Korean J Physiol Pharmacol
Vol 6: 287–291, December, 2002

Changes in Excitability of Neurons in Rat Medial Vestibular Nucleus Following Vestibular Neurectomy

Sang Woo Chun, Jeong Hee Choi, Shin Hyung Lee[1], and Byung Rim Park[1]

Department of Oral Physiology, Wonkwang University College of Dentistry, [1]Department of Physiology, Wonkwang University School of Medicine & Vestibulocochlear Research Center at Wonkwang University, Iksan 570–749, Korea

Intrinsic excitabilities of acutely isolated medial vestibular nucleus (MVN) neurons of rats with normal labyrinth and with undergoing vestibular compensation from 30 min to 24 h after unilateral vestibular deafferentation (UVD) were compared. In control rats, proportions of type A and B cells were 30 and 70%, respectively, however, the proportion of type A cells increased following UVD. Bursting discharge and irregular firing patterns were recorded from 2 to 12 h post UVD. The spontaneous discharge rate of neurons in the ipsilesional MVN increased significantly at 2 h post-UVD and remained high until 12 h post-UVD in both type A and type B cells. After-hyperpolarization (AHP) of the MVN neurons decreased significantly from 2 h post-UVD in both types of cells. These results suggest that the early stage of vestibular compensation after peripheral neurectomy is associated with an increase in intrinsic excitability due to reduction of AHP in MVN neurons.

Key Words: Intrinsic excitability, Vestibular compensation, MVN, Ca^{2+}-activated K^+ current, Rat

INTRODUCTION

Damage to the vestibular receptor or vestibular nerve leads to severe oculomotor and postural symptoms, including spontaneous nystagmus, head tilt to lesioned side, contralesional limb extension and ipsilesional limb flexion, and circular walking. Unilateral vestibular deafferentation (UVD) decreases neuronal activity in the ipsilesional medial vestibular nucleus (MVN), due to loss of primary vestibular afferent input and enhanced commissural inhibition from contralesional MVN neurons. An imbalance of resting activity between the ipsilesional and contralesional MVN neurons causes the vestibular symptoms, and the subsequent recovery from these symptoms is called vestibular compensation (Smith & Curthoys, 1989; Curthoys & Halmagyi, 1995; Ris & Godaux, 1998a). Vestibular compensation is known to occur, because resting discharge in ipsilesional type I MVN cells is recovered, hyperactivity in contralesional cells is decreased, and excitability in the MVN neurons of the two sides is rebalanced.

Until recently, the mechanism for vestibular compensation was proposed to involve replacement of the diminished afferent input in ipsilesional MVN neurons by an external cue (Dieringer, 1995; Park et al, 1995; Kim et al, 1997; Kitahara et al, 1997). However, a recent hypothesis suggests that intrinsic membrane excitability in MVN neurons, which normally contributes to the spontaneous resting discharge, is modified during vestibular compensation (Darlington & Smith, 1996). Dutia and colleagues (Cameron & Dutia, 1997; Yamanaka et al, 2000) observed that the intrinsic *in vitro* firing rate of rostral ipsilesional MVN neurons was increased within 4 h post-UVD and lasted for over 24 h. They suggested that the increased firing rate might overcome an excessive commissural inhibition and disfacilitation in the early stage of unilateral labyrinthectomy (UL). Moreover, the increased firing rate might have been due to down-regulation of GABA receptors on ipsilesional MVN cells. Vibert et al. (1999b) also reported that an increase in the neuronal excitability of MVN neurons occurred 7 days post-UL in the guinea pig. Him & Dutia (2001) recently proposed that the increase in intrinsic excitability was due to a change in ion channel expression, in particular up-regulation of the low voltage activated Ca^{2+} conductances in type B MVN neurons as well as down-regulation in GABA receptor efficacy. In the present study, changes in neuronal excitability of acutely isolated neurons from the ipsilesional MVN following UVD were examined, in order to investigate whether vestibular compensation could be induced by changes in the intrinsic membrane excitability of MVN neurons.

METHODS

Animal preparation

Fourteen- to 17-day-old Sprague-Dawley rats of both sexes were used. All animals were anesthetized with ether. In UVD group, the unilateral VIIIth nerve was cut near the internal auditory porus through a ventral approach, following destruction of the ampulla. Control (intact vesti-

Corresponding to: Byung Rim Park, Department of Physiology, Wonkwang University School of Medicine, Iksan 570-749, Korea. (Tel) 82-63-850-6773, (Fax) 82-63-852-6108, (E-mail) byungp@wonkwang.ac.kr

ABBREVIATIONS: MVN, medial vestibular nuclei; UVD, unilateral vestibular deafferentation; UL, unilateral labyrinthectomy; RMP, resting membrane potential; AHP, after-hyperpolarization.

bular nerves) and experimental UVD groups were investigated at 30 min, 2 h, 12 h, and 24 h post-UVD.

Cell preparation

MVN neurons were dissociated according to the method of Kay& Wong (1986) with minor modifications. Briefly, animals were anesthetized with ether and decapitated. The brainstem was rapidly dissected and submerged in 4°C artificial cerebrospinal fluid (ACSF). The slices of brainstem 400 μm thick were cut coronally with a vibroslicer (752 M, Campden Instruments, UK). These slices were pre-incubated in ACSF, saturated with 95% O_2-5% CO_2, at room temperature for 1 h. Thereafter, slices were treated with 0.2 mg/ml pronase for 30~60 min at 32°C and subsequently exposed to 0.2 mg/ml thermolysin under the same condition. Micropunching was used to remove a portion of the ipsilesional MVN neurons, which were then dissociated into single cells by pipetting with a Pasteur pipette. The dissociated neurons were transferred into a recording chamber (volume of chamber, 0.5 ml) mounted on an inverted microscope (CK-40, Olympus, Japan).

Solutions

The ionic composition of the ACSF (in mM) was 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 10 glucose, and 24 $NaHCO_3$ and was continuously bubbled with 95% O_2-5% CO_2. The patch pipettes were filled with a solution containing (in mM) 140 KCl, 1 $MgCl_2$, 0.1 $CaCl_2$, 2 MgATP, 0.5 ethylene glycol-bis (-amin-ethylether)-N,N,N',N'-tetra-acetic acid (EGTA), and 10 N-2-hydroxy ethylpiperazine-N'-2-ethanesulfonic acid (HEPES). The pH of the internal solution was adjusted to 7.25 using KOH.

Recording procedures

Patch pipettes were pulled from 1.5 mm OD glass tube (WPI, USA) using a vertical microelectrode puller (PP83, Narishige, Japan). Recording electrodes had 2~3 MΩ of resistance in recording solution. Recordings were made by using the whole cell configuration of the patch clamp technique in current clamp mode with an Axopatch 1D Amplifier (Axon Instruments, USA). The experiment was terminated if the seal resistance dropped below 1 GΩ during the recording. All experiments were performed at room temperature (20~25°C). After filtration at 5 KHz using a low-pass filter, data were obtained using a Digidata 1200 interface and pCLAMP software (Version 6.0.3, Axon Instruments, USA) and later analyzed with pCLAMP. ANOVA (analysis of variance) was used to compare neuronal excitability between each group. The results are expressed as mean±SE.

RESULTS

Deviation of the eye and head toward the ipsilesional side and ipsilesional limb flexion and contralesional limb extension were evident after UVD, and these symptoms lasted until up to 24 h post-UVD. Circling movement and barrel rolling to the ipsilesional side were observed until up to 12 h post-UVD. In the control group, eight of 87 MVN neurons did not show spontaneous activity, but did exhibit a stable resting membrane potential (RMP), ranging from −40 to −53 mV, and the remaining 79 cells were spontaneously active and the RMP ranged from −41 to −67 mV. MVN neurons were categorized into types A and B: the type A had a single deep after-hyperpolarization(AHP) and the type B had an early fast and a delayed slow AHP (Fig. 1. A2, B2). Our results indicated that the early fast AHP was caused by a large conductance Ca^{2+}-activated K^+ current, and the delayed slow AHP was caused by a small conductance Ca^{2+}-activated K^+ current, in agreement with the previous report (Chun et al, 2000). Of the 79 control cells recorded, there were 28 type A cells (35%) and 51 type B cells (65%) (Fig. 1C). The RMP for the type A and B cells was −50.2±1.2 mV and −51.7±1.0 mV, respectively. The AHP amplitude was 14.9±0.5 mV in the type A control cells and 10.4±0.4 mV in the type B control cells, and the frequency of spontaneous discharge was 7.4±0.9 spikes/s in the type A and 7.8±0.6 spikes/s in the type B. Some MVN neurons from 14-day-old rats showed features of immature type A cells, such as action potential of long duration and incomplete single deep AHP. Moreover, some neurons exhibited short isopotentiality, which suggested the presence of immature type B cells (Fig. 1. A1, B1). After

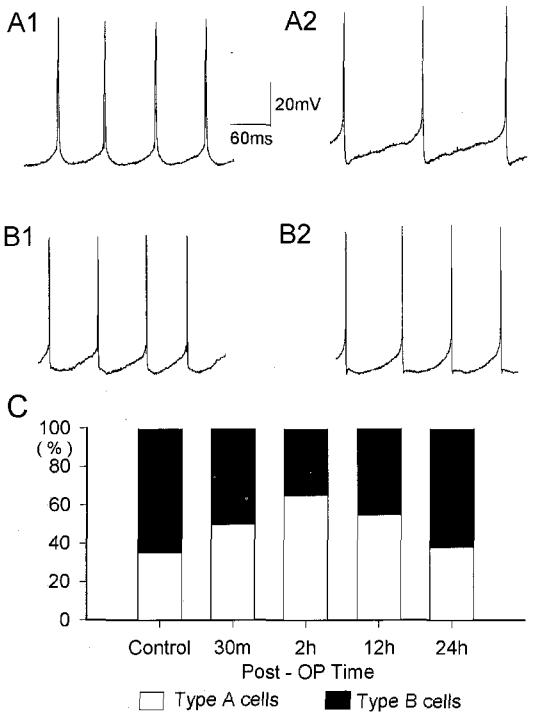

Fig. 1. Shapes of action potential and relative proportions of type A and type B MVN neurons following unilateral vestibular deafferentation. A1, B1, a typical response of immature type A cell and type B cell, respectively, recorded from a 14-day-old rat. A2, B2 a typical response of mature type A cell and type B cell, respectively, recorded from a 16-day-old rat. Note the single deep AHP pattern of type A cells, and the period of isopotentiality followed by the delayed slow AHP in immature type B cell. Both the early fast AHP and the delayed slow AHP are present in the type B cells. C, histogram showing the relative proportions of type A and type B cells after vestibular deafferentation. The sample sizes in each time after vestibular deafferentation are 79 cells in control, 19 cells in 30 min post-op, 34 cells in 2 h, 22 cells in 12 h, and 26 cells in 24 h.

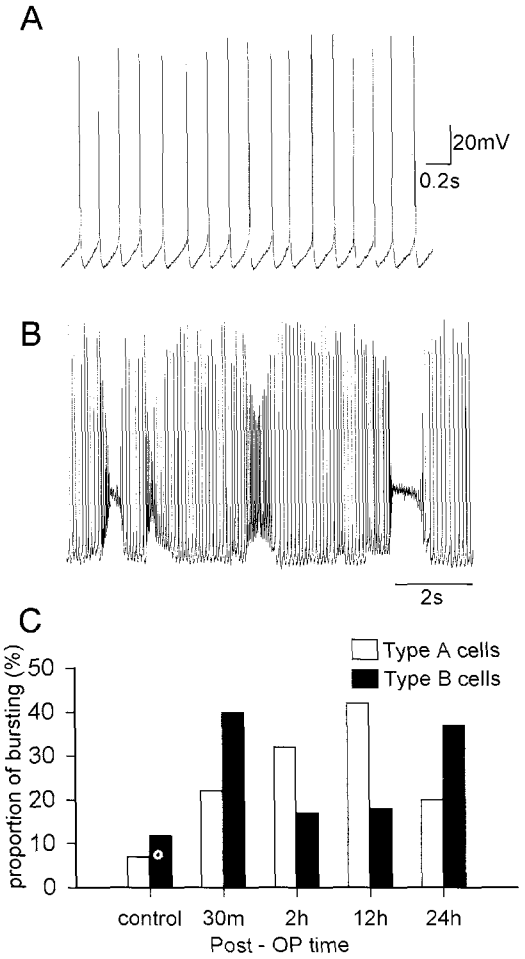

Fig. 2. Regularity of the resting discharge patterns of MVN neurons. A, a typical response of regular and repetitive firing pattern recorded from a 15-day-old control rat. B, a typical response of irregular firing pattern recorded from cells undergoing vestibular compensation after unilateral vestibular deafferentation. C, histogram showing the proportion of irregular firing after vestibular deafferentation. Note the increase of the proportion of irregular firing cells, in particular in the type A cells.

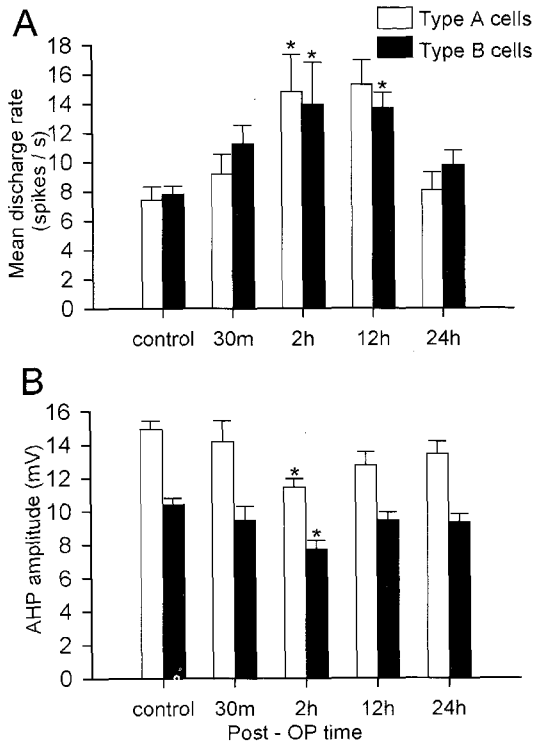

Fig. 3. Histograms showing mean discharge rates (A) and mean after hyperpolarization amplitude (B) of the type A and B cells in the medial vestibular nucleus after vestibular deafferentation. Asterisks indicate values significantly different from control ($p < 0.05$).

UVD, the proportion of the type A cells increased. At 2 h post-UVD, the type A cells accounted for 65% (n=22) and the type B cells for 35% (n=12). The increased proportion of the type A cells lasted until up to 12 h post-UVD, thereafter, the proportion of each cell type was similar to intact control levels at 24 h post-UVD (Fig. 1C).

In the control group, repeated and regular firing was observed in 25 out of 28 type A cells recorded and 45 out of 51 type B cells recorded (Fig. 2). Irregular firing activity or firing activity in bursts was increased in the type A cells after UVD, and was evident in the type B cells. At 12 h post-UVD, 5 of 12 type A cells (42%) showed this type of firing activity.

Fig. 3 shows the frequency of spontaneous discharge and AHP amplitude as the time after UVD lapsed. In comparison with the control group, the frequency in the both type A and B cells increased from 30 min post-UVD. At 2 h post-UVD, the frequency of spontaneous discharge significantly increased to 14.8 ± 2.6 spikes/s and 13.9 ± 2.9 spikes/s in the type A and B cells, respectively ($p < 0.05$), and lasted until 12 h post-UVD. The AHP amplitude decreased in the both type A and B cells after UVD, and at 2 h post-UVD there was a significant difference, showing 11.5 ± 0.5 mV and 7.7 ± 0.5 mV, respectively ($p < 0.05$).

DISCUSSION

The present study suggests that vestibular compensation is generated by changes in the intrinsic membrane properties of single neurons, and also support the hypothesis that significant changes in the electrophysiological properties of type B MVN neurons are possible (Him & Dutia, 2001).

Labyrinthectomy, which destroys peripheral vestibular receptors, has been used as a tool to remove vestibular input (Park et al, 1995; Kim et al, 1997). However, because of difficulty in confirming the vestibular receptor in rats around 15 days old, vestibular neurectomy was used in this study to remove vestibular input. The RMP and AHP amplitudes measured in this study were in good agreement with those derived from mice aged 5~30 days (Dutia & Johnston, 1998). However, the spontaneous firing frequency of MVN neurons recorded in this study (7~8 spikes/s) was lower than that recorded in brain slices (10~20 spikes/s) and *in vivo* (37 spikes/s) (Darlington et al, 1995; Ris & Godaux, 1998a). Thus, the excitability of single neurons lacking synaptic connectivity was decreased. External excitatory input has been reported to influence spontaneous

firing frequency as much as twice intrinsic membrane excitability (Ris & Godaux, 1998a). It is quite possible that our data were influenced by both dissociation procedures employed (enzymatic and mechanical) as well as recording conditions, such as temperature and intracellular solutions used.

Ris & Godaux (1998b) reported that the irregularity of spontaneous discharge increased during the period of 1 to 10 h after labyrinthectomy, due to an increase in external synaptic input. Vibert et al. (1999a) observed bursting behavior 3 days post-UVD in 33% of ipsilesional MVN neurons isolated from guinea pig brain, and Him and Dutia (2001) also reported that burst firing increased in MVN neurons after labyrinthectomy, particularly in type B cells. Our results showed an increase in irregular activity in cells of post-UVD animals (Fig. 2C). However, the increase in irregularity in a single cell, where synaptic input was excluded, was attributed to changes in membrane conductance in MVN neurons. Apamin treatment of MVN neurons was reported to alter their regular and rhythmic firing to bursting behavior due to the suppression of small conductance Ca^{2+}-activated K^+ currents (de Waele et al, 1993; Wang et al, 1999; Chun et al, 2000). In the present study, the proportion of type A cells increased (Fig. 1C), and type A cells exhibiting bursting behavior also increased after UVD (Fig. 2C). These changes were likely due to transformation of type B cells into type A cells, and this process probably occured, because of a decrease in the delayed slow AHP, in conjunction with the suppression of small conductance Ca^{2+}-activated K^+ currents during vestibular compensation.

After UVD, a reduction in AHP amplitude resulted in an increase in spontaneous discharge, and the associated changes with the lapse of time post-UVD tended to be stable. Bliss & Collingridge (1993) explained the long term potentiation process to be due to calcium influx through the NMDA channel, leading to increased $[Ca^{2+}]i$ in the early stage, which activated PKC, PKA, and Ca^{2+}/calmodulin-dependent protein kinase, and the resulting suppressed potassium channel activity would increase neuronal excitability. An increase in $[Ca^{2+}]i$ in MVN neurons by stimulating the ipsilateral afferent and commissural fiber has been observed (Takahashi et al, 1994). An increase in $[Ca^{2+}]i$ after unilateral labyrinthectomy has not been reported, although PKC activity (suggested to be activated by an increase in $[Ca^{2+}]i$) is reportedly altered following labyrinthectomy (Balaban & Romero, 1996). Hence, it can be presumed that UVD increases $[Ca^{2+}]i$ through NMDA channel-activated PKC or Ca^{2+}/calmodulin-dependent protein kinase, which suppresses Ca^{2+}-activated K^+ channels, reduces hyperpolarization amplitude, and ultimately results in increased neuronal excitability.

Fig. 3A showed that the spontaneous firing rate increased 30 min post-UVD and peaked at 2 h post-UVD. This result differs from other earlier studies, which observed no change in spontaneous firing at 2 h post-UVD and a peak in spontaneous firing at 4 h post-UVD (Cameron & Dutia, 1997; Yamanaka et al, 2000). This discrepancy might have been due to difference in the postnatal age of the rats used. However, the results are compatible with studies to assess c-Fos protein expression after unilateral labyrinthectomy; c-Fos protein expression in the bilateral MVN peaked at 2 h post-UVD (Kaufman et al, 1992). Since c-Fos expression plays roles in activation of the NMDA receptor, increased $[Ca^{2+}]i$, activation of calcium-dependent protein kinase, and protein phosphorylation (Hughes & Dragunaw, 1995), the increased excitability of MVN neurons post-UVD may be related to the process of c-Fos expression.

In conclusion, the presently described results suggest that the increase in intrinsic excitability following UVD is related to the early stage of vestibular compensation, and that the increase in excitability is caused mainly by a decrease in the delayed slow AHP in type B neurons. Nevertheless, to confirm changes in membrane conductance relevant to an increase in neuronal excitability, followup voltage-clamp studies are needed.

ACKNOWLEDGEMENT

This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ1-PG10-21402--0001).

REFERENCES

Balaban CD, Romero GG. Inferior olive ablation does not affect nodulus protein kinase C responses to unilateral labyrinthectomy. *Soc Neurosci Abstr* 22: 18−31, 1996

Bliss TVP, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31−39, 1993

Cameron SA, Dutia MB. Cellular basis of vestibular compensation: changes in intrinsic excitability of MVN neurons. *Neuroreport* 8: 2595−2599, 1997

Chun SW, Jun JW, Park BR. Roles of Ca2+-Activated K+ conductances on spontaneous firing patterns of isolated rat medial vestibular nucleus neurons. *Korean J Physiol Pharmacol* 4: 1−8, 2000

Curthoys IS, Halmagyi GM. Vestibular compensation: a review of the oculomotor, neural, and clinical consequences of unilateral vestibular loss. *J Vestibular Res* 5: 67−107, 1995

Darlington CL, Gallagher JP, Smith PF. In vitro electrophysiological studies of the vestibular nucleus complex. *Prog Neurobiol* 45: 335−46, 1995

Darlington CL, Smith PF. The recovery of static vestibular function following peripheral vestibular lesions in mammals: The intrinsic mechanism hypothesis. *J Vestibular Res* 6: 185−201, 1996

Dieringer N. 'Vestibular compensation': Neural plasticity and its relations to functional recovery after labyrinthine lesions in frogs and other vertebrates. *Prog Neurobiol* 46: 97−129, 1995

Dutia MB, Johnston AR. Development of action potentials and apamin-sensitive after-potentials in mouse vestibular nucleus neurons. *Exp Brain Res* 118: 148−154, 1998

Him A, Dutia MB. Intrinsic excitability changes in vestibular nucleus neurons after unilateral deafferentation. *Brain Res* 908: 58−66, 2001

Hughes P, Dragunaw M. Induction of immediate early genes and the control of neurotransmitter-regulated gene expression within the nervous system. *Pharmacol Rev* 47: 133−178, 1995

Kaufman GD, Anderson JH, Beitz AJ. Brainstem Fos expression following acute unilateral labyrinthectomy in the rat. *Neuroreport* 3: 829−832, 1992

Kay AR, Wong RK. Isolation of neurons suitable for patch-clamping from adult mammalian central nervous systems. *J Neurosci Meth* 16 (3): 227−238, 1986

Kim MS, Jin BK, Chun SW, Lee MY, Lee SH, Kim JH, Park BR. Role of vestibulocerebellar N-methyl-D-aspartate receptors for behavioral recovery following unilateral labyrinthectomy. *Neurosci Lett* 222: 171−174, 1997

Kitahara T, Takeda N, Saika T, Kubo T, Kiyama H. Role of the flocculus in the development of the vestibular compensation: immunohistochemical studies with retrograde tracing and flocculectomy using Fos expression as a marker in the rat brainstem. *Neuroscience* 76: 571−580, 1997

Park BR, Suh JS, Kim MS, Jeong JY, Chun SW, Lee JH. Effect of sensory deprivation or electrical stimulation on acute vestibular symptoms following unilateral labyrinthectomy in rabbits. *Acta Otolaryngol Suppl* 519: 162–167, 1995

Ris L, Godaux E. Neuronal activity in the vestibular nuclei after contralateral or bilateral labyrinthectomy in the alert guinea pig. *J Neurophysiol* 80: 2352–2367, 1998a

Ris L, Godaux E. Spike discharge regularity of vestibular neurons in labyrinthectomized guinea pigs. *Neuroscience Letters* 253: 131–134, 1998b

Smith PF, Curthoys IS. Mechanisms of recovery following unilateral labyrinthectomy: a review. *Brain Res Rev* 14: 155–180, 1989

Takahashi Y, Takahashi M, Tsumoto T, Doi K, Matsunaga T. Synaptic input-induced increase in intraneuronal calcium in the medial vestibular nucleus of young rats. *Neurosci Res* 21: 59–69, 1994

Vibert N, Babalian A, Serafin M, Gasc JP, Muhlethaler M, Vidal PP. Plastic changes underlying vestibular compensation in the guinea pig persist in isolated, in vitro whole brain preparations. *Neuroscience* 93: 413–432, 1999a

Vibert N, Bantikyan A, Babalian A, Serafin M, Muhlethaler M, Vidal PP. Post-lesional plasticity in the central nervous system of the guinea-pig: a "Top-down" adaptation process. *Neuroscience* 94: 1–5, 1999b

de Waele C, Serafin M, Khateb A, Yabe T, Vidal PP, Muhlethaler M. Medial vestibular nucleus neurons in the guinea pig: apamin induced rhythmic burst firing an in vitro and in vivo study. *Exp Brain Res* 95: 213–222, 1993

Wang GY, Olshousen BA, Chalupa LM. Differential effects of apamin and charybdotoxin sensitive K^+ conductances on spontaneous discharge patterns of developing retinal ganglion cells. *J Neurosci* 19(7): 2609–2618, 1999

Yamanaka T, Him A, Cameron SA, Dutia MB. Rapid compensatory changes in GABA receptor efficacy in rat vestibular neurones after unilateral labyrinthectomy. *J Physiol* 523 (2): 413–424, 2000