An Increase in Glycinergic Quantal Amplitude and Frequency During Early Vestibular Compensation in Mouse

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INTRODUCTION

Vestibular compensation is a form of adult neuronal plasticity that occurs in response to the unilateral damage or destruction of the peripheral vestibular organs. Initially, a broad set of behavioral symptoms follow unilateral labyrinthectomy (UL), including head tilt and postural deficits toward the lesioned side, spontaneous nystagmus, and asymmetry of the vestibulococular reflex (VOR). Typically static symptoms that occur in the absence of movement, such as head tilt and spontaneous nystagmus, abate within 1 wk (Darlington and Smith 2000). However, dynamic symptoms such as asymmetry in the VOR have a longer time course of recovery and are never fully restored (Aleisa et al. 2007; Maioli et al. 1983; Newlands et al. 2005).

The central mechanisms that underlie vestibular compensation are poorly understood. In vivo studies have shown in the acute phase of vestibular compensation, immediately after UL, neurons in the ipsilesional medial vestibular nucleus (MVN) become silent, whereas contralesional MVN neurons have discharge rates higher than those of control (Ris et al. 1995; Shimazu and Precht 1965; Smith and Curthoys 1988a,b). Under normal conditions, type I MVN neurons are inhibited during contralateral horizontal rotation. Contralesional neurons of the commissural pathway are responsible for this inhibition (Kasahara et al. 1968; Mano et al. 1968; Shimazu and Precht 1966). Immediately after UL, the discharge rate of type I ipsilesional MVN neurons is diminished due to both the removal of primary vestibular input and increased inhibition from contralateral commissural neurons (Markham et al. 1977). Approximately 2–3 days after UL, the discharge rates between ipsi- and contralateral sides are comparable and by 1 wk, discharge rates on both sides are equivalent to those prior to ablation (Ris et al. 1995; Smith and Curthoys 1988a,b). It has been suggested that restoration of pre-UL discharge rates may be mediated, in part, by mutually inhibitory commissural connections between the bilateral MVNs (Bergquist et al. 2008; Galliana et al. 1984).

Inhibition in the vestibular nucleus arises from commissural fibers (Precht et al. 1973), cerebellum (Bagnall et al. 2009; de Zeeuw and Berrebi 1996), and inhibitory interneurons (Holstein et al. 1999b). Inhibitory commissural neurons connecting the two MVNs are GABAergic and glycinergic (Bagnall et al. 2007; Holstein et al. 1999a; Precht et al. 1973); thus the role of inhibitory receptors and their transmitters during vestibular compensation has been investigated using a variety of techniques with inconsistent results. In situ hybridization, Western blots, and immunohistochemistry in rats have shown no changes in γ-aminobutyric acid types A and B (GABA_A, GABA_B) or glycine receptor expression levels after UL (Eleore et al. 2004, 2005; Gliddon et al. 2005a). Contrary evidence, however, suggests enhanced GABA transmission such as increases in mRNA of the α1 subunit of the GABA_A receptor and for GABA precursor enzymes, GAD65 and 67, after UL (Horii et al. 2003). Pharmacological studies show modified sensitivity of discharge rate to inhibitory amino acid agonists and antagonists during vestibular compensation (Johnston et al. 2001; Vibert et al. 2000; Yamanaka et al. 2000). Furthermore, elevated GABA release after UL has been reported by microdialysis (Bergquist et al. 2008), as well as increases in the number of GABA immunoreactive neurons in the MVN (Tighilet and Lacour 2001). In this study we aim to resolve the discrepant role of fast inhibitory transmission after UL, by investigating synaptic GABA_A and glycine receptors in ipsi- and contralateral MVN neurons at three time points after UL. Our results show evidence of changes in both presynaptic and postsynaptic inhibitory glycinergic synaptic transmission that occur during early vestibular compensation.

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METHODS

Unilateral labyrinthectomy

All experiments were conducted according to The University of Newcastle Animal Care and Ethics Committee Regulations. Prior to surgery, mice were habituated to jelly pellets containing pain medication, buprenorphine (Reckitt and Colman Pharmaceuticals, Sydney, NSW, Australia). A previous study in rodents has shown that inclusion of sublingual buprenorphine into jelly cubes is an effective, noninvasive means of analgesic delivery (Volker et al. 2000).

Forty-four mice (C57BL6) approximately 4 wk old of both sexes underwent UL to the left ear in a procedure previously described by Gacek and Khertarpal (1998). Briefly, under 2.5% isoflurane anesthesia, a subcutaneous injection of carprofen (rimadyl; Novartis, 2.5 mg/kg) was administered to minimize inflammation and pain responses. A postauricular incision was made to expose the external ear canal. The tympanic membrane was ruptured and the malleus and incus were removed. A small corneal rust ring remover with 0.5 mm burr (Algerbrush II; Alger Equipment, Lago Vista, TX) was used to remove the promonotory region of the tympanic bone, thereby better exposing the stapes and the intersecting stapedial artery. The stapedial artery was coagulated on either side of the footplate using a unipolar cautery. The stapes was then removed using fine forceps and a hook was inserted into the oval window to out-fracture the bone. Forceps were used to remove the exposed horizontal and anterior ampullae and a right-angled hook was used to disrupt the remaining vestibular organs. The vestibule was filled with Gelfoam and the surgical incision closed using interrupted sutures. Subcutaneous injections of buprenorphine (0.05 mg/kg) and lactated Ringer solution (0.5–1 ml) were administered 30 min prior to the removal of isoflurane anesthesia. Mice recovered quickly from anesthesia and immediately displayed symptoms consistent with damage to the peripheral organs, including head tilt, circling, and barrel rolling (Gacek and Khertarpal 1998). In all cases, a postmortem examination of the bony labyrinth confirmed horizontal and anterior ampullae destruction and that Gelfoam had filled much of the vestibular labyrinth.

In vitro slice preparation

Three time points were investigated after UL: 4 hours, 2 days, and 7 days. Mice were injected with an intraperitoneal injection of ketamine (150 mg/kg) and decapitated. The head was placed in ice-cold sucrose-based Ringer solution containing (in mM): 236 sucrose, 25 NaHCO3, 11 glucose, 2.5 KCl, 1 NaH2PO4, 1 MgCl2, and 2.5 CaCl2 (Camp et al. 2006). The brain stem containing MVN was dissected as previously described (Camp et al. 2006). Coronal sections (300 μm thick) containing MVN were cut using a Leica microtome (VT1000S). We made a small nick to the right ventral side of the section with micro scissors to identify the contralesional side. Sections were then transferred to a humidified oxygenated incubation chamber containing normal Ringer solution identical to the sucrose-based Ringer solution described earlier, except 118 mM NaCl was substituted for sucrose. Sections were incubated for 1 h prior to recording.

Electrophysiology

Voltage-clamp recordings of miniature inhibitory postsynaptic currents (mIPSCs) were made from MVN neurons that were located within the rostral half of the nucleus. They were visualized using infrared differential interference contrast optics. Observations were made at room temperature (23–25°C) on slices that were continually superfused with normal Ringer solution oxygenated with carbogen (95% O2,5% CO2). Room temperature was selected so that direct comparison could be made with data previously reported by this laboratory (Camp et al. 2006) and with the majority of other studies of inhibitory postsynaptic quantal currents, also recorded at room temperature (21–23°C) (Graham et al. 2006; Lim et al. 1999; Lu et al. 2008; O’Brien and Berger 1999; also see Discussion). Patch electrodes (borosilicate glass; 3–5 MQ) were filled with a CsCl-based internal solution containing (in mM): CsCl 130, MgCl2 1, EGTA 10, HEPES 10, ATP-Mg 2, and GTP-Na 0.2. To isolate inhibitory mIPSCs, antagonists of sodium-dependent action potentials (tetrodotoxin [TTX]; 1 μM; Alomone, Jerusalem, Israel) and glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (6-cyano-7-nitroquinoxaline-2,3-dione [CNQX]; 10 μM; Sigma–Aldrich, Castle Hill, NSW, Australia) were added to the perfusate. Drugs were applied for a minimum of 3 min prior to data acquisition. Neurons were held at a membrane potential of −70 mV; thus the normal Mg2+ block of the N-methyl-D-aspartate (NMDA) receptor prevents activation of NMDA receptors from contributing to synaptic events. GABAergic mIPSCs were isolated by the addition of the glycine receptor antagonist, strychnine (1 μM; Sigma–Aldrich). In experiments isolating glycineergic mIPSCs, bicuculline (5 μM; Tocris Bioscience, Bristol, UK) was added to the TTX and CNQX perfusate. In some cells, the frequency of glycineergic mIPSCs was low. If in the presence of bicuculline, the frequency was <0.03 Hz (<1 event per 30 s), we classified these neurons as receiving GABAergic synaptic inputs and we analyzed mSPC data previously recorded in the presence of TTX and CNQX only.

Series resistance was routinely monitored before, during, and after the addition of drugs. Data were rejected if series resistance changed by >20%. Synaptic currents were recorded and filtered on-line (2–5 kHz, sampling rate 5–10 kHz) using a Multiclamp 700A amplifier (Axon CNS, MDS Analytical Technologies, ITC1600 digital interface [Instrutech], and Axograph X (Axograph Scientific, Sydney, Australia) or Axopatch 1D amplifier (Axon Instruments), an ITC-16 digital interface (Instrutech) and Axograph 4.8 (Axon Instruments). No significant difference was observed between the two data acquisition systems.

Data analysis

Quantal synaptic events were detected using the sliding template function of Axograph X (see Clements and Bekkers 1997), using parameters previously determined by Camp et al. (2006). Peak amplitude, kinetics (rise time; 10–90% of peak amplitude and decay time constant; single exponential 20–80% of decay phase), and frequency were measured for each neuron. The decay time of mIPSCs was best fit by a single exponential. Quantal events within a minimum of 3-min recording time were used to calculate the frequency. To calculate the interevent intermittent cumulative probability distribution for cells in the 4 h post UL group, the data were normalized and pooled using Igor Pro graphical analysis package (v6.0.2, WaveMetrics). Mann–Whitney U nonparametric tests (SPSS Statistics 17.0; Chicago, IL) were used for comparisons of mIPSC characteristics. Data are presented as means ± SE.

RESULTS

Behavioral observations after UL

After UL, all mice showed typical behaviors consistent with damage to the peripheral vestibular organs, including head tilt and postural deficits toward the lesioned side, circular ambulation, and occasional barrel rolling. Although we did not measure the degree of spontaneous nystagmus, we video-recorded and observed the behavior of mice during the week after UL. Within 3 days of UL, there was a significant improvement in head tilt and postural lean as well as reduced circling and rolling behaviors. One week after UL, with the exception of a slight head tilt, mice displayed few asymmetric behaviors. The gait was normal and sure-footed and some mice were observed to crawl upside-down on the underside of their
cage cover with no apparent impairment. The time course of improvements in posture and gait were consistent with those described previously by Gacek and Kheterpal (1998).

**Electrophysiological recordings**

Recordings were obtained from a total of 181 mouse MVN neurons (52 mice including control animals). GABA\(_{\text{A}}\)-ergic and glycineric mIPSCs were recorded from MVN neurons at three time points after UL: 4 h, 2 days, and 7 days. Previously, it has been shown that only 30% of MVN neurons receive glycineric input (Camp et al. 2006). However, to allow comparisons of quantal event properties for both GABA\(_{\text{A}}\)-ergic and glycineric mIPSCs, with approximately equal sample sizes, we increased the glycineric data set.

**GABA\(_{\text{A}}\)-ergic mIPSCs during vestibular compensation**

In all, 89 neurons from 19 mice receiving GABA\(_{\text{A}}\)-ergic inputs were recorded. These were a combination of exclusively GABA\(_{\text{A}}\)-ergic and mixed inhibitory inputs (Camp et al. 2006). Figure 1A shows representative data of GABA\(_{\text{A}}\)-ergic mIPSCs from a control neuron. The top panel (Fig. 1A) is a continuous trace of GABA\(_{\text{A}}\)-ergic mIPSCs in the presence of TTX, CNQX, and strychnine. Figure 1A (inset) is the averaged mIPSC profile (n = 159 mIPSCs), whereas the bottom panel in Fig. 1A is the amplitude distribution of GABA\(_{\text{A}}\)-ergic mIPSCs for this neuron. All other panels share the same layout. Figure 1, B and C shows representative data of GABA\(_{\text{A}}\) mIPSCs from both ipsilesional (B\(_1\)–B\(_4\)) and contralesional (C\(_1\)–C\(_4\)) MVN neurons at each of the three time points (4 h: B\(_1\) and C\(_1\); 2 days: B\(_2\) and C\(_2\); 7 days: B\(_3\) and C\(_3\)) after UL. Our results showed that although there appeared to be an increase in GABA\(_{\text{A}}\) mIPSC amplitude in ipsilesional MVN neurons, at 4 h post UL it was not significant. Group data (Fig. 1D) showed that after UL there was no significant change in the mean GABA\(_{\text{A}}\) mIPSC amplitude compared with control for either ipsi- or contralesional MVN neurons, or between the ipsi- and contralesional sides. Analysis of other quantal parameters including rise time, decay time, and frequency (see Table 1) also showed no significant changes in the kinetics or frequency of GABA\(_{\text{A}}\) mIPSC quantal events between experimental and control MVN neurons, or between ipsi- and contralesional MVN neurons at any time point investigated after UL.

**Glycinergic mIPSCs during vestibular compensation**

Glycinergic mIPSCs were recorded from a total of 92 neurons (25 mice). These mIPSCs were from a population of glycine only and mixed inhibitory inputs. Compared with GABA\(_{\text{A}}\) mIPSCs, glycinergic mIPSCs have larger mean amplitudes, faster kinetics, and lower frequencies, consistent with results previously reported (Camp et al. 2006). Figure 2A shows representative data of glycinergic mIPSCs from a control neuron. The top panel of Fig. 2A is a continuous trace of glycinergic mIPSCs in the presence of TTX, CNQX, and bicuculline. Figure 2A (inset) is the averaged mIPSC profile (n = 63 mIPSCs), whereas Fig. 2A (bottom) is the amplitude distribution of glycinergic mIPSCs for this neuron. All other panels share the same layout. Figure 2, B and C shows representative data of glycinergic mIPSCs after UL, presented in the same format as GABA\(_{\text{A}}\)-ergic mIPSCs described earlier. Four hours after UL there was a significant increase in the mean amplitude of glycinergic mIPSCs in contralesional MVN neurons compared with control (70.2 ± 16.1 vs. 37.3 ± 6.6 pA; P < 0.05; Fig. 2D). This increase in amplitude of glycinergic mIPSCs from contralesional MVN neurons represents an almost twofold increase compared with control (Fig. 2, C\(_1\) vs. A) and indicates a change in postsynaptic receptor subunit composition or cluster size.

An increase in mean glycine receptor amplitude in contralesional MVN neurons, 4 h post UL, may be due to the alteration of glycine receptor subunits. This would be seen as a change in decay time kinetics of mIPSCs. A comparison of decay time constants between contralesional and control MVN neurons shows no significant change 4 h after UL (5.9 ± 0.5 vs. 6.0 ± 0.7 ms; P > 0.05). Similarly, there was no significant difference in the decay time kinetics between ipsi- and contralesional neurons or control neurons at any time point after UL (see Table 2). Therefore these data suggest that UL does not modify glycine receptor subunit composition. Alternatively, as mentioned earlier, the increase in glycine receptor amplitude may be due to changes in the size of glycine receptor clusters. It is possible that there is an increase in the size of the “synaptic” glycine receptor cluster area that would have been difficult to detect in previous immunolabeling studies. Confirmation, however, would require labeling of both pre- and postsynaptic specializations and is beyond the scope of this study.

Our results also show a shift in the amplitude distribution of glycinergic mIPSCs in contralesional MVN neurons at 4 h post UL toward larger quantal events compared with control. For example, the cell illustrated in Fig. 2C shows a typical increased mean amplitude and skewed amplitude distribution (filled histogram) when compared with control (open histogram; Fig. 2, C\(_1\) vs. A; mean mIPSC amplitude: −73.7 vs. −31.7 pA; range: −17 to −343 vs. −9.8 to −93.2 pA). This shift toward larger quantal events after UL suggests there may be a preferential activation of receptors from large synaptic sites (Lim et al. 2003). This possibility is supported by changes in the frequency of glycinergic mIPSCs addressed in the following text.

Although the above-cited results suggest evidence for postsynaptic modulation of synaptic strength, our data also imply presynaptic effects. This may be mediated by changes in the transmitter release, as evidenced by alterations in mIPSC frequency. Even though we did not observe a change in GABA\(_{\text{A}}\) mIPSC frequency after UL (Table 1), however, there is a significant increase in glycine mIPSC frequency in both ipsi- and contralesional MVN neurons, 4 h after UL compared with control (ipsilesional 0.6 ± 0.2 Hz; contralesional 0.7 ± 0.2 Hz; control 0.2 ± 0.03 Hz; P < 0.01; Fig. 3A). The increased frequency of mIPSCs reflects a decrease in the interevent intervals of quantal events. Figure 3B illustrates a higher proportion of short-latency interevent intervals in ipsi- and contralesional MVN neurons compared with control, 4 h post UL. Between 75 and 85% of interevent intervals are <2.5 s in ipsi- and contralesional MVN neurons, whereas <60% of interevent intervals are <2.5 s in control neurons. This increase in the frequency of glycinergic mIPSCs most likely reflects an increase in the probability of presynaptic release of glycine onto MVN neurons within 4 h of UL. Although this increase in frequency of glycinergic mIPSCs in contralesional MVN neurons, 4 h post UL, is not correlated with increases in mIPSC amplitude, it should be emphasized...
that presynaptic modulation of synaptic strength has not been reported during vestibular compensation.

In summary, although our results show no change in GABA_A receptor function in MVN neurons 1 wk post UL, however, we observed a distinct, brief alteration in inhibitory glycinergic transmission 4 h after UL. Modifications in both pre- and postsynaptic transmission, such as frequency of quantal events and mean current amplitude, respectively, suggest there is more than one mechanism responsible for initiating and/or maintaining vestibular compensation.

**FIG. 1.** γ-Aminobutyric acid type A (GABA_A) receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs) after unilateral labyrinthectomy (UL). A: representative current trace (top), amplitude distribution (bottom), and averaged quantal events (inset) of control GABA_Aergic mIPSCs (see METHODS). The layout of A is the same for the panels in B (B1, B2, B3) and C (C1, C2, C3). Representative ipsilesional recordings of GABA_A mIPSCs at 4 h (B1), 2 days (B2), and 7 days (B3). Representative contralesional recordings of GABA_A mIPSCs at 4 h (C1), 2 days (C2), and 7 days (C3). D: bar graph shows the average GABA_A receptor-mediated mIPSC amplitude recorded from ipsi- and contralesional medial vestibular nuclei (MVN) neurons at 4 h, 2 days, and 7 days post UL. There were no significant differences at any time point post UL. Data are expressed as means ± SE.
Asymmetric discharge rates in MVN neurons immediately after UL are attributed not only to loss of primary afferent input but also to a concomitant change in fast inhibitory synaptic drive mediated by GABA\textsubscript{A} and glycine receptors (Bergquist et al. 2008; Gliddon et al. 2005b). Whether inhibitory synaptic connections have a role in restoring discharge rates between the two sides has not yet been established. This study has investigated fast inhibitory synaptic transmission in MVN neurons at various time points after UL, to determine their role in vestibular compensation.

Synaptic inhibition in mouse MVN is mediated by both GABA\textsubscript{A}ergic and glycine receptors (Camp et al. 2006). It has been proposed that recovery from the asymmetric discharge rate of MVN neurons after UL is partially due to GABAergic and glycineergic commissural connections between the two sides (Bergquist et al. 2008). It is thus possible that altered inhibitory synaptic transmission, due to changes in neurotransmitter release and/or functional modifications of postsynaptic receptors, could differentially modulate the discharge rate of MVN neurons during vestibular compensation.

**GABA\textsubscript{A} receptor-mediated mIPSCs**

Studies have reported that MVN neurons have an altered sensitivity to GABA\textsubscript{A}-receptor agonists and antagonists after UL (Johnston et al. 2001; Vibert et al. 2000; Yamanaka et al. 2000), which may be reflected in functional changes of receptor properties. It was an aim of this study to determine whether UL could induce a change in inhibitory receptor function. These potential changes would influence the discharge rate of MVN neurons and consequently help explain previous pharmacological results. To determine whether receptor properties were altered after UL, we recorded quantal synaptic currents, at three time points after UL. Our results indicate that properties of GABA\textsubscript{A} receptors, including amplitude, kinetics, and mIPSC frequency, in labyrinthectomized animals were not significantly altered compared with control or between ipsi- and contralateral sides. Thus we see no evidence for an alteration in subunit composition that may account for reduced agonist sensitivity reported previously (Johnston et al. 2001; Vibert et al. 2000; Yamanaka et al. 2000).

The properties of GABA\textsubscript{A} postsynaptic receptors are comparable with those previously reported by Camp et al. (2006), with the exception of lower frequency of GABA\textsubscript{A}ergic events in this study. The lower frequency might be due to sampling neurons that receive both GABA\textsubscript{A}-only and mixed GABA\textsubscript{A}-ergic–glycinergic inputs, whereas the data reported by Camp et al. (2006) represent the frequency of mIPSCs that originate from GABA\textsubscript{A}-only inputs to MVN neurons. Therefore neurons that possess GABA\textsubscript{A}-only receptors may have a greater number of GABAergic inhibitory inputs or a higher rate of GABA release, i.e., higher mIPSC frequency, than that of MVN neurons that have both GABA\textsubscript{A} and glycine receptors, although this has yet to be determined.

Our results did not show any changes in GABA\textsubscript{A} receptor-mediated synaptic transmission after UL and is consistent with previous immunohistochemical and Western blot studies of GABA\textsubscript{A} receptor expression (Eleore et al. 2004, 2005). These results, however, are in contrast with those of Horii et al. (2003), which show an increase in GABA\textsubscript{A} receptor alpha-1 subunit after UL. Higher concentrations of GABA\textsubscript{A} agonist muscimol are required to attenuate discharge rate of MVN neurons after UL (Vibert et al. 2000; Yamanaka et al. 2000), suggesting alterations in GABA\textsubscript{A} receptors. Both these studies may reflect an up-regulation of extrasynaptic receptors, not addressed in this study.

Given the lack of change in GABA\textsubscript{A} receptor-mediated quantal transmission in mouse, how does this reconcile with previous findings regarding increases in GABA immunoreactivity and release after UL? Studies in cats have shown an increase in the number of GABA immunoreactive neurons in MVN after UL (Tighilet and Lacour 2001). Alterations in the number of inhibitory GABA\textsubscript{A}ergic neurons may be a means by which neuronal excitability is modulated. It is unknown, however, whether these are inhibitory interneurons operating within the MVN or inhibitory projection neurons. If it were the former, we would have expected to see an increase in GABA\textsubscript{A} receptor-mediated quantal events on MVN neurons, but this was not the case. We thus suggest that these may be neurons projecting out of the nucleus. However, we cannot discount the possibility that increased GABA\textsubscript{A}ergic neurons may be acting on GABA\textsubscript{B} receptors that are also known to modulate discharge rate of MVN neurons (Johnston et al. 2001; Yamanaka et al. 2000).

A possible reason we did not observe a change in GABA\textsubscript{A} receptor-mediated synaptic transmission, post UL, may be due to highly effective GABA uptake mechanisms. GABA transporters appear to minimize fluctuations in GABA concentrations from the MVN, as suggested in a recent microdialysis study by Bergquist et al. (2008). Indeed, increased mRNA expression of GABA transporter, GAT, has been shown in MVN, 6–50 h post UL (Horii et al. 2003) and supports this notion. It should be noted that Bergquist et al. (2008) reported a brief increase in GABA release at 24 h post UL that may be due to the transporters being transiently overwhelmed. Al-
though not significant, our results suggest at 4 h, there is elevated GABA release as indicated by an increase in the frequency of GABAergic synaptic currents. However, since we did not record at a 24-h post UL time point we cannot confirm the findings of Bergquist et al. (2008).

Our study has shown that despite the predominance of GABAergic synaptic transmission in the MVN, there is no change in postsynaptic GABA receptor function, subunit composition, or presynaptic release probability after sensory ablation. Therefore our results strongly suggest that vestibular...
compensation in the MVN does not involve alterations in GABAergic synaptic input during the first week after UL.

**Glycine receptor-mediated mIPSCs**

In contrast to our GABA<sub>A</sub> receptor results described earlier, we have shown that inhibitory glycinergic synaptic transmission is altered during the early phase of vestibular compensation. We found a significant increase in the amplitude of glycine receptor-mediated mIPSCs in contralesional MVN neurons, 4 h after UL. This indicates postsynaptic receptor changes, such as alterations in receptor cluster size or the up-regulation of receptor subunits. A previous report (Eleore et al. 2004) found no differences in the immunofluorescence of the inhibitory receptor anchoring protein gephyrin, or glycine receptor α1, α2, α3, and β subunits between ipsi- and contralesional MVN. It should be noted, however, that the study represents immunofluorescence of a total population of receptors both synaptic and extrasynaptic and did not quantify possible ultrastructural changes in receptor cluster area or alteration of receptor number or somatodendritic localization.

Although the effects of UL were induced in vivo, at physiological temperature, the effects of sensory removal on inhibitory synaptic transmission were investigated in vitro in a brain slice recording. Recordings from brain slices were done at room temperature to allow direct comparisons of data between

### TABLE 2. Quantal parameters of glycinergic mIPSCs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Ipsi</th>
<th>Contra</th>
<th>Ipsi</th>
<th>Contra</th>
<th>Ipsi</th>
<th>Contra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude, pA</td>
<td>37.3 ± 6.6</td>
<td>45.1 ± 6.0</td>
<td>70.2 ± 16.1*</td>
<td>57.5 ± 11.2</td>
<td>53.5 ± 7.8</td>
<td>42.9 ± 7.1</td>
<td>42.1 ± 7.3</td>
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<td>Rise time, ms</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.07</td>
<td>0.8 ± 0.05</td>
<td>0.7 ± 0.05</td>
<td>0.8 ± 0.04</td>
<td>0.9 ± 0.07</td>
<td>0.9 ± 0.05</td>
</tr>
<tr>
<td>Decay time, ms</td>
<td>5.9 ± 0.5</td>
<td>6.5 ± 0.9</td>
<td>6.0 ± 0.7</td>
<td>5.7 ± 0.6</td>
<td>5.2 ± 0.4</td>
<td>6.5 ± 0.5</td>
<td>7.1 ± 1.0</td>
</tr>
<tr>
<td>Frequency, Hz</td>
<td>0.2 ± 0.03</td>
<td>0.6 ± 0.2*</td>
<td>0.7 ± 0.2*</td>
<td>0.1 ± 0.03</td>
<td>0.5 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>CV, amplitude</td>
<td>0.6 ± 0.05</td>
<td>0.7 ± 0.04</td>
<td>0.6 ± 0.06</td>
<td>0.5 ± 0.05</td>
<td>0.7 ± 0.06</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.07</td>
</tr>
<tr>
<td>Number of neurons</td>
<td>14</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>13</td>
<td>12</td>
<td>19</td>
</tr>
</tbody>
</table>

Values are means ± SE. UL, unilateral labyrinthectomy; Ipsi, ipsilesional; Contra, contralesional. *P < 0.05.
this and other laboratories. The vast majority of studies that recorded inhibitory quantal synaptic currents were done at room temperature (see Jonas et al. 1998; Lu et al. 2008; Nusser et al. 1998; Sebe et al. 2003). The advantage of recording quantal synaptic currents at room temperature allows stable physiological recordings, particularly important when investigating changes that occur following application of various antagonists, such as bicuculline and strychnine. Furthermore, voltage clamp of neurons with extensive dendritic trees is potentially problematic due to space-clamp distortions, although recording at room temperature can reduce these errors (Williams and Mitchell 2008). As a consequence of recording at room temperature, it is established that synaptic currents have slower kinetics, smaller magnitude, and lower frequencies than those recorded at physiological temperature (Awatramani et al. 2004; Fatt and Katz 1952; Zhang and Trussell 1994).

However, it has been shown that the proportion of time that glycine receptors translocate between synaptic and extrasynaptic domains is not significantly different between room temperature and 37°C (Dahan et al. 2003). Therefore our findings that glycinergic mIPSC amplitude and frequency increase during early vestibular compensation, reported here at room temperature, would presumably be even greater in brain slices at, or near, physiological temperatures and in vivo, although this has yet to be established.

Previous studies have not addressed the possibility that alterations in presynaptic signaling at glycinergic synapses may also be involved in vestibular compensation. Our results showed a significant increase in the mean frequency of glycinergic mIPSCs in both ipsi- and contralesional MVN neurons, 4 h post UL. This suggests an elevated rate of quantal glycine release and is a presynaptic means of modulating synaptic strength (Lim et al. 2003). Increased presynaptic glycine release, together with larger-amplitude glycinergic postsynaptic currents would provide elevated levels of inhibition to contralesional MVN during a period of intensive excitation immediately following UL, seen in vivo (Smith and Curthoys 1988a).

What is the potential role of enhanced glycinergic inhibition ipsilaterally early in vestibular compensation? Here we discuss two possibilities. First, inhibition may exert a modulatory affect on discharge rate by an unusual mechanism known as firing rate potentiation (FRP; Nelson et al. 2003). Initially, neuronal discharge is prevented by stimulation of inhibitory fibers, or application of GABA or glycine. Later, release from this inhibition results in long-lasting potentiation of discharge rate (Nelson et al. 2003). Increased glycinergic drive ipsilaterally therefore may act to enhance and restore low spontaneous discharge rates that are evident after UL.

Second, the involvement of excitatory NMDA receptors in synaptic plasticity (Bliss and Collingridge 1993) has led to the proposal that they also contribute to mechanisms underlying vestibular compensation (Flohr and Luneburg 1993; Kinney et al. 1994; Vidal et al. 1996). It should be noted that NMDA receptors require glycine as a coagonist. Since we have shown an increase in the frequency of quantal glycinergic currents, this raises the possibility that elevated levels of glycine might “spill over” to activate and/or potentiate NMDA receptors. Indeed, blockade of glycine uptake has been shown to potentiate NMDA receptors in the brain stem (Lim et al. 2004). Like GABA transporters (Bergquist et al. 2008), it is possible that glycine transporters are similarly overwhelmed during vestibular compensation, resulting in activation of NMDA receptors. Further experiments are required to determine the effect of increased glycine release on NMDA receptors after UL.

An interplay between excitation and inhibition is necessary to maintain complex neuronal discharge patterns. Removing a significant sensory input induces profound changes in neuronal signaling and processing as seen in the visual system. This model in particular has been used to study homeostatic plasticity. Most studies, however, have focused on synaptic scaling of excitatory inputs, that is, an alteration of mEPSC amplitude as a result of sensory deprivation (Desai et al. 2002; Goel and Lee 2007; Turrigiano et al. 1998). Fewer studies of homeostatic plasticity have addressed how deprivation of sensory inputs affects inhibitory synaptic connections. In layer IV of the visual cortex, 2 days of monocular deprivation significantly potentiates the inhibitory synaptic connection between fast-spiking basket cells and star pyramidal cells and results in a significantly reduced discharge rate of star pyramidal cells (Maffei et al. 2006). Our data show that permanent loss of sensory input can also enhance inhibitory synaptic transmission. This enhancement of glycine receptor-mediated synaptic transmission coincides with a reduction in discharge rates of MVN neurons. Modulation of inhibitory inputs appears to be an important mechanism of altering neuronal excitability. In vivo patch-clamp methods will be necessary to unequivocally establish the influence of inhibitory GABA_A and glycine receptors on the discharge rate of MVN neurons after UL.

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