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ADAPTATION OF CHICKEN VESTIBULAR NUCLEUS NEURONS TO UNILATERAL VESTIBULAR GANGLIONECTOMY

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Abstract—Vestibular compensation refers to the behavioral recovery after a unilateral peripheral vestibular lesion. In chickens, posture and balance deficits are present immediately following unilateral vestibular ganglionectomy (UVG). After three days, most operated chickens begin to recover, but severe deficits persist in others. The tangential nucleus is a major avian vestibular nucleus whose principal cells are vestibular reflex projection neurons. From patch-clamp recordings on brain slices, the percentage of spontaneous spike firing principal cells, spike discharge rate, ionic conductances, and spontaneous excitatory postsynaptic currents (sEPSCs) were investigated one and three days after UVG. Already by one day after UVG, sEPSC frequency increased significantly on the lesion side, although no differences were detected in the percentage of spontaneous spike firing cells or discharge rate. In compensated chickens three days after UVG, the percentage of spontaneous spike firing cells increased on the lesion side and the discharge rate increased bilaterally. In uncompensated chickens three days after UVG, principal cells on the lesion side showed increased discharge rate and increased sEPSC frequency, whereas principal cells on the intact side were silent. Typically, silent principal cells exhibited smaller persistent sodium conductances and higher activation thresholds for the fast sodium channel than spiking cells. In addition, silent principal cells on the intact side of uncompensated chickens had larger dendrotoxin-sensitive potassium conductance, with a higher ratio of Kv1.1 surface/cytoplasmic expression. Increased sEPSC frequency in principal cells on the lesion side of uncompensated chickens was accompanied by decreased Kv1.2 immunolabeling of presynaptic terminals on principal cell bodies. Thus, both intrinsic ionic conductances and excitatory synaptic inputs play crucial roles at early stages after lesions. Unlike the principal cells in compensated chickens which showed similar percentages of spontaneous spike firing cells, discharge rates, and sEPSC frequencies bilaterally, principal cells in uncompensated chickens displayed gross asymmetry in these properties bilaterally.

Key words: plasticity, brain slice, sodium and potassium channels.

Vestibular compensation is the popular term for the behavioral recovery which occurs following unilateral labyrinthectomy, or unilateral vestibular ganglionectomy (UVG) (for review, see Straka et al., 2005). Most vestibular ganglion cells do not recover their spike activity after labyrinthectomy (Sirkin et al., 1984), and the primary vestibular fibers degenerate centrally after UVG (Li et al., 1995; Aldrich and Peusner, 2002). Accordingly, the mechanisms underlying adaptation to vestibular deafferentation are thought to reside within the central nervous system.

The cellular mechanisms underlying vestibular compensation have been investigated most extensively on medial vestibular nucleus (MVN) neurons from rat and guinea pig using both *in vivo* and *in vitro* preparations. In studies performed on whole animals, the immediate response to vestibular deafferentation is decreased spontaneous spike discharge rate in neurons on the lesion side, while the rate increases in neurons on the intact side (Smith and Curthoys, 1988a,b). Overall, behavioral recovery after labyrinthectomy coincides with the restoration of symmetric discharge rates in MVN neurons bilaterally, despite a certain degree of dissociation (see Ris et al., 1997). In brain slice preparations, the spontaneous spike firing rate increases on the lesion side within hours after labyrinthectomy (Cameron and Dutia, 1997; Vibert et al., 1999; Beraneck et al., 2003), whereas the discharge rate remains unchanged, or decreases on the intact side (Cameron and Dutia, 1997; Beraneck et al., 2004). Like *in vivo* studies, MVN neurons in brain slice preparations show a gradual recovery of symmetric spontaneous discharge rate bilaterally (Cameron and Dutia, 1997). *In vitro* studies indicate that vestibular nuclei neurons on the lesion side exhibit increased subthreshold potentials, which could explain the increased spontaneous spike discharge recorded (Him and Dutia, 2001). Furthermore, the relative proportions of the two main MVN neuron subclasses change after labyrinthectomy, with type B MVN neurons increasing on the intact side, while type A neurons increases on the lesion side (Beraneck et al., 2003, 2004). MVN neuron types are classified according to their spike waveform, without knowledge of their axonal output as projection, commissural, interneuron, vestibulocerebellar, vestibulothalamic, or a combination of these functional subtypes. Since neurons in

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Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, afterhyperpolarization; CNQX, 6-cyano-7-nitroquinoxaline-2, 3-dione; CV, coefficient of variation; DTX, dendrotoxin; H, hatching day; I_{DS}, dendrotoxin-sensitive current; IgG, antibody; I_{Na}, fast sodium current; I_{NaP}, persistent sodium current; ISI, interspike interval; Kv1.1, Kv1.2, sustained potassium channel subunit with high sensitivity to dendrotoxin; MAP2, microtubule-associated protein 2; mEPSC, miniature excitatory postsynaptic current; MVN, medial vestibular nucleus; NA, numerical aperture; PBS, phosphate-buffered saline; sEPSC, spontaneous excitatory postsynaptic current; TTX, tetrodotoxin; UVG, unilateral vestibular ganglionectomy; VOC, vestibuloocular collic; VOR, vestibuloocular reflex.

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other sensory systems respond differentially to deafferentation depending on the neural circuitry in which they participate (e.g. see Francis and Manis, 2000), it is conceivable that vestibular neuron subtypes also respond differentially to vestibular deafferentation. Thus, it could be informative to record from a single functional class of vestibular nucleus neurons after vestibular deafferentation.

The tangential nucleus is a major avian vestibular nucleus in the chicken. Its principal cells represent a morphologically distinctive class of glutamatergic (unpublished observations, Popratiloff and Peusner) vestibular reflex projection neurons, which receive monosynaptic input from the primary vestibular fibers (Peusner and Giaume, 1994). While some principal cells are vestibulo-ocular reflex (VOR) neurons which project to the oculomotor (Wold, 1978; Labandeira-Garcia et al., 1989, 1991; Petursdottir, 1990), trochlear (Evinger and Erichsen, 1986), or abducens nucleus (Gottesman-Davis and Peusner, 2008), other principal cells are vestibulo-ocular collic (VOC) neurons, whose axons terminate in the abducens nucleus with collaterals descending to high cervical spinal cord (Cox and Peusner, 1990a). Thus, all principal cells are VOR or VOC neurons. The distribution of VOR and VOC neuronal classes among the vestibular nuclei in chicken differs from mammals, since most VOR and VOC neurons are situated more laterally within the chicken medulla oblongata, with heavy involvement of neurons in the tangential, ventrolateral vestibular, and descending vestibular nuclei, and, unlike mammals, only a modest contribution from the MVN (Gottesman-Davis and Peusner, 2008). It will be important to determine how spontaneous spike discharge rate in principal cells is affected by UVG, and compare this to what was found after averaging responses from different MVN neuron classes after labyrinthectomy. Most important, recording from a homogeneous class of VOR and VOC neurons will broaden our understanding of the specific role of this neuron class in adaptation to vestibular deafferentation.

The adult vestibular system exhibits plasticity in response to changing environmental demands (e.g. Mandl et al., 1981). However, the juvenile vestibular system is characterized by a more complete and rapid functional recovery after injury and lesions than the adult system (e.g. Kaga, 1999). Accordingly, here UVG was performed on four day-old hatchling chickens (hatching day [H] 4), which were sacrificed one (H5) or three days (H7) after surgery to trace the sequence of cellular events during early recovery. In preliminary experiments, some operated chickens did not begin to recover by three days, despite similar surgical and postsurgical treatment for all operated animals (see Curthoys and Hamalgii, 2007). Therefore, this new group of uncompensated animals was included in the present study.

To identify which intrinsic membrane conductances underlie changes in the spontaneous spike discharge rate after UVG, we focused on the sodium and potassium channels, I_{NaP}, I_{Na}, and $I_{DS,}$ which are critically involved in the emergence of spontaneous spike discharge in developing principal cells (Gamkrelidze et al., 1998, 2000; Popratiloff et al., 2003; Shao et al., 2006a,b). In addition, spontaneous excitatory postsynaptic currents (sEPSCs), which are crucial events in the assembly of the neural circuitry, were investigated (Shao et al., 2003, 2004). This work represents the first voltage-clamp study of sEPSCs, and sodium and potassium conductances, in combination with immunolabeling of potassium channels in vestibular nuclei neurons after UVG. Part of this work was presented in abstract form (Peusner et al., 2007; Popratiloff et al., 2007; Shao et al., 2007).

EXPERIMENTAL PROCEDURES

Experimental animals

Chick embryo eggs (*Gallus gallus*) were purchased from CBT Farm (Chesterton, MD, USA), and placed until hatching in an egg incubator equipped with circulated air, egg rotation unit, and temperature and humidity controls (model 1502, G.Q.F. Manufacturing Co., Savannah, GA, USA). Twenty-four hours after hatching, the chicken was identified as a one day-old hatchling (H1). Chickens were housed in cages equipped with heating lamps at the University's Animal Research Facility until the appropriate ages (H4–H7). Animal protocols were approved by the Institution Animal Care and Use Committee of the George Washington University. The experiments conformed to the guidelines for the care and use of animals in research (National Research Council, 2003), and all efforts were made to minimize the number of animals used and their suffering.

UVG

UVG was performed on H4 chickens, which were sacrificed one or three days later. The surgical protocol was adapted from previous studies (Aldrich and Peusner, 2002; Pollack et al., 2004). Briefly, under general anesthesia (ketamine, 100 mg/kg, Fort Dodge Animal Health, Fort Dodge, IO, USA; xylazine, 20 mg/kg; Akorn Inc., Decatur, IL, USA), an incision was made in the skin on the left side of the head, posterior to the external auditory meatus. The exoccipital and squamosal bones were removed to expose the lateral part of the bony vestibule of the inner ear, which after removal exposed the membranous labyrinths. The membranous labyrinths and bony medial wall of the vestibule were removed to expose the dura mater overlying the vestibular ganglion. The dura mater was cut, so that both the anterior and posterior portions of the vestibular ganglion and the auditory nerve were revealed and extirpated. A curved, flame-sharpened tungsten wire was used to cut the vestibular nerve between the vestibular ganglion and lateral brain surface. After achieving deep anesthesia in sham-operated chickens, the skin was cut on the left side of the head posterior to the external auditory meatus, several blood vessels in the fascia were coagulated, and the skin was sutured closed. Control, operated, and sham-operated chickens were housed under the same conditions.

After UVG, the survival rate was about 90%. Operated chickens received daily i.m. injections of 0.25 ml penicillin-G prophylactically (Bimeda Inc., Le Sueur, MN, USA). Dehydration was treated by s.c. injections of sterile, lactated Ringer's solution (Braun Medical, Inc., Irvine, CA, USA), which was started about 1–2 h after surgery when the operated chickens regained consciousness. The volume of replacement fluid was determined by the weight loss (National Research Council, 2003). On the second day after surgery, chickens which could not eat independently were given rice cereal for babies (Gerber, Fremont, MI, USA) via gavage every 3 hours, four times a day, until sacrificed. Operated chickens, which could stand, drink, and eat on their own three days after UVG were considered compensated, while those which could not perform these activities were considered uncompensated.