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Effects of Chronic Infusion of a $GABA_A$ Receptor Agonist or Antagonist into the Vestibular Nuclear Complex on Vestibular Compensation in the Guinea Pig

Catherine M. Gliddon, Cynthia L. Darlington, and Paul F. Smith

Vestibular Research Group, Department of Pharmacology and Toxicology, School of Medical Sciences, University of Otago Medical School, Dunedin, New Zealand

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ABSTRACT

The aim of this study was to determine the effects of chronic infusion of a $GABA_A$ receptor agonist/antagonist into the ipsilateral or contralateral vestibular nuclear complex (VNC) on vestibular compensation, the process of behavioral recovery that occurs after unilateral vestibular deafferentation (UVD). This was achieved by a mini-osmotic pump that infused, over 30 h, muscimol or gabazine into the ipsilateral or contralateral VNC. Spontaneous nystagmus (SN), yaw head tilt (YHT), and roll head tilt (RHT) were measured. Infusion of muscimol or gabazine into either the ipsilateral or the contralateral VNC had little effect on SN compensation. In contrast, infusion of muscimol (250, 500, and 750 ng) into the contralateral VNC and gabazine (31.25, 62.5, and 125 ng) into the ipsilateral VNC significantly affected YHT and RHT ($p < 0.05$), but not their rate of compensation ($p > 0.05$). Interestingly, the effects of muscimol and gabazine on YHT and RHT were consistent throughout the first 30 h post-UVD. Infusion of muscimol (62.5, 125, and 250 ng) into the ipsilateral VNC and gabazine (125, 375, and 750 ng) into the contralateral VNC had little effect on YHT and RHT or their rate of compensation. These results suggest that the ipsilateral gabazine and contralateral muscimol infusions are modifying the expression of the symptoms without altering the mechanism of compensation. Furthermore, the neurochemical mechanism responsible for vestibular compensation can cope with the both the $GABA_A$ receptor-mediated and the UVD-induced decrease in resting activity.

γ-Aminobutyric acid (GABA) mediates inhibitory synaptic transmission via its interaction with $GABA_A$, $GABA_B$, and $GABA_C$ receptors (for review, see Chebib and Johnston, 2000). $GABA_A$ and $GABA_B$ receptors are involved in the vestibular reflex pathways, and it has been suggested that these receptors are involved in the behavioral recovery process that occurs after the loss of sensory input from one vestibular labyrinth (i.e., vestibular compensation) (for review, see Gliddon et al., 2005a). This loss of sensory input (unilateral vestibular deafferentation; UVD) results in a severe ocular motor and postural syndrome that compensates over time (for review, see Smith and Curthoys, 1989). It is generally accepted that the UVD-induced neuronal imbalance in the resting activity between the two vestibular nuclear complexes (VNCs) generates the behavioral syndrome and that the restoration of the resting activity in the ipsilateral VNC plays a causal role in the compensation of the static symptoms (Ris et al., 1997). Many premotor neurons in the VNC (e.g., type I neurons) are believed to receive GABAergic input from interneurons (type II neurons) that in turn receive excitatory inputs from the contralateral VNC (Gliddon et al., 2005a). Any modification in GABAergic transmission within the ipsilateral VNC could bring about the restoration of resting activity. Modification of the GABAergic transmission within the ipsilateral VNC could involve changes in either the $GABA_A$ or $GABA_B$ receptors. This article focuses on the $GABA_A$ receptor.

Clinical evidence suggests that $GABA_A$ receptor ligands can reduce spontaneous nystagmus (SN) frequency and the subjective feelings of vertigo in patients (Ehrenberger et al., 1982; Ehrenberger and Felix, 1996). Furthermore, animal studies suggest that diazepam can reduce SN frequency (McCabe et al., 1973; Bernstein et al., 1974; but see Peppard, 1986; Martin et al., 1996 for conflicting data). Magnusson et al. (2000, 2002) have reported complex effects of systemic

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ABBREVIATIONS: UVD, unilateral vestibular deafferentation; VNC, vestibular nuclear complex; SN, spontaneous nystagmus; THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol hydrochloride; YHT, yaw head tilt; RHT, roll head tilt; SR 95531, 2-(3′carboxy-2′-propyl)-3-amino-6-*p*-methoxyphenylpyridazinium bromide; ANOVA, analysis of variance.

injections of the partial $GABA_A$ receptor agonist 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol hydrochloride (THIP) and i.c.v.-injected muscimol, on SN compensation. THIP reduced the mean SN frequency at days 2 and 3, but not at 1, 4, or greater than 4 days post-UVD (Magnusson et al., 2002). Muscimol did not induce SN in compensated animals (Magnusson et al., 2002).

There has been no systematic investigation of the effects of $GABA_A$ receptor agonists or antagonists on postural compensation during the early stages of vestibular compensation. However, during 6 to 144 days post-UVD, it has been demonstrated that muscimol injections could not alter the chronic residual head deviation toward the ipsilateral side nor the circling induced by lifting the animals by the tail (Magnusson et al., 2000).

In vitro evidence suggests that early on in compensation, there is a down-regulation and an up-regulation of the muscimol and [^{3}H]flunitrazepam binding sites in the ipsilateral and contralateral VNCs, respectively (Calza et al., 1989, 1992; Vibert et al., 2000; Yamanaka et al., 2000). Late compensation is associated with no change in the muscimol or [^{3}H]flunitrazepam binding sites (Calza et al., 1989, 1992; Johnston et al., 2001; Giardino et al., 2002). Results from polymerase chain reaction studies suggest that there is a significant increase in the $GABA_A$ receptor α_1 subunit mRNA expression in the ipsilateral VNC compared with the contralateral VNC, at 6 h, but not at 50 h post-UVD (Horii et al., 2003). Microarray techniques have demonstrated a change in the rho 2 subunit of the $GABA_A$ receptor at 6 h post-UVD (Horii et al., 2004). In contrast, Eleore et al. (2005) reported no significant difference in α_1, α_5, β_1, β_2, β_3, and γ_2 mRNA expression between the ipsilateral and contralateral medial vestibular nuclei at 5 h, and 1, 3, and 8 days post-UVD. Compensation is not associated with changes in the protein expression of the α_1, β_1, β_2, and γ_2 subunits (Eleore et al., 2005; Gliddon et al., 2005b).

The limitation of the behavioral studies conducted to date is that only systemic or i.c.v. injections have been used. Neither of these methods provides information on the site of drug action. Furthermore, some of the behavioral studies have used THIP and diazepam, and neither are full $GABA_A$ receptor agonists (McCabe et al., 1973; Bernstein et al., 1974; Peppard, 1986; Martin et al., 1996; Magnusson et al., 2002). Neither has there been any systematic investigation of the role of $GABA_A$ receptors in postural compensation. Consequently, the aim of the experiments described here was to determine the effect of activating or blocking ipsilateral and contralateral VNC $GABA_A$ receptors on the compensation of SN, yaw head tilt (YHT), and roll head tilt (RHT) in guinea pig.

Materials and Methods

Subjects. Data were obtained from a total of 92 male pigmented guinea pigs (*Cavia porcellus*) (250–350 g). Animals were divided into UVD and labyrinthine-intact groups (Table 1). It was possible that the behavioral effects observed with the $GABA_A$ receptor agonist/antagonist were not due to the drugs interacting with the neurochemical mechanisms of vestibular compensation but instead were solely due to their effects on the vestibulo-ocular and -spinal reflex pathways. To determine whether this was the case, the highest dose of the $GABA_A$ receptor agonist/antagonist was infused into the VNC of labyrinthine-intact animals and any resulting SN, RHT, and YHT was measured. Animals were obtained from the Department of Laboratory Animal Sciences, Ethics No. 29/02.

TABLE 1

Experimental groups

Animals were allocated to two experimental groups: UVD (A) and labyrinthine intact (B).

Group No.	Cannula Site	Drug and Total Dose over 30-h Infusion Period	Sample Size
A1	Ipsilateral VNC	75 ng of muscimol	5
A2	Ipsilateral VNC	125 ng of muscimol	5
A3	Ipsilateral VNC	250 ng of muscimol	5
A4	Ipsilateral VNC	2 ng of NaOH	5
A5	Ipsilateral VNC	31.25 ng of gabazine	5
A6	Ipsilateral VNC	62.5 ng of gabazine	5
A7	Ipsilateral VNC	125 ng of gabazine	5
A8	Ipsilateral VNC	Deionized water	5
A9	Contralateral VNC	250 ng of muscimol	5
A10	Contralateral VNC	400 ng of muscimol	5
A11	Contralateral VNC	750 ng of muscimol	5
A12	Contralateral VNC	8.1 ng of NaOH	5
A13	Contralateral VNC	125 ng of gabazine	5
A14	Contralateral VNC	375 ng of gabazine	5
A15	Contralateral VNC	750 ng of gabazine	5
A16	Contralateral VNC	Deionized water	5
B1	Ipsilateral VNC	750 ng of muscimol	4
B2	Ipsilateral VNC	8.1 ng of NaOH	4
B3	Ipsilateral VNC	750 ng of gabazine	4

Surgical Procedures. All surgical procedures were performed under strict aseptic conditions. Lidocaine hydrochloride (1%) with 1 in 100,000 adrenaline (Xylocaine; AstraZeneca Pharmaceuticals LP, Wilmington, DE) was injected into all wound margins and pressure points. Wounds were sutured and covered with antibiotic cream [2% (w/w) mupirocin (Bactroban, GlaxoSmithKline, Welwyn Garden City, Hertfordshire, UK]. Each animal in the UVD group underwent three surgical procedures: cannulation, osmotic pump implantation, and UVD. The labyrinthine-intact group underwent only cannulation and osmotic pump implantation. The first procedure for both groups was cannulation. The cannula was an L-shaped stainless steel cannula (30 gauge) with an inner obturator (0.125-mm wire). Animals were anesthetized with 0.4 ml/kg i.m. fentanyl citrate (0.4 mg/ml), azaperone (3.2 mg/ml), and xylazine hydrochloride (58.3 mg/ml) (Fentazin; Parnell, Aukland, New Zealand). With the use of a stereotaxic apparatus, bregma was used as the reference point for the VNC stereotaxic coordinates (caudal, 12 mm; lateral, 1 mm; ventral, 10.3 mm; Gliddon et al., 2000). The cannula was inserted into the brainstem through the cerebellum and secured to the skull by dental cement. At 10 h postcannulation, the animals were observed for SN, YHT, and RHT. If the animals were in good health (eating, drinking, and defecating) and exhibited no vestibular symptoms (i.e., SN $<$ 1 beat/15 s, RHT $<$ 10°, and YHT $<$ 10°), they were included in the study.

Seven days later, in both the UVD and labyrinthine-intact groups, a mini-osmotic pump (pumping rate 1 $\pm$ 0.15 μl/h, model 1003D; Alzet, Cupertino, CA) was implanted between the shoulder blades under isoflurane anesthesia. The advantage of using mini-osmotic pumps is that, unlike injections, they are not associated with frequent handling of the animal, which can lead to stress and modify the animal's vestibular, proprioceptive, and visual information.

In the UVD group, immediately after mini-osmotic pump implantation, a surgical UVD was performed. The UVD was performed in accordance with the procedure of Smith et al. (1986). Briefly, with the aid of an operating microscope (OPM 199; Carl Zeiss, Jena, Germany), a high-speed dental drill was used to expose the ampullae of the horizontal and anterior semicircular canals. The horizontal and anterior canal ampullae, and the maculae of the utricle and saccule, were probed and aspirated. The posterior canal ampulla was blindly probed and aspirated. During the operation, the heart rate was monitored via ECG electrodes that were inserted into the forelimbs.

Drug Protocol. Muscimol, a full $GABA_A$ receptor agonist, was used due to its higher potency for the $GABA_A$ receptor compared with isoguvacine; also, it is not a partial $GABA_A$ receptor agonist like THIP and piperidine-4-sulfonic acid (Woodward et al., 1993; Chang et al., 2000). All of the available $GABA_A$ receptor agonists interact with the $GABA_C$ receptor, and muscimol is also a $GABA_C$ receptor agonist (Woodward et al., 1993; Chang et al., 2000). No previous study has infused muscimol into the ipsilateral or contralateral VNC during compensation. Furthermore, only one study has infused muscimol (62.5 and 250 ng/h) via a mini-osmotic pump into brain tissue (Martin et al., 2000); therefore, the muscimol dose used was based on previous studies that have delivered either muscimol or GABA into the brain via a mini-osmotic pump (Brailowsky et al., 1986; Silva-Barrat et al., 1989). Brailowsky and colleagues have used a high GABA dose (1000 μg/h) that induces a "GABA withdrawal syndrome" characterized by spontaneous epileptic activity in the area surrounding the infusion. It was postulated that this was due to a reduction in the number and/or efficacy of the $GABA_A$ receptors as a consequence of prolonged GABA infusion (Silva-Barrat et al., 1989). Muscimol is 4 times more potent than GABA (Woodward et al., 1993; Chang et al., 2000), and therefore the dose was kept below 250 μg/h to avoid possible changes in $GABA_A$ receptor physiology.

It was hypothesized that chronic infusion of muscimol into the ipsilateral VNC would increase the severity of the vestibular syndrome. For ethical reasons, doses were required to demonstrate whether this hypothesis was correct without causing distress to the animal. A pilot study used high doses of muscimol (i.e., 4.2 μg/h and 41.6 ng/h); however, the animals were clearly distressed and therefore the doses were reduced to 2.1, 4.2, and 8.3 ng/h. These doses correspond to a total final dose of 75, 125, and 250 ng of muscimol for the 30-h infusion time. It was hypothesized that chronic infusion of muscimol into the contralateral VNC would decrease the severity of the vestibular syndrome. The first muscimol dose evaluated on the contralateral side was 250 ng/30 h. This dose did not alter compensation and therefore higher doses were used (i.e., 500 and 750 ng/30 h).

As recommended by Seutin and Johnson (1999), neither bicuculline nor its salts were used due to their nonselectivity; nor was picrotoxin used due to its action on the glycine receptor (Chattipakorn and McMahon, 2002). Gabazine (SR 95531) was chosen; however, gabazine is also a weak $GABA_C$ receptor antagonist. Like muscimol, gabazine has not been chronically infused into the VNC. Nor has there been any study that has chronically infused gabazine into other brain regions. The initial dose of gabazine, therefore, was based on the medium muscimol dose for the ipsilateral side (i.e., 125 ng/30 h). This dose infused into the ipsilateral VNC reduced the severity of the vestibular syndrome; therefore, lower doses were used (i.e., 31.25 and 62.5 ng/30 h). On the contralateral side, the 125 ng/30 h dose had no effect, and therefore the doses were increased to 375 and 750 ng/30 h.

Muscimol and gabazine (Tocris Cookson Inc., Bristol, UK) were dissolved in a solution of 100 mM NaOH or deionized water. The drugs were aliquoted, frozen at −20°C, and defrosted when required. Both drugs were diluted to the required concentration using modified cerebrospinal fluid (124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, and 1.3 mM $MgSO_4$) (Gliddon et al., 2000). Drug solutions were tested for pH and changed to a pH 7.0 if necessary.

Muscimol or gabazine was infused into either the ipsilateral or contralateral VNC via an osmotic mini-pump for the first 30 h post-UVD. The osmotic mini-pump assembly was modified from the method suggested by Hagg (1994). A coiled catheter of polyethylene tubing (0.75 i.d.; 1.45 mm o.d.) was filled with either the drug or vehicle solution and connected to the modified flow moderator. The mini-osmotic pump, modified flow moderator, and a portion of the catheter were filled with dyed sterile phosphate-buffered solution. The osmotic mini-pump was primed overnight in sterile saline solution at 37°C. Following the movement of the dyed PBS solution down the catheter ensured a constant rate of infusion.

Behavioral Observations. Behavioral observations were made without restraining the animal because restraint can induce stress that can alter SN frequency. All behavioral observations were recorded by a Panasonic (model NV-MO50) videocamera with a zoom lens. The observations were replayed through a Mitsubishi (model HS-641V) videorecorder to a Sony Trinitron color monitor (model PVN 14N2A).

The frequency of SN was measured visually in the contralateral eye and defined as the number of quick phases (beats) to the contralateral side within a 15-s period (Gilchrist et al., 1993). If the quick phases were directed to the ipsilateral side then the SN frequency was given a negative value. This method is a quick, noninvasive, and accurate method of quantifying SN with an estimated measurement error of ±1 beat/15 s (Gilchrist et al., 1993). SN was counted visually five times. The measurements were made while the animal's head was stationary to avoid contamination of the SN with head movement-induced vestibulo-ocular and optokinetic reflex nystagmus. The five SN measurements at each observation time were averaged.

YHT (also known as lateral head deviation) is defined as the angle, in degrees, between a line that passes through the midscapular point and the sacrum and a line from the midscapular point to the center of the guinea pig's snout in the horizontal plane (Curthoys et al., 1988). If head nystagmus was present, YHT was measured as the greatest angle. RHT (also known as longitudinal twist of the head) is defined as the angle, in degrees, between a line passing through the center of the guinea pig's head and a line perpendicular to the floor in the vertical plane (Curthoys et al., 1988). YHT and RHT were recorded with videocameras that were mounted above and in front of the guinea pig, respectively. All postural symptoms were measured from the monitor screen using a protractor device that fitted over the screen. The measurement error for both of the above-mentioned parameters is ±10° (Gilchrist et al., 1993). YHT and RHT were measured once at each individual observational time.

To monitor the possible effects of the $GABA_A$ receptor agonist or antagonist, the direction of head tilt was given either a positive value if the head was tilted to the ipsilateral side (i.e., as would occur in an untreated animal after an ipsilateral UVD) or a negative value if the head was directed toward the contralateral side. Furthermore, in some cases, the drug resulted in the animal being unable to stand and hence YHT and RHT could not be measured. There are two ways with which to treat such animals for the purposes of data analyses. Either exclude the animal from the data analyses or give the animal an arbitrary YHT or RHT value. It was decided to give the YHT and RHT a 180° value. The direction of this arbitrary YHT and RHT cannot be measured due to the animal being pinned to the ground and therefore the direction of the YHT and RHT was based on the first measurement that could be made when the animal could stand. There are limitations to this method. For example, 180° does not represent a physiological value, and the direction was based on other readings. However, this method allows for the animal to be included in the two-way analysis of variance (ANOVA) with repeated measures.

This experiment was not conducted blind. Although this would have made a stronger experimental design, it was not practical. There was a component of blindness, however, because the site of the cannula tip was not known until the experiment was completed, and histological analysis of the cannula sites was performed. It was possible, therefore, that the cannula tip was positioned in another region of the brain, such as the cerebellum. In addition, because this was the first study to investigate the role of the ipsilateral and contralateral VNC $GABA_A$ receptors using chronic injections of $GABA_A$ receptor ligands into the VNC, the outcome of the experiment could not be predicted.

Cardiac Perfusion and Histology. After the last observation at 50 h post-UVD, the animals were anesthetized with sodium pentobarbitone (30 mg/kg i.p., Pentobarb 300; National Veterinary Suppliers Ltd., Middleton, Christchurch, New Zealand), and a cardiac

perfusion was performed. For histological verification of the site of the cannula tip, Alcian Blue was injected into the cannula. The animal was then decapitated and the cerebellum and brainstem were dissected and stored in 10% buffered formalin until histology was performed. Sections were stained either using Toluidine Blue or Thionin Stain.

Statistical Analysis for the Behavioral Data. In all behavioral experiments, the data obtained were analyzed using a two-factor ANOVA with repeated measures. The advantage of this statistical test is that it can detect the following: effect of the treatment on the severity of a symptom, independently of time (factor A); change in the severity of a symptom over time and independently of the treatment (factor B); and interaction between the treatment effect and time (factor AB) in one statistical test (Gilchrist et al., 1993). The factor AB is used as an index of the treatment effect on the rate of vestibular compensation (Gilchrist et al., 1993). Mauchley's test of sphericity was also performed to determine whether the assumption of sphericity was violated. If this was so, then the Huynh-Feldt correction was used; the Greenhouse-Geisser correction was not used because it is too conservative for small sample sizes (Howell, 1992). When the two-factor ANOVA with repeated measures indicated a significant result, the Scheffé post hoc test was performed. The significance level was set a 0.05 for all comparisons. Statistical analysis and graphs were obtained using the statistical and graphic software packages SPSS (SPSS Inc., Chicago, IL) and Prism (GraphPad Software Inc., San Diego, CA), respectively.

Results

For the placement of cannula tip lesion sites for all experimental groups, refer to Fig. 1. The Mauchley's test of sphericity indicated that sphericity was always violated, and therefore the Huynh-Feldt correction was used. Note that all doses are expressed as the total dose that was given over 30 h.

Effects of Chronic Infusion of Muscimol or Gabazine into the Ipsilateral or Contralateral VNC on the Compensation of SN. Three doses of muscimol (62.5, 125, and 250 ng) or its vehicle (2 ng of NaOH) were infused into the ipsilateral VNC for the first 30 h post-UVD (Fig. 2). There was no significant effect on SN frequency ($p > 0.05$), or on its rate of compensation ($p > 0.05$). At 30 h, the pump was disconnected, and SN frequency was measured at 31, 33, and 50 h post-UVD. At 30 h post-UVD, minimal SN (<2 beats/15

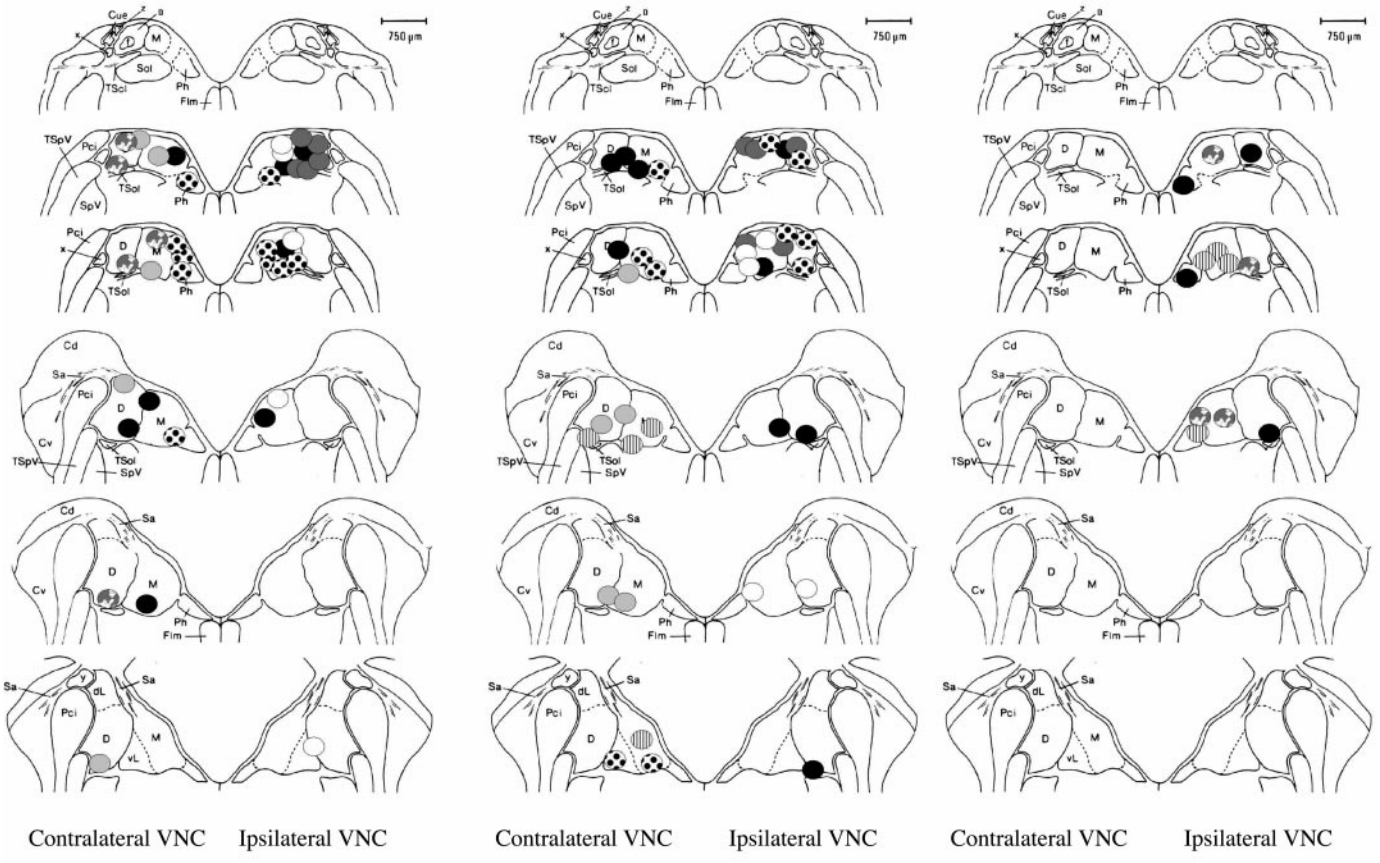

Fig. 1. Schematic illustration of the cannula tip lesion sites in the ipsilateral and contralateral VNC for all experimental groups. Left, cannula tip lesion sites in the contralateral and ipsilateral VNCs (left and right, respectively) for the vehicle (black dots), 62.5-ng (dark gray dots), 125-ng (white dots), 250-ng (black spotted dots), 500-ng (light gray dots), or 750-ng (dark gray and white dots) muscimol groups during the first 30 h post-UVD. Middle, cannula tip lesion sites in the ipsilateral and the contralateral VNCs for the vehicle (black dots), 31.25-ng (dark gray dots), 62.5-ng (white dots), 125-ng (spotted dots), 375-ng (light gray dots), and 750-ng (dots with vertical lines) gabazine groups during the first 30 h post-UVD. Right, cannula tip lesion sites in the VNC for the vehicle (black dots), 750-ng muscimol (dark gray and white dots), and 750-ng gabazine (dots with vertical lines) labyrinthine-intact groups. Histology figures represent transverse sections of the guinea pig VNC. The top drawing is the most caudal. Modified from Gstoettner and Burian (1987). M, medial vestibular nucleus; sol, nucleus tractus solitarii; Ph, prepositus hypoglossi nucleus; Tsol, tractus solitarius; Flm, fasciculus longitudinalis medialis; D, descending vestibular nucleus; Cue, nucleus cuneatus externus; XII, hypoglossus nucleus; Pci, restiform body; TSpV, tractus nucleus spinalis nervis trigemini; SpV, nucleus spinalis nervis trigemini; Cd, nucleus cochlearis dorsalis; Sa, stria acustica; Cv, nucleus cochlearis ventralis; dL, dorsal portion of the caudal most part of the lateral vestibular nucleus; x and y, cells in close relation to the descending vestibular nucleus.

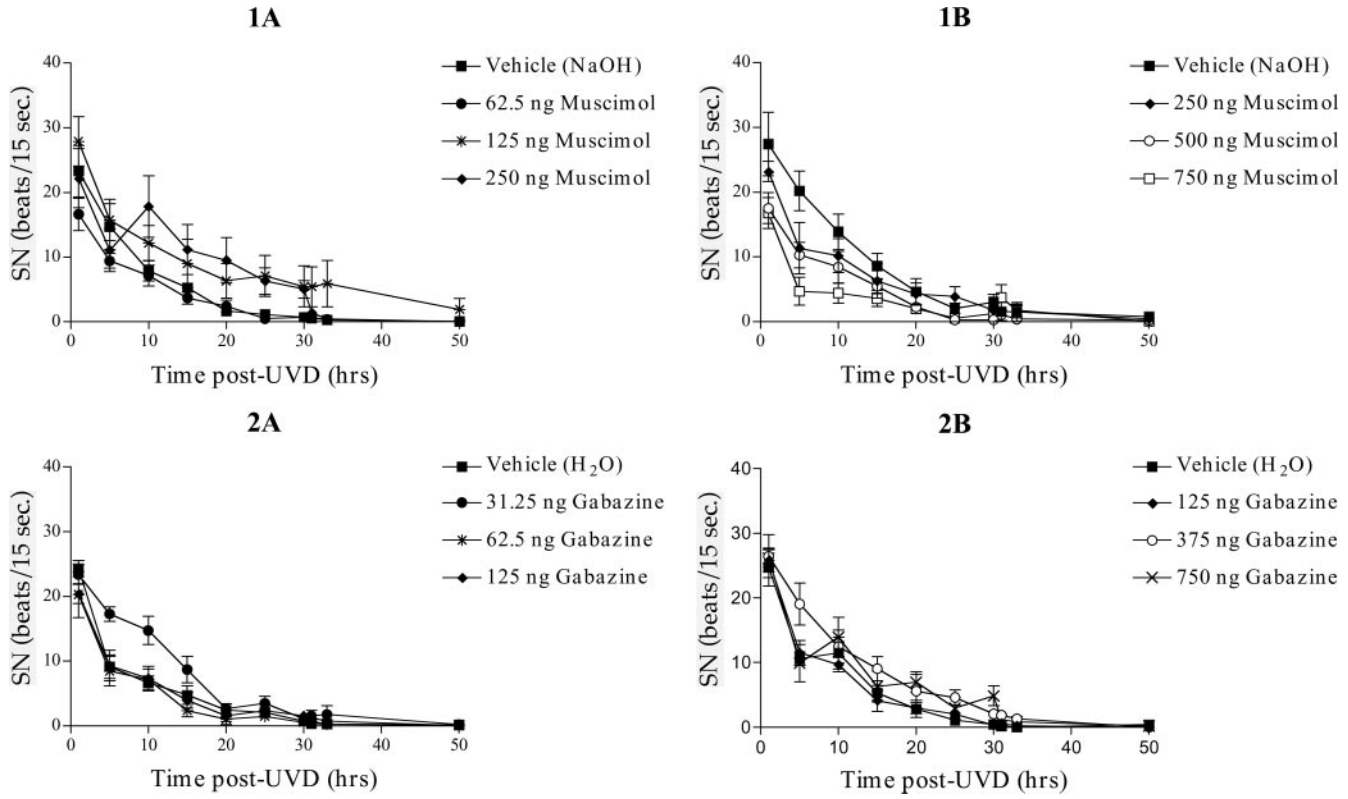

Fig. 2. Effects of chronic infusion of muscimol or gabazine into either the ipsilateral or contralateral VNC on the compensation of SN. Muscimol (62.5, 125, and 250 ng) or its vehicle (2 ng of NaOH) were infused into the ipsilateral VNC during the first 30 h post-UVD (1A). There was no significant effect on SN frequency ($p > 0.05$) or its rate of compensation ($p > 0.05$). Gabazine (31.25, 62.5, and 125 ng) or its vehicle (H_2O) were infused into the ipsilateral VNC during the first 30 h post-UVD (2A). There was a significant change in the SN frequency ($p < 0.05$) and its rate of compensation ($p < 0.05$). Muscimol (250, 500, and 750 ng) or its vehicle (8.1 ng of NaOH) were infused into the contralateral VNC for the first 30 h post-UVD (1B). There was a significant change in SN frequency ($p < 0.05$) and its rate of compensation ($p < 0.05$). Gabazine (125, 375, and 750 ng) or its vehicle (H_2O) were infused into the contralateral VNC during the first 30 h post-UVD (2B). There was no significant effect on SN frequency ($p > 0.05$) or its rate of compensation ($p > 0.05$). Data are expressed as mean ± S.E.M.

s) was exhibited by all experimental groups. Discontinuation of the drug treatment did not increase the SN frequency at 31, 33, and 50 h post-UVD in any of the experimental groups.

Three doses of gabazine (31.25, 62.5, and 125 ng) or its vehicle (H_2O) were infused into the ipsilateral VNC for the first 30 h post-UVD (Fig. 2). Infusion of gabazine into the ipsilateral VNC significantly altered the SN frequency ($p < 0.01$) and its rate of compensation ($p < 0.05$). Post hoc testing indicated that at 5 h, there was a significant increase in the mean SN frequency in the 31.25-ng gabazine group (19.2 ± 0.4 beats/15 s) compared with the 62.5-ng gabazine group (7.8 ± 0.6 beats/15 s). Furthermore, at 10 h post-UVD, there was a significant increase in the mean SN frequency in the 31.25-ng gabazine group (14.7 ± 2.2 beats/15 s) compared with the vehicle group (6.6 ± 1.1 beats/15 s). At 30 h post-UVD, minimal SN (>2 beats/15 s) was exhibited by all experimental groups. Discontinuation of the drug treatment did not increase the SN frequency at 31, 33, and 50 h post-UVD in any of the experimental groups.

Three doses of muscimol (250, 500, and 750 ng) or its vehicle (8.1 ng of NaOH) were infused into the contralateral VNC for the first 30 h post-UVD (Fig. 2). There was a dose-dependent significant effect on SN frequency ($p < 0.05$) and its rate of compensation ($p < 0.05$). Post hoc analyses indicated that at 5 h post-UVD, there was a significant decrease in the SN frequency exhibited by the 750-ng gabazine group (4.7 ± 2 beats/15 s) compared with the vehicle group (20.5 ± 5 beats/15 s). At 30 h post-UVD, there was minimal SN (>3.5 beats/15 s) exhibited by all experimental groups. Discontinuation of the drug treatment did not increase the SN frequency at 31, 33, and 50 h post-UVD.

Three doses of gabazine (250, 500, and 750 ng) or its vehicle (H_2O) were infused into the contralateral VNC for the first 30 h post-UVD (Fig. 2). There was no significant effect on SN frequency ($p > 0.05$) or its rate of compensation ($p > 0.05$). At 30 h, the pump was disconnected and SN frequency was measured at 31, 33, and 50 h post-UVD. At 30 h post-UVD, minimal SN (>5 beats/15 s) was exhibited by all experimental groups. Discontinuation of the drug treatment did not increase the SN frequency at 31, 33, and 50 h post-UVD.

Effects of Chronic Infusion of Muscimol or Gabazine into the Ipsilateral or Contralateral VNC on the Compensation of YHT. Three doses of muscimol (62.5, 125, and 250 ng) or its vehicle (2 ng of NaOH) were infused into the ipsilateral VNC for the first 30 h post-UVD (Fig. 3). There was a dose-dependent significant effect on YHT ($p < 0.05$) but not its rate of compensation ($p > 0.05$). Post hoc analyses indicated that there was a significant increase in the mean YHT exhibited by the 250-ng muscimol group (40.6 ± 7.7°) compared with the 62.5-ng muscimol group (8.6 ± 6.1°) at 20 h post-UVD. Furthermore, there was a significant increase in the mean YHT exhibited by the 250-ng muscimol group (50.7 ± 3.9°) compared with the vehicle group (4.6 ±

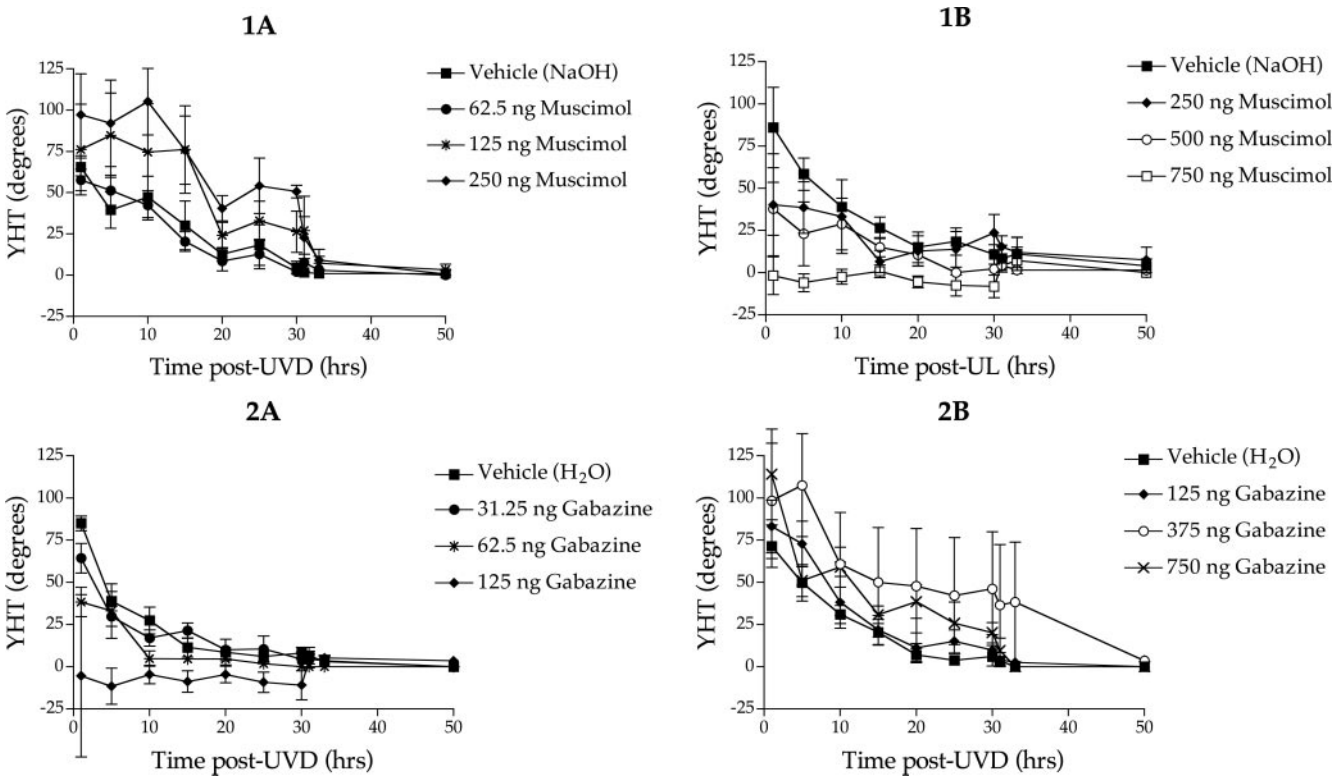

Fig. 3. Effects of infusion of chronic muscimol or gabazine into either the ipsilateral or contralateral VNC on the compensation of YHT. Muscimol (62.5, 125, and 250 ng) or its vehicle (2 ng of NaOH) were infused into the ipsilateral VNC during the first 30 h post-UVD (1A). There was a significant effect on YHT ($p < 0.05$) but not its rate of compensation ($p > 0.05$). Gabazine (31.25, 62.5, and 125 ng) or its vehicle (H_2O) were infused into the ipsilateral VNC during the first 30 h post-UVD (2A). There was a significant effect on YHT ($p < 0.05$) but not its rate of compensation ($p > 0.05$). Muscimol (250, 500, and 750 ng) or its vehicle (8.1 ng of NaOH) were infused into the contralateral VNC for the first 30 h post-UVD (Graph 1B). There was a significant effect on YHT ($p < 0.05$) but not its rate of compensation ($p > 0.05$). Gabazine (125, 375, and 750 ng) or its vehicle (H_2O) were infused into the contralateral VNC during the first 30 h post-UVD (2B). There was no significant effect on YHT ($p > 0.05$) or its rate of compensation ($p > 0.05$) (2B). Data are expressed as mean ± S.E.M.

3.9°) at 30 h post-UVD. At 30 h post-UVD, there was a significant increase in the mean YHT exhibited by the 250-ng muscimol group compared with the vehicle group. Discontinuation of the drug treatment at 30 h post-UVD resulted in a reduction of the mean YHT exhibited by the 125- and 250-ng muscimol-treated groups, so that by 33 h post-UVD, all experimental groups exhibited similar YHT.

Three doses of gabazine (31.25, 62.5, and 125 ng) or its vehicle (H_2O) were infused into the ipsilateral VNC for the first 30 h post-UVD (Fig. 3). There was a dose-dependent significant effect on YHT ($p < 0.01$) but not on its rate of compensation ($p > 0.05$). Post hoc analyses indicated that there was a significant decrease in the mean YHT exhibited by the 250-ng gabazine group compared with the vehicle group at 5 h (i.e., 38.8 ± 5.7° vehicle compared with −11.6 ± 10.7° 250-ng gabazine) and 10 h (i.e., 27.4 ± 7.9° vehicle compared with −4.6 ± 5.4° 250-ng gabazine) post-UVD. Furthermore, there was a significant decrease in the mean YHT exhibited by the 250-ng gabazine group (−8.8 ± 6.4°) compared with the 31.25-ng gabazine group (21.4 ± 4.5) at 15 h post-UVD. Discontinuation of the drug treatment at 30 h post-UVD, resulted in the mean YHT of the 250-ng gabazine group (−11 ± 8.6° at 30 h post-UVD) returning to positive values (3.1 ± 4.1° at 31 h post-UVD). By 33 h, the magnitude of the mean YHT was similar between all experimental groups.

Three doses of muscimol (250, 500, and 750 ng) or its vehicle (8.1 ng of NaOH) were infused into the contralateral VNC for the first 30 h post-UVD (Fig. 3). There was a dose-dependent significant effect on YHT ($p < 0.05$) but not on its rate of compensation ($p > 0.05$). Post hoc analyses indicated that there was a significant decrease in the mean YHT exhibited by the 750-ng muscimol group compared with the vehicle group at 5 h (i.e., 58.4 ± 5.3° vehicle compared with −6 ± 9.5° 750-ng muscimol) and 15 h (i.e., 26.6 ± 6.4° vehicle compared with 0.8 ± 3.7° 750-ng muscimol) post-UVD. Furthermore, there was a significant decrease in the mean YHT exhibited by the 750-ng muscimol group (−8.2 ± 6.7°) compared with the 250-ng muscimol group (23.5 ± 11°) at 30 h post-UVD. Discontinuation of the drug treatment at 30 h post-UVD, resulted in the mean YHT of the 750-ng muscimol group (−3.2 ± 5.5° at 30 h post-UVD) returning to positive values (i.e., 4.2 ± 1.1° at 31 h post-UVD). By 33 h, the magnitude of the mean YHT was similar between all experimental groups.

Three doses of gabazine (125, 375, and 750 ng) or its vehicle (H_2O) were infused into the contralateral VNC during the first 30 h post-UVD (Fig. 3). There was, however, no significant effect on YHT ($p > 0.05$) or its rate of compensation ($p > 0.05$). Discontinuation of the drug treatment at 30 h post-UVD did not alter the severity of YHT. By 50 h post-UVD, the magnitude of YHT was similar between all experimental groups.

Effects of Chronic Infusion of Muscimol or Gabazine into the Ipsilateral or Contralateral VNC on the Compensation of RHT. Three doses of muscimol (62.5, 125, and 125 ng) or its vehicle (2 ng of NaOH) were infused into the ipsilateral VNC for the first 30 h post-UVD (Fig. 4). There

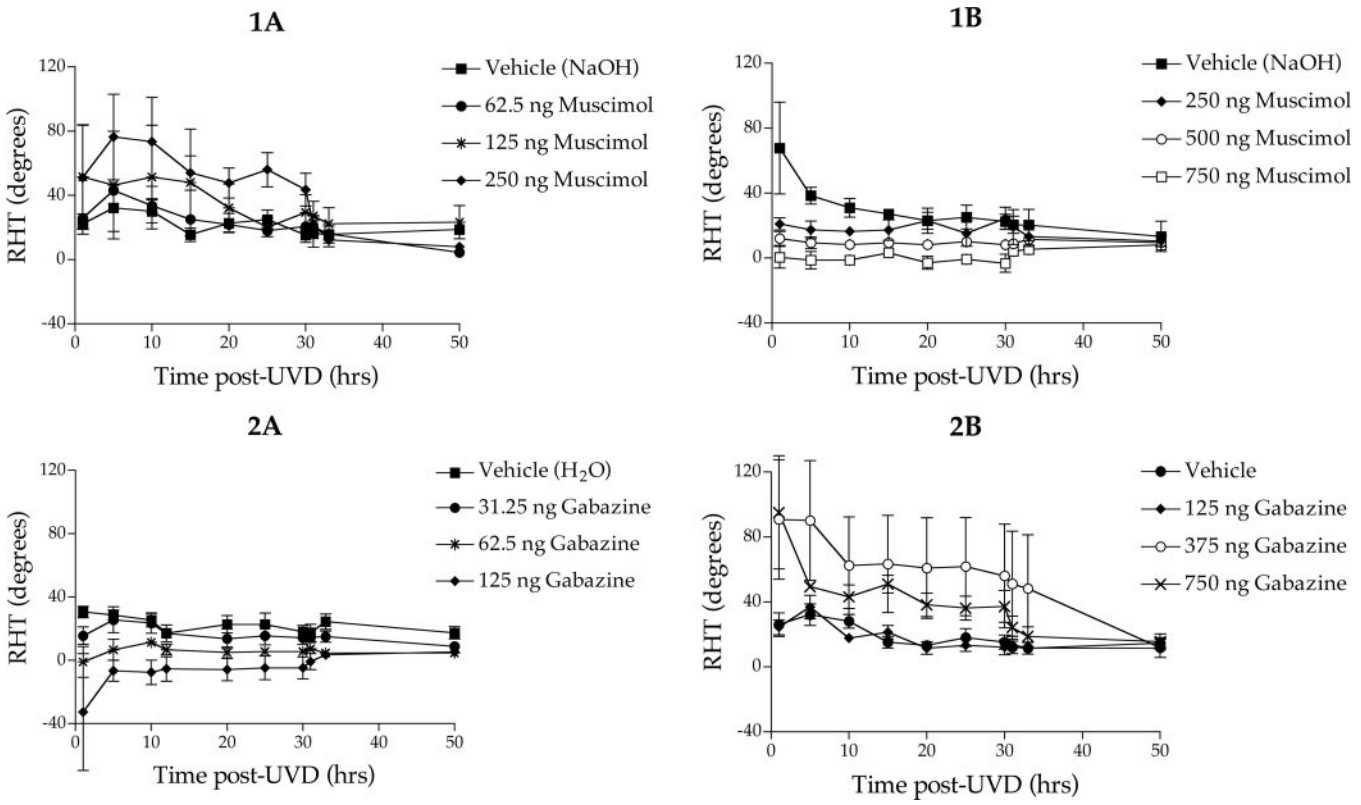

Fig. 4. Effects of chronic infusion of muscimol or gabazine into either the ipsilateral or contralateral VNC on the compensation of RHT. Muscimol (62.5, 125, and 250 ng) or its vehicle (2 ng of NaOH) were infused into the ipsilateral VNC during the first 30 h post-UVD (1A). There was no significant effect on RHT ($p > 0.05$) or its rate of compensation ($p > 0.05$). Gabazine (31.25, 62.5, and 125 ng) or its vehicle (H_2O) were infused into the ipsilateral VNC during the first 30 h post-UVD (2A). There was a significant effect on RHT ($p < 0.05$) but not its rate of compensation ($p > 0.05$). Muscimol (250 ng, 500, and 750 ng) or its vehicle (8.1 ng NaOH) were infused into the contralateral VNC for the first 30 h post-UVD (Graph 1B). There was a significant effect on RHT ($p < 0.05$) but not its rate of compensation ($p > 0.05$). Gabazine (125, 375, and 750 ng) or its vehicle (H_2O) were infused into the contralateral VNC during the first 30 h post-UVD (2B). There was no significant effect on RHT ($p > 0.05$) or its rate of compensation ($p > 0.05$). Data are expressed as mean ± S.E.M.

was no significant effect on RHT ($p > 0.05$) or its rate of compensation. Discontinuation of the drug treatment at 30 h post-UVD resulted in the mean RHT exhibited being similar between experimental groups.

Three doses of gabazine (31.25, 62.5, and 125 ng) or its vehicle (H_2O) were infused into the ipsilateral VNC for the first 30 h post-UVD (Fig. 4). There was a dose-dependent significant effect on RHT ($p < 0.05$) but not its rate of compensation ($p > 0.05$). Furthermore, there was no significant time effect. Post hoc analyses indicated that there was a significant decrease in the mean RHT exhibited by the 250-ng gabazine group compared with the vehicle group at 5 h (i.e., 28.6 ± 3.2° vehicle compared with −6.6 ± 6.7° 250-ng gabazine), 10 h (i.e., 25.4 ± 4.3° vehicle compared with −7.6 ± 7.8° 250-ng gabazine), 20 h (i.e., 22.8 ± 5.5° vehicle compared with −5.8 ± 7.1° 250-ng gabazine), 25 h (i.e., 22.8 ± 7.1° vehicle compared with −4.8 ± 7.5° 250-ng gabazine), and 30 h (i.e., 18.0 ± 4° vehicle compared with −4.8 ± 6.8° 250-ng gabazine) post-UVD. Furthermore, there was a significant decrease in the mean RHT exhibited by the 250-ng gabazine group compared with the 31.25-ng gabazine group at 5 and 10 h post-UVD. Discontinuation of the drug treatment at 30 h post-UVD resulted in the mean RHT of the 250-ng gabazine group (i.e., −4.8 ± 6.8° at 30 h post-UVD) returning to positive values (i.e., 3.6 ± 1.6° at 33 h post-UVD). By 50 h, the magnitude of the mean RHT was similar between all experimental groups.

Three doses of muscimol (250, 500, and 700 ng) or its vehicle (8.1 ng of NaOH) were infused into the contralateral VNC for the first 30 h post-UVD (Fig. 4). There was a significant dose-dependent effect on RHT ($p < 0.05$) but not its rate of compensation. Nor was there a significant time effect. Post hoc testing indicated that there was a significant decrease in the mean RHT exhibited by the 750-ng muscimol group compared with the vehicle group at 1 h (67.8 ± 28.3° vehicle compared with 0.5± 6.7° 750-ng muscimol), 5 h (38.4 ± 5.1° vehicle compared with −1.3 ± 5.3° 750-ng muscimol), 10 h (31.0 ± 5.7 compared with −1.3 ± 2.8° 750-ng muscimol), 15 h (27.1 ± 3.0° vehicle compared with 3.1 ± 2.4° 750-ng muscimol), 20 h (23.0 ± 5.2° vehicle compared with −2.9 ± 4.0° 750-ng muscimol), 25 h (25.2 ± 7.2° vehicle compared with −0.7 ± 1.9° 750-ng muscimol), and 30 h (22.4 ± 4.6° vehicle compared with −3.2 ± 5.5° 750-ng muscimol) post-UVD. Furthermore, there was a significant decrease in the mean RHT exhibited by the 500-ng muscimol group compared with the vehicle group at 5 h (−9.4 ± 3.7° 500-ng muscimol), 10 h (8.3 ± 2.5° 500-ng muscimol), and 15 h (9.4 ± 7.2° 500-ng muscimol) post-UVD. Discontinuation of the drug treatment at 30 h post-UVD, resulted in the mean RHT of the drug-treated groups returning to vehicle-treated values so that by 50 h, the magnitude of the mean RHT was similar between all experimental groups.

Three doses of gabazine (125, 375, and 750 ng) or its vehicle (H_2O) were infused into the contralateral VNC during

the first 30 h post-UVD (Fig. 4). There was no significant effect on RHT ($p > 0.05$) or its rate of compensation ($p > 0.05$). At 30 h, the pump was disconnected and RHT was measured at 31, 33, and 50 h post-UVD. Discontinuation of the drug treatment at 30 h post-UVD resulted in the mean RHT of the drug-treated groups returning to vehicle-treated values so that by 50 h, the magnitude of the mean RHT was similar between all experimental groups.

Effects of the Infusion of a $GABA_A$ Receptor Agonist/Antagonist on Ocular-Motor and Postural Reflexes in Labyrinthine-Intact Animals. It is possible that the behavioral effects observed with the $GABA_A$ receptor ligands were not due to the drugs interacting with the neurochemical mechanisms of vestibular compensation but were solely due to their effects on the vestibulo-ocular and -spinal pathways. To determine whether this was the case, the highest doses of muscimol (750 ng) or gabazine (750 ng) were infused into the VNC of labyrinthine-intact guinea pigs. Only the 750-ng muscimol labyrinthine-intact group exhibited YHT and RHT (Fig. 5). There was a significant difference in the expression of YHT ($p < 0.05$) that was constant over time ($p > 0.05$). Post hoc testing indicated that there was a higher mean YHT in the muscimol group compared with both the vehicle and the gabazine groups at 1, 5, 10, 15, and 20 h but not at 25 or 30 h post-pump connection. Chronic infusion of muscimol did induce RHT in labyrinthine-intact animals; however, the degree of RHT was not significantly different compared with the gabazine or vehicle groups nor did it change over time ($p > 0.05$). Only one animal, at a 10-h post-pump connection in the 750-ng muscimol group, exhibited SN. There was, however, no difference in the SN frequency between the groups ($p > 0.05$), nor did SN frequency change over time ($p > 0.05$). At 30 h, the pump was disconnected and SN, YHT, and RHT were measured at 31, 33, and 50 h post-pump connection. Discontinuation of the drug treatment resulted in the 750-ng muscimol group exhibiting no SN, YHT, or RHT.

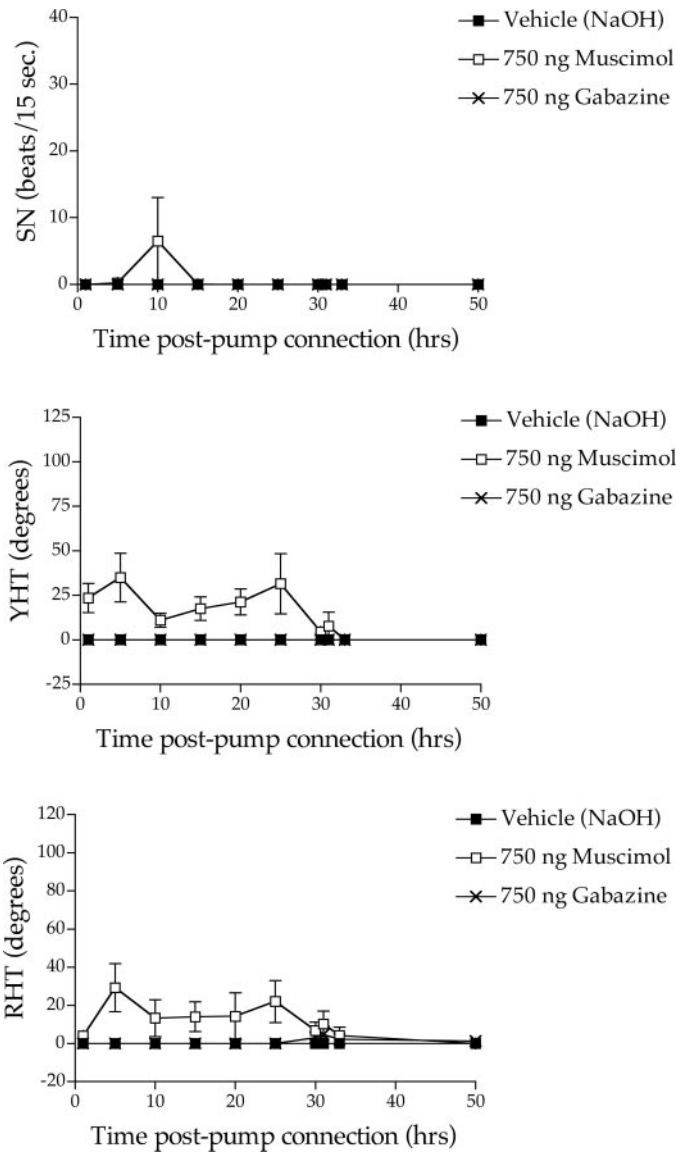

Fig. 5. Effects of chronic muscimol or gabazine infusion on the expression of SN, YHT, and RHT in labyrinthine-intact animals. The effects of 750-ng muscimol ($n = 4$), 750-ng gabazine ($n = 4$), and 8.1-ng NaOH ($n = 4$) infusion over 30 h on the expression of SN (top), YHT (middle), and RHT (bottom) in labyrinthine-intact animals. Only the infusion of 750 ng of muscimol over 30 h could induce YHT and RHT; SN was exhibited by one animal at 10 h post-pump connection.

Discussion

Vestibular compensation is defined as the reduction in the severity of the ocular motor and postural symptoms over time after UVD. A drug can reduce the symptoms by either interfering with the neurochemical mechanisms of compensation or by reducing the expression of the symptoms independently of this mechanism. From a clinical perspective, this distinction may not be important because the end result is the beneficial reduction in the severity of the symptoms for the patient. From a mechanistic perspective, the distinction is important and should be kept in mind when interpreting the following results.

It was hypothesized that the infusion of muscimol and gabazine into the contralateral and ipsilateral VNCs, respectively, would reduce the asymmetry in the resting activity between the two VNCs and thereby decrease SN, YHT, and RHT, and possibly increase their rate of compensation. Furthermore, it was hypothesized that the infusion of muscimol and gabazine into the ipsilateral and contralateral VNCs, respectively, would increase the asymmetry in the resting activity between two VNCs and thereby increase SN, YHT, and RHT, and decrease their rate of compensation. This hypothesis is based on two assumptions. First, that the infusion of a $GABA_A$ receptor agonist/antagonist would alter the resting activity of the VNC neurons. In support of this, both in vivo and in vitro electrophysiological studies have demonstrated that $GABA_A$ receptor agonists/antagonists modify the resting activity of the VNC neurons (for review, see Gliddon et al., 2005a). What is not known is the extent of the change in resting activity induced by the doses used in this study. The second assumption is that any change in the animal's SN, YHT, and RHT is due to modifications of the resting activity of the VNC neurons. In support of this, it is generally accepted that the UVD-induced decrease in resting activity in the ipsilateral VNC generates SN, YHT, and RHT. Furthermore, the direction of the quick phase beats of SN and the tilt of the head for YHT and RHT provide information as to which VNC has the lowest resting activity. For example, a right UVD, as used in this study, would result in the resting

activity of the right VNC being lower than that of the left VNC. This asymmetry in the resting activity between the two VNCs would generate the quick phase beats of SN toward the left side and tilt of the head for YHT and RHT toward the right side.

Infusion of muscimol and gabazine into either the ipsilateral or the contralateral VNC had complex effects on SN compensation. Only the infusion of muscimol into the contralateral VNC and gabazine into the ipsilateral VNC significantly affected SN frequency and its rate of compensation. Furthermore, only muscimol infusions into the contralateral VNC induced the predicted dose-dependent decrease in SN frequency and increased its rate of compensation. In contrast to what was hypothesized, the 31.25-ng dose of gabazine infused into the ipsilateral VNC significantly retarded SN compensation but not the 62.5- and 125-ng gabazine doses. It is possible that the use of a higher dose of muscimol and gabazine may have affected SN compensation and further investigation is required to determine whether this is so. In the present study, higher doses of muscimol were not infused into the ipsilateral VNC because even the 250-ng dose significantly worsened YHT and, as detailed under *Materials and Methods*, we were attempting to use doses that were high enough to induce an effect without causing the animals distress. In labyrinthine-intact animals, neither muscimol nor gabazine induced SN, except for one animal that exhibited SN at a 10-h post-pump connection, suggesting that these doses do not generate SN. Overall, the results suggest that $GABA_A$ receptors in the ipsilateral and contralateral VNCs do not play a substantial role in the compensation of SN.

The lack of effect of ipsilateral gabazine and contralateral muscimol infusion on SN and its rate of compensation was a surprise because the change in the direction of YHT and RHT suggests that the contralateral VNC had a higher resting activity than the ipsilateral VNC. Furthermore, the infusion of muscimol into the VNC of labyrinthine-intact animals induced YHT and RHT, suggesting that there was an asymmetry in the resting activity between the two VNCs. This asymmetry, however, was not enough to induce SN. The reason for this is unclear.

The lack of effect on SN compensation with $GABA_A$ receptor ligands is consistent with Magnusson et al. (2000, 2002). This may be due to the complex circuitry involved in generating eye movement. This result is further supported by those obtained from the effects of $GABA_A$ receptor ligands on eye movement in labyrinthine-intact animals. Depending on the site of injection within the VNC, $GABA_A$ receptor ligands can induce neural integrator failure independent of SN, SN in combination with neural integrator failure or SN without neural integrator failure (Arnold et al., 1999; Mestdagh and Wulfert, 1999). Furthermore, injections of bicuculline into the medial vestibular nucleus induced SN without any neural integrator failure (Mettens et al., 1994). The direction of the bicuculline-induced SN slow phase was either toward or away from the injected side (Mettens et al., 1994; Arnold et al., 1999). This bidirectional bicuculline-induced SN is somewhat surprising since bicuculline should increase the resting activity on the injected side, thereby generating SN with a slow phase contralateral to the injection. The reason why bicuculline induces either an ipsilaterally or contralaterally directed horizontal slow phase, is unclear, but it could be due to the complex effect on $GABA_A$ receptors within the circuitry responsible for generating eye movement. The method used in this study to measure SN did not allow for the determination of whether the $GABA_A$ receptor ligands were inducing neural integrator failure.

The results suggest that the $GABA_A$ receptor-mediated decrease in the asymmetry in resting activity between the two VNCs facilitates the reduction in the severity of the postural symptoms. For example, the infusion of muscimol and gabazine into the contralateral and ipsilateral VNCs, respectively, reduced the YHT and RHT but not their rate of compensation. The 750-ng muscimol dose infused into the contralateral VNC and 125-ng gabazine dose infused into the ipsilateral VNC generated YHT and RHT that tilted away from the side of the UVD. The extent of the head tilt away from the lesion was not large, but it suggests that the infusion of these $GABA_A$ receptor ligands modified the resting activity to such a degree that now the ipsilateral VNC had a higher resting activity than the contralateral VNC. Interestingly, the effects of muscimol and gabazine on YHT and RHT were consistent throughout the first 30 h post-UVD. At 30 h post-UVD, the pumps were disconnected. In both experimental groups, the value and direction of the YHT and RHT returned to vehicle levels. These results suggest that the effects of ipsilateral gabazine and contralateral muscimol infusions on the resting activity of the VNC neurons are independent of the effects of the UVD on the resting activity of the VNC neurons. It can be concluded, therefore, that the ipsilateral gabazine and contralateral muscimol infusions are modifying the expression of the postural symptoms without altering the neurochemical mechanism of compensation. In support of this, the infusion of muscimol into the VNC of labyrinthine-intact animals induced YHT and RHT. The infusion of gabazine into the VNC of labyrinthine-intact animals, however, did not induce YHT and RHT. It is possible that VNC neurons from labyrinthine-intact animals are less susceptible to the changes in the resting activity mediated by the $GABA_A$ receptor ligands than VNC neurons from UVD animals.

The results observed are most likely due to muscimol/gabazine acting on $GABA_A$ receptors within the VNC. However, the extent of the diffusion of muscimol/gabazine was not measured, and the possibility that both drugs were also acting on an area outside the VNC cannot be excluded. The highest drug concentration is obtained at the site of the cannula tip (i.e., the VNC) and as the distance from the cannula tip site increases, the drug concentration decreases in an exponential function (Kasamatsu and Schmidt, 1997). If a proportion of the dose of muscimol/gabazine reached an adjacent brainstem structure, it would therefore be at a smaller concentration.

The results of this study suggest that the $GABA_A$ receptor-mediated increase in the asymmetry in the resting activity between the two VNCs had little effect on the compensation of the postural symptoms. This was the first study to infuse muscimol and gabazine directly into the VNC and therefore there was no information available on which to base the dose; it is possible that higher doses of muscimol and gabazine may have had different effects. It would also be interesting to determine the effect of the combined infusion of muscimol and gabazine on compensation. However, it is possible that the mechanism responsible for vestibular compensation can

cope with the both the $GABA_A$ receptor-mediated and the UVD-induced decrease in resting activity.

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References

Arnold DB, Robinson DA, and Leigh RJ (1999) Nystagmus induced by pharmacological inactivation of the brainstem ocular motor integrator in monkey. *Vision Res* **39:**4286–4295.

Bernstein P, McCabe BF, and Ryu JH (1974) The effect of diazepam on vestibular compensation. *Laryngoscope* **84:**267–272.

Brailowsky S, Knight RT, Blood K, and Scabini D (1986) γ-Aminobutyric acid-induced potentiation of cortical hemiplegia. *Brain Res* **362:**322–330.

Calza L, Giardino L, Zanni M, and Galetti G (1992) Muscarinic and gamma-aminobutyric acid-ergic receptor changes during vestibular compensation. A quantitative autoradiographic study of the vestibular nuclei complex in the rat. *Eur Arch Otorhinolaryngol* **249:**34–39.

Calza L, Giardino L, Zanni M, Galetti R, Parchi P, and Galetti G (1989) Involvement of cholinergic and GABA-ergic system in vestibular compensation, in *Vestibular Compensation: Facts Theories and Clinical Perspectives* (Lacour M, Toupet M, Denise P and Christen Y eds) pp 189–199, Elsevier, Paris.

Chang Y, Covey DF, and Weiss DS (2000) Correlation of the apparent affinities and efficacies of γ-aminobutyric $acid_C$ receptor agonists. *Mol Pharmacol* **58:**1375–1380.

Chattipakorn SC and McMahon LL (2002) Pharmacological characterization of glycine-gated chloride currents recorded in rat hippocampal slices. *J Neurophysiol* **87:**1515–1525.

Chebib M and Johnston GA (2000) GABA-activated ligand gated ion channels: medicinal chemistry and molecular biology. *J Med Chem* **43:**1427–1447.

Curthoys IS, Smith PF, and Darlington CL (1988) Postural compensation in the guinea pig following unilateral labyrinthectomy. *Prog Brain Res* **76:**375–384.

Ehrenberger K, Benkoe E, and Felix D (1982) Suppressive action of picrotoxin, a GABA antagonist, on labyrinthine spontaneous nystagmus and vertigo in man. *Acta Otolaryngol* **93:**269–273.

Ehrenberger K and Felix D (1996) Receptor pharmacological models for the therapy of labyrinthine vertigo. *Acta Otolaryngol* **116:**189–191.

Eleore L, Vassias I, Bernat I, Vidal P, and Waele C (2005) An in situ hybridization and immunofluorescence study of $GABA_A$ and $GABA_B$ receptors in the vestibular nuclei of the intact and unilaterally labyrinthectomized rat. *Exp Brain Res* **160:** 166–179.

Giardino L, Zanni M, Fernandez M, Battaglia A, Pignataro O, and Calza L (2002) Plasticity of GABA(a) system during ageing: focus on vestibular compensation and possible pharmacological intervention. *Brain Res* **929:**76–86.

Gilchrist DP, Darlington CL, and Smith PF (1993) Effects of flunarizine on ocular motor and postural compensation following peripheral vestibular deafferentation in the guinea pig. *Pharmacol Biochem Behav* **44:**99–105.

Gliddon CM, Darlington CL, and Smith PF (2005a) GABAergic systems in the vestibular nucleus and their contribution to vestibular compensation. *Prog Neurobiol* **75:**53–81.

Gliddon CM, Darlington CL, and Smith PF (2005b) $GABA_A$ receptor subunit expression in the guinea pig vestibular nucleus during the development of vestibular compensation. *Exp Brain Res*, in press.

Gliddon CM, Sansom AJ, Smith PF, and Darlington CL (2000) Effects of intravestibular nucleus injection of the group I metabotropic glutamate receptor antagonist AIDA on vestibular compensation in guinea pigs. *Exp Brain Res* **134:** 74–80.

Gstoettner W and Burian M (1987) Vestibular nuclear complex in the guinea pig: a cytoarchitectonic study and map in three planes. *J Comp Neurol* **257:**176–188.

Hagg T (1994) Continuous central nervous system infusion with Alzet osmotic pumps, in *Methods in Neuroscience* (Flanagon T, Emerich D, and Winn S eds) pp 201–213, Academic Press Inc., San Diego.

Horii A, Kitahara T, Smith PF, Darlington CL, Masumura C, and Kubo T (2003) Effects of unilateral labyrinthectomy on GAD, GAT1 and GABA receptor gene expression in the rat vestibular nucleus. *Neuroreport* **14:**2359–2363.

Horii A, Masumura C, Smith PF, Darlington CL, Kitahara T, Uno A, Mitani K, and Kubo T (2004) Microarray analysis of gene expression in the rat vestibular nucleus complex following unilateral vestibular deafferentation. *J Neurochem* **91:**975–982.

Howell DC (1992) *Statistical Methods for Psychology*, Wadsworth, Belmont, CA.

Johnston AR, Him A, and Dutia MB (2001) Differential regulation of GABA(A) and GABA(B) receptors during vestibular compensation. *Neuroreport* **12:**597–600.

Kasamatsu T and Schmidt ER (1997) Continuous and direct infusion of drug solutions in the brain of awake animals: implementation, strengths and pitfalls. *Brain Res Brain Res Protoc* **1:**57–69.

Magnusson AK, Lindstrom S, and Tham R (2000) $GABA_B$ receptors contribute to vestibular compensation after unilateral labyrinthectomy in pigmented rats. *Exp Brain Res* **134:**32–41.

Magnusson AK, Ulfendahl M, and Tham R (2002) Early compensation of vestibulo-oculomotor symptoms after unilateral vestibular loss in rats is related to GABA(B) receptor function. *Neuroscience* **111:**625–634.

Martin J, Gilchrist DP, Smith PF, and Darlington CL (1996) Early diazepam treatment following unilateral labyrinthectomy does not impair vestibular compensation of spontaneous nystagmus in guinea pig. *J Vestib Res* **6:**135–139.

Martin JH, Donarummo L, and Hacking A (2000) Impairments in prehension produced by early postnatal sensory motor cortex activity blockade. *J Neurophysiol* **83:**895–906.

McCabe BF, Sekitani T, and Ryu JH (1973) Drug effects on postlabyrinthectomy nystagmus. *Arch Otolaryngol* **98:**310–313.

Mestdagh N and Wulfert E (1999) Bicuculline increases Ca^{2+} transients in rat cerebellar granule cells through non-$GABA_A$ receptor associated mechanisms. *Neurosci Lett* **265:**95–98.

Mettens P, Godaux E, Cheron G, and Galiana HL (1994) Effect of muscimol microinjections into the prepositus hypoglossi and the medial vestibular nuclei on cat eye movements. *J Neurophysiol* **72:**785–802.

Peppard SB (1986) Effect of drug therapy on compensation from vestibular injury. *Laryngoscope* **96:**878–898.

Ris L, Capron B, de Waele C, Vidal PP, and Godaux E (1997) Dissociations between behavioural recovery and restoration of vestibular activity in the unilabyrinthectomized guinea-pig. *J Physiol (Lond)* **500:**509–522.

Seutin V and Johnson SW (1999) Recent advances in the pharmacology of quaternary salts of bicuculline. *Trends Pharmacol Sci* **20:**268–270.

Silva-Barrat C, Champagnat J, Brailowsky S, Menini C, and Naquet R (1989) Relationship between tolerance to $GABA_A$ agonist and bursting properties in neocortical neurons during GABA-withdrawal syndrome. *Brain Res* **498:**289–298.

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

Smith PF, Darlington CL, and Curthoys IS (1986) The effect of visual deprivation on vestibular compensation in the guinea pig. *Brain Res* **364:**195–198.

Vibert N, Beraneck M, Bantikyan A, and Vidal PP (2000) Vestibular compensation modifies the sensitivity of vestibular neurones to inhibitory amino acids. *Neuroreport* **11:**1921–1927.

Woodward RM, Polenzani L, and Miledi R (1993) Characterization of bicuculline/baclofen-insensitive (ρ-like) γ-aminobutyric acid receptors expressed in *Xenopus* oocytes. II. Pharmacology of γ-aminobutyric $acid_A$ and γ-aminobutyric $acid_B$ receptor agonists and antagonists. *Mol Pharmacol* **43:**609–625.

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

Address correspondence to: Dr. P. F. Smith, Vestibular Research Group, Department of Pharmacology and Toxicology, School of Medical Sciences, University of Otago Medical School, University of Otago, Dunedin, New Zealand. E-mail: paul.smith@stonebow.otago.ac.nz