulation at the PC with -50 μA, i.e., a relative lack of increase in utricular, HC, and AC afferent activity, and increases in PC and saccular afferent firing rates greater than 200% of their baseline. Also, the only recorded neurons to show increases in firing rates of this magnitude even at cathodal currents less than 25 μA were PC and saccular afferent neurons. Two of three recorded saccular afferent neurons did not show a large increase in firing, which might be due to the location of their hair cells in the anterior portion of the saccular macula.

Galvanic stimulation at the PC of -50 μA or more in the alert electrical stimulus group was sufficient to induce upregulation of Fos in the dmcc. Two of the five alert animals exposed to PC galvanic stimulation that received less than -50 μA resulted in no dmcc Fos expressing neurons. These responses in the dmcc are summarized in Table 2. This suggests the presence of a threshold of activity necessary to produce IEG activation for some neurons in the dmcc, a characteristic that has been noted in ITF expression in single cell preparations [30], despite the observation that some PC and saccular afferent neurons did respond robustly to galvanic stimulation of as little as -25 μA.

The presence of a response threshold is supported by the lack of dmcc labeling in the two awake head restrained animals that underwent protracted exposure to a 1 G stimulus in the supine position. The novel orientation of the head with respect to the earth's gravitational vector was not sufficient to induce dmcc labeling in these animals.

Dmcc Fos IL was concurrent with a significant increase in ipsilateral MVN and contralateral PrH IL. It is known that dmcc output consists primarily of climbing fibers to the cerebellar uvula and possibly the nodulus [2,5,28]. This suggests the presence of an adaptation/control functional circuit from vestibular afferent neurons to the cerebellum, with sites of potential integration and/or control at the ipsilateral MVN and the dmcc, with inputs from the PrH.

A factor to be considered in this study is the effect of anesthesia on the firing rates of the recorded neurons. While the presence of anesthesia has been shown in the gerbil to affect the variability and to some extent frequency of vestibular afferent average firing rates [25], it would not be expected to affect the observed pattern of differential activation seen across the labyrinth. This vari-

Table 2
The number of Fos IL cells counted in the inferior olivary dmcc in six animals after the galvanic current intensities shown

Number of immunolabeled cells in dmcc	Stimulation applied to PC (μA)
0	0 (surgical control)
0	−25
0	−40
71	−50
118	−50
86	−100

able does make determination of an absolute threshold for brainstem genomic activity difficult with the present design.

The present work builds on previous studies that suggest that sustained, novel inertial force of sufficient magnitude applied to the otolith organs results in an increase in genomic activation at specific sites in the brainstem, most notably in the dmcc. While the relative contributions of utricular and saccular afferent neurons remains to be determined, an increase primarily in saccular afferent activity appears to be sufficient to upregulate transcription of Fos in the dmcc independently of other olivary nuclei. Adaptation to novel gravitational and inertial environments, as determined by IEG upregulation, therefore appears to involve olivo-cerebellar pathways mediated by both saccular and utricular activity, with inputs from the dmcc, the MVN, and the PrH.

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