Inhibitory synaptic transmission in neurons of the medial vestibular nucleus after unilateral labyrinthectomy

R. Lim, R.J. Callister and A.M. Brichta, Hunter Medical Research Institute, School of Biomedical Sciences, The University of Newcastle, University Drive, Callaghan, NSW 2308, Australia.

Introduction. Loss of peripheral balance (vestibular) organs from one side of the head or unilateral labyrinthectomy (UL) causes stereotypical behaviour in all animals and includes head tilt toward the ablated side, circling, barrel-rolling, and spontaneous nystagmus (eye flicking). Within a few days after UL, many of these symptoms abate. This recovery is referred to as "vestibular compensation" and is due entirely to changes in central activity since there is no regeneration of peripheral organs within this period. Precisely how and where this compensation occurs is unknown.

It has been suggested that much of this "compensation" occurs in the vestibular nuclei, the primary target for incoming vestibular afferents. *In vivo* studies have shown in the acute phase of vestibular compensation, ipsilesional neurons in the medial vestibular nucleus (MVN) are silent while contralateral MVN neurons have markedly raised background discharge rates (Smith & Curthoys, 1988a & b). Within a week, as behavioural symptoms subside, similar firing rates between the ipsi- and contralateral sides are re-established. (Ris *et al.*, 1995). It is this correlation between behaviour and neuronal activity that made the vestibular nuclei a proposed site for compensation. Studies have shown that there are significant commissural inhibitory inputs between the two MVN and they may play an important role in compensation by restoring firing rate after unilateral vestibular loss. Therefore, we have investigated whether there are changes in inhibitory GABAergic and glycinergic quantal synaptic transmission during vestibular compensation.

Methods. *Unilateral Labyrinthectomy:* All experiments were conducted according to The University of Newcastle Animal Care and Ethics Committee Regulations. Mice (C57BL6) approximately 4 weeks old underwent UL in a procedure described by Gacek & Khetarpal (1998). Under isoflurane anaesthetic, both the horizontal and anterior ampullae were removed, the vestibule was filled with Gelfoam and the surgical incision closed using interrupted sutures. Animals recovered after anaesthetic and immediately displayed behavioural symptoms consistent with UL. *In vitro slice preparation:* At three time points following UL (4 hours, 2 days and > 7 days), mice were anaesthetised using Ketamine (100 mg/kg, i.p) and decapitated. Brainstem sections were prepared as previously described (Camp *et al.*, 2006). Post-mortem examination of the bony labyrinth confirmed the removal and destruction of the ampullae. *Whole-cell patch-clamp recordings*: Glycine and $GABA_A$ receptor mediated quantal synaptic currents were recorded using CsCl-based internal solution and in the presence of various antagonists including tetrodotoxin (1 μM), CNQX (10 μM), bicuculline (5 μM) and strychnine (1 μM).

Results & Discussion. Inhibitory quantal currents were recorded from a total of 157 MVN neurons. We measured quantal amplitude, rise time, decay time and frequency from neurons in the ipsi-lesioned and contra-lesioned MVN. Our results show that there are no significant changes in GABAergic quantal events (including amplitude, kinetics or frequency) between ipsilesional, contralesional, or control medial vestibular nucleus neurons (MVNn) at any time point investigated after UL. In contrast, we have found a significant increase at 4 hours after UL, in the amplitude and frequency of glycine receptor mediated mIPSCs from contra-lesioned MVNn (amplitude: 70.2 ± 16.1pA, $p < 0.05$; frequency: 0.7 ± 0.2 Hz, $p < 0.05$) and an increase in the frequency of mIPSCs in ipsi-lesioned MVNn (frequency: 0.6 ± 0.2 Hz, $p < 0.05$) compared to control MVNn (amplitude: 37.3 ± 6.6 pA; frequency 0.2 ± 0.03 Hz). Since there were no changes in glycinergic mIPSC kinetics, increases in amplitude were not due to receptor subunit changes. Therefore our results suggest that GABAergic synaptic transmission has a limited role in MVNn during early vestibular compensation. In contrast, fast inhibitory synaptic transmission mediated by glycine receptors plays a significant a role in altering neuronal excitability during early vestibular compensation.

Camp AJ, Callister RJ & Brichta AM. (2006) *Journal of Neurophysiology*, **95:** 3208-18.
Gacek RR & Khetarpal U. (1998) *Laryngoscope* **108:** 671-8.
Ris L, de Waele C, Serafin M, Vidal PP & Godaux E. (1995) *Journal of Neurophysiology*, **74:** 2087-99.
Smith PF & Curthoys IS. (1988a) *Brain Research*, **444:** 295-307.
Smith PF & Curthoys IS. (1988b) *Brain Research*, **444:** 308-19.

Funding provided by NHMRC, Garnett Passe and Rodney Williams Memorial Foundation and HMRI