Neuroscience 160 (2009) 716–730

STRESS AXIS PLASTICITY DURING VESTIBULAR COMPENSATION IN THE ADULT CAT

B. TIGHILET,* C. MANRIQUE AND M. LACOUR

Université Aix-Marseille, UMR 6149 Université de Provence/CNRS, Neurobiologie Intégrative et Adaptative, Pôle 3C, Comportement, Cerveau, Cognition, Centre de St Charles, Case B, 3 Place Victor Hugo, 13331 Marseille Cedex 3, France

Abstract—The postural, ocular motor, perceptive and neurovegetative syndromes resulting from unilateral vestibular neurectomy (UVN) symptoms could generate a stress and thereby activate the hypothalamo-pituitary–adrenal (HPA) axis. This study was aimed at determining whether UVN causes changes in the activity of the HPA axis, and if so, evaluating the time course of changes associated with UVN syndrome. At the cellular level, corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) immunoreactivity (Ir) were analyzed and quantified in the paraventricular nucleus (PVN) and the vestibular nuclei (VN) complex of cats killed early (1 and 7 days) or late (30 and 90 days) after UVN. Dopamine-β-hydroxylase (DβH), the enzyme synthesizing noradrenaline was examined in the locus coeruleus (LC) in these same cats. At the behavioral level, the time course of recovery of the postural and locomotor functions was quantified at the same postoperative delays in another group of UVN cats. Results showed a significant bilateral increase in the number of both AVP-Ir and CRF-Ir neurons in the PVN and an increase of DβH-Ir neurons in the LC at 1, 7 and 30 days after UVN. This increased number of neurons was no longer observed at 90 days. Conversely, a significant bilateral decrease of CRF-Ir neurons was observed in the VN at these same postlesion times, with a similar return to control values at 90 days. Our behavioral observations showed strong posturo-locomotor functional deficits early after UVN (1 and 7 days), which had recovered partially at 30 days and completely by 90 days postlesion. We demonstrate a long-lasting activation of the HPA axis, which likely reflects a chronic stress, experienced by the animals, which corresponds to the time course of full vestibular compensation, and which is no longer present when the animals are completely free of posturo-locomotor symptoms at 90 days.

Key words: vestibular deafferentation, hypothalamic–pituitary–adrenal axis, corticotropin-releasing factor, arginine vasopressin, noradrenaline, cat.

Unilateral lesion of the peripheral vestibular system results in damage on several levels, from basic reflex problems such as postural and ocular motor syndromes to higher order deficits including perceptive syndromes. These syndromes are accompanied by severe vegetative symptoms like nausea and vomiting (Curthoys and Halmagyi, 1995; Curthoys, 2000; Lacour, 2006; Borel et al., 2008), but whereas the vegetative syndrome disappears quickly after unilateral vestibular loss, recovery of vestibular function takes considerably longer (Lacour et al., 1989; Smith and Curthoys, 1989; Darlington and Smith, 2000). As a general rule, behavioral compensation of static deficits such as ocular nystagmus, head tilt and posture instability, and dynamic deficits like eye and head/body stabilization problems, requires several weeks or months depending on the species (Dieringer, 1995; Darlington and Smith, 2000; Lacour, 2006).

All the symptoms seen after unilateral vestibular lesion most likely alter homeostasis and initiate an adaptive stress response. Stressors, in turn, are known to activate the hypothalamo-pituitary–adrenal (HPA) axis by a well-understood pathway: inputs converging on the paraventricular nucleus (PVN) of the hypothalamus, a nucleus in which the synthesis of corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) takes place. From the PVN, these neuropeptides are released into the portal vasculature where they stimulate the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland, ACTH in turn stimulates the synthesis and release of glucocorticoids (cortisol in humans and corticosterone in rodents) from the adrenal cortex (Herman and Cullinan, 1997; Herman et al., 2003). Stress also activates catecholaminergic neurons in brain stem structures, notably in the solitary tract nucleus and locus coeruleus (LC), and these neurons respond by increasing their noradrenaline (NA) release. Glucocorticoid and catecholamine release induces adaptive physiological responses by increasing energy substrates that tend to facilitate the rapid restoration of homeostasis (Keller-Wood and Dallman, 1984; Zinder and Dar, 1999). These hormones also act on the pituitary gland and hypothalamus in a classic negative feedback loop to decrease CRF and ACTH release (de Kloet et al., 1998).

Activation of the HPA axis has been reported after electrical stimulation of the vestibular nerve and caloric stimulation of the peripheral labyrinth (Azzena et al., 1993; Liu et al., 1997), and both electrical and caloric vestibular stimulation increase plasma vasopressin levels in the rat (Horii et al., 2001). Interestingly, Gustave Dit Duflo et al. (1999) reported Fos-like immunoreactivity changes in most of the cat nervous structures known for their implication in both stress response and vestibular response, notably the

*Corresponding author. Tel: +33-0488576841; fax: +33-0488576818.
E-mail address: brahim.tighilet@univ-provence.fr (B. Tighilet).

Abbreviations: ACTH, adrenocorticotrophic hormone; ANOVA, analysis of variance; AVP, arginine vasopressin; BSA, bovine serum albumin; CRF, corticotropin-releasing factor; DβH, dopamine-β-hydroxylase; HPA, hypothalamo-pituitary–adrenal; Ir, immunoreactive; LC, locus coeruleus; Max. P., maximal locomotor balance performance; NA, noradrenaline; PB, phosphate buffer; PBS, phosphate buffer saline; PVN, paraventricular nucleus; UL, unilateral labyrinthectomy; UVN, unilateral vestibular neurectomy/neurectomized; VN, vestibular nuclei.

0306-4522/09 $ - see front matter © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2009.02.070

autonomic nuclei, LC, hypothalamus, hippocampus, vestibular nuclei (VN), and inferior olive (IO). Modifications of Fos-expression were also found after exposure to gravito-inertial force changes, which comprise an experimental condition known to modify vestibular input and to induce stress in the rat (Kaufman et al., 1991; Gustave Dit Duflo et al., 2000) and the gerbil (Marshburn et al., 1997). However, very few works have investigated the neuroendocrine response after vestibular deafferentation and during the vestibular compensation process (Seemungal et al., 2001; Gliddon et al., 2003b for review). Experimental evidence linking unilateral vestibular deafferentation to activation of the HPA axis includes the observation of a significant increase in Fos-like immunoreactive (Ir) neurons in the PVN of the hypothalamus in both the unilateral labyrinthectomized rat (Cameron and Dutia, 1999) and the unilateral vestibular neurectomized (UVN) cat (Gustave Dit Duflo et al., 1999). This up-regulation of Fos expression points to an increased neuronal activity in the PVN, and can be seen as a mechanism of neuroendocrine plasticity underlying adaptive physiological responses to stress induced by the vestibular deafferentation.

Those brain stem nuclei immunostained after hypergravity exposure showed similar immunolabeling after unilateral vestibular deafferentation, in several species including the rat (Saika et al., 1991: rat, Kaufman et al., 1993; Cirelli et al., 1996; Darlington et al., 1996: guinea pig, Kitahara et al., 1998; Gustave Dit Duflo et al., 1999: cat).

Vestibular deafferentation has also been found to induce a significant increase in salivary cortisol concentration in the guinea pig (Gliddon et al., 2003a). Moreover, it has been suggested that body's response to stress could be implicated in the vestibular compensation process via activation of the HPA axis (Gliddon et al., 2003a for review). In addition, daily systemic injections of ACTH accelerate the recovery of postural and ocular motor deficits after unilateral vestibular deafferentation in animal models (Flohr and Luneburg, 1982; Gilchrist et al., 1990: guinea pig). On the other hand, clinical investigations have indicated a possible link between HPA axis activation and vestibular pathology. Plasma AVP levels were increased in patients with peripheral vestibular vertigo (Takeda et al., 1995; Kumagami et al., 1998), and the administration of AVP induced endolymphatic hydrops—a pathological state that can be reduced by vasopressin antagonists—in animal experiments (Takeda et al., 2000). Patients with vestibular dysfunction also exhibit modifications of their stress hormones profiles, noted by changes in cortisol and ACTH blood concentration (Horner and Cazals, 2005) and further reinforced by evidence that glucocorticoid agonists showed some effectiveness in the treatment of Menière's disease (Sennaroglu et al., 1999; Garduno-Anaya et al., 2005).

Despite this relatively broad background, there has been no investigation on the stress-related hormones AVP and CRF, nor on catecholamines like NA during vestibular compensation. The present study was aimed at determining the kinetics of such neuroendocrine plasticity, if indeed it occurs, during the course of recovery after UVN in the cat. This was achieved using immunohistochemistry, notably by quantifying the Ir neurons expressing CRF and AVP in the PVN of the hypothalamus, and those expressing dopamine-β-hydroxylase (DβH) in the LC. CRF-Ir was also examined in the VN, in which neurons are known to express glucocorticoid receptors (Lindsay et al., 2005; Zhang et al., 2005) and contribute to vestibular compensation. The changes in CRF-Ir, AVP-Ir and DβH-Ir were investigated during the course of recovery in four groups of cats, killed at periods of 1 day, 1 week (the acute stage), 1 month (the compensatory stage) or 3 months (the compensated stage) after UVN. To assess the functional relevance of vestibular lesion-induced neuroendocrine plasticity, the kinetics of the behavioral recovery was quantified in another group of cats. Quantification of posture was performed to test the compensation of static deficits, while locomotion performance on a rotating beam was used to assess the more dynamic aspect of compensation.

EXPERIMENTAL PROCEDURES

The experiments were performed on 24 (2 years old) adult pigmented domestic cats (3–4 kg) obtained from the Centre d'Élevage du Contigné, Bray-Lu, an approved supplier in France. All experimental procedures were carried out in line with the guidelines Animals (Scientific Procedures) Act of 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publications No. 8023, revised 1978). Every attempt was made to minimize both the number and the suffering of animals used in these experiments. The cats were housed under a constant 12-h light/dark cycle with free access to food and water.

Sixteen adult animals were subjected to UVN on the left side and killed at four recovery times: 1 day (n=4), 1 week (n=4), 1 month (n=4) and 3 months (n=4). Four sham-operated animals were used as control group; these were submitted to anesthesia and surgical approach without sectioning of the vestibular nerve. Our previous studies in the cat model (Tighilet et al., 2006, 2007a,b) showed very weak inter-individual variations after UVN for both the cellular markers and the behavioral parameters. This justifies the sample size of four cats per group. The recovery periods were chosen based on our previous behavioral and electrophysiological investigations, which showed major postural and ocular motor deficits in acute cats between 1 and 7 days post-neurectomy, a partial recovery at 1 month, and complete recovery by 3 months (Lacour et al., 1997). Four additional UVN cats were used for the behavioral investigations performed at the same postoperative delays (1, 7, 30 and 90 days).

Vestibular neurectomy

Animals underwent a left side vestibular nerve section performed under fluothane anesthesia (2%) and aseptic conditions. Vestibular neurectomy was performed under visual control through a dissecting microscope. The vestibular nerves were sectioned at a post-ganglion level after mastoidectomy, partial destruction of the bony labyrinth, and surgical exposure of the internal auditory canal (Xerri and Lacour, 1980). The classic postural, locomotor and ocular motor deficits displayed in the days following UVN were used as criteria to evaluate the effectiveness of the vestibular nerve lesion, but completeness of UVN produced by this technique has been confirmed by histological procedures in previous studies (Lacour et al., 1976). All animals including the sham group were treated post-operatively with antibiotics (ampicillin 20 mg/kg i.m.) for 7 days and with analgesics (tolfenamic acid 4 mg/kg i.m.) for 1–2 days.

Tissue preparation

Cats were deeply anesthetized with ketamine dihydrochloride (20 mg/kg i.m., Rhône Poulenc Mérieux) and perfused under halothane (2%), through the ascending aorta, with 1 L of normal saline solution (0.9%) containing 0.1% heparin, and thereafter with 2 L of ice cold 0.1 M phosphate buffer (PB, pH 7.4) including 4% paraformaldehyde and 0.2% picric acid.

After removal from the skull, the brain was cut into several blocks and post-fixed overnight at 4 °C in the same solution. The blocks were then rinsed and cryoprotected by increasing concentrations (10%, 20%, and 30%) of sucrose solution in 0.1 M PB for 72 h at 4 °C. Blocks containing the VN, PVN, and LC were rapidly frozen with CO_2 gas, and coronal sections 20 μm thick were cut in a cryostat (Leica, Rueil Malmaison, France).

Immunohistochemistry

Free-floating sections were first incubated three times for 5 min in PBS (phosphate buffer saline 0.1 M), then for 1 h in 10% bovine serum albumin (BSA) and 0.25% Triton X-100. Thereafter, sections were incubated under continuous agitation 24 h at 4 °C with a goat polyclonal anti-CRF antiserum (1:500 in PBS containing 2% BSA and 0.25% Triton, Santa Cruz Biotechnology, CA, USA), a rabbit polyclonal antibody to DβH (1:1000 in PBS containing 2% BSA and 0.25% Triton, Santa Cruz Biotechnology), or 72 h at 4 °C with a rabbit anti-AVP antiserum (1:10,000 in PBS containing 2% BSA and 0.25% Triton; ICN Biomedicals). After several rinses (3×5 min in PBS with 2% BSA and 3×5 min in PBS with 5% BSA) sections were incubated for 1 h respectively in biotinylated rabbit anti-goat IgG, or goat anti-rabbit IgG secondary antibody (1:200 in PBS containing 2% BSA; from Vector Laboratories, Burlingame, CA, USA). After rinses (3×5 min in PBS with 2% BSA and 3×5 min in PBS with 5% BSA), the sections were processed for immunodetection through the following incubation: 1 h in horseradish peroxidase avidin D (Vector Laboratories), 10 min in a solution containing 0.02% diaminobenzidine, and 5 more minutes in the diaminobenzidine solution with 0.03% hydrogen peroxide added.

Double staining for CRF and AVP. We used indirect immunofluorescence to achieve double immunocytochemical staining and examine potential colocalization of CRF and AVP. Sections were incubated first for 72 h at 4 °C with the rabbit anti-AVP antiserum (1:10,000), and after 48 h of incubation we added the goat polyclonal anti-CRF antiserum (1:500) in PBS containing 2% BSA and 0.3% Triton X-100 for another 24 h. After washing (3×5 min in PBS with 2% BSA and 3×5 min in PBS with 5% BSA), the sections were reincubated in a mixture of Alexa Fluor 594–conjugated donkey anti-goat IgG (1:200, Interchim) and Alexa Fluor 488–conjugated donkey anti rabbit IgG (1:200, Interchim) in PBS containing 5% BSA and 0.3% Triton X-100. Following several rinses, sections were mounted under coverslips on glass slides in a 1:3 mixture of 0.1 M PB and glycerol. Sections were then examined under an epifluorescence microscope equipped with corresponding-exciting filters.

Data quantification. The VN, the PVN and the LC were identified through Berman's stereotaxic atlases (Berman, 1968; Berman and Jones, 1982). Forty serial sections were quantified in the PVN of each animal, with eight sections being used for each of the five main levels examined (rostrocaudal planes of A11, A11.8, A12, A12.6 and A12.9 for the PVN). The four VN (medial, inferior, superior and lateral; hereinafter MVN, IVN, SVN and LVN, respectively), and the LC were analyzed in serial sections collected from the dorsal (0.9) to the caudal (12.1) part of the brainstem, the number depending on each region's rostrocaudal extent. The immunoreactivity quantification was performed on both sides.

A quantitative analysis of the number of AVP, CRF and DβH Ir neurons was performed via computer-assisted image analysis using a DMLB microscope (Leica Microsystems, Wetzlar, Germany) equipped with a DXM 1200 Nikon high-resolution digital camera (1024×1024 pixels; Nikon, Tokyo, Japan) interfaced to a PC computer employing image software for capturing and processing the images (Lucia G, Nikon, Champigny-sur-Marne, France). Quantification of the immunolabeled neurons was performed via a grey-level method by adjusting a threshold brightness value, and only cells labeled with a grey value above this threshold were taken into account. Reproducibility was assessed by comparing the same data analyzed independently by two researchers blind to the animals' groups. The specific immunolabeling was quantified in each section as the number of labeled neurons, and was automatically computed and evaluated thereafter as the mean (±SEM) for each side, each cat, and each subgroup of cats (Tighilet et al., 2007a). To eliminate quantification problems due to potentially asymmetric slides, data were collected from symmetrical slides only, and symmetry was assessed on the basis of the number of cells stained with Cresyl Violet on each side.

Statistical analysis. Statistical analysis was performed in the control group (sham-operated cats) and in each experimental group corresponding to a specific post-operative sacrifice date. Results were evaluated by analysis of variance (ANOVA) to test the effects of the lesion (sham-operated vs. UVN cats), the post-operative recovery period, the side (deafferented vs. intact), and the structure (MVN, IVN, SVN, LVN, LC and PVN) on CRF-Ir/AVP-Ir/DβH-Ir, and to determine whether any interactions between these variables are present. ANOVA was followed by post hoc analysis with the Scheffe test (StatView II, SAS Software, Inc., Cary, NC, USA).

Behavioral investigations

Posture recovery. Posture deficits and recovery were evaluated by measuring the support surface delimited by the four legs of the cat while it was standing erect and stationary. Support surface can be regarded as a good estimate of postural control because it reflects the cat's behavioral adaptation in compensating static vestibulospinal deficits induced by the vestibular lesion (Tighilet et al., 2006, 2007b). In general, the support surface is small in the normal cat (about 50 cm^2) and greatly increased in the days following unilateral vestibular lesion. To quantify support surface, cats were placed in a device with a graduated transparent ground that allowed them to be photographed from underneath. Five repeated measurements were taken for each cat at each postoperative time (1, 7, 30 and 90 days) and an average was calculated for each experimental session. The support surface was measured as the surface delimited by the four legs, using an image analysis system (Canvas 9TM, Deneba Software, Miami, FL, USA). Data recorded after vestibular lesion were compared to pre-lesion values using individual references, that is, each animal acted as its own control.

Equilibrium function recovery. Locomotor balance was quantified using the rotating beam experimental device (see Xerri and Lacour, 1980 for details). In this apparatus, two compartments (0.5×0.6×0.5 m) are connected by a horizontal beam (length: 2 m; diameter: 0.12 m) situated in a tunnel whose walls are covered by a pseudo-random visual pattern. The beam, placed 1.2 m off the ground, can be rotated about its longitudinal axis with a constant angular velocity ranging from 0° to 750°/s (linear tangential speed: 0 to 0.785 m s^{-1}). Cats were conditioned with reward (a small piece of meat) to cross the beam at the presentation of a light signal in the arrival compartment. The tunnel was equipped with a safety net to ensure the animals were protected in case they fell. First crossings were made on the immobile beam and, thereafter, on the rotating beam. Rotation velocity of the beam was progressively increased after four consecutive trials

without fall, and equilibrium performance was characterized as the highest speed of beam rotation that did not induce a fall. This maximal rotation speed determined the maximal locomotor balance performance (Max. P.).

Preoperative training on the rotating beam necessitated 6 to 10 training periods of 1 h per day, depending on the cat. Behavioral training on the rotating beam consisted of depriving the animals of food for 24 h before the first training session; thereafter, they were fed at the end of each of the ensuing sessions, and this procedure was sufficient to motivate the cats and to condition them relatively rapidly (8 days). Training was ceased when the cats' Max. P. was reached and stabilized at its highest level, and this level was remarkably similar from one cat to another.

Four trained animals were subjected to a UVN on the left side, after which postoperative locomotor balance was measured every 2 days beginning on the second postoperative day and continuing until complete recovery. It was verified that animal's food preferences did not change postoperatively. As in the posture evaluation, data recorded after vestibular lesion (Max. P.) were compared to individual pre-operative values such that each animal acted as its own control.

Statistical analysis. Statistical analysis consisted of an ANOVA to test for changes at the different post-lesion delays (1, 7, 30 and 90 days) for both the support surface and the maximal equilibrium performance of the cats. Results were considered significant at $P<0.05$.

RESULTS

Immunohistochemical observations

CFR and AVP immunoreactivity in the paraventricular nuclei of the hypothalamus. Control cats. A large number of CRF-Ir neurons (Fig. 1A) and AVP-Ir neurons (Fig. 1C) were observed in the PVN of the hypothalamus. The CRF-Ir cells were medium-sized and few showed proximal dendrite characteristics (Fig. 1B) while most of the AVP-Ir cells exhibited spine-like processes over their somata and proximal dendrites (Fig. 1D). In the PVN, no significant differences were found between the left and the right sides in the number of both CRF-Ir and AVP-Ir neurons. The double immunohistochemistry staining for CRF and AVP revealed a complete lack of colocalization for CRF-Ir and AVP-Ir in the cytoplasm of individual cells in the sham-operated control cats (Fig. 1E).

In the UVN cats. Vestibular lesion-induced changes in number of cells were seen for both the CRF-Ir and AVP-Ir neurons of the PVN. ANOVA revealed no inter-individual differences between the cats in each group. Fig. 2A shows photomicrographs of CRF-Ir sections taken from the PVN of four representative animals: one control and three UVN cats killed at days 1, 30 and 90 after a left UVN. Compared to controls, which exhibited a moderate and symmetric number of CRF-Ir neurons, UVN induced a strong bilateral increase in CRF-Ir neurons in the PVN at both the 1 day and 1 month postlesion times. Three months later, the number of CRF-Ir neurons in PVN had returned to control levels on both sides. Fig. 2B illustrates the quantitative analysis of the CRF-Ir neurons in the PVN for the four groups of neurectomized cats. The mean number of CRF-Ir neurons in the PVN of control animals was 72.9±9.0. This number increased bilaterally by 1 day postlesion (158.0±18.5 and 157.8±18.6 for the ipsilateral and contralateral sides, $P<0.0001$) and remained unchanged at the 1 week (130.1±14.4 and 128.8±15.0 for the ipsilateral and contralateral sides, $P<0.0001$) and 1 month (128.0±12.8 and 132.1±14.2 for the ipsilateral and contralateral sides, $P<0.0001$) recovery times. Three months after UVN, the mean number of CRF-Ir neurons in the PVN had returned to control values on both the intact (79.5±4.7) and lesioned (76.5±4.6) sides.

Fig. 3A shows photomicrographs of AVP-Ir sections taken from the PVN of four representative animals: one control and three UVN cats killed at the same postlesion times (1 day, 1 month and 3 months). Neurectomy induced a strong and bilateral increase in the number of AVP-Ir neurons in this structure. Fig. 3B illustrates the quantitative analysis for the four groups of UVN cats. Compared to controls, which exhibited a mean number of AVP-Ir neurons of 60.0±2.5, the 1 day postlesion group displayed a 100% increase with a mean number of 119.0±5.5 and 122.1±9.5 for the ipsilateral and contralateral PVN, respectively ($P<0.0001$). This number of AVP-Ir neurons remained unchanged at 1 week (115.7±14.0 and 110.8±12.1 for the ipsilateral and contralateral sides, $P<0.0001$) and 1 month (113.2±7.7 and 111.7±11.7, $P<0.0001$) postlesion times for the ipsilateral and contralateral sides. As shown with CRF-Ir neurons, the mean number of AVP-Ir neurons in the PVN returned to control values at 3 months postlesion on both the intact (65.2±3.3) and lesioned (64.9±2.1) sides. Double staining for CRF and AVP revealed a complete lack of colocalization for CRF-Ir and AVP-Ir in neurectomized cats from all groups (Fig. 1E), as shown in the control cats.

DβH immunoreactivity in the LC. Control cats. A moderate number of DβH-Ir neurons were found in the LC of the control cats (Fig. 4A), equally distributed on the left and right sides.

UVN cats. As with CRF-Ir and AVP-Ir neurons of the PVN, the number of DβH-Ir neurons in the LC showed substantial bilateral increases at 1 day, 1 week, and 1 month after UVN, and returned to control values at 3 months. Fig. 4A shows photomicrographs of DβH-Ir sections taken from the LC of one representative control cat, and of three representative lesioned cats at 1 day, 1 month, and 3 months after lesion. Quantification of the data is shown in the Fig. 4B. The average number of DβH-Ir neurons was 45.4±0.4 in the LC of control cats, 80.9±3.5 and 79.3±3.2 for the ipsilateral and contralateral sides of cats examined 1 day after UVN ($P<0.0001$), 76.1±2.3 and 78.1±2.7 at 1 week ($P<0.0001$) and 76.0±3.5 and 77.4±3.3 at 1 month ($P<0.0001$). Three months after UVN, the mean number of DβH-Ir neurons in the LC had returned to control values on both the intact (46.7±0.6) and lesioned (46.8±0.5) sides.

CRF and AVP immunoreactivity in the VN. Control cats. There was a total lack of AVP-Ir in the VN complexes. By contrast, a moderate CRF-Ir was observed in the four main VN (superior: SVN; medial: MVN; lateral: LVN and inferior: IVN) (Fig. 5). The CRF-Ir neurons were medium in size and round or oval in shape, and in the MVN

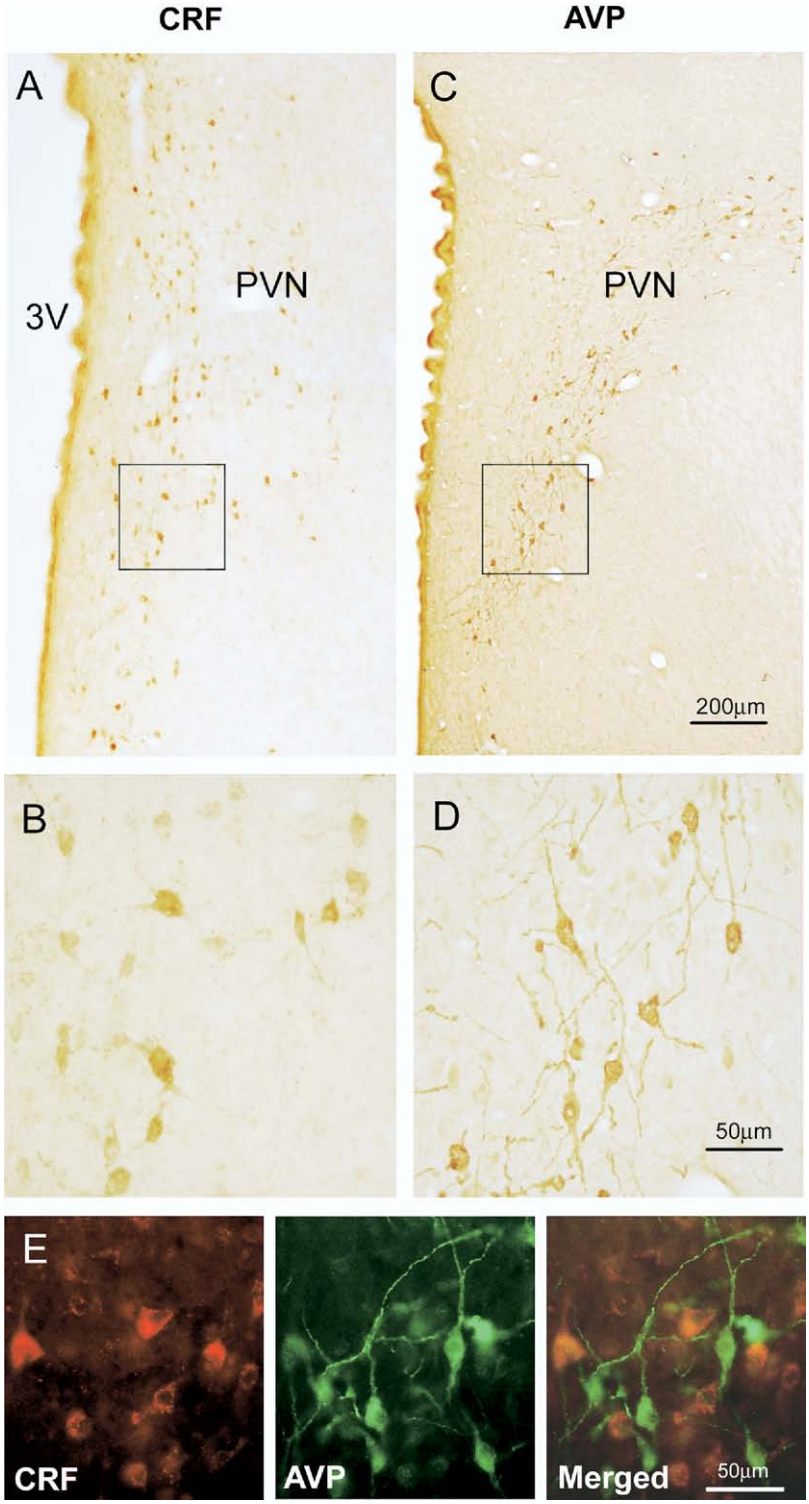

Fig. 1. Illustration of CRF and AVP Ir neurons in the PVN of control cats. Illustration of CRF (A, B) and AVP (C, D) Ir neurons in the PVN of unlesioned control cats. (E) Illustration of double immunostaining for CRF (red) and AVP (green) neurons showing an absence of colocalization of CRF and AVP immunoreactivity in the same section in PVN of control cats (3 V: third ventricle).

and IVN, most of the cells appeared to be grouped and located along the periphery of the fourth ventricle. Some CRF-immunonegative neurons in the LVN and the SVN were, however, surrounded with CRF terminals. Among the VN complexes, the IVN showed the highest number of CRF-Ir neurons (19.3±0.5) whereas the other three exhibited lower numbers: 16.2±1.4 in the SVN, 13.1±3.1 in the LVN and 12.7±1.7 in the MVN.

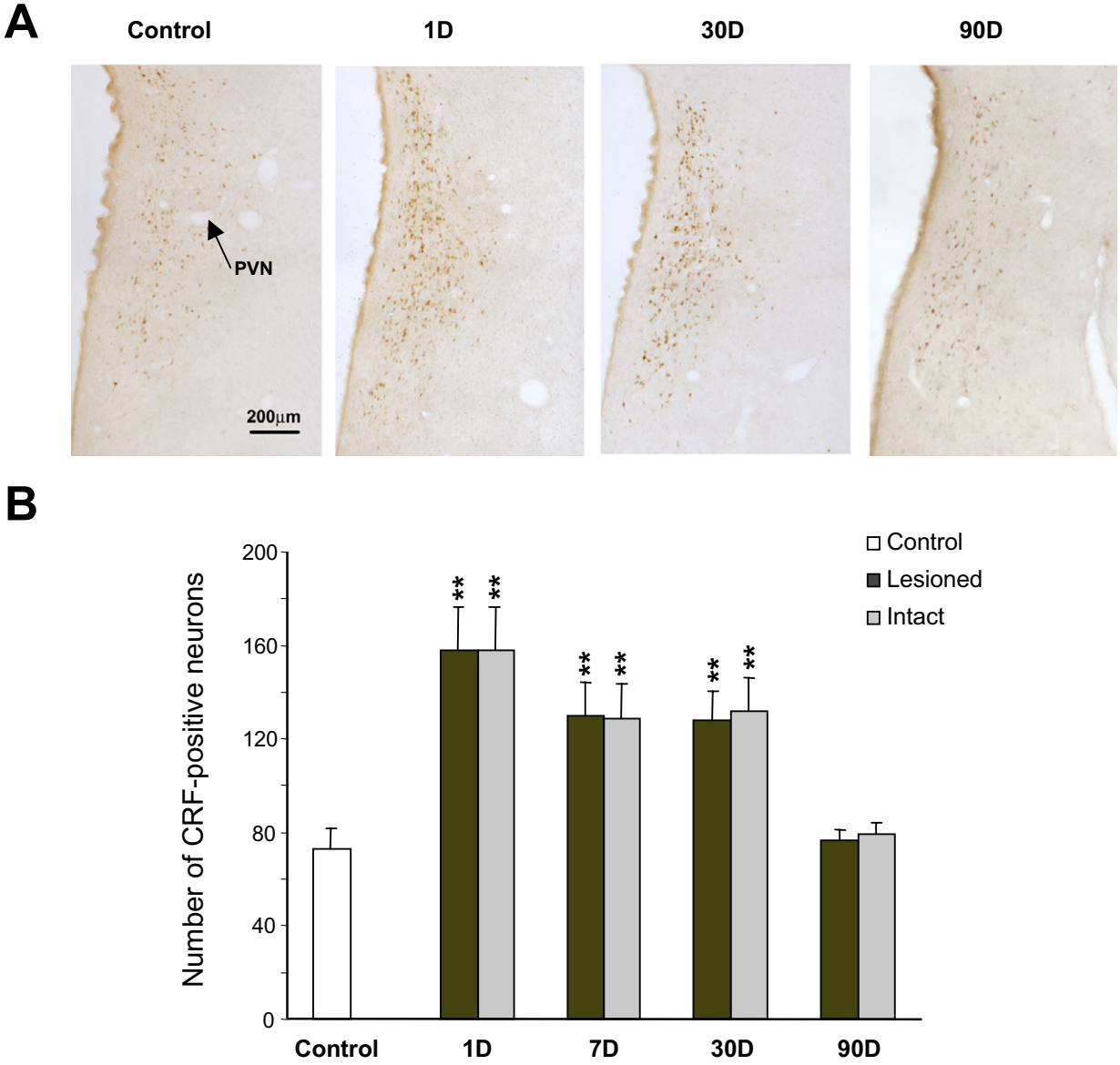

Fig. 2. (A) Illustration of the lesion-induced changes of CRF-Ir neurons in the PVN. Illustration of typical labeling in a representative control cat and in three experimental animals observed at 1 D, 30 D, and 90 D after UVN. Note that vestibular nerve section induced a strong increase in the number of CRF-Ir neurons. Three months after UVN, the number of CRF-Ir neurons had returned to control levels on both sides. (B) Quantitative evaluation of the effects of a UVN on CRF-Ir neurons in the PVN. Data are mean values (±SEM) of the number of CRF-Ir neurons in the PVN for control cats (n=4) and unilateral neurectomized cats examined at 1 D (n=4), 30 D (n=4) and 90 D (n=4) after lesion. Values recorded on the lesioned (black histograms) and intact (grey histograms) sides are given separately for all subgroups of UVN cats. Data from both sides of control cats were pooled (white histograms) to provide a direct comparison with the subgroups of UVN cats. ** P<0.0001 versus control and 90 D post-lesion (1 D: 1 day; 30 D: 30 days; 90 D: 90 days).

In the UVN cats. Contrary to the effects seen in PVN, the number of CRF-Ir neurons was greatly reduced bilaterally in all nuclei of the VN complex from 1 day to 1 month after UVN, and returned to control values at the same 3 month postlesion time. Fig. 6A illustrates these changes in the IVN and the MVN. Quantitative analysis of the data is shown in Fig. 6B for the IVN and MVN, displayed here as representative nuclei of the VN complex. The mean numbers of CRF-Ir neurons in these nuclei were 19.3±0.5 and 12.7±1.7, respectively, in controls. A significant bilateral decrease was observed 1 day postlesion in the IVN (9.5±0.4 and 8.2±0.7 for the ipsilateral and contralateral sides, P<0.0001) and the MVN (6.1±0.9 and 5.6±0.5 for ipsi- and contralateral, P<0.004). This decrease was accentuated significantly at 1 week postlesion in both the IVN (4.1±0.3 and 4.0±0.6 on the intact and lesioned sides, respectively: P<0.0001) and the MVN (3.0±0.3 and 2.6±0.2 for the ipsi- and contralateral sides, P<0.0001), and remained significant in cats examined 1 month after UVN in both sides of IVN (6.0±0.5 for ipsi- and 6.4±0.8 for contralateral, respectively, P<0.0001) and MVN (5.8±0.7 and 4.5±0.7, respectively, P<0.0001). Three months after

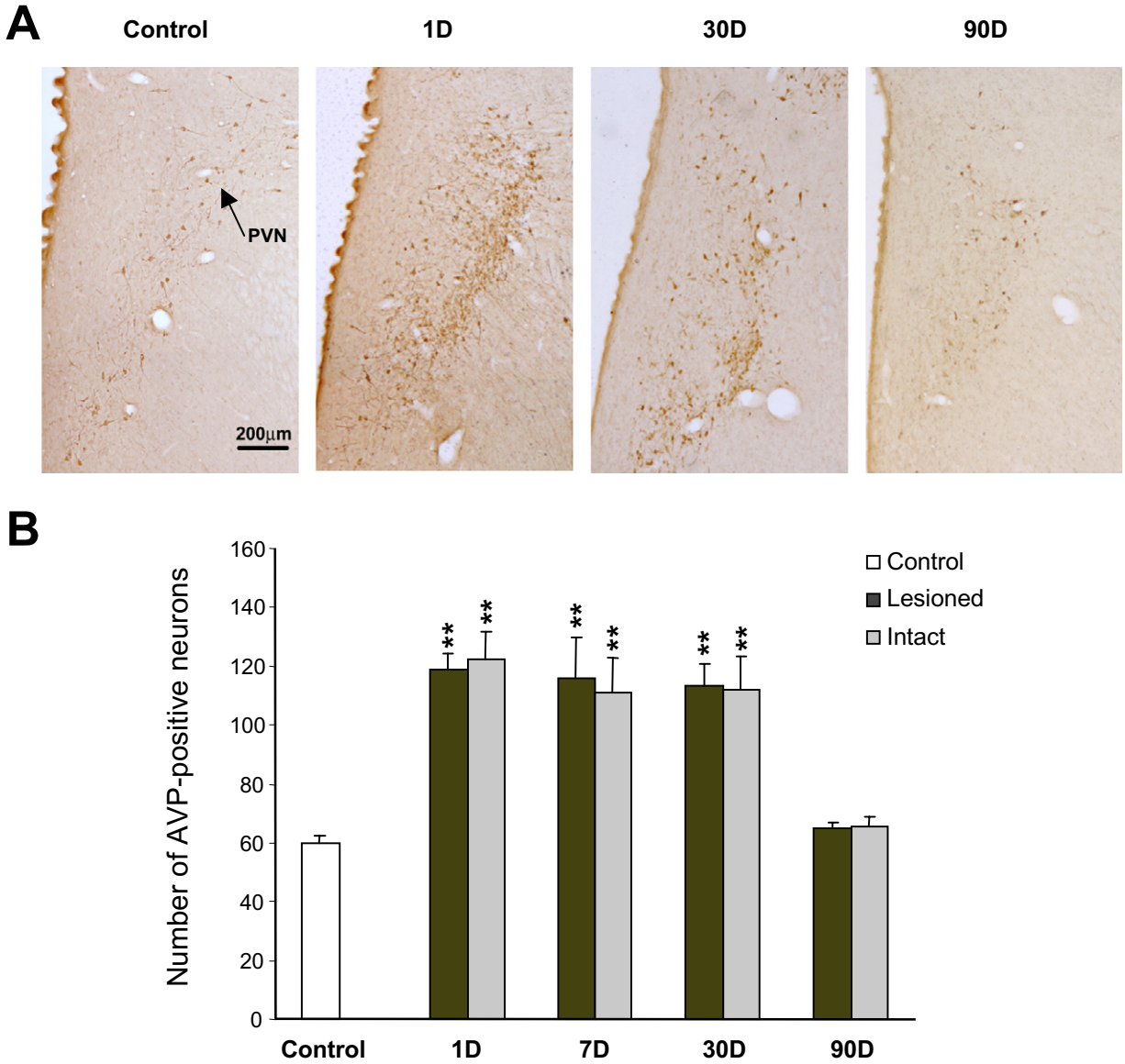

Fig. 3. (A) Illustration of lesion-induced changes in AVP-Ir neurons in the PVN. Illustration of the typical labeling in a representative control cat and in three experimental animals subjected to UVN and observed 1 D, 30 D, and 90 D after the lesion. Note that vestibular nerve section induced a strong increase in the number of AVP-Ir neurons. Three months after UVN, the number of AVP-Ir neurons had returned to control levels on both sides. (B) Quantitative evaluation of the effects of a UVN on AVP-Ir neurons in the PVN. Data are mean values (±SEM) of the number of AVP-Ir neurons in the PVN for control cats (n=4) and unilateral neurectomized cats examined at 1 D (n=4), 30 D (n=4) and 90 D (n=4) after the lesion. The values recorded on the lesioned (black histograms) and intact (grey histograms) sides are given separately for all subgroups of UVN cats. The data from both sides were pooled for the controls (white histograms) to provide a direct comparison with the subgroups of UVN cats. ** P<0.0001 versus control and 90 D post-lesion (1 D: 1 day; 30 D: 30 days; 90 D: 90 days).

UVN, near normal values were recorded in the IVN (17.2± 0.7 and 16.9±0.7 for the ipsi- and contralateral sides, *NS*) and the MVN (10.3±1.2 and 9.6±1.2, *NS*).

Behavioral correlates

Behavioral observations. During the acute period (1 week) after UVN, all cats exhibited the typical vestibular syndromes consisting of ocular motor, postural and posturo-locomotor deficits. The ocular motor symptoms consisted of a strong ocular nystagmus with its slow phase directed to the lesioned side; the main component of the nystagmus was in the horizontal plane, but vertical and torsional nystagmus were also present and associated with a skew deviation in the vertical plane. Postural signs consisted of head tilt toward the lesioned side, postural asymmetry with an increased support surface, and falls to the lesioned side. These symptoms were observed throughout the first postlesion week, but the cats had recovered sufficiently within 2 or 3 days to feed themselves. All the UVN cats showed a near complete behavioral recovery at 3 months postlesion. Spontaneous nystagmus observed in light completely disappeared by the

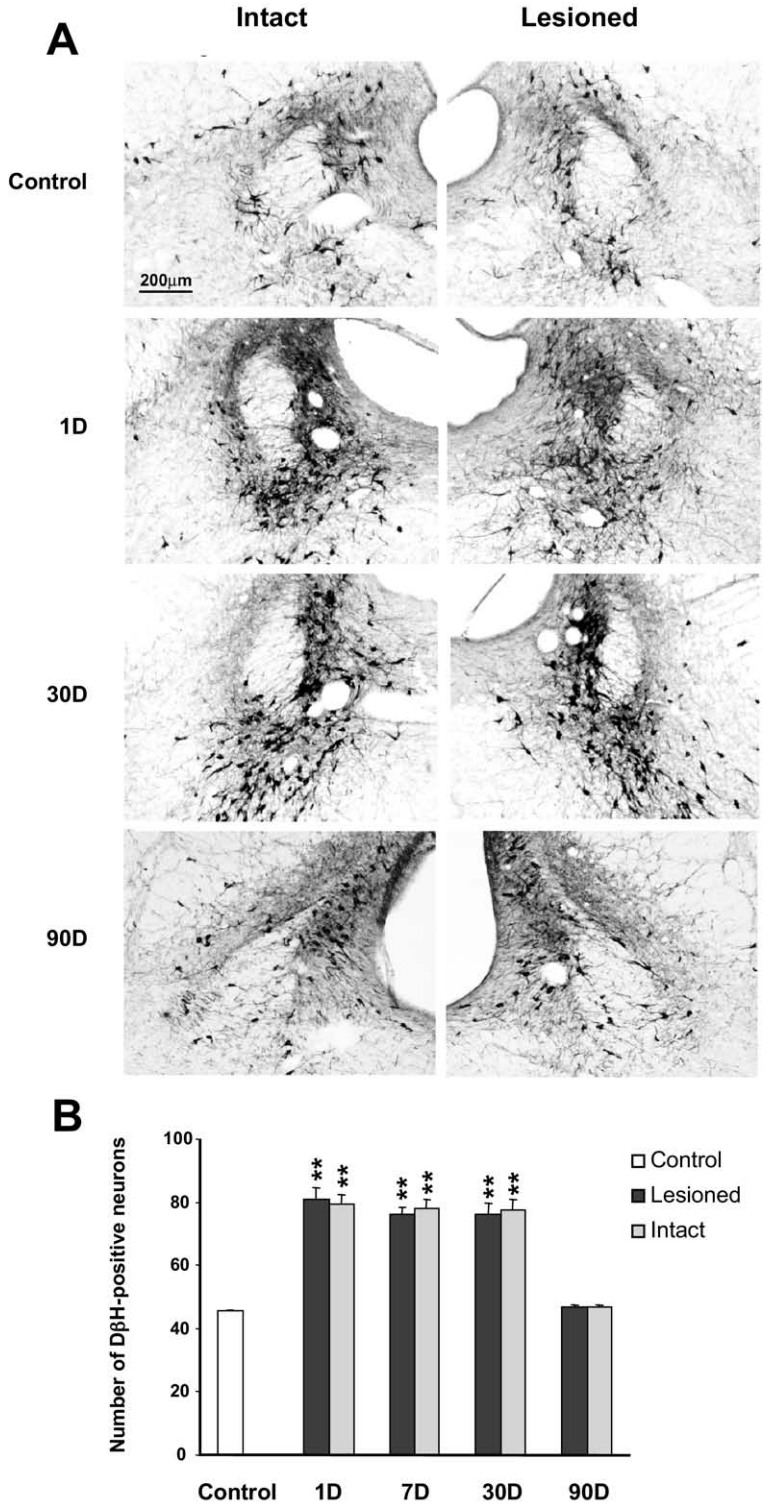

Fig. 4. (A) Illustration of lesion-induced changes of DβH-Ir neurons in the LC. Illustration of the number of DβH-Ir neurons in the LC on the intact (left panel) and lesioned (right panel) sides of a representative control cat. The number of DβH-Ir neurons increased on both the intact and the lesioned side in the LC of cats examined at 1, 30 and 90 D after UVN. This increased number of neurons was no longer observed at 3 months. (B) Quantitative evaluation of the effects of a UVN on DβH-Ir neurons in the LC (LC). Data are expressed as mean values (±SEM). The values recorded on the lesioned (black histograms) and intact (gray histograms) sides are given separately for all lesioned cats. The data from both sides of control cats were pooled (white histograms) to provide a direct comparison with the subgroups of cats sacrificed 1, 3, 30 and 90 D after a left UVN. ** $P<0.0001$ versus control and 90 D post-lesion (1 D: 1 day; 30 D: 30 days; 90 D: 90 days).

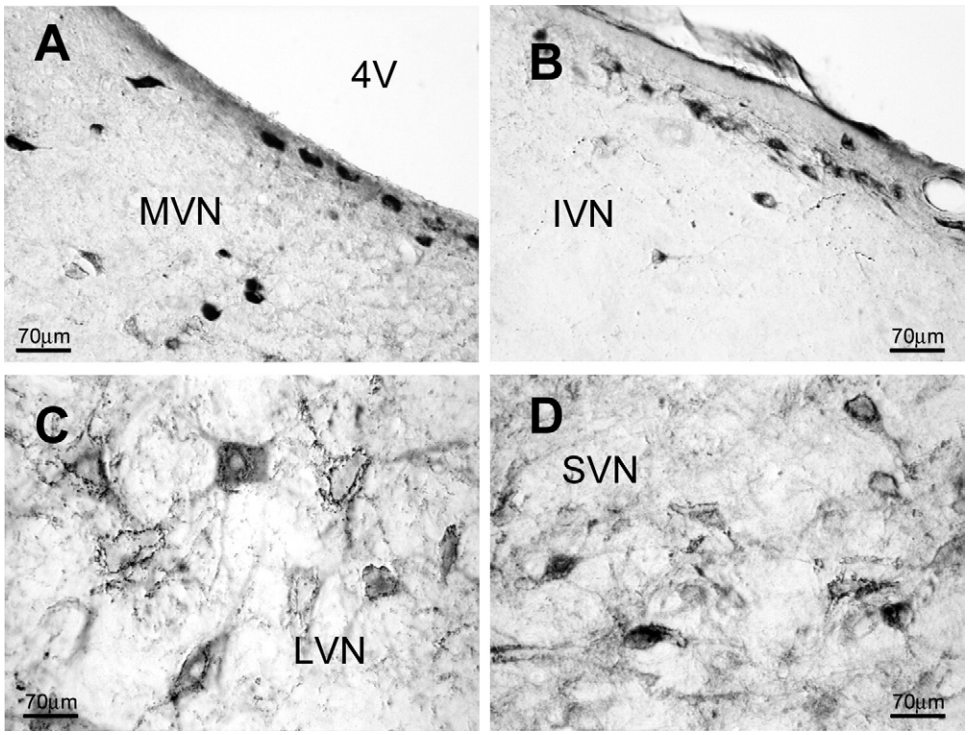

Fig. 5. Illustration of CRF Ir neurons in the VN of control cats. Illustration of CRF Ir neurons from unlesioned control cats in the medial vestibular nucleus (A), the IVN (B), the LVN (C) and the SVN (D). (4 V: Fourth ventricle; MVN: medial vestibular nucleus; IVN: inferior vestibular nucleus; LVN: lateral vestibular nucleus; SVN: superior vestibular nucleus.)

end of the first week postlesion, while posture and locomotor equilibrium did not fully recover until 6 weeks postlesion. Cats killed at 1 day or 1 week therefore fully experienced any stress induced by UVN syndrome, whereas those killed at the 1 and 3 month recovery times were likely free of stress, or at least relatively so, since they were almost completely compensated and no longer exhibiting symptoms. As expected, the sham-operated cats did not show any ocular motor or postural deficits.

Posture function recovery. Fig. 7A shows the time course of posture function recovery as revealed by changes in the support surface delimited by the four legs of the resting, standing cat. The mean normalized value was strongly increased acutely after UVN since it was five times that recorded preoperatively (5.0 ± 0.2; $P<0.0001$). At 7 days post-lesion, the support surface was still strongly increased compared to preoperative values (4.6 ± 0.2; $P<0.0001$), and it remained significantly larger at 1 month after UVN (2.2 ± 0.04; $P<0.0001$). A return of the support surface toward preoperative values was observed 3 months after UVN, corresponding to a full functional recovery of posture.

Locomotor balance recovery. Fig. 7B shows the recovery time course of the locomotor balance function. The UVN cats were unable to cross the beam up to 8 days after vestibular lesion; maximal performance observed at 1 and 7 days post-lesion was 0% ($P<0.0001$). At 30 days post-UVN, Max. P. Increased to 50% of the preoperative value but was still significantly lower ($P<0.0001$). Locomotor balance had fully recovered to 100% performance by 3 months post-lesion.

DISCUSSION

This study demonstrated that UVN induces a significant bilateral increase in the number of both AVP-Ir and CRF-Ir neurons in the PVN of the hypothalamus, and a similar increase of DβH-Ir neurons in the LC. Conversely, a significant bilateral decrease of CRF-Ir neurons was observed in the VN. These changes were detectable as early as 1 day after vestibular lesion, and persisted through both the acute stage (at 1 week) and the compensatory stage (at 1 month) of vestibular compensation. The number of AVP-Ir, CRF-Ir and DβH-Ir neurons in the PVN, the VN and the LC returned to control values found in the sham group by 3 months after UVN, when the animals were fully compensated from a behavioral point of view.

Considered together, these results provide strong evidence of neuroendocrine plasticity in response to the stress induced by vestibular deafferentation. They point to a persistent activation of the HPA axis, which very likely reflects a chronic stress to which the animals were subjected until they become totally free of their posturo-locomotor symptoms.

Activation of the cat HPA axis after UVN

The distribution of CRF-Ir and AVP-Ir neurons shown herein in the PVN of cat hypothalamus is generally consistent with earlier reports in the same species (Reaves

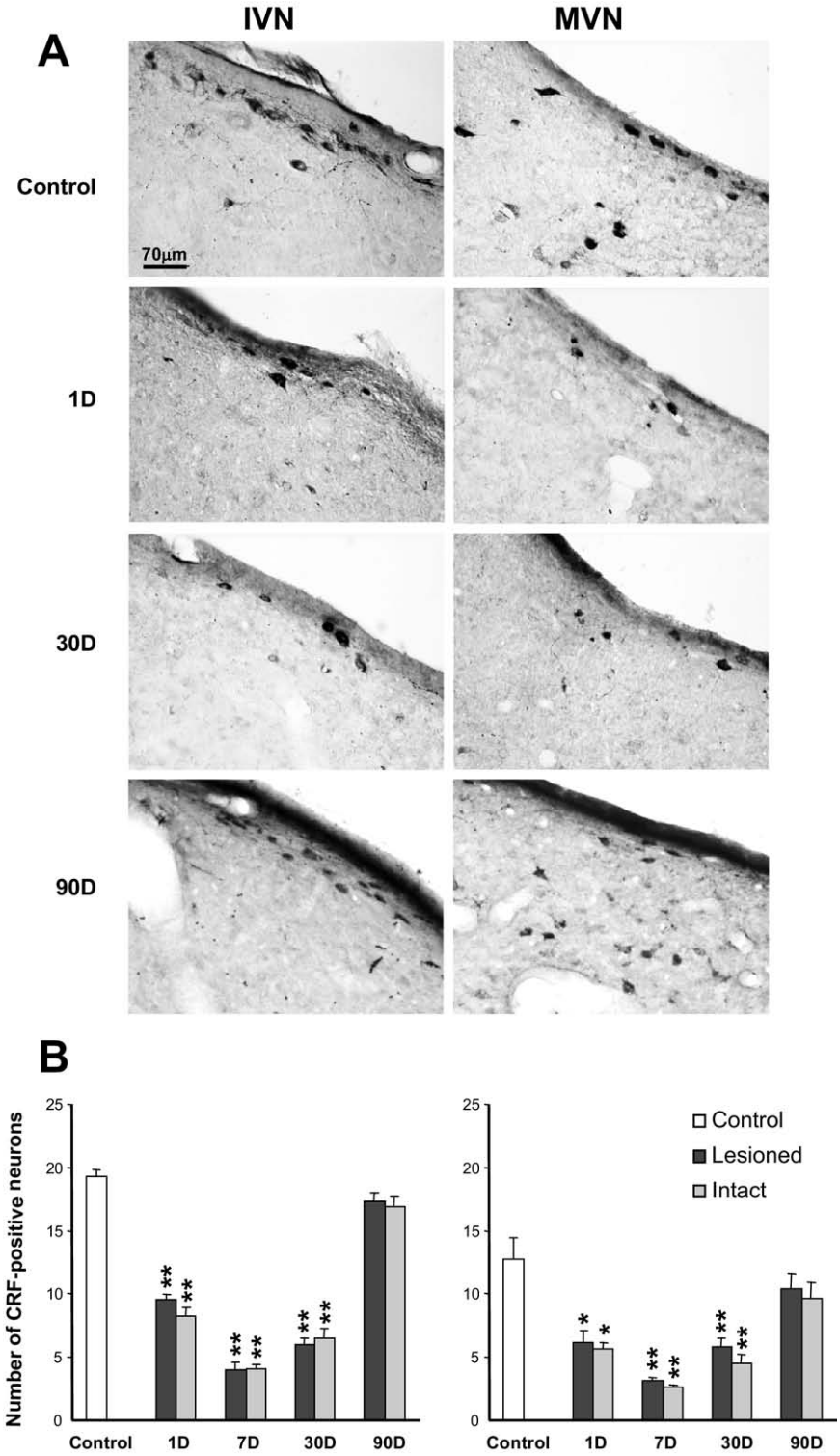

Fig. 6. (A) Illustration of the lesion-induced changes of CRF-Ir neurons in the IVN and the MVN. Illustration of the typical labeling in a representative control cat and in three experimental animals subjected to UVN and observed at 1 D, 30 D, and 90 D after lesion. Note that the vestibular nerve section induced a strong decrease in the number of CRF-Ir neurons. Three months after UVN, the number of CRF-Ir neurons returned to control levels on both sides. (B) Quantitative evaluation of the effects of a UVN on CRF-Ir neurons in the IVN and the MVN. Data are mean values (±SEM) of the number of CRF-Ir neurons in the IVN and the MVN for control cats (n=4) and unilateral neurectomized cats examined at 1 D (n=4), 30 D (n=4) and 90 D (n=4) after lesion. Values recorded on the lesioned (black histograms) and intact (grey histograms) sides are given separately for all subgroups of UVN cats. The data from both sides were pooled for the controls (white histograms) to provide a direct comparison with the subgroups of UVN cats. * $P<0.005$, ** $P<0.0001$ versus control and 90 D post-lesion (1 D: 1 day; 30 D: 30 days; 90 D: 90 days; IVN: inferior vestibular nucleus; MVN: medial vestibular nucleus).

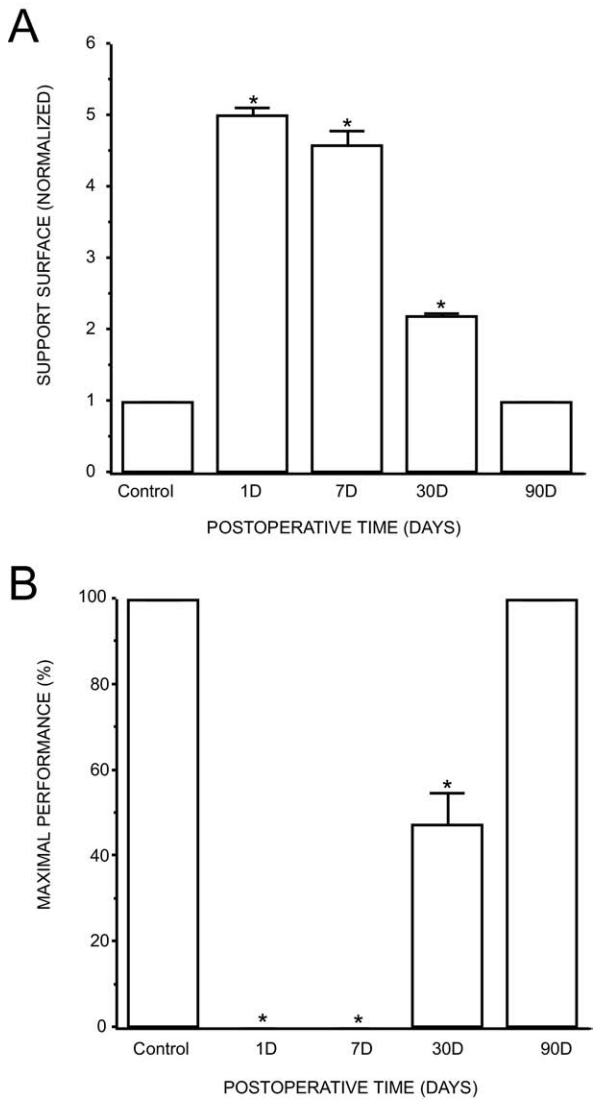

Fig. 7. Quantitative evaluation of the effects of a UVN on posture function and locomotor balance recovery. (A) Mean postoperative development of the support surface in the UVN group of cats. The support surface was evaluated in cm^2 and normalized with respect to the preoperative values (where unity is approximately 50 cm^2), and is reported here as a function of the postoperative time in days. Each histogram represents the mean value (n=5 measurements per cat) calculated in a UVN group (n=4). Standard error in the mean is shown as a vertical line. Note the strong increase in support surface in the days following UVN and the complete recovery by 90 D after UVN. (B) Mean recovery histograms illustrating maximal performance of cats on the rotating beam, expressed as a percentage of preoperative maximum performance (on the ordinates) as a function of the postoperative time in days (on the abscissa). Standard error in the mean is shown as a vertical line. Note the poor performance at 1 and 7 days after UVN and the partial recovery at 30 D post-UVN. A complete recovery is observed 90 D after UVN. * $P<0.0001$ versus control and 90 D post-lesion (1 D: 1 day; 30 D: 30 days; 90 D: 90 days).

and Hayward, 1979; Kawata et al., 1982; Carlson and Gann, 1987; Caverson et al., 1987; Li et al., 1997). Both CRF-Ir and AVP-Ir were strongly increased in the bilateral PVN of the hypothalamus just after UVN (1 day) and during the acute stage (1 week), suggesting that UVN syndrome could be responsible for the HPA axis activation. These data corroborate the increased Fos-like immunoreactivity seen acutely in the PVN after vestibular deafferentation in the rat (Cameron and Dutia, 1999) and the cat (Gustave Dit Duflo et al., 1999), and support the notion of functional connections between the vestibular system and PVN neurons. These connections are further suggested by studies of galvanic stimulation of the vestibular nerve and caloric stimulation of the labyrinth (Azzena et al., 1993; Liu et al., 1997).

The up-regulation of AVP, a known stress hormone, in the PVN may also explain the increased level of plasma AVP found in rats subjected to electrical/caloric inner ear stimulation (Horii et al., 2001, 2004), in patients with peripheral vestibular vertigo (Takeda et al., 1995) and in humans subjected to experimental motion sickness (Kim et al., 1997). On the other hand, up-regulation of CRF in the PVN supports those data showing an increase in night salivary cortisol concentration recorded after unilateral vestibular deafferentation in the guinea pig (Gliddon et al., 2003b), as well as an elevation of cortisol and ACTH in patients with peripheral vestibular dysfunction (Horner and Cazals, 2005).

The notion that the HPA axis is modulated by vestibular inputs has been recently reinforced by anatomical evidence of VN complex projections to the PVN in the rat (Markia et al., 2008). It has been suggested that the vestibular-paraventricular connection may serve as a feedback route in the central organization of responses to stressful vestibular stimuli. Vestibular stress inputs to the hypothalamic PVN also originate from noradrenergic neurons in the LC (Habib et al., 2001), and NA release from the LC is associated with glucocorticoid release when stress stimuli activate the HPA axis. The up-regulation of NA observed in the bilateral LC thereby further reinforces the argument that catecholaminergic plasticity after lesion is induced by UVN syndrome.

The role of brain histamine in stress response has been emphasized (Bergquist and Dutia, 2006) for review, and its importance is suggested by an increased histamine turnover rate reported in rodent hypothalamus after exposure to stressful situations (Ito, 2000). Brain histamine increases the release of ACTH and AVP (Kjaer et al., 1991; Van de Kar and Blair, 1999) via the activation of hypothalamic AVP and CRF neurons (Kjaer et al., 1994). Interestingly, we have previously reported a post-UVN up-regulation of histidine decarboxylase mRNA expression measured with *in situ* hybridization methods, suggesting that expression of this enzyme, which is essential to the synthesis of histamine, is increased bilaterally in the hypothalamus at 1 and 3 weeks postlesion before returning to control levels by 3 months after neurectomy (Tighilet et al., 2006, 2007b). Taken together, this strongly suggests a link between stressor signals (the UVN syndrome), brain histamine release and HPA axis activation, since CRF and AVP neurons seem to be under histaminergic control (Li and Hatton, 1996).

The present findings clearly demonstrate that UVN syndrome-induced activation of the HPA axis persists at 1

month of postlesion time. AVP and CRF in the PVN, as well as DβH in the LC, remain up-regulated at this postlesion time, a finding which suggests that animals continued to have stress factors and experience a chronic stress. Our behavioral observations seem to corroborate this assumption. While the spontaneous nystagmus in light had totally disappeared by 1 week postlesion, both static (support surface) and dynamic (locomotor equilibrium) posturo-locomotor functions remained only partially recovered by 1 month postlesion (Xerri and Lacour, 1980; Tighilet et al., 2006). Persistence of postural instability and falls very likely constitute stressful situations for the cat and serve to maintain chronic activation of the HPA axis. Indeed, when the animals are totally free of posturo-locomotor deficits at the 3 months postlesion time, they no longer exhibit HPA axis activation. One important remaining question concerns whether this chronic stress is deleterious or beneficial for vestibular compensation; in other words, what is the overarching role of stress hormones in this process?

Neuroendocrine plasticity and vestibular compensation

Behavioral investigations in animal models provide evidence that glucocorticoids contribute to the neurochemical mechanisms of vestibular compensation (Gliddon et al., 2003a) and interfere with the recovery of both static and dynamic functions. In the rabbit, glucocorticoid receptor agonists like dexamethasone accelerated ocular motor and postural compensation, whereas glucocorticoid receptor antagonists such as RU38486 slowed it down (Yamanaka et al., 1995). In the rat, RU38486 delayed circular walking compensation (Cameron and Dutia, 1999), and in the guinea pig glucocorticoids accelerated the compensation of post-rotatory nystagmus (Shimogori and Yamashita, 2000). The literature on the effects of steroids on vestibular compensation is however more complicated than suggested by these aforementioned studies. Jerram et al. (1995) reported a reduction of spontaneous nystagmus following unilateral labyrinthectomy (UL) in guinea pigs after methylprednisolone treatment. In the same animal model (UL guinea pig) submitted to similar pharmacological treatments (methylprednisolone, dexamethasone), Alice et al. (1998) found no effects on spontaneous nystagmus compensation.

Rebalancing of bilateral VN complex activity is considered a key process in behavioral recovery, at least for the static functions (Lacour, 2006). The restoration of horizontal head posture in the guinea pig after UL takes 1 week, and the restoration of normal spontaneous discharge of the VN cells on the lesioned side occurs over the same time period (Ris and Godaux, 1998). Correspondingly, the increased support surface observed in the UVN cats returns to pre-lesion baseline values after 6 weeks, a time period over which the resting discharge rates of the two VN complexes rebalance (Zennou-Azogui et al., 1993). Electrophysiological studies strongly suggest that glucocorticoids are involved in the restoration of a spontaneous electrical activity in the deafferented VN. The glucocorticoid receptor antagonist RU38486 blocked the increase in the intrinsic excitability of the deafferented MVN neurons when injected just before vestibular deafferentation (Cameron and Dutia, 1999). Moreover, *in vitro* recordings in brain slices from rats kept under anesthesia after vestibular deafferentation—animals which thereby did not experience the consequent lesion-induced stress—failed to show the same firing rate increase as did deafferented MVN neurons in non-anesthetized animals. In contrast, injection of dexamethasone in the anesthetized animals restored the resting activity of the deafferented MVN cells. However, Alice et al. (1998) showed that only a minority of MVN neurons responded to both dexamethasone and methylprednisolone. Steroids could be efficient in the treatment of acute vestibular disorders arising from inflammatory origin (Ariyasu et al., 1990), but not for improving vestibular compensation after UL (Alice et al., 1998).

The interaction of glucocorticoids with GABA is of particular interest here, since changes in GABA receptor function in the deafferented MVN neurons have been proposed as a possible mechanism underlying vestibular compensation (Cameron and Dutia, 1997; Yamanaka et al., 2000). Steroids modulate MVN neuronal activity through their actions on $GABA_A$ and AMPA/kainate receptors (Seemungal et al., 2001; Grassi et al., 2007). Glucocorticoid neuroendocrine plasticity after UVN in the cat could thereby modulate the resting discharge rates in the deafferented VN, and contribute to restoration of a balance of bilateral activity. This view is supported by the presence of CRF-Ir terminals in the entire VN complex, and by the identification of glucocorticoids receptors throughout the VN complexes (Lindsay et al., 2005; Zhang et al., 2005). Given that complete rebalancing of VN cell activity on both sides requires at least 6 weeks after UVN in the cat (Zennou-Azogui et al., 1993), it could be hypothesized that persistent activation of the HPA axis seen at the 1 month recovery point reflects a long-lasting neuroendocrine plasticity mechanism necessary for a full functional recovery.

In clinical practice, glucocorticoids can be used to treat vertigo in patients with vestibular dysfunction (Adour et al., 1981), in Menière's disease (Sennaroglu et al., 1999) and in vestibular neuronitis (Ohbayashi et al., 1993). Structural analogues of histamine are also effective in the pharmacological treatment of Menière's disease (Lacour and Sterkers, 2001, Bergquist and Dutia, 2006). The efficacy of these substances might therefore be explained, at least in part, by their implication in the neuroendocrine response to vestibular stress.

CRF plasticity in the VN

Our data in the normal cat confirm previous immunohistochemical investigations into the distribution of CRF-containing neurons in the VN complexes in the rat and cat. CRF-Ir neuronal cell bodies have been reported in these species in the IVN and MVN (Merchenthaler, 1984; Sakanaka et al., 1987; Cummings, 1989; Ikeda et al., 1992). We have now extended these data to the SVN and LVN in the cat.

A significant and sustained bilateral decrease of CRF-Ir neurons was observed in the MVN and IVN after

UVN. This down-regulation in the intraneuronal level of CRF, observed during both the acute (1 day, 1 week) and compensatory stages (1 month), may reflect a negative glucocorticoid feedback loop in these structures. Increased steroid binding to glucocorticoid receptors on VN neurons' membranes might induce such a CRF down-regulation by means of neuronal pathways (CRF-Ir terminals ending in the VN) or neurohormones reaching the VN via the cerebrospinal fluid of the fourth ventricle (Saxon and Beitz, 2000).

Once activation of the HPA axis returns to normal levels—in this case, by 3 months in the behaviorally compensated cats—this negative feedback loop would cause intraneuronal CRF level to return toward normal values. It should be noted that an excess of glucocorticoids has been shown to produce neuronal damage after stress or neuronal injury, and their sustained high levels in a nervous structure can lead to neuronal death (Antonawich et al., 1999). In contrast, beneficial effects of the moderate expression of glucocorticoids, including neuroprotective actions, have been reported (Jeanneteau et al., 2008). We can therefore speculate that this down-regulation of CRF expression in the VN may serve to prevent apoptosis and promote neurogenesis, one important mechanism of plasticity we have recently described in this same structure (Tighilet et al., 2007a).

Acknowledgments—This study was supported by grants from the Ministère de l'enseignement supérieur et de la recherche and CNRS (UMR Université de Provence, CNRS No. 6149). The authors thank Valerie Gilbert and Catherine Marra for taking care of the animals.

REFERENCES

Adour KK, Sprague MA, Hilsinger RL Jr (1981) Vestibular vertigo. A form of polyneuritis? JAMA 246:1564–1567.

Alice C, Paul AE, Sansom AJ, Maclennan K, Darlington CL, Smith PF (1998) The effects of steroids on vestibular compensation and vestibular nucleus neuronal activity in the guinea pig. J Vestib Res 8:201–207.

Antonawich FJ, Miller G, Rigsby DC, Davis JN (1999) Regulation of ischemic cell death by glucocorticoids and adrenocorticotropic hormone. Neuroscience 88:319–325.

Ariyasu L, Byl FM, Sprague MS, Adour KK (1990) The beneficial effect of methylprednisolone in acute vestibular vertigo. Arch Otolaryngol Head Neck Surg 116:700–703.

Azzena GB, Melis F, Caria MA, Teatini GP, Bozzo G (1993) Vestibular projections to hypothalamic supraoptic and paraventricular nuclei. Arch Ital Biol 131:127–136.

Bergquist F, Dutia MB (2006) Central histaminergic modulation of vestibular function—a review. Sheng Li Xue Bao 58:293–304.

Berman AL (1968) A cytoarchitechtonic atlas with stereotaxic coordinates. The brain stem of the cat. Madison: Wisconsin University Press.

Berman AL, Jones EG (1982) A cytoarchitectonic atlas with stereotaxic coordinates. The thalamus and basal telencephalon of the cat. Madison: Wisconsin University Press.

Borel L, Lopez C, Peruch P, Lacour M (2008) Vestibular syndrome: a change in internal spatial representation. Clin Neurophysiol 38: 375–389.

Cameron SA, Dutia MB (1997) Cellular basis of vestibular compensation: changes in intrinsic excitability of MVN neurones. Neuroreport 8:2595–2599.

Cameron SA, Dutia MB (1999) Lesion-induced plasticity in rat vestibular nucleus neurones dependent on glucocorticoid receptor activation. J Physiol 518 (Pt 1):151–158.

Carlson DE, Gann DS (1987) Factors from paraventricular nucleus mediating adrenocorticotropin release in cats. Am J Physiol 252:R109–R121.

Caverson MM, Ciriello J, Calaresu FR, Krukoff TL (1987) Distribution and morphology of vasopressin-, neurophysin II-, and oxytocin-immunoreactive cell bodies in the forebrain of the cat. J Comp Neurol 259:211–236.

Cirelli C, Pompeiano M, D'Ascanio P, Arrighi P, Pompeiano O (1996) C-fos expression in the rat brain after unilateral labyrinthectomy and its relation to the uncompensated and compensated stages. Neuroscience 70:515–546.

Cummings SL (1989) Distribution of corticotropin-releasing factor in the cerebellum and precerebellar nuclei of the cat. J Comp Neurol 289:657–675.

Curthoys IS (2000) Vestibular compensation and substitution. Curr Opin Neurol 13:27–30.

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

Darlington CL, Lawlor P, Smith PF, Dragunow M (1996) Temporal relationship between the expression of fos, jun and krox-24 in the guinea pig vestibular nuclei during the development of vestibular compensation for unilateral vestibular deafferentation. Brain Res 735:173–176.

Darlington CL, Smith PF (2000) Molecular mechanisms of recovery from vestibular damage in mammals: recent advances. Prog Neurobiol 62:313–325.

De Kloet ER, Vreugdenhil E, Oitzl MS, Joels M (1998) Brain corticosteroid receptor balance in health and disease. Endocr Rev 19:269–301.

Dieringer N (1995) "Vestibular compensation": neural plasticity and its relations to functional recovery after labyrinthine lesions in frogs and other vertebrates. Prog Neurobiol 46:97–129.

Flohr H, Luneburg U (1982) Effects of ACTH4-10 on vestibular compensation. Brain Res 248:169–173.

Garduno-Anaya MA, Couthino De Toledo H, Hinojosa-Gonzalez R, Pane-Pianese C, Rios-Castaneda LC (2005) Dexamethasone inner ear perfusion by intratympanic injection in unilateral Meniere's disease: a two-year prospective, placebo-controlled, double-blind, randomized trial. Otolaryngol Head Neck Surg 133:285–294.

Gilchrist DP, Smith PF, Darlington CL (1990) ACTH(4–10) accelerates ocular motor recovery in the guinea pig following vestibular deafferentation. Neurosci Lett 118:14–16.

Gliddon CM, Darlington CL, Smith PF (2003a) Activation of the hypothalamic-pituitary-adrenal axis following vestibular deafferentation in pigmented guinea pig. Brain Res 964:306–310.

Gliddon CM, Smith PF, Darlington CL (2003b) Interaction between the hypothalamic-pituitary-adrenal axis and behavioural compensation following unilateral vestibular deafferentation. Acta Otolaryngol 123:1013–1021.

Grassi S, Frondaroli A, Dieni C, Dutia MB, Pettorossi VE (2007) Neurosteroid modulation of neuronal excitability and synaptic transmission in the rat medial vestibular nuclei. Eur J Neurosci 26:23–32.

Gustave Dit Duflo S, Gestreau C, Lacour M (2000) Fos expression in the rat brain after exposure to gravito-inertial force changes. Brain Res 861:333–344.

Gustave Dit Duflo S, Gestreau C, Tighilet B, Lacour M (1999) Fos expression in the cat brainstem after unilateral vestibular neurectomy. Brain Res 824:1–17.

Habib KE, Gold PW, Chrousos GP (2001) Neuroendocrinology of stress. Endocrinol Metab Clin North Am 30:695–728, vii–viii.

Herman JP, Cullinan WE (1997) Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci 20:78–84.

Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, Cullinan WE (2003) Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Front Neuroendocrinol 24:151–180.

Horii A, Kitahara T, Uno A, Kondoh K, Morihana T, Okumura S, Nakagawa A, Mitani K, Masumura C, Kubo T (2004) Vestibular function and vasopressin. Acta Otolaryngol Suppl 553:50–53.

Horii A, Koike K, Uno A, Uno Y, Kubo T (2001) Vestibular modulation of plasma vasopressin levels in rats. Brain Res 914:179–184.

Horner KC, Cazals Y (2005) Stress hormones in Meniere's disease and acoustic neuroma. Brain Res Bull 66:1–8.

Ikeda M, Houtani T, Ueyama T, Sugimoto T (1992) Distribution and cerebellar projections of cholinergic and corticotropin-releasing factor-containing neurons in the caudal vestibular nuclear complex and adjacent brainstem structures. Neuroscience 49:635–651.

Ito C (2000) The role of brain histamine in acute and chronic stresses. Biomed Pharmacother 54:263–267.

Jeanneteau F, Garabedian MJ, Chao MV (2008) Activation of Trk neurotrophin receptors by glucocorticoids provides a neuroprotective effect. Proc Natl Acad Sci U S A 105:4862–4867.

Jerram AH, Darlington CL, Smith PF (1995) Methylprednisolone reduces spontaneous nystagmus following unilateral labyrinthectomy in guinea pig. Eur J Pharmacol 275:291–293.

Kaufman GD, Anderson JH, Beitz A (1991) Activation of a specific vestibulo-olivary pathway by centripetal acceleration in rat. Brain Res 562:311–317.

Kaufman GD, Anderson JH, Beitz AJ (1993) Otolith-brain stem connectivity: evidence for differential neural activation by vestibular hair cells based on quantification of FOS expression in unilateral labyrinthectomized rats. J Neurophysiol 70:117–127.

Kawata M, Hashimoto K, Takahara J, Sano Y (1982) Immunohistochemical demonstration of the localization of corticotropin releasing factor-containing neurons in the hypothalamus of mammals including primates. Anat Embryol (Berl) 165:303–313.

Keller-Wood ME, Dallman MF (1984) Corticosteroid inhibition of ACTH secretion. Endocr Rev 5:1–24.

Kim MS, Chey WD, Owyang C, Hasler WL (1997) Role of plasma vasopressin as a mediator of nausea and gastric slow wave dysrhythmias in motion sickness. Am J Physiol 272:G853–G862.

Kitahara T, Takeda N, Kiyama H, Kubo T (1998) Molecular mechanisms of vestibular compensation in the central vestibular system—review. Acta Otolaryngol Suppl 539:19–27.

Kjaer A, Knigge U, Olsen L, Vilhardt H, Warberg J (1991) Mediation of the stress-induced prolactin release by hypothalamic histaminergic neurons and the possible involvement of vasopressin in this response. Endocrinology 128:103–110.

Kjaer A, Larsen PJ, Knigge U, Moller M, Warberg J (1994) Histamine stimulates c-fos expression in hypothalamic vasopressin-, oxytocin-, and corticotropin-releasing hormone-containing neurons. Endocrinology 134:482–491.

Kumagami H, Loewenheim H, Beitz E, Wild K, Schwartz H, Yamashita K, Schultz J, Paysan J, Zenner HP, Ruppersberg JP (1998) The effect of anti-diuretic hormone on the endolymphatic sac of the inner ear. Pflugers Arch 436:970–975.

Lacour M (2006) Restoration of vestibular function: basic aspects and practical advances for rehabilitation. Curr Med Res Opin 22: 1651–1659.

Lacour M, Roll JP, Appaix M (1976) Modifications and development of spinal reflexes in the alert baboon (Papio papio) following an unilateral vestibular neurotomy. Brain Res 113:255–269.

Lacour M, Sterkers O (2001) Histamine and betahistine in the treatment of vertigo: elucidation of mechanisms of action. CNS Drugs 15:853–870.

Lacour M, Sun J, Harlay F (1997) Kinematic analysis of locomotion in unilateral vestibular neurectomized cats. J Vestib Res 7:101–118.

Lacour M, Toupet M, Denise P, Christen Y, eds (1989) Vestibular compensation: facts, theories and clinical perspectives. Paris: Elsevier.

Li J, Hand GA, Potts JT, Mitchell JH (1997) Identification of hypothalamic vasopressin and oxytocin neurons activated during the exercise pressor reflex in cats. Brain Res 752:45–51.

Li Z, Hatton GI (1996) Histamine-induced prolonged depolarization in rat supraoptic neurons: G-protein-mediated, Ca(2+)-independent suppression of K^+ leakage conductance. Neuroscience 70:145–158.

Lindsay L, Liu P, Gliddon C, Zheng Y, Smith PF, Darlington CL (2005) Cytosolic glucocorticoid receptor expression in the rat vestibular nucleus and hippocampus following unilateral vestibular deafferentation. Exp Brain Res 162:309–314.

Liu F, Inokuchi A, Komiyama S (1997) Neuronal responses to vestibular stimulation in the guinea pig hypothalamic paraventricular nucleus. Eur Arch Otorhinolaryngol 254:95–100.

Markia B, Kovacs ZI, Palkovits M (2008) Projections from the vestibular nuclei to the hypothalamic paraventricular nucleus: morphological evidence for the existence of a vestibular stress pathway in the rat brain. Brain Struct Funct 213:239–245.

Marshburn TH, Kaufman GD, Purcell IM, Perachio AA (1997) Saccule contribution to immediate early gene induction in the gerbil brainstem with posterior canal galvanic or hypergravity stimulation. Brain Res 761:51–58.

Merchenthaler I (1984) Corticotropin releasing factor (CRF)-like immunoreactivity in the rat central nervous system. Extrahypothalamic distribution. Peptides 5 (Suppl 1):53–69.

Ohbayashi S, Oda M, Yamamoto M, Urano M, Harada K, Horikoshi H, Orihara H, Kitsuda C (1993) Recovery of the vestibular function after vestibular neuronitis. Acta Otolaryngol Suppl 503:31–34.

Reaves TA Jr, Hayward JN (1979) Immunocytochemical identification of vasopressinergic and oxytocinergic neurons in the hypothalamus of the cat. Cell Tissue Res 196:117–122.

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

Saika T, Kiyama H, Senba E, Tohyama M (1991) Expression of c-fos-like protein following unilateral labyrinthectomy. Neurosci Res 16 (Suppl):S36.

Sakanaka M, Shibasaki T, Lederis K (1987) Corticotropin releasing factor-like immunoreactivity in the rat brain as revealed by a modified cobalt-glucose oxidase-diaminobenzidine method. J Comp Neurol 260:256–298.

Saxon DW, Beitz AJ (2000) Neuropeptides associated with the vestibular nuclei. In: Neurochemistry of the vestibular system (Beitz AJ, Anderson JH, eds), pp 183–196. Boca Raton: CRC Press.

Seemungal BM, Gresty MA, Bronstein AM (2001) The endocrine system, vertigo and balance. Curr Opin Neurol 14:27–34.

Sennaroglu L, Dini FM, Sennaroglu G, Gursel B, Ozkan S (1999) Transtympanic dexamethasone application in Meniere's disease: an alternative treatment for intractable vertigo. J Laryngol Otol 113:217–221.

Shimogori H, Yamashita H (2000) Efficacy of intracochlear administration of betamethasone on peripheral vestibular disorder in the guinea pig. Neurosci Lett 294:21–24.

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

Takeda T, Kakigi A, Saito H (1995) Antidiuretic hormone (ADH) and endolymphatic hydrops. Acta Otolaryngol Suppl 519:219–222.

Takeda T, Takeda S, Kitano H, Okada T, Kakigi A (2000) Endolymphatic hydrops induced by chronic administration of vasopressin. Hear Res 140:1–6.

Tighilet B, Brezun JM, Sylvie GD, Gaubert C, Lacour M (2007a) New neurons in the vestibular nuclei complex after unilateral vestibular neurectomy in the adult cat. Eur J Neurosci 25:47–58.

Tighilet B, Mourre C, Trottier S, Lacour M (2007b) Histaminergic ligands improve vestibular compensation in the cat: behavioural, neurochemical and molecular evidence. Eur J Pharmacol 568: 149–163.

Tighilet B, Trottier S, Mourre C, Lacour M (2006) Changes in the histaminergic system during vestibular compensation in the cat. J Physiol 573:723–739.

Van de Kar LD, Blair ML (1999) Forebrain pathways mediating stress-induced hormone secretion. Front Neuroendocrinol 20:1–48.

Xerri C, Lacour M (1980) [Compensation deficits in posture and kinetics following unilateral vestibular neurectomy in cats. The role of sensorimotor activity]. Acta Otolaryngol 90:414–424.

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

Yamanaka T, Sasa M, Amano T, Miyahara H, Matsunaga T (1995) Role of glucocorticoid in vestibular compensation in relation to activation of vestibular nucleus neurons. Acta Otolaryngol Suppl 519:168–172.

Zennou-Azogui Y, Borel L, Lacour M, Ez-Zaher L, Ouaknine M (1993) Recovery of head postural control following unilateral vestibular neurectomy in the cat. Neck muscle activity and neuronal correlates in Deiters' nuclei. Acta Otolaryngol Suppl 509:1–19.

Zhang R, Smith PF, Darlington CL (2005) Immunocytochemical and stereological study of glucocorticoid receptors in rat medial vestibular nucleus neurons and the effects of unilateral vestibular deafferentation. Acta Otolaryngol 125:1258–1264.

Zinder O, Dar DE (1999) Neuroactive steroids: their mechanism of action and their function in the stress response. Acta Physiol Scand 167:181–188.

(Accepted 24 February 2009)
(Available online 11 March 2009)