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I. BASIC MECHANISMS

THE ROLE OF VOLTAGE-GATED HAIR-CELL CURRENTS IN VESTIBULAR TRANSDUCTION

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Voltage-gated currents were studied in type I hair cells from the central zone and type II hair cells from the peripheral zone of the turtle posterior crista. As previously reported for other preparations (Rennie and Correia, 1994; Rusch and Eatock, 1996), type I cells have an outward current with a hyperpolarized activation range, slow activation kinetics, large maximal conductances, and almost no fast inactivation. Type II outward currents, besides their more depolarized activation range, have fast activation kinetics, small maximal conductances, and can show fast inactivation. To study transduction in the frequency range of natural head movements, we measured voltage responses to sinusoidal currents superimposed on a steady depolarizing current intended to simulate the resting mechanoelectric transducer current. The steady current inactivated type II outward currents. Input impedances were high and Bode plots agreed with a passive, RC model. Inactivation was much less complete in type I cells, which had lower input impedances and phase leads indicative of active, outward currents. To study inactivation in more detail, voltage was stepped from -67 to -47 mV for 60 s. Because of differences in peak whole-cell conductances and in fast and slow inactivation, steady-state conductances were 40x lower in type I than in peripheral type II cells. Voltage responses to transducer currents are inversely proportional to conductance. This implies a qualitative difference in the voltage gains for the two kinds of hair cells.

DETERMINANTS OF THE RESPONSE DYNAMICS OF THE VESTIBULAR SEMICIRCULAR CANALS IN THE TOADFISH, OPSANUS TAU

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The vestibular semicircular canals sense angular acceleration of the head, transducing this acceleration into a frequency code that is transmitted over the VIIIth nerve to the brain. The responses of VIIIth nerve afferents to this acceleration are diverse, varying in both amplitude and phase from the periphery to the center of the crista and also across the frequency bandwidth of stimuli naturally encountered. Canal afferent fibers can be divided into three broad categories based upon these response dynamics. They are: (1, 2) low and high gain velocity-sensitive, and (3) acceleration-sensitive fibers. The origins of these responses can be viewed as originating in five broad categories associated with the canal morphology leading

from head acceleration to afferent impulse initiation. These categories are: (1) the biomechanics or cupular and hair cell stereocilliary motion across the dimensions of the crista, (2) mechano-transduction, hair cell transduction and basolateral currents, (3) voltage sensitive hair cell channel dynamics, (4) hair cell synapse, presynaptic vesicle binding and release, and (5) post synaptic membrane dynamics and / or afferent dendritic structure. We have attempted to study the relative contributions of these five categories to the construction of the three classes of afferent responses. Present results indicate that the three afferent fiber classes employ differential contributions of canal biomechanics and post transduction current mechanisms to configure the three afferent classes.

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DYNAMIC PROPERTIES OF NMDA RECEPTORS - A POTENTIALLY IMPORTANT FACTOR IN VESTIBULAR SIGNALING

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Recent studies have established that NMDA-mediated synaptic potentials play an important role in transmission of information between vestibular afferents and second order vestibular neurons. We are using whole-cell patch recording from vestibular neurons in rat brain stem slices and computer modeling to examine how these synaptic potentials could contribute to the processing of vestibular signals. One possibility is that calcium influx through NMDA channels could be involved in long-term plastic adaptive modification of transmission in vestibular circuits. Another is that the non-linear voltage-current curves resulting from magnesium block of NMDA channels could provide a mechanism for multiplicative regulation of the gain of transmission between vestibular afferents and second order neurons, such as that occurring in the VOR when gaze is shifted from a far to a near target.

Participation of NMDA-mediated transmission in regulation of gain of vestibuloocular responses requires that this transmission be able to function effectively at the high discharge rates characteristic of vestibular afferent discharge. Our recordings of NMDA-mediated responses to stimulation of the vestibular nerve at frequencies ranging from 1 - 100 Hz have revealed that neurons in the medial vestibular nucleus vary widely in the ability of their NMDA-mediated transmission to follow repetitive stimulation. We expect that this reflects the expression of different forms of NR1 and NR2 channel components in these neurons, perhaps related to the particular function they subserve. To explore this we have fitted our synaptic potentials with a kinetic equation reflecting the binding and unbinding of transmitter and desensitization of NMDA channels. Results suggest that the differences in frequency following could be related to differential expression of NR2A and NR2C molecules in these neurons.

Our kinetic fits also provide a way of introducing realistic NMDA-mediated transmission into model vestibular neurons to explore the potential importance of

such transmission in vestibular signaling. When NMDA and AMPA channels were included in such neurons in a ratio of 1:2, the dynamic properties of the NMDA channels present had a very strong influence on the spike frequency output of these neurons produced by a sinusoidally modulated afferent spike train. Gains varied by a factor of more than 2 and significant phase shifts were observed between responses of neurons containing rapidly and slowly desensitizing forms of the NMDA receptor. Thus it appears that the type of NMDA receptor channels expressed by a neuron can play an important role in determining its behavior.

FIRST STEPS IN CENTRAL VESTIBULAR INFORMATION PROCESSING

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Semicircular canal-related afferent inputs of second order vestibular neurons (2° VN) were studied in the isolated frog brain following electrical stimulation of individual labyrinthine nerve branches via suction electrodes. Postsynaptic potentials were recorded intracellularly before, during and after a reversible blockade of membrane receptors by specific antagonists. Most of the recorded 2° VN (about 90%) received a monosynaptic excitatory input from only one of the three ipsilateral semicircular canals (Straka et al., *J Neurophysiol.*, **78**: 1363- 1372, 1977).

The afferent nerve input was mediated by glutamate or a related substance and by at least two different subtypes of glutamate receptors, i.e. AMPA-R and NMDA-R. Evidence based on the recruitment order from thick to thin axons after electrical pulse stimulation indicates that all afferent fibers activated AMPA-R whereas NMDA-R were only activated by a subpopulation of thick vestibular nerve afferent fibers. Practically each 2° VN was excited via AMPA-R as well as via NMDA-R, some 2° VN were in addition electrically coupled with thick vestibular nerve afferent fibers (Straka et al., *Neuroscience*, **70**: 697-707, 1996).

Monosynaptic EPSPs were superimposed by small, disynaptic IPSPs that were mediated by glycine (in 90% of the 2° VN), by GABA (in 76% of the 2° VN) or by both glycine and GABA (in 62% of the 2° VN). The converging monosynaptic excitatory and disynaptic inhibitory signals of 2° VN had the same semicircular canal specificity (Straka and Dieringer, *J. Neurophysiol.*, **76**: 3087-3101, 1996; Straka et al., *J. Neurophysiol.*, **78**: 1363-1372, 1997). In addition, disynaptic inhibitory inputs were activated by thick vestibular nerve afferent fibers. Therefore, the feedforward inhibition is appropriate to control the activation of voltage-dependent NMDA-R. Ongoing experiments indicate that the kinetics of NMDA-R of 2° VN differ strongly from those e.g. of hippocampal NMDA-R in mammals. In particular, receptor desensitization is small even at firing rates of afferent fibers above 60 Hz. Because of the longlasting decay of NMDA-mediated EPSPs this component facilitates temporal summation already at low firing frequencies.

CELLULAR MECHANISMS OF VESTIBULAR COMPENSATION

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In recent experiments we have examined changes in intrinsic properties of medial vestibular nucleus (MVN) cells during the early stage of vestibular compensation, over the 48 hours immediately after the surgical ablation of one labyrinth (Cameron and Dutia, *NeuroReport*, **8**: 2595-2599, 1997). Experiments were carried out on rat MVN cells in vitro, using slices prepared from animals that had undergone unilateral labyrinthectomy (UL) at various times previously (see 1 for details). MVN cells in the rostral region of the nucleus ipsilateral to the UL developed a significant increase in their intrinsic excitability between 2 - 4 h after UL, which was sustained for the following 24 h. We have proposed that this increase in intrinsic excitability is an important mechanism for the recovery of resting activity in the ipsilateral MVN cells, as it would help to overcome the excessive commissural inhibition that silences them after UL in vivo. We have now shown that this increase in intrinsic excitability is accompanied by a marked down-regulation of the functional efficacy of both $GABA_A$ and $GABA_B$ receptors in the rostral region of the ipsilateral MVN, within 4 hours after UL. Simultaneously in the contralateral MVN, there is a significant up-regulation of the functional efficacy of $GABA_A$ receptors. Thus, a key process in the initial recovery from the static vestibular symptoms appears to be the reduction of the imbalance in commissural inhibition after UL, by synergistic compensatory changes in GABAergic synaptic efficacy in both the ipsilateral and contralateral vestibular nuclei.

Using intracellular whole-cell patch clamp recordings from MVN cells in slices from animals that had been labyrinthectomised 4h previously, the resting membrane potentials, input resistance and spike amplitude of identified Type A and Type B MVN cells (Serafin et al., *Exp. Brain Res.*, **84**: 417-425, 1991; Johnston et al., *J. Physiol., Lond.*, **481**: 61-77, 1994) were found to be unchanged. However there were significant changes in the spontaneous action potentials and dynamic firing characteristics of Type B cells, but not Type A cells, in the rostral MVN on the ipsilateral side. The action potential fall-time in Type B cells was prolonged, suggesting a down-regulation of K and/or Ca conductances after UL. Spike frequency adaptation during depolarising current injection in Type B cells was increased, and the steady-state gain (spikes/nA current injected) was also increased significantly.

The compensatory changes in intrinsic excitability of the MVN neurones were found to be dependent on the activation of glucocorticoid receptors (GR; Cameron and Dutia, *Soc. Neurosci.*, Abstr., **23**: p. 752, 1997). The increase in intrinsic excitability was abolished in animals that were administered the GR antagonist RU48386 (5 mg/Kg i.p.). They also did not occur in animals that were kept anaesthetised with urethane for the 4- or 6-h period after UL, so that they did not experience the stress that normally follows UL. Significantly, in animals that were kept anaesthetised but which were given the GR agonist dexamethasone, to "simulate" the activation of the hypothalamo-pituitary-adrenal stress axis after UL, the

compensatory changes in intrinsic excitability were restored. These results therefore show that the secretion of stress hormones that normally occurs following UL, is an essential requirement for the expression of neuronal plasticity in MVN cells. In parallel experiments we have shown that there is a high level of immunoreactivity for c-Fos in the paraventricular nucleus of the hypothalamus over the first 6 h after UL, significantly higher than in sham-operated controls, confirming a strong activation of the HPA stress axis during the initial stage of vestibular compensation.

VESTIBULAR PLASTICITY: A STUDY IN THE *IN VITRO* WHOLE BRAIN PREPARATION OF ADULT GUINEA PIG

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The mechanisms involved in vestibular compensation are still controversial. Unilateral labyrinthectomy might induce permanent changes in the efficacy of the other sensory inputs reaching the vestibular nuclei. The compensation could also depend on fast modifications of the intrinsic properties in the vestibular-related networks. If this last hypothesis is correct, correlates of such robust plastic process should be detected early on *in vitro*. Since we expected changes to occur both at vestibular nuclei level and elsewhere in the vestibular related-network, we investigate that question both in brainstem slices and in a preparation of an adult *in vitro* isolated whole brain (IWB).

An initial labyrinthectomy was performed in adult guinea-pigs, before the brain was removed for *in vitro* recordings 1 to 7 days later. Using IWB (or slices) dissected out from previously lesioned animals, offered an advantage: the vestibular-related networks had a different history of sensory deafferentation on both sides of the brain. On one side (the "compensated" one), the deafferented VNn had 1 to 7 days to compensate *in vivo* for the initial labyrinthectomy. On the other side (the "newly deafferented" one), the VNn were disconnected from the ipsilateral labyrinth only at the removal of the brain. *In vivo*, following compensation from an initial labyrinthectomy, the deafferentation of the contralesional VNn triggers what is known as the Bechterew's phenomenon.

Most VNn were spontaneously active in slices. Their median discharge rate, in synaptic uncoupling condition, was symmetric between both sides of D1 and D3 slices and always stayed in the control range. However, a clear-cut asymmetry was revealed on D7 slices, due to the higher than normal median discharge displayed by the VNn on the compensated side. This asymmetry was in the same direction as the one causing *in vivo* the Bechterew's phenomenon. The modifications of the VNn activity demonstrates therefore that, in the guinea pig, vestibular compensation is associated, 7 day after the lesion, with changes in the intrinsic properties of the deafferented neurons.

Nevertheless, we felt that earlier traces of this process might appear in the IWB, because that preparation preserves the integrity of the central vestibular-related networks. Indeed, the vestibular-related pathways of D3 and D7 brains displayed prominent asymmetries between both sides of the preparation. Importantly, the observed pattern of asymmetries was coherent with the known organization of the vestibular-related network. The second-order VNn were hypoactive on the newly deafferented side, whereas neurons on the compensated side were more active than in control brains. Accordingly, the spontaneous discharge of the abducens nerve fibers excitable from the newly deafferented side was lower than normal, whereas a high level of activity was detected in the nerve activated by the hyperactive VNn on the compensated side. Finally, this asymmetry was reflected in the responses evoked in the abducens nerves by contralateral vestibular nerve stimulation. While the asymmetries were non-significant in D1 brains, they became more prominent in D3 brains, and were the largest in D5-7 brains. Therefore, the pattern of neuronal asymmetries observed *in vitro* not only mimicked the Bechterew's phenomenon induced *in vivo*, but also followed the same time course. This was true to the extend that a spontaneous nystagmus could be recorded in some of the IWB.

The fact that, in the guinea pig, a clear imbalance of the VNn resting activity could be observed 3 days after the lesion in the IWB while it take 7 days to record a minor imbalance on slices demonstrates that during the first week, resting discharge recovery of the deafferented VNn is subserved by changes in the vestibular network outside of the vestibular nuclei and the adjacent reticular formation. On the other hand, the asymmetry observed in synaptic uncoupling conditions between the VNn discharges on both sides of D7 slices suggest that between days 3 and 7, vestibular compensation begins to rely on changes in the intrinsic membrane properties of the deafferented VNn. From 7 days on to several weeks later, the deafferented VNn would undergo changes in their physiological and pharmacological properties progressively allowing them to maintain a normal resting discharge in the absence of any excitatory drive.

Altogether, we propose that vestibular compensation in guinea pig might follow a "top to bottom" strategy. It would first rely on external cues given by the intact sensory systems, then on overall changes in the activity of vestibular-related networks embedding the deafferented cells, and finally on modifications of the intrinsic properties of the deafferented cells themselves.

FOS EXPRESSION IN THE RAT BRAIN AFTER EXPOSURE TO GRAVITO-INERTIAL FORCE CHANGES

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Immediate early genes are generally expressed in response to sensory stimulation or deprivation and can be used for mapping brain functional activity and studying the molecular events underlying adaptation to novel environments.

We immunohistochemically investigated Fos protein induction in the rat brain after exposure to gravito-inertial force changes, with special reference to the vestibular nuclei (VN) and related structures. Fos-like immunoreactivity was analyzed in both hypergravity and hypogravity subgroups of rats. The hypergravity subgroup was composed of rats born and housed for 60 days in normal gravity, and thereafter subjected to either 2G or 4G hypergravity environment for 90 min by means of a centrifuge. The hypogravity subpopulation was made of rats born in 2G hypergravity environment, housed in the centrifuge for 60 days, and there after exposed to normal gravity for 90 min. Data from these experimental groups of rats were quantified with computer-assisted image analysis and statistically evaluated by comparison with control (1G) non-rotated rats

Results showed that Fos immunoreactivity was near totally lacking in the control unrotated rats. By contrast, Fos protein was rapidly and consistently expressed after short-duration (90 min) exposition to modified gravito-inertial forces. Both the hypergravity and the hypogravity rats exhibited a significant increase in Fos expression compared to the controls, but they displayed a rather different spatial pattern of Fos induction. The hypergravity rats (no significant differences between the 2G and 4G animals) showed a bilateral increase in the number of Fos-positive cells in several brain stem structures. Strong labelling was observed in the caudal vestibular complexes (medial and inferior VN), the prepositus hypoglossi nuclei, the inferior olive (dorsomedial cell column) and the dorsolateral periaqueductal gray. By contrast, Fos induction was never found in these nuclei in the hypogravity rats. However, Fos protein was similarly expressed in suprabulbar structures (diencephalic nuclei, visual, temporal and parietal cortical areas) in both the hypergravity and hypogravity subpopulations of rats.

We conclude that Fos induction in the VN and related structures results from the activation of the otolith system. This result corroborates previous investigations (Kaufman et al., 1992; Marshburn et al., 1997) suggesting that otolith-olivo-cerebellar pathways are implicated in adaptation to novel inertial environments. Fos expression in the suprabulbar regions can be explained by the activation (or disinhibition) of vestibulocortical projections. The multiple representations of vestibular information in the cerebral cortex support this finding.

CANAL AND OTOLITH INPUTS TO SINGLE VESTIBULAR NEURONS IN CATS

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Convergence from both afferents of the posterior semicircular canal (PC) and of the saccular (SAC) macula were studied using intracellular recordings from identified vestibular neurons in 11 decerebrated cats. Stimulating electrodes were placed on the left PS and on the left SAC nerves and they were covered by

Vaseline-paraffin mixture. Other vestibular nerves were transected. Three electrodes were inserted into the C1 segment to stimulate the left and right lateral (L) and medial (M) vestibulospinal tracts (VST). Another monopolar electrode was placed in the oculomotor nucleus.

Stimulation of the PC nerve at an intensity of approximately 1-3 x N1 threshold evoked monosynaptic EPSPs (<1.2 ms) in 67 vestibular neurons [15 vestibulospinal (VS) neurons sent axons to the LVST (l); 11 VS neurons sent axons to the MVST (m); 10 vestibulo-ocular (VO) neurons sent axons to the 3rd nucleus but not to the spinal cord; 11 vestibulo-oculo-spinal (VOS) neurons and 20 unidentified vestibular neurons (UV) were not activated antidromically from the 3rd nucleus and the spinal cord].

Stimulation of the SAC nerve at an intensity of around 1-5 x N1 threshold was applied to test a convergence. Eight of the 15 (53%) VS1 and 6 of the 11 (55%) Vsm neurons received excitatory and/or inhibitory inputs from the SAC nerve. Five of 15 (33%) VOS neurons demonstrated excitatory and/or inhibitory convergence. About half of the excitatory inputs from SAC afferents to VS1, VSm and VOS neurons was monosynaptic. On the contrary, only 2 of 14 (14%) VO and 5 of 34 (15%) UV neurons received excitatory and/or inhibitory inputs from the SAC nerve. In conclusion, this convergence may contribute to the vestibulocollic reflex including vestibulospinal reflex by sending excitatory inputs to the neck and other muscles during combined vertical angular and linear acceleration.

SPATIO-TEMPORAL CONVERGENCE RESPONSES IN VESTIBULAR NUCLEAR NEURONS

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Vestibular response have been characterized by a response vector with a spatial (showing the direction of maximal response) and temporal (response phase) components. Within the vestibular nuclei, convergence of responses which differ in their spatial properties (e.g. arising from multiple semicircular canals) or temporal properties (e.g. canals and otolith organs) have been reported by numerous investigators. When the convergence involves differing spatial and temporal properties, a novel set of responses (here called STC, or spatio-temporal convergence) can be demonstrated. Two characteristics of these responses are a deviation from the "cosine rule" of spatial tuning (commonly described as the absence of a "null" response when stimulating in a plane) and a difference between clockwise and counterclockwise OVAR or "wobble" responses.

Several different criteria have been advanced to determine if a neuron shows an STC response. One is to calculate a "tuning ratio", the ratio between the minimal and maximal response amplitudes (Angelaki, 1992), and to say the response is STC if the ratio is larger than some value, say 0.1. Another is to compare the amplitudes to clockwise and counterclockwise "wobble" (Wilson et al., 1986), and consider the response STC if the ratio is by greater than, say, 2:1.

We here propose an alternative test, based on the statistical properties of the response. Consider, for example, a neuron whose response has a S/N ratio of 1.5 and, in fact, has no STC response. While the expected value of the tuning ratio will be 0, its standard deviation (assuming gain and phase are estimated from 64 samples/cycle) will be 0.12. Thus a conservative criterion to consider this neuron to show STC is to observe a tuning ratio greater than 0.24. Similar criteria exist for clockwise/counterclockwise responses.

POSTNATAL DEVELOPMENT OF THE SPATIAL CODING CAPACITY OF OTOLITH NEURONS: PHYSIOLOGICAL PROPERTIES AND C-FOS EXPRESSION

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During slow head movements, spatial and temporal signals arising from the otolith organs are centrally integrated in brainstem neurons. To map the central otolith neuronal circuit that is activated during constant velocity off-vertical axis rotation (OVAR) in complete darkness, the distribution of c-fos protein immunoreactivity in conscious young (7-, 14- and 21-day-old) and adult (10-week-old) rats was examined. That a significant labelling of neurons was evident in the vestibular nuclear complex and oculomotor-related nuclei indicates that information about otolith stimulus is transmitted from the receptors to the brainstem by the end of the first postnatal week.

The developmental profile of the electrophysiological properties of vestibular nuclear neurons was also assessed in young and adult rats decerebrated under halothane anaesthesia.

Two groups of neurons were identified according to their response sensitivities to OVAR in the clockwise (CW) and counterclockwise (CCW) directions. One-dimensional neurons showed symmetric and stable bidirectional sensitivity to velocity while two-dimensional neurons showed asymmetric and variable bidirectional sensitivity to velocity. Two-dimensional neurons that showed a greater gain during ipsilateral rotations and those during contralateral rotations displayed distinct spontaneous discharge regularities. In 7-day-old rats, two- dimensional neurons constitute a significantly greater proportion of the sampled neuronal population than in older rats. This proportion progressively decreased in the course of development. The extent of directional asymmetry of two-dimensional neurons also decreased with age. This directional asymmetry may provide directional coding across an ensemble of neurons during postnatal development. In adult rats, the vector orientations of the one-dimensional and two-dimensional neurons were found to point in both roll and pitch directions on the horizontal plane. In 7-day-old rats, however, the vector orientations were distributed predominantly along the roll directions on the horizontal plane. It may be inferred that the neonatal rat's ability to encode head positions with respect to gravity would be more restricted than the adults.

Taken together, our results suggest that during the first few postnatal weeks central vestibular neurons of rats gradually achieve the adult capacity in coding head movements.

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A 3-D MODEL OF OTOLITH-OCULAR BRAINSTEM CONNECTIONS: THE EFFECT OF PERIPHERAL AND BRAIN STEM LESIONS

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Static eye position is modified by graviceptive inputs to the otoliths: ocular counterroll, equivalent to a shift of Listing's plane, is induced by roll tilt of the head. Likewise, a tilt of Listing's plane is induced by pitching the head forward or backward. Both changes in static eye position are relatively small in amplitude. Clinical signs observed after unilateral peripheral or central lesions of the vestibulo-ocular pathways such as ocular torsion and skew deviation are larger in amplitude but show similar properties. Therefore, they are usually attributed to lesions of the otolith-ocular pathways.

To simulate gravity-induced eye position deviations, the necessary connections from both utricles to the eye muscles have been modeled. Since otolith-ocular pathways are, to a large extent, yet unrevealed, the well-known canal-ocular pathways through the brainstem forming the vestibulo-ocular reflex arc are used. Lesions are then simulated by disconnecting the modeled pathways at different locations ranging from the periphery to the brainstem. The model simulations quantitatively match data from patients with different types of peripheral and central disorders such as vestibular neuropathy or brainstem lesions affecting the central pathways.

Thus, model simulations-suggest that pathological ocular torsion and skew deviation can be attributed to lesions of the otolith-ocular pathways. Moreover, modeling otolith-ocular interconnections as sharing the same pathways as canal-ocular inter-connections appears to be appropriate for the simulation of static vestibular brainstem syndromes.

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THE LOCATION OF MOTONEURONS INNERVATING SLOW AND FAST EXTRAOCULAR EYE MUSCLE FIBRES IN MONKEY

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The location of motoneurons innervating the extraocular eye muscles has been the subject of numerous studies. Lateral rectus motoneurons lie in the abducens

nucleus (VI), superior oblique motoneurons lie in the trochlear nucleus (IV), while those of the inferior oblique, inferior and superior recti are located within the oculomotor nucleus (III). However extraocular eye muscles contain 'fast' and 'slow' types of muscle fibres, but the location of motoneurons innervating each fibre-type is not known. The 'slow' fibres are characterized by their long contraction time, and multiple small motor-endplates along the length of the fibre up to the distal tendon ('en grappe' terminals). The 'fast' fibres have large motor-endplates arranged in discrete bands across the muscle.

In a series of experiments on monkeys, we attempted to retrogradely label (i.e. back-fill) the motoneurons of only 'slow' muscle fibres. We found groups of labelled motoneurons mostly outside, or at least around the boundaries of, the classical VI, IV or III nuclei. The motoneurons innervating the 'slow fibres' in lateral rectus lay around the dorsal, medial and ventral perimeter of VI, those of the superior oblique lay in a compact group within dorsal IV. Medial and inferior rectus motoneurons were mainly within the dorsomedial C-group of III, while superior rectus and inferior oblique clustered along the midline between the nuclei of III. These perinuclear motoneurons, which were assumed to innervate the 'slow' muscle fibres component of the extraocular eye muscles, have a slightly smaller diameter than the motoneurons within the classical VI, IV and III, which label the 'fast' muscle fibres. The physical separation of motoneurons controlling 'slow' and 'fast' extraocular muscle fibres has important implications for understanding their independent control by afferent pathways.

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THREE-DIMENSIONAL VESTIBULAR SIGNAL PROCESSING IN THE ROSTRAL FASTIGIAL NUCLEUS

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The cerebellum plays an important role in the processing of signals deriving from the vestibular nuclei. Not only the vestibular cerebellum (flocculus, nodulus) but also the vermis receives a mossy fiber input from the vestibular nuclei. Purkinje cells, as the only output element of the vermis, project from the anterior vermis to the rostral and from the dorsal vermis to the caudal fastigial nucleus (FN), the most medial of the deep cerebellar nuclei. This anatomical subdivision of FN is also reflected in a functional sense. From single unit recordings it is known that units in the caudal FN respond to eye movement related tasks, whereas neurons in the rostral FN show no eye movement related activity, but are modulated during vestibular stimulation and have been labeled 'vestibular only' neurons. The efferent connections of the rostral FN suggest an important role in vestibulo-spinal mechanisms. Accordingly, animals with experimental lesions in the FN show a falling tendency to the ipsilateral side. In order to control postural reflexes correctly, vestibular signals during natural head movements in 3-dimen-

sional space, have to be processed. In a previous study it was shown that during vertical stimulation around an earth fixed horizontal axis neuronal responses could be related to canal planes (LARP, RALP) but canal-convergence including pitch responses and otolith-related responses were common.

In the present study we investigated 'vestibular only' neurons in rostral FN at different frequencies (0.06 - 1.4 Hz) and amplitudes (±2.5° - ±15°) of sinusoidal vertical vestibular stimulation. Monkeys were chronically prepared for single unit recordings, and sat erect with the head fixed in a primate chair during the experiments. Initially the response vector orientation (optimal response) was determined at 0.6 Hz (±15°). For this a platform with the monkey was slowly (2°/s) moved to different orientations (pitch to roll) during vertical stimulation. Following, different frequencies and amplitudes were applied at the response vector orientation. Most neurons responded at all frequencies and amplitudes. Response to different amplitudes: The majority of neurons showed a monotonically decreasing sensitivity over the tested amplitude range. The remaining neurons had either a constant sensitivity at all amplitudes or showed a clear non linear behavior with a high sensitivity at small amplitudes and a considerable decrease in sensitivity at large amplitudes. For all neurons phase remained unchanged either with head velocity or position over the whole amplitude range. Response to different frequencies: Most of the head velocity related neurons (response in phase with head velocity) had a roughly constant sensitivity (relative to stimulus velocity) over the frequency range, whereas many head position related neurons showed a pronounced decrease in sensitivity to higher stimulus frequencies. A considerable number of neurons, however, had intermediate sensitivity characteristics many of which changed their phase relation relative to stimulus velocity systematically with frequency. None of the neurons increased sensitivity with increasing frequency.

These results suggest that abundant information about head movements in 3-dimensional space is being projected to or processed in the FN of the monkey. The response characteristics of many neurons resemble the behavior of neurons which can be found upstream to the FN (vestibular nuclei, vestibular nerve afferents). Responses of many other neurons, however, have not been described previously in other structures. Vertical canal and canal-otolith interaction is required to explain responses of many neurons and the complex behavior of some neurons is not readily understood in many cases.

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MODULATION OF VESTIBULAR NEURON ACTIVITY: ACTIVE VERSUS PASSIVE HEAD MOVEMENTS

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The activity of vestibular nuclei neurons has been well characterized in the head-restrained monkey. Here we describe the activity of vestibular neurons dur-

ing active voluntary head movements. Neuronal discharges were first characterized in two rhesus monkeys during headrestrained paradigms: fixation, sinusoidal smooth pursuit, passive whole-body rotation in the dark (VORd) and VOR cancellation (VORc). Once the head-restrained characterization of a neuron was completed, the monkey's head was released and the same neuron was then characterized during voluntary combined eye-head gaze shifts and gaze pursuit. In this report we focus on two classes of neurons: 1) type I Position-Vestibular-Pause (PVP) neurons which increased their discharge in response to ipsilateral passive head rotation (VORd and VORc), contralateral eye movements, and paused during ipsilateral saccades, and 2) type I "Pure" Vestibular neurons which also increased their discharge during ipsilateral passive head rotation, but were not sensitive to eye motion.

The head-related discharge of type I PVP neurons was modulated during behaviours where the monkey actively redirected its axis of gaze in space. During voluntary combined eye-head gaze shifts, the discharges of type I PVP neurons were poorly described by a linear model based on a neuron's head motion sensitivity during VORd and eye motion sensitivities during head-restrained paradigms (model 1); the "active" head velocity signal carried by these neurons was significantly reduced during gaze shifts (64±12% for large (50-60 deg) gaze shifts). Similarly, model 1 poorly described the discharges of these cell types during VORc and gaze pursuit paradigms during which the animal also voluntarily redirected its gaze in space; the head movement signal was attenuated (23±9 % and 22±13%, respectively). In contrast, during "active" voluntary head motion where the monkey's gaze was stable in space, the head velocity signal carried by type I PVP neurons was comparable to that measured during passive VORd. For example, during the period immediately following a gaze shift where gaze is stabile while the monkey's head continues to move, the activity of type I PVP neurons was well described by model 1. Type I pure vestibular neurons, which did not carry eye movement related signals, behaved differently. These neurons demonstrated identical responses to passive whole-body rotation (VORc (gaze redirected) versus VORd (gaze stabilized)). On average, the head velocity signal carried by these neurons dramatically decreased during all active head movements, regardless of whether the animal was involved in stabilizing or redirecting its gaze in space. During active head movement paradigms the head movement sensitivity of these neurons was greatly attenuated, not only during gaze pursuit and gaze shifts 95±24% versus 47±15% for large (50-60 deg), respectively, but also during active head movements when gaze was stable in space (e.g. immediately following gaze shifts). Our data suggest that the vestibular neurons which mediate the vestibulo-ocular reflex (i.e. type I PVP neurons), do not transmit vestibular information to motoneurons in a stereotyped manner during either passive or active head movements (as did the pure vestibular neurons), but rather in a manner that depends on an animal's current gaze strategy (stabilization vs. redirection).

SIGNAL PROCESSING IN THE VESTIBULAR NUCLEI DURING ACTIVE AND PASSIVE HEAD MOVEMENTS

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The firing behavior of 109 horizontal canal related neurons in the vestibular nuclei were studied in alert squirrel monkeys during active voluntary and reflexive head movements. The signals generated during active head movements were compared to signals recorded during passive whole body rotation (WBR) with the head restrained from moving and during forced passive rotation of the head on the body. Both active and passive head movements were angular rotations that were primarily confined to the plane of the horizontal semicircular canal. Most of the vestibular units studied were activated at monosynaptic latencies following electrical stimulation of the ipsilateral vestibular nerve. Approximately half (57/109) of the units had firing behavior that was related to eye position or to smooth pursuit eye velocity. The remaining 52 non-eye-movement (NEM) related units included eight units that were antidromically activated following electrical stimulation of the cervical spinal cord. The vestibular sensitivity of each unit was estimated by its response to sinusoidal passive WBR at 0.5 and 2.3 Hz. This vestibular sensitivity was compared to the signals generated during active head movements and during forced head and neck rotation. Vestibular units exhibited a variety of responses during active head movements. Three general classes of responses were observed during saccade related head movements: 1) inhibition or pauses during active head movements (28%); 2) cancellation of vestibular signals related to active head movements (33%); and 3) attenuation of vestibular signals during active head movements (39%). Many of the units that exhibited attenuated responses during active head movements were simply less sensitive to active head movement in one direction or within a small range of head velocities; e.g., they carried signals related to on direction head movements whose peak velocity was less than 200°/s, but signals related to head movements in the off direction were less vigorous than predicted. Units that paused or were inhibited during active head movements also exhibited asymmetric responses. For example, the inhibition of position-vestibular-pause units was present throughout the duration of active head movements in the vestibular on direction, but was terminated at the end of the gaze shift when the head movement was in the vestibular off direction. The responses of vestibular units during active head smooth pursuit following movements and during the vestibulo-collic reflex (VCR) related head movements was also studied. Units whose vestibular signals were canceled during saccade related head movements also tended to have canceled signals during smooth active head movements as well. Althongh the spontaneous firing rate of most units was changed during these smooth movements, units were rarely silenced, even those that usually exhibited pauses during saccades. During WBR in head free animals the VCR produced a difference in the velocity of the head and body in space. The firing behavior of many vestibular units, particularly NEM units, was better related to body velocity in space than to head velocity in space.

The contribution of neck afferent inputs to the signals produced by vestibular units during active head movements was investigated by examining the response to forced rotation of the head and neck and to passive rotation of the body with the head fixed in space. Virtually every unit tested was sensitive to neck rotation. In most units this neck afferent input was too small to account for the reduction in sensitivity to active head movements and passive WBR; although the asymmetry in the responses of attenuated units was often attributable entirely to neck proprioceptive inputs. The reduction in unit sensitivity to active head movements was also not attributable to a change in the synaptic inputs to secondary vestibular neurons from the vestibular nerve, since when passive and active head movements were combined, most vestibular units, including units that exhibited canceled vestibular signals, continned to be sensitive to passive perturbation of the head. These observations suggest that both reafferent neck proprioceptive and efference copy signals related to active head movements are used to modify the responses of secondary vestibular neurons to self generated head movements. These head movement related signals, as well as signals related to eye movements, profoundly modify signal processing in the vestibular nuclei during active head movements. In some units neck inputs function to transform vestibular signals related to head movement in space to signals related to body movement. In many units the addition of a neck efference copy signals allows passive and active movements of the head to be distinguished. We suggest that the function and synaptic weight of neck reafferent and efference copy inputs to different neurons in the vestibular nuclei varies depending on their functional role.

EXPLAINING CHANGES IN VESTIBULAR CELL CHARACTERISTICS DURING ACTIVE VS PASSIVE HEAD MOVEMENT

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The vestibulo-ocular reflex (VOR) during angular rotation is usually evaluated in two ways: At the behavioural level, experimentalists normally examine the ratio of eye velocity to head velocity during perturbations, or the gain during passive sinusoidal head rotation in the dark. At the central level, the modulation in activity of premotor cells, after correction for eye position sensitivity (when appropriate), is examined in various protocols. Typical test protocols include passive rotation in the dark, passive rotation in the light with head-fixed or room-fixed targets, and active head rotation during gaze reorientation or visual pursuit. These data analysis approaches produce labile estimates of VOR gain such that the apparent vestibular sensitivity of premotor cells in the vestibular nuclei (VN) can vary widely, depending on the head rotation context. As a result, the VOR can appear to be switched 'ON' or 'OFF' at the VN level, especially when comparing the characteristics of central responses during passive versus active head turns.

Using a previously published model of eye-head coordination (Galiana and

Guitton, *Ann. N.Y. Acad. Sci.*, **656**: 452-471, 1992), various vestibular protocols are simulated to examine the resulting behavioural and central VN responses. The model duplicates the characteristics of labile central VOR responses, including the 'apparent' loss of VOR sensitivity during active head turns. Mathematical analysis and computer simulations show clearly that changes in central responses can simply be attributed to the changes in premotor circuitry (loops) in the various protocols. The addition of visual motor errors at VN levels during VOR tests in the light (or with imagined targets) is sufficient to produce enhanced or decreased VN modulations during passive rotation. During active head turns, the head velocity profile reaching VN cells via the canals is now also imbedded in a feedback loop, since the same visual goals can influence both eye and head trajectories. As a result, the characteristics of premotor VN cells can change from head-velocity modolation (passive VOR) to gaze-position modulation during active head turns. The VOR may appear 'OFF' when examining the cell's modulation, without recourse to some cortical intervention; furthermore, responses to head perturbation during active head turns can remain totally intact. Hence, despite the characteristics of VN cell modulations, proper function of the VOR during active head turns can remain intact.

LAST-ORDER PREMOTOR NEURONS TERMINATING ON HORIZONTAL OCULAR MOTONEURONS

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Lateral rectus (LR) and medial rectus (MR) motoneurons (MNs) are final common pathways for various kinds of horizontal eye movements. Various new anatomical tracing and electro-physiological methods have been used in determing the neural organization of supranuclear connections with LR and MR MNs. Among them, most commonly used is the retrograde labeling of neurons after injection of HRP into the oculomotor nucleus. However, with this technique, it is difficult to prove a direct projection of supranuclear origin onto ocular motoneurons, especially onto MR MNs, because of the unavoidable uptake of HRP by terminals in or near the injected nuclens. Therefore, our knowledge about exact locations of premotoneuronal cell groops terminating on LR and MR MNs has been still limited because of the technical difficulties.

Afferent connections of LR and MR MNs were examined in the cat by means of transneuronal labelina with wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP). WGA-HRP was injected into muscle nerves of LR or MR muscles. This method appears to limit uptake of WGA-HRP to last-order interneurons which terminate directly upon retrogradely-labeled motoneurons. In addition to retrogradely-labelled motoneurons, transneuronally-labeled neurons were observed in the following areas. After LR nerve injection, labeled cells were found in the

bilateral vestibular nuclei (VN), a rather wide area in the paramedian pontine reticular formation (PPRF) just rostral to the ipsilateral abducens nucleus (AbdN), a restricted area in the PPRF just ventrocaudal to the contralateral AbdN and the ipsilateral prepositus hypoglossi. After MR nerve injection, labeled cells were found in the bilateral VN and the contralateral AbdN.

NEURAL ORGANIZATION FROM THE SUPERIOR COLLICULUS TO HORIZONTAL OCULAR MOTONEURONS

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The superior colliculus (SC) plays an important role in control of visually-triggered orienting eye movement. The present study was performed to determine the pathways from the SC to ocular motoneurons (MNs) in the horizontal saccadic system, using electrophysiological and morphological techniques. Intracellular potentials were recorded from lateral rectus (LR), internuclear neurons in the abducens nucleus (INNs) and medial rectus (MR) MNs, and effects of stimulation of the bilateral SCs on them were examined in anaesthetized cats. Stimulation of the contralateral (contra) SC produced excitation and stimulation of the ipsilateral (ipsi) SC produced inhibition in LR MNs. The latency of EPSPs in LR MNs evoked by stimulation of the contra SC was 1.6 msec, suggesting that these EPSPs were disynaptic. The latency of IPSPs evoked by stimulation of the ipsi SC was almost comparable to that of the EPSPs, suggesting that the shortest inhibitory pathway is disynaptic from the SC. EPSPs and IPSPs with latencies similar to those in LR MNs were evoked in INNs by stimulation of the contra and ipsi SC, respectively. Stimulation of the ipsi SC produced excitation in MR MNs at the shortest latency of 2.6 msec, but stimulation of the contra SC produced no response in them. Conditioning stimulation of the ipsi SC facilitated trisynaptic excitation in the MR MNs from the ipsilateral vestibular afferents, indicating that the trisynaptic excitation from the ipsi SC is mediated to MR MNs via contra INNs.

To determine the location of last-order premotor neurons mediating disynaptic EPSPs and IPSPs to LR MNs and INNs, such premotor neurons were transneuronally labelled by injection of WGA-HRP into the abducens nerve, and biocytin was injected into a site in the contra SC from which horizontal eye movements were evoked by microstimulation. Labeled axon terminals from the contra SC were distributed in the PPRF where transneuronally labeled neurons terminating on ipsi LR MNs were observed, and also in the area ventrocaudal to the abducens nucleus where transneuronally -labeled neurons terminating on contra LR MNs were observed. Furthermore, labeled axon terminals of SC neurons made apparent contact with cell bodies and proximal dendrites of the transneuronally labeled neurons. These morphological data confirmed the above electrophysiological conclusion.

ACTIVITY IN COLLICULAR BUILDUP NEURONS DURING INTERRUPTED SACCADES

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Because saccades have a very stereotypical relationship between their duration and velocity profile and their size, it is difficult to relate neural discharge that may be correlated with saccade dynamics with causality. The interrupted saccade paradigm potentially can overcome the difficulty by perturbing saccades in midflight, and thus altering their usually set trajectories. This type of perturbation can be produced by stimulation in the region of the omnipause neurons (OPNs) at the time of saccade onset. The movement is stopped in midflight and then resumes after the end of stimulation and finally ends near the original target location. This paradigm was applied to the study of saccade-related burst neurons in the superior colliculus, a type of cell whose discharge has been shown to be correlated with dynamic motor error during saccades. The results obtained during interrupted saccades suggested that these neurons had to receive feedback information about the trajectory perturbation of the ongoing saccade (Keller and Edelman, 1994). However, unexpectedly the stimulation immediately silenced all active collicular burst neurons, a result which raised the question of how the accurate resumed movement was controlled if the population activity in the colliculus was momentarily erased by the perturbation. Another more recent study also recorded the activity of collicular neurons during saccades interrupted by stimulation in the rostral fixation zone of the colliculus itself (Munoz et al., 1996). This study also reported that active burst neurons in the colliculus were silenced by the stimulation, but then resumed their discharge for the postinterruption movement. In addition, a few collicular buildup cells were recorded in this study. Their discharge was also reported to be momentarily silenced by the stimulation.

We have extended these results by studying the activity in a large number of buildup cells located over a wide extent of the colliculus during saccades interrupted by stimulation in the OPNs. In agreement with the previous study involving fixation zone stimulation, we found that most buildup neurons showed a transient decrease in their activity produced by the stimulation. However, in contrast to the previous report, most buildup cells were not silenced completely, and about 10% of these cells showed no disturbance in their low-level discharge during the perturbation. The spatial distribution of the population activity in buildup cells during saccade interruption was examined to determine if it was sufficient to retain a code of the remaining motor error or the desired final location of the saccade even in the face of the perturbation.

ABDUCENS NEURON ACTIVITY DURING HEAD-UNRESTRAINED GAZE SHIFTS: UNEXPECTED ASSOCIATIONS BETWEEN FIRING AND GAZE, NOT EYE

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The role of premotor elements in saccade generation has been revealed almost exclusively in animals unable to move their heads. Recently, a number of studies have revisited the brain stem in animals with their heads unrestrained, concluding that gaze movement signals abound. We show here that all is not always what it seems since it may appear that gaze signals are present even on neurons in the abducens nucleus.

In the head-restrained animal, abducens motoneurons and internuclear neurons discharge as a function of both eye position and eye velocity. Thus, during saccades in the pulling direction of the innervated muscle, they exhibit a burst-tonic pattern of activity to first make the saccade and hold the eye steady in its deviated position. In head-unrestrained animals, gaze initially is shifted by a rapid eye saccade and as the slower head rotates toward the target, the VOR causes compensatory counter rotations of the eye in the orbit. Curiously, the neurons in the abducens nucleus still exhibit a burst-tonic discharge pattern, i.e., very little modulation of firing during eye counter rotation. Similarly, for off-direction saccades, motoneurons show a pause followed by an essentially constant discharge rate. This seemingly paradoxical activity can be explained in terms of a trade-off between the position and velocity sensitivity.

The burst of motoneurons derives from excitatory burst neurons (EBNs) in the brain stem, which exhibit an intense burst during saccades and are silent during fixation. During headrestrained saccades, the number of EBN spikes is highly linearly correlated to saccade amplitude. We found a similar linear relationship between the number of spikes and saccade size in the burst of abducens neurons. With the head unrestrained, large gaze shifts cause eye movement to saturate while gaze amplitude continues to increase. In this situation, the number of spikes in the burst of EBNs continues to increase, leading to the suggestion that EBNs specify the amplitude of the gaze shift. However, similar increases in the number of spikes occur for abducens neurons, casting doubt on this interpretation. It seems that some spikes are consumed by the mechanical constraints of the plant.

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