

Effects of chronic infusion of a GABA<sub>A</sub> receptor agonist or antagonist into the vestibular nuclear complex on vestibular compensation in the guinea pig.

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Running title page

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Non-standard abbreviations

MVN = medial vestibular nucleus

SN = spontaneous nystagmus

THIP = 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol hydrochloride

RHT = roll head tilt

YHT = yaw head tilt

VNC = vestibular nuclear complex

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## Abstract

The aim of the experiment was to determine the effects of chronic infusion of a GABA<sub>A</sub> receptor agonist/antagonist into the ipsilateral or contralateral vestibular nuclear complex (VNC) on vestibular compensation, the process of behavioural recovery that occurs following unilateral vestibular deafferentation (UVD). This was achieved by an mini-osmotic pump that infused, over 30 hs, 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 hs 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<sub>A</sub> receptor-mediated and the UVD-induced decrease in resting activity.

## Introduction

$\gamma$ -Aminobutyric acid (GABA) mediates inhibitory synaptic transmission via its interaction with GABA<sub>A</sub>, GABA<sub>B</sub>, and GABA<sub>C</sub> receptors (for review Chebib and Johnston, 2000). GABA<sub>A</sub> and GABA<sub>B</sub> receptors are involved in the vestibular reflex pathways, and it has been suggested that these receptors are involved in the behavioural recovery process that occurs following the loss of sensory input from one vestibular labyrinth (i.e., vestibular compensation) (for review Gliddon et al., 2005, in press). This loss of sensory input (unilateral vestibular deafferentation (UVD)) results in a severe ocular motor and postural syndrome that compensates over time (for review, 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 behavioural 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.*, 2005, in press). 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<sub>A</sub> or GABA<sub>B</sub> receptors. This paper will focus on the GABA<sub>A</sub> receptor.

Clinical evidence suggests that GABA<sub>A</sub> 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) (see Peppard, 1986; Martin et al., 1996 for conflicting data). Magnusson *et al* (2000, 2002) have reported complex effects of systemic injections of the partial GABA<sub>A</sub> 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<sub>A</sub> receptor agonists or antagonists on postural compensation during the early stages of vestibular compensation. However, during 6-144 days post-UVD, it has been demonstrated that muscimol injections could not alter the chronic residual head deviation towards 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 [<sup>3</sup>H]-flunitrazepam binding sites in the ipsilateral and contralateral VNCs, respectively (Calza et al., 1989; Calza et al., 1992; Vibert et al., 2000; Yamanaka et al., 2000). Late compensation is associated with no change in the muscimol or [<sup>3</sup>H]-flunitrazepam binding sites (Calza et al., 1989; Calza et al., 1992; Johnston et al., 2001; Giardino et al., 2002). Results from PCR studies suggest that there is a significant increase in the GABA<sub>A</sub> receptor  $\alpha_1$  subunit mRNA expression in the ipsilateral VNC compared to the contralateral VNC, at 6 hs, but not at 50 hs post-UVD (Horii et al., 2003). Microarray techniques have demonstrated a change in the rho 2 subunit of the GABA<sub>A</sub> receptor at 6 hs post-UVD (Horii et al., 2004). In contrast, Eleore *et al.*, (2005) reported no significant difference in  $\alpha_1$ ,  $\alpha_5$ ,  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ , and  $\gamma_2$  mRNA expression between the ipsilateral and contralateral medial vestibular nuclei at 5 hs, 1, 3, and 8 days post-UVD. Compensation is not associated with changes in the protein expression of the  $\alpha_1$ ,  $\beta_1$ ,  $\beta_2$ , and  $\gamma_2$  subunits (Eleore et al., 2005; Gliddon et al., 2005, submitted).

The limitation of the behavioural 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 behavioural studies have used THIP and diazepam, and neither are full GABA<sub>A</sub> 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<sub>A</sub> 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<sub>A</sub> receptors on the compensation of SN, yaw head tilt (YHT), and roll head tilt (RHT) in guinea pig.

## Methods

### *Subjects*

Data were obtained from a total of 92 male pigmented guinea pigs (250-350g) (*Cavia porcellus*). Animals were divided into UVD and labyrinthine-intact groups (Table 1). It was possible that the behavioural effects observed with the GABA<sub>A</sub> receptor agonist/antagonist were not due to the drugs interacting with the neurochemical mechanisms of vestibular compensation, but solely due to their effects on the vestibulo-ocular and -spinal reflex pathways. To determine if this was the case, the highest dose of the GABA<sub>A</sub> 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 Number 29/02.

### *Surgical procedures*

All surgical procedures were performed under strict aseptic conditions. Lidocaine hydrochloride (1%) with 1 in 100,000 adrenaline (XYLOCAINE, AstraZeneca) was injected into all wound margins and pressure points. Wounds were sutured and covered with antibiotic cream (2% w/w mupirocin (BACTROBAN, SmithKline Beecham)). 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 anaesthetized with 0.4 ml/kg, i.m of fentanyl citrate 0.4 mg/ml, azaperone 3.2 mg/ml and xylazine hydrochloride 58.3 mg/ml (FENTAZIN, Parnell, 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 hs post-cannulation, 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 sec.; RHT < 10°; YHT < 10°), they were included in the study.

Seven days later, in both the UVD and labyrinthine-intact groups, a mini-osmotic pump (Alzet, model 1003D, pumping rate  $1 \pm 0.15$   $\mu$ l/h) was implanted between the shoulder blades under isoflurane anaesthesia. 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 following mini-osmotic pump implantation, a surgical UVD was performed. The UVD was performed in accordance with the procedure of Smith *et al* (Smith et al., 1986). Briefly, with the aid of an operating microscope (Zeiss, OPM 199), a high speed dental drill was used to expose the ampullae of the horizontal and anterior semi-circular 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<sub>A</sub> receptor agonist, was used due to its higher potency for the GABA<sub>A</sub> receptor compared to isoguvacine; also, it is not a partial GABA<sub>A</sub> receptor agonist like THIP and piperidine-4-sulfonic acid (Woodward et al., 1993; Chang et al., 2000). All of the available GABA<sub>A</sub> receptor agonists interact with the GABA<sub>C</sub> receptor and muscimol is also a GABA<sub>C</sub> 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  $\mu\text{g/h}$ ) that induces a 'GABA withdrawal syndrome' characterised 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<sub>A</sub> receptors as a consequence of prolonged GABA infusion (Silva-Barrat et al., 1989). Muscimol is four times more potent than GABA (Woodward et al., 1993; Chang et al., 2000), and therefore the dose was kept below 250  $\mu\text{g/h}$  to avoid possible changes in GABA<sub>A</sub> 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 if this hypothesis was correct without causing distress to the animal. A pilot study used high doses of muscimol (i.e., 4.2  $\mu\text{g/h}$  and 41.6  $\text{ng/h}$ ); however the animals were clearly distressed and therefore the doses were reduced to 2.1, 4.2, and 8.3  $\text{ng/h}$ . These doses correspond to a total final dose of 75, 125 and 250  $\text{ng}$  of muscimol for the 30  $\text{hs}$  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  $\text{ng}/30 \text{ hs}$ . This dose did not alter compensation and therefore higher doses were used (i.e., 500 and 750  $\text{ng}/30 \text{ hs}$ ).

As recommended by Seutin & Johnson (1999), neither bicuculline nor its salts were used due to their non-selectivity; nor was picrotoxin used due to its action on the glycine receptor (Chattipakorn and McMahon, 2002). Gabazine (SR 95531 (2-(3'-carboxy-2'-propyl)-3-amino-6-*p*-methoxyphenylpyridazinium bromide)) was chosen, however, gabazine is also a weak GABA<sub>C</sub> 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 hs). 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 hs). On the contralateral side, the 125 ng/30 hs dose had no effect, and therefore the doses were increased to 375 and 750 ng/30 hs.

Muscimol and gabazine (Tocris Cookson Ltd, UK) were dissolved in a solution of NaOH (100 mM) or deionised water. The drugs were aliquoted out, and then frozen at -20°C and defrosted when required. Both drugs were diluted to the required concentration using modified cerebrospinal fluid (in mM: NaCl 124 mM, KCl 5 mM, KH<sub>2</sub>PO<sub>4</sub> 1.2 mM and MgSO<sub>4</sub> 1.3 mM) (Gliddon et al., 2000). Drug solutions were tested for pH and changed to a pH of 7.0 if necessary.

Muscimol or gabazine were infused into either the ipsilateral or contralateral VNC via an osmotic mini-pump for the first 30 hs post-UVD. The osmotic mini-pump assembly was modified from the method suggested by Hagg (Hagg, 1994). A coiled catheter of polyethylene tubing (ID = 0.75; OD = 1.45 mm) 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.

### ***Behavioural observations***

Behavioural observations were made without restraining the animal because restraint can induce stress that can alter SN frequency. All behavioural observations were recorded by a Panasonic (model NV-MO50) video camera with a zoom lens. The observations were

replayed through a Mitsubishi (model HS-641V) video recorder to a Sony Trinitron colour 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 sec 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, non-invasive, and accurate method of quantifying SN with an estimated measurement error of  $\pm 1$  beat/15 sec (Gilchrist et al., 1993). SN was counted visually five times. The measurements were made whilst 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 centre 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 centre 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 video cameras 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 abovementioned parameters is  $\pm 10$  degrees (Gilchrist et al., 1993). YHT and RHT were measured once at each individual observational time.

To monitor the possible effects of the GABA<sub>A</sub> 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 following an ipsilateral UVD) or a negative

value if the head was directed towards 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° degrees 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 ANOVA with repeated measures.

This experiment was not conducted blind. While 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<sub>A</sub> receptors using chronic injections of GABA<sub>A</sub> receptor ligands into the VNC, the outcome of the experiment could not be predicted.

### ***Cardiac perfusion and histology***

Following the last observation at 50 hs post-UVD, the animals were anaesthetised with sodium pentobarbitone (30 mg/kg, i.p: PENTOBARB 300, National Veterinary Suppliers Ltd) 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 behavioural data***

In all behavioural experiments the data obtained were analysed using a two factor analysis of variance (ANOVA) with repeated measures. The advantage of this statistical test is that it can detect the following: the effect of the treatment on the severity of a symptom, independent of time (factor A); change in the severity of a symptom over time and independent of the treatment (factor B); and the 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). The Mauchley's Test of Sphericity was also performed to determine if 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 Scheffe *post hoc* test was performed. The significance level was set at 0.05 for all comparisons. Statistical analysis and graphs were obtained using the statistical and graphic software packages, SPSS and Prism, 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 hs.

### *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 NaOH) were infused into the ipsilateral VNC for the first 30 hs post-UVD (Fig 2). There was no significant effect on SN frequency ( $p > 0.05$ ), nor on its rate of compensation ( $p > 0.05$ ). At 30 hs, the pump was disconnected and SN frequency was measured at 31, 33, and 50 hs post-UVD. At 30 hs post-UVD, minimal SN ( $< 2$  beats/15 sec.) was exhibited by all experimental groups. Discontinuation of the drug treatment did not increase the SN frequency at 31, 33, and 50 hs post-UVD in any of the experimental groups.

Three doses of gabazine (31.25, 62.5, 125 ng) or its vehicle ( $H_2O$ ) were infused into the ipsilateral VNC for the first 30 hs 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 hs, there was a significant increase in the mean SN frequency in the 31.25 ng gabazine group (19.2 beats/15 sec.  $\pm$  0.4) compared to the 62.5 ng gabazine group (7.8 beats/15 sec.  $\pm$  0.6). Furthermore, at 10 hs post-UVD, there was a significant increase in the mean SN frequency in the 31.25 ng gabazine- group (14.7 beats/15 sec.  $\pm$  2.2) compared to the vehicle group (6.6 beats/15 sec.  $\pm$  1.1). At 30 hs post-UVD, minimal SN ( $> 2$  beats/15 sec) was exhibited by all experimental groups. Discontinuation of the drug treatment did not increase the SN frequency at 31, 33, and 50 hs post-UVD in any of the experimental groups.

Three doses of muscimol (250, 500, and 750 ng) or its vehicle (8.1 ng NaOH) were infused into the contralateral VNC for the first 30 hs 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 hs post-UVD, there was a significant decrease in the SN frequency exhibited by the 750 ng gabazine group (4.7 beats/15 sec  $\pm$  2) compared to the vehicle group (20.5 beats/15 sec  $\pm$  5). At 30 hs post-UVD, there was minimal SN ( $> 3.5$  beats/15 sec) exhibited by all experimental groups. Discontinuation of the drug treatment did not increase the SN frequency at 31, 33, and 50 hs 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 hs post-UVD (Fig 2). There was no significant effect on SN frequency ( $p > 0.05$ ), nor its rate of compensation ( $p > 0.05$ ). At 30 hs the pump was disconnected and SN frequency was measured at 31, 33, and 50 hs post-UVD. At 30 hs post-UVD, minimal SN ( $> 5$  beats/15 sec) was exhibited by all experimental groups. Discontinuation of the drug treatment did not increase the SN frequency at 31, 33, and 50 hs 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 NaOH) were infused into the ipsilateral VNC for the first 30 hs 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 ( $40.6^\circ \pm 7.7$ ) compared to the 62.5 ng muscimol group ( $8.6^\circ \pm 6.1$ ) at 20 hs post-UVD. Furthermore, there was a significant increase in the mean YHT exhibited by the 250 ng muscimol group ( $50.7^\circ \pm 3.9$ ) compared to the vehicle group ( $4.6^\circ \pm 3.9$ ) at 30 hs post-UVD. At 30 hs post-UVD, there was a significant increase in the mean

YHT exhibited by the 250 ng muscimol group compared to the vehicle group. Discontinuation of the drug treatment at 30 hs post-UVD resulted in a reduction of the mean YHT exhibited by the 125 and 250 ng muscimol-treated groups, so that by 33 hs post-UVD, all experimental groups exhibited similar YHT.

Three doses of gabazine (31.25, 62.5, and 125 ng) or its vehicle (H<sub>2</sub>O) were infused into the ipsilateral VNC for the first 30 hs 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 to the vehicle group at 5 (i.e.,  $38.8^\circ \pm 5.7$  vehicle compared to  $-11.6^\circ \pm 10.7$  250 ng gabazine) and 10 (i.e.,  $27.4^\circ \pm 7.9$  vehicle compared to  $-4.6^\circ \pm 5.4$  250 ng gabazine) hs post-UVD. Furthermore, there was a significant decrease in the mean YHT exhibited by the 250 ng gabazine group ( $-8.8^\circ \pm 6.4$ ) compared to the 31.25 ng gabazine group ( $21.4 \pm 4.5$ ) at 15 hs post-UVD. Discontinuation of the drug treatment at 30 hs post-UVD, resulted in the mean YHT of the 250 ng gabazine group ( $-11^\circ \pm 8.6$  at 30 hs post-UVD) returning to positive values ( $3.1^\circ \pm 4.1$  at 31 hs post-UVD). By 33 hs, 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 NaOH) were infused into the contralateral VNC for the first 30 hs 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 decrease in the mean YHT exhibited by the 750 ng muscimol group compared to the vehicle group at 5 (i.e.,  $58.4^\circ \pm 5.3$  vehicle compared to  $-6^\circ \pm 9.5$  750 ng muscimol group) and 15 (i.e.,  $26.6^\circ \pm 6.4$  vehicle group compared to  $0.8^\circ \pm 3.7$  750 ng muscimol group) hs post-UVD. Furthermore, there was a significant decrease in the mean YHT exhibited by the 750 ng muscimol group ( $-8.2^\circ \pm 6.7$ ) compared to the 250 ng muscimol group ( $23.5^\circ \pm 11$ ) at 30 hs post-UVD. Discontinuation of



the drug treatment at 30 hs post-UVD, resulted in the mean YHT of the 750 ng muscimol group ( $-3.2^{\circ} \pm 5.5$  at 30 hs post-UVD) returning to positive values (i.e.,  $4.2^{\circ} \pm 1.1$  at 31 hs post-UVD). By 33 hs, 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 hs post-UVD (Fig. 3). There was, however, no significant effect on YHT ( $p > 0.05$ ), nor its rate of compensation ( $p > 0.05$ ). Discontinuation of the drug treatment at 30 hs post-UVD, did not alter the severity of YHT. By 50 hs 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 NaOH) were infused into the ipsilateral VNC for the first 30 hs post-UVD (Fig. 4). There was no significant effect on RHT ( $p > 0.05$ ), nor its rate of compensation. Discontinuation of the drug treatment at 30 hs 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 hs 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 groups compared to the vehicle group at 5 (i.e.,  $28.6^{\circ} \pm 3.2$  vehicle compared to  $-6.6^{\circ} \pm 6.7$  250 ng gabazine), 10 (i.e.,  $25.4^{\circ} \pm 4.3$  vehicle compared to  $-7.6^{\circ} \pm 7.8$  250 ng gabazine), 20 (i.e.,  $22.8^{\circ} \pm 5.5$  vehicle compared to  $-5.8^{\circ} \pm 7.1$  250 ng gabazine), 25 (i.e.,  $22.8^{\circ} \pm 7.1$  vehicle compared to  $-4.8^{\circ} \pm 7.5$  250 ng gabazine), and 30 (i.e.,  $18.0^{\circ} \pm 4$  vehicle compared to  $-4.8^{\circ} \pm 6.8$  250 ng

gabazine) hs post-UVD. Furthermore, there was a significant decrease in the mean RHT exhibited by the 250 ng gabazine group compared to the 31.25 ng gabazine group at 5 and 10 hs post-UVD. Discontinuation of the drug treatment at 30 hs post-UVD, resulted in the mean RHT of the 250 ng gabazine group (i.e.,  $-4.8^{\circ} \pm 6.8$  at 30 hrs post-UVD) returning to positive values (i.e.,  $3.6^{\circ} \pm 1.6$  at 33 hrs post-UVD). By 50 hs, 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 NaOH) were infused into the contralateral VNC for the first 30 hs 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 to the vehicle group at 1 ( $67.8^{\circ} \pm 28.3$  vehicle compared to  $0.5^{\circ} \pm 6.7$  750 ng muscimol), 5 ( $38.4^{\circ} \pm 5.1$  vehicle compared to  $-1.3^{\circ} \pm 5.3$  750 ng muscimol), 10 ( $31.0^{\circ} \pm 5.7$  compared to  $-1.3^{\circ} \pm 2.8$  750 ng muscimol), 15 ( $27.1^{\circ} \pm 3.0$  vehicle compared to  $3.1^{\circ} \pm 2.4$  750 ng muscimol), 20 ( $23.0^{\circ} \pm 5.2$  vehicle compared to  $-2.9^{\circ} \pm 4.0$  750 ng muscimol), 25 ( $25.2^{\circ} \pm 7.2$  vehicle compared to  $-0.7^{\circ} \pm 1.9$  750 ng muscimol), and 30 ( $22.4^{\circ} \pm 4.6$  vehicle compared to  $-3.2^{\circ} \pm 5.5$  750 ng muscimol) hs post-UVD. Furthermore, there was a significant decrease in the mean RHT exhibited by the 500 ng muscimol group compared to the vehicle group at 5 ( $-9.4^{\circ} \pm 3.7$  500 ng muscimol), 10 ( $8.3^{\circ} \pm 2.5$  500 ng muscimol) and 15 ( $9.4^{\circ} \pm 7.2$  500 ng muscimol) hs post-UVD. Discontinuation of the drug treatment at 30 hs post-UVD, resulted in the mean RHT of the drug treated groups returning to vehicle treated values so that by 50 hs, 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 hs post-UVD (Fig. 4). There was no significant effect on RHT ( $p > 0.05$ ), nor its rate of compensation ( $p > 0.05$ ). At 30 hs the pump was

disconnected and RHT was measured at 31, 33, and 50 hs post-UVD. Discontinuation of the drug treatment at 30 hs post-UVD, resulted in the mean RHT of the drug treated groups returning to vehicle treated values so that by 50 hs, the magnitude of the mean RHT was similar between all experimental groups.

***Effects of the infusion of a GABA<sub>A</sub> receptor agonist/antagonist on ocular-motor and postural reflexes in labyrinthine-intact animals***

It is possible that the behavioural effects observed with the GABA<sub>A</sub> receptor ligands were not due to the drugs interacting with the neurochemical mechanisms of vestibular compensation, but solely due to their effects on the vestibulo-ocular and -spinal pathways. To determine if 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 to both the vehicle and the gabazine groups at 1, 5, 10, 15, and 20 hs, but not at 25 or 30 hs, post-pump connection. Chronic infusion of muscimol did induce RHT in labyrinthine-intact animals, however, the degree of RHT was not significantly different compared to the gabazine or vehicle groups, nor did it change over time ( $p > 0.05$ ). Only one animal, at 10 hs 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 hs the pump was disconnected and SN, YHT, and RHT were measured at 31, 33, and 50 hs post-pump connection. Discontinuation of the drug treatment resulted in the 750 ng muscimol group exhibiting no SN, YHT or RHT.

## Discussion

Vestibular compensation is defined as the reduction in the severity of the ocular motor and postural symptoms over time following 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 point of view, 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<sub>A</sub> 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<sub>A</sub> receptor agonists/antagonists modify the resting activity of the VNC neurons (for a review Gliddon *et al.*, in press, 2005). 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, provides information as to which VNC has the lowest resting activity. For example, a right UVD as employed 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 towards 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 if 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 in the Methods section, 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 10 hs post-pump connection, suggesting that these doses do not generate SN. Overall, the results suggest that GABA<sub>A</sub> 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<sub>A</sub> 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<sub>A</sub> receptor ligands on eye movement in labyrinthine-intact animals. Depending on the site of injection within the VNC, GABA<sub>A</sub> 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 towards 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 could be due to the complex effect on GABA<sub>A</sub> receptors within the circuitry responsible for generating eye movement. The method employed in this study to measure SN did not allow for the determination of whether the GABA<sub>A</sub> receptor ligands were inducing neural integrator failure.

The results suggest that the GABA<sub>A</sub> 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 which tilted away from the side of

the UVD. The extent of the head tilt away from the lesion was not large, but suggests that the infusion of these GABA<sub>A</sub> 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 hs post-UVD. At 30 hs 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<sub>A</sub> receptor ligands than VNC neurons from UVD animals.

The results observed are most likely due to muscimol/gabazine acting on GABA<sub>A</sub> 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 & Schmidt, 1997). If a proportion of the dose of muscimol/gabazine reached an adjacent brainstem structure, it would therefore be at smaller concentration.

The results of this study suggest that the GABA<sub>A</sub> 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<sub>A</sub> receptor-mediated and the UVD-induced decrease in resting activity.

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#### Footnotes

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### Legends for Figures:

**Fig. 1:** Schematic illustration of the cannula tip lesion sites in the ipsilateral and contralateral VNC for all experimental groups.

The left figure illustrates the cannula tip lesion sites in the contralateral and ipsilateral VNCs (left and right, respectively) for the vehicle (black dots), 62.5 ng (dark grey dots), 125 ng (white dots), 250 ng (black spotted dots), 500 ng (light grey dots), or 750 ng (dark grey and white dots) muscimol groups during the first 30 hs post-UVD. The middle figure illustrates the cannula tip lesion sites in the ipsilateral and the contralateral VNCs for the vehicle (black dots), 31.25 ng (dark grey dots), 62.5 ng (white dots), 125 ng (spotted dots), 375 ng (light grey dots), and 750 ng (dots with vertical lines) gabazine groups during the first 30 hs post-UVD. The right figure illustrates the cannula tip lesion sites in the VNC for the vehicle (black dots), 750 ng muscimol (dark grey 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). Abbreviations: 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 NaOH) were infused into the ipsilateral VNC during the first 30 hs post-UVD (Graph 1A). There was no significant effect on SN frequency ( $p > 0.05$ ), nor 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 hs post-UVD (Graph 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 NaOH) were infused into the contralateral VNC for the first 30 hs post-UVD (Graph 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 hs post-UVD (Graph 2B). There was no significant effect on SN frequency ( $p > 0.05$ ), nor its rate of compensation ( $p > 0.05$ ). Data are expressed as mean  $\pm$  SEM.

**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 NaOH) were infused into the ipsilateral VNC during the first 30 hs post-UVD (Graph 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 hs post-UVD (Graph 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 NaOH) were infused into the contralateral VNC for the first 30 hs 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 hs post-UVD (Graph 2B). There was no significant effect on YHT ( $p > 0.05$ ), nor its rate of compensation ( $p > 0.05$ ) (Graph 2B). Data are expressed as mean  $\pm$  SEM.

**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 NaOH) were infused into the ipsilateral VNC during the first 30 hs post-UVD (Graph 1A). There was no significant effect on RHT ( $p > 0.05$ ), nor 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 hs post-UVD (Graph 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 hs 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 hs post-UVD (Graph 2B). There was no significant effect on RHT ( $p > 0.05$ ), nor its rate of compensation ( $p > 0.05$ ). Data are expressed as mean  $\pm$  SEM.

**Fig. 5:** Effects of chronic muscimol or gabazine infusion on the expression of SN, YHT, and RHT in labyrinthine-intact animals.

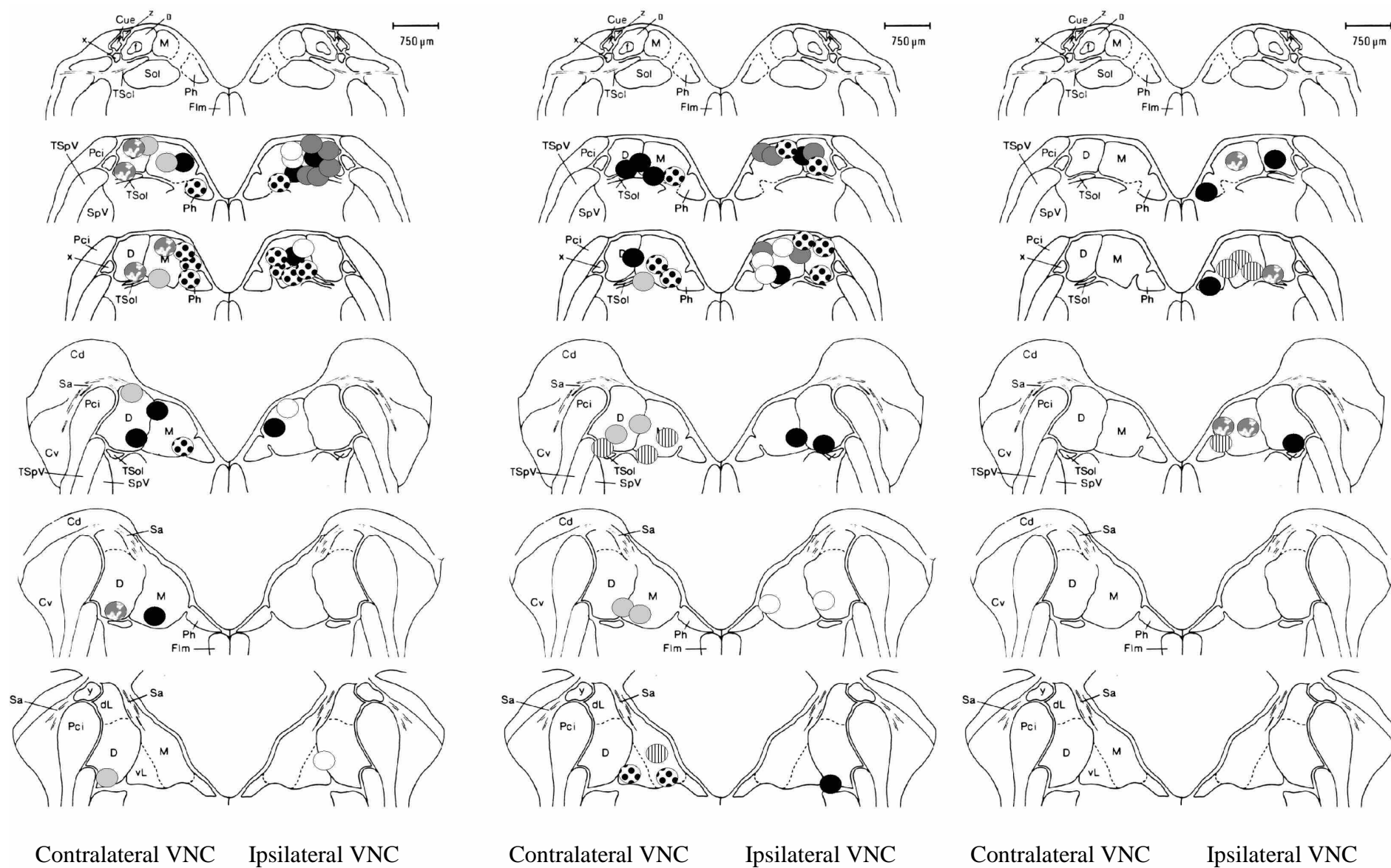
The effects of 750 ng muscimol ( $n = 4$ ), 750 ng gabazine ( $n = 4$ ), and 8.1 ng NaOH ( $n = 4$ ) infusion over 30 hs on the expression of SN (upper graph), YHT (middle graph) and RHT (lower graph) in labyrinthine-intact animals. Only the infusion of 750 ng muscimol over 30 hs could induce YHT and RHT; SN was exhibited by 1 animal at 10 hs post-pump connection.

TABLE 1: Experimental groups

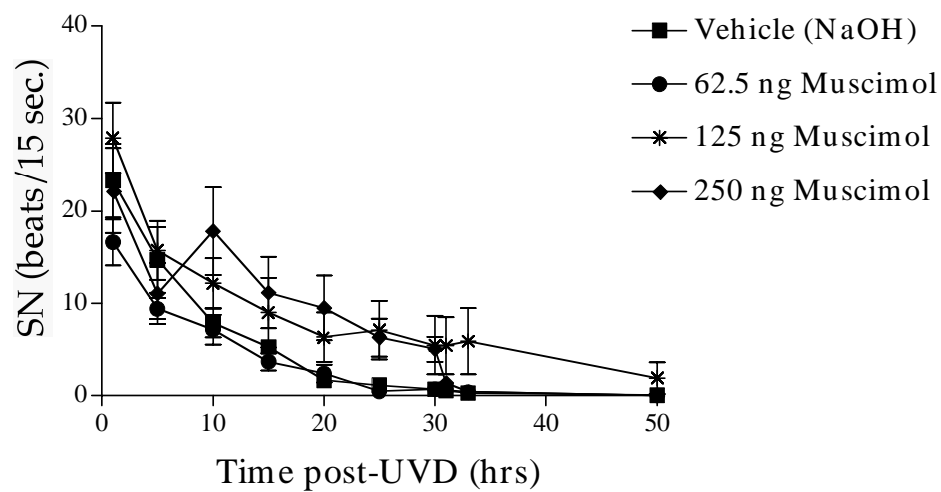
Animals were allocated to two experimental groups: (A) UVD and (B) labyrinthine-intact.

Group Number	Cannula site	Drug and total dose over the 30 hrs infusion period	Sample size
A1	Ipsilateral VNC	75 ng muscimol	5
A2	Ipsilateral VNC	125 ng muscimol	5
A3	Ipsilateral VNC	250 ng muscimol	5
A4	Ipsilateral VNC	2 ng NaOH	5
A5	Ipsilateral VNC	31.25 ng gabazine	5
A6	Ipsilateral VNC	62.5 ng gabazine	5
A7	Ipsilateral VNC	125 ng gabazine	5
A8	Ipsilateral VNC	Deionised water	5
A9	Contralateral VNC	250 ng muscimol	5
A10	Contralateral VNC	400 ng muscimol	5
A11	Contralateral VNC	750 ng muscimol	5
A12	Contralateral VNC	8.1 ng NaOH	5
A13	Contralateral VNC	125 ng gabazine	5
A14	Contralateral VNC	375 ng gabazine	5
A15	Contralateral VNC	750 ng gabazine	5
A16	Contralateral VNC	Deionised water	5
B1	Ipsilateral VNC	750 ng muscimol	4

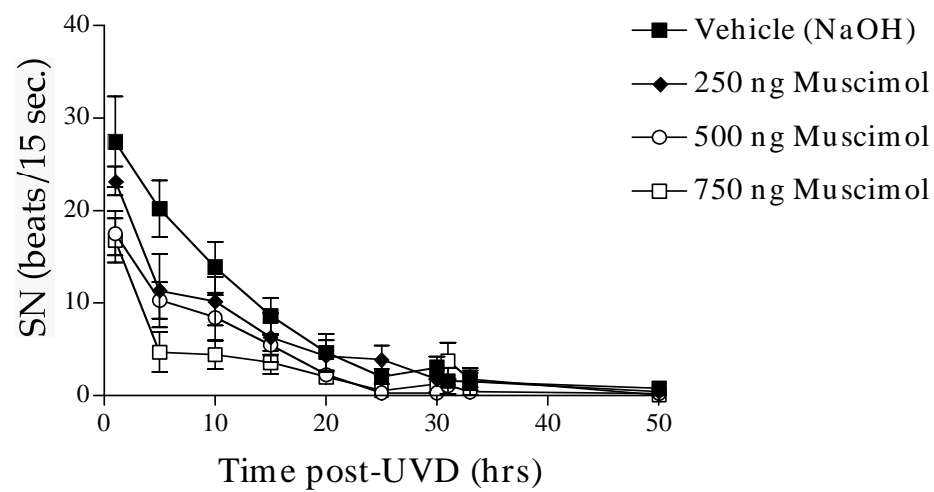
B2	Ipsilateral VNC	8.1 ng NaOH	4
B3	Ipsilateral VNC	750 ng gabazine	4



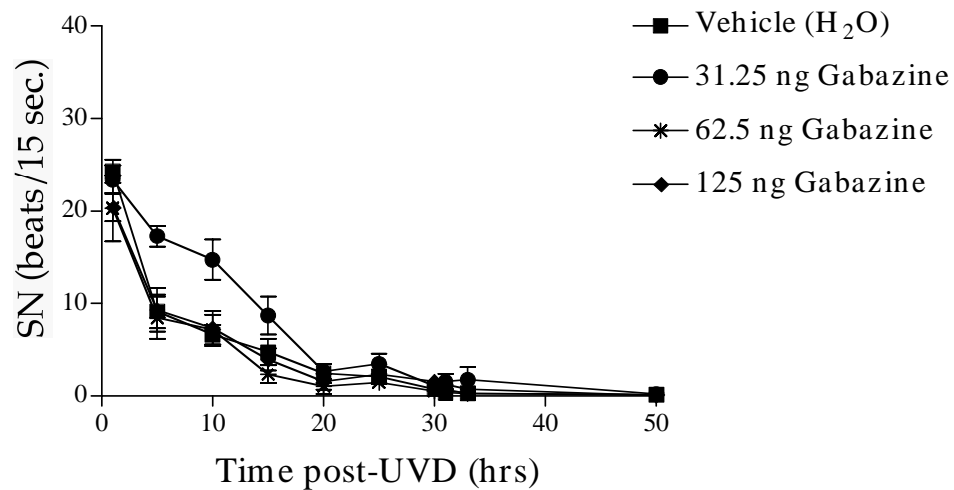
**1A**



**1B**



**2A**



**2B**

