Presynaptic and postsynaptic ion channel expression in vestibular nuclei neurons after unilateral vestibular deafferentation

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Abstract. Vestibular compensation refers to the recovery of function occurring after unilateral vestibular deafferentation, but some patients remain uncompensated. Similarly, more than half of the operated chickens compensate three days after unilateral vestibular ganglionectomy (UVG), but the rest remain uncompensated. This review focuses on the studies performed on the principal cells of the chick tangential nucleus after UVG. The tangential nucleus is a major avian vestibular nucleus whose principal cells are all second-order, vestibular reflex projection neurons participating in the vestibulocular and vestibulocollic reflexes controlling posture, balance, and eye movements. Using whole-cell patch-clamp approach in brain slice preparations, spontaneous spike firing, ionic conductances, and spontaneous excitatory postsynaptic currents (sEPSCs) are recorded in principal cells from controls and operated chickens three days after UVG. In compensated chickens, the proportion of spontaneous spike firing principal cells and their spike discharge rate are symmetric on the lesion and intact sides, with the rates increased over controls. However, in the uncompensated chickens, the spike discharge rate increases on the lesion side, but not on the intact side, where only silent principal cells are recorded. In all the experimental groups, including controls, silent principal cells are distinguished from spontaneous spiking cells by smaller persistent sodium conductances and higher activation thresholds for the fast sodium channel. In addition, silent principal cells on the intact side of uncompensated chickens have larger dendrotoxin-sensitive potassium conductances, with a higher ratio of immunolabeling for surface/cytoplasmic expression of a dendrotoxin-sensitive, potassium channel subunit, Kv1.1. Finally, in compensated chickens, sEPSC frequency is symmetric bilaterally, but in uncompensated chickens sEPSC frequency increased only on the lesion side, where the expression of Kv1.2 decreased in synaptotagmin-labeled terminal profiles on the principal cell bodies. Altogether, the specific sodium and potassium channels important for the development of spike firing pattern and/or presynaptic glutamate release on vestibular reflex projection neurons may be critically involved in changing postsynaptic neuron excitability after vestibular deafferentation.

Keywords: Adaptation, brain slice, action potential, synaptic transmission

1. Introduction

Vestibular nuclei neurons are important models to investigate adaptation to unilateral peripheral vestibular lesions, commonly referred to as “vestibular compensation.” In vertebrates, vestibular nuclei neurons usually generate spontaneous spike discharge at rest, with rates that are symmetric bilaterally. However, shortly after unilateral labyrinthectomy (UL), single unit extracellular recordings from whole animals reveal that the spontaneous spike discharge rate decreases in vestibular nuclei neurons on the lesion side, and increases on the intact side. Often, the recovery of posture and balance coincides with the restoration of bilaterally symmetric firing rates in these neurons [46,47]. Other events may coincide with the change in spike discharge rate, includ-
ing the reorganization of synaptic inputs to vestibular nuclei neurons (for review [49]). Until now, vestibular compensation studies have been performed primarily on medial vestibular nucleus (MVN) neurons from rat and guinea pig, which are recorded without knowledge of their output pathways or their neurotransmitter profiles. In contrast, the chick tangential nucleus is a major vestibular nucleus, with its principal cells representing 80% of the neuron population. Principal cells are readily distinguished morphologically from other neuron types in the nucleus. Principal cells are all vestibular reflex projection neurons (e.g. [8,17]). Recent studies show major changes in their spontaneous spike firing, intrinsic ionic conductances, and sEPSCs after UVG [43]. This review focuses on these studies, and presents the rationale for performing vestibular compensation experiments on the hatchling chicken.

2. Behavioral and postural deficits in animal models after UL and UVG

In the clinic, patients with peripheral vestibular neuropathies are treated using diverse surgical approaches [15]. At present, some of these approaches are applied to experimental animal models to understand the mechanisms underlying recovery of function after peripheral vestibular lesions. UL involves removing the peripheral vestibular endorgans containing the vestibular nerve endings, while UVG entails removing the vestibular ganglion by sectioning the vestibular nerve at the lateral brain surface [1,28]. After UL, some vestibular ganglion cells and central vestibular axons survive [7,12], so that ectopic spike activity is detected centrally [24]. Thus, some vestibular nuclei neurons may retain some abnormal primary vestibular inputs after UL. However, after UVG, complete degeneration of the primary vestibular fibers occurs in the brainstem, so that all the vestibular nuclei neurons undergo identical complete loss of primary vestibular inputs. Accordingly, UVG offers a more consistent model for studying vestibular nuclei neurons responses to lesions [1,27,28]. So far, UVG has been performed on two animal models, the rat and chicken. In the rat, the behavioral deficits are more severe after UVG compared to UL, with dramatic asymmetry in muscle tone for neck, trunk and limb muscles [28]. In the chicken, about half the operated animals compensate three days after UVG, since they can stand, feed and drink independently. The remaining operated chickens, which fail to perform these tasks, are considered uncompensated [44].

3. Age of animal models in vestibular compensation studies

So far, cellular electrophysiological studies on vestibular nuclei neurons after vestibular deafferentation have been performed most often on medial vestibular nucleus (MVN) neurons from rat and guinea pig in vitro, and in one case on the tangential principal cells from chicken in vitro preparations (e.g. [5,22,44]). In rat and guinea pig experiments, the animals are usually 30 days postnatal (P30), whereas chickens are 4–7 day old hatchlings (H4–H7). In all species, vestibular nuclei neurons acquire their mature spike firing pattern gradually during development, including spontaneous spike firing and repetitive spike firing on depolarization. In the rat, the frequency of spontaneous spike firing in MVN neurons continues to increase to P20 [25], and evoked spike firing on depolarization shows little adaptation after P17 [30]. Thus, spike generation in rat MVN neurons attains the mature pattern within a week after eye opening, which occurs around P14. Compared to the rat, maturation of spontaneous spike firing and repetitive spike firing on depolarization follows a precocious and more compressed schedule in the chicken [43]. Within hours of birth, hatchlings open their eyes, and can stand and feed themselves [40]. Also, hatchlings can produce vestibuloocular reflex adaptive changes like those recorded in 4–6 week chickens [52]. Moreover, the spike firing rate of vestibular nuclei neurons in one week old hatchlings is similar to that reported for three-to-four week old rats and guinea pigs [43]. Thus, one week old hatchling chickens show a similar vestibular performance to that observed in one month old rodents.

4. Targets selection is based on their roles in maturation of spike firing pattern

During embryonic development (E10-E16), spontaneous spike firing is not recorded from tangential principal cells, but is present after hatching [13,14,42–44]. In contrast, repetitive spike firing on depolarization is observed in a small percentage of principal cells at E16, and appears in most principal cells by H5–H7. Several intrinsic ionic conductances underlie the developmental changes, including the fast (I_{Na}) and persistent (I_{NaP}) sodium conductances, which enhance the ability of principal cells to fire spontaneous spikes, and dendrotoxin-sensitive (DTX) potassium conductance (I_DS), which blocks repetitive spike firing on depo-
Fig. 1. Spontaneous spike firing in principal cells of the chick tangential nucleus before and after UVG. A₁, Spontaneous spike firing and A₂, silent principal cell from H7 control chickens. Note the sEPSPs in the trace (*). B, Percentage of spontaneous spike-firing principal cells before and after UVG. Three days after UVG in compensated chickens, a significantly higher percentage of principal cells are silent fire spontaneously compared to controls (p < 0.05). Moreover, on the intact side of uncompensated chickens three days after UVG, all recorded principal cells are silent, which is highly significantly different from all other groups (p < 0.01). C₁, Spike discharge rate calculated by combining spontaneous spike firing and silent principal cells, and C₂, Spike discharge rate calculated from spontaneous spike firing principal cells only. Both calculations made in C₁ and C₂ reveal similar patterns. Three days after UVG, spike discharge rate is significantly higher on the lesion and intact sides in the compensated chickens, as well as on the lesion side of uncompensated chickens compared to controls (p < 0.05).

larization in principal cells. Another striking difference between embryonic and hatchling principal cells is the increased sEPSC frequency which appears after birth [41]. It is interesting that in response to injury or disease, mature cells in other systems can repeat temporarily certain aspects of their developmental phenotype. For example, during gliosis, spinal-cord astrocytes switch from inward to outwardly rectifying potassium channels, which characterize their immature phenotype [29]. In atherosclerotic lesions, smooth muscle cells reexpress a developmentally-regulated gene and repeat certain early developmental events [21]. From this, we suggest that vestibular nuclei neurons should be tested to determine whether the ionic conductances, which are important during the development of firing pattern, are reexpressed transiently during functional recovery after UVG.

5. Percentage of spontaneous spike firing cells and spike discharge rate

Although vestibular nuclei neurons are usually characterized by spontaneous spike discharge at rest, some of them show no spike discharge at resting membrane potential (Fig. 1A). In fact, silent vestibular nuclei neurons are recorded from normal animals in diverse preparations, including the intact cat [45], whole brain preparations from the guinea pig [2], and brain slices from both guinea pig and chicken [4,43,44]. While 56% of principal cells fire spikes spontaneously in controls (Fig. 1B), by three days after UVG, the percentage of spontaneous spike firing principal cells is elevated on the lesion (94%) and intact sides (67%) of the compensated chickens, and on the lesion side of the uncompensated chickens (83%). However, on the intact
side of the uncompensated chickens, all of the principal cells are silent. Change in the percentage of silent neurons also occurs in vestibular nuclei neurons on the lesion and intact sides after UL performed on adult whole animals [36,38,39] and in vitro [5]. Accordingly, there is a capacity for bidirectional transformation between spontaneous spike firing and silent vestibular nuclei neurons, and these transformations are sensitive to lesions.

Besides modifications in the number of spontaneous spike firing cells, the rate of spike discharge is subject to change after vestibular deafferentation. Spontaneous spike discharge rate may be calculated by averaging the responses from all the recorded neurons, including the spontaneous spike firing and silent cells (Fig. 1C), or by averaging the responses from the spontaneous spike firing cells only (Fig. 1C2), with both calculations revealing similar patterns. In the case of the tangential principal cells, spontaneous spike firing neurons on the lesion (32 spikes/sec) and intact sides (35 spikes/sec) in the compensated chickens, as well as on the lesion side (38 spikes/sec) in the uncompensated chickens exhibit increased spike discharge rates compared to controls (18 spikes/sec). Similar results are obtained from MVN neurons recorded on the lesion side after UL performed on the rat [6] and guinea pig [51]. However, MVN neurons on the intact side show decreased or unchanged spike discharge rates [5,6]. It is interesting that the mean spike discharge rate for spontaneous spike firing principal cells in chickens is within the same range as that found by averaging the responses from different classes of spontaneous spike firing MVN neurons from adult rat, mouse and guinea pig brain slice preparations [3,25,39]. This finding suggests that spike discharge rate of vestibular nuclei neurons may not be a sufficient indicator of the critical events regulating recovery of function after vestibular lesions. In addition to this, there is a dissociation in some systems between the capacity for vestibular nuclei neurons to recover symmetric spontaneous spike firing bilaterally and the process of vestibular compensation [39]. For example, one day after UVG, principal cells exhibit symmetric spontaneous spike firing on both sides, but the chickens show extremely severe postural deficits [44]. Apparently, recovery from the postural deficits must involve activity from more than one group of vestibular nuclei neurons.

The global output of a vestibular nucleus may provide a more accurate assessment of the extent of recovery. This could be calculated by weighting the percentage of spontaneous spike firing neurons in a formula with spike discharge rate. Change in either factor, or both, will modify the global output of the nucleus. Finally, other components besides vestibular nuclei neurons must be instrumental in determining how the lesion affects overall vestibular reflex function at different times after the lesion.

6. Intrinsic membrane conductances

Spontaneous spike activity is generated by the spatiotemporal integration of excitatory and inhibitory synaptic inputs and/or intrinsic membrane conductances of the postsynaptic neuron [43]. Within one day after UL or UVG, spontaneous spike discharge is not abolished by applying a cocktail of excitatory and inhibitory synaptic transmission antagonists [19,43]. Accordingly, shortly after vestibular deafferentation, intrinsic membrane conductances must be involved critically in the changing neuronal excitability. Passive membrane properties, including resting membrane potential and input resistance, also change after vestibular deafferentation. Compared to controls, vestibular nuclei neurons on the lesion side have depolarized resting membrane potentials and/or higher input resistances, either of which would increase neuronal excitability. In contrast, vestibular nuclei neurons on the intact side exhibit the opposite set of changes, which would tend to decrease their excitability [4,5,22,44].

There have been few studies focused on identifying the intrinsic ionic conductances in vestibular nuclei neurons from vestibular deafferented animals. So far, all the investigations have been performed on brain slice preparations and focus on the conductances important for spike generation during development [9,34,43]. For example, after UL, the number of MVN neurons which express low-threshold calcium spikes increases on the lesion side [22], which could increase their responsiveness to the remaining synaptic inputs. While no calcium spikes are detected in the principal cells on the lesion side after UVG, the percentage of spontaneous spike firing principal cells increases and exhibit larger $I_{Na}$ (Fig. 2A) with lower activation thresholds for $I_{Na}$ compared to the silent cells (Fig. 2B). Altogether, these modifications allow some principal cells to generate spontaneous spike firing more readily. Thus, by increasing the number of spontaneous spike firing principal cells after UVG, $I_{Na}$ and $I_{Na}$ contribute to an overall increase in the global output of the tangential nucleus. From studies measuring the transcriptional level of sodium channels after UL [32,50], there is no
Fig. 2. $I_{\text{NaP}}$ and $I_{\text{Na}}$ in spontaneous spike firing and silent principal cells. A, averaged I/V curves for $I_{\text{NaP}}$ in spontaneous spike firing and silent principal cells recorded from all experimental groups. Regardless of experimental group, the spontaneous spike firing principal cells exhibit larger amplitude $I_{\text{NaP}}$ compared to the silent principal cells ($p < 0.05$). B, At $-50$ mV (*), there is a significant difference in $I_{\text{Na}}$ amplitude between spontaneous spike firing and silent principal cells.

Evidence for sodium channel involvement in vestibular compensation. However, the latter approach does not address translational modifications or surface targeting of sodium channel proteins at specific cellular microdomains, which could greatly influence neuronal excitability. Indeed, sodium and calcium channels are heavily involved in increasing neuronal excitability in the ventral posterolateral nucleus of the thalamus after spinal cord injury [20,53].

Like the majority of their embryonic counterparts [13,35], some principal cells on the intact side of the uncompensated chickens fail to generate repetitive spike firing on depolarization. Three days after UVG, about half the principal cells on the intact side generate either single spikes on depolarization or require higher than normal current injections to generate repetitive spike discharge [44]. In addition, in voltage-clamp recordings, the I/V curve for $I_{\text{DS}}$ is steeper, and $I_{\text{DS}}$ amplitude and its percentage of the total outward current at $-30$ mV are higher for principal cells on the intact side compared to principal cells from all other experimental groups (Fig. 3A–C), supporting the presence of larger $I_{\text{DS}}$ conductances, with greater control over principal cell activity on the intact side. All of these modifications contribute to the decreased spike discharge recorded in principal cells on the intact side.

Immunolabeling and confocal imaging show that Kv1.1 expression is stronger in principal cell body cytoplasm from the lesion and intact sides of uncompensated chickens compared to controls (Fig. 4A–J). Also, Kv1.1 surface expression increases for principal cell bodies on both the lesion and intact side of the uncompensated chickens compared to controls, with a significant increase on the intact side compared to the lesion side (Fig. 4L). However, the ratio for Kv1.1 cell surface/cytoplasmic expression in principal cells increases significantly only on the intact side of uncompensated chickens, indicating a higher efficiency of surface expression on that side (Fig. 4M). In summary, intrinsic membrane conductances are a major contributor to the changing excitability recorded in vestibular nuclei neurons at early stages after vestibular deafferentation.
7. Changes in presynaptic glutamate release after vestibular deafferentation

The inhibitory system, including GABA and glycine, has been studied intensely after vestibular deafferentation [31], but there have been few studies focused on characterizing the excitatory glutamatergic system. Using microdialysis, changes in glutamate concentration occur in rat MVN neurons on both sides immediately after UL, but returns to normal levels after 12 hours [23]. At present, there are no data to support pharmacological changes in NMDA [26,48], or transcriptional changes in AMPA receptor subunits up to 8 days after UL [37]. However, on stimulating the primary vestibular fibers after UL, rat MVN neurons exhibit NMDA receptor-dependent long-term potentiation on the lesion side and NMDA receptor-dependent long-term depression on the intact side more frequently than observed in controls, suggesting that these receptors are involved in vestibular compensation [18, 33]. In whole-cell voltage-clamp recordings, sEPSCs frequency in the tangential principal cells increases almost four-fold on the lesion side of the uncompensated chickens (Fig. 5A) at the same time that Kv1.2 expression in synaptotagmin-positive terminal profiles on the principal cell bodies decreases significantly (Fig. 5B–K). Decreased Kv1.2 expression in somatic terminal profiles could explain the increased sEPSC frequency recorded, because Kv1.2 is known to control aberrant spike firing at other synaptic terminals [11]. Since no new terminals are detected on the principal cell bodies using transmission electron microscopy at 1–56 days after UVG [1], it is possible that the change in excitatory synaptic events occurs at preexisting terminals. In addition, Kv1.2 expression may change at the terminals on the principal cell dendrites, which have not been investigated. In frogs which undergo partial unilateral vestibular neurectomy, on stimulating the unlesioned primary vestibular fibers, the amplitude of the evoked postsynaptic excitatory potentials in vestibular nuclei neurons on the lesion side increase significantly, suggesting that new synapses form from the remaining inputs [16]. The rewiring of the preexisting vestibular neural circuitry could be functionally appropriate and enhance recovery, or inappropriate so that recovery is impaired [10]. The latter explanation may underlie
why the principal cells on the lesion side of the uncompensated chickens exhibit highly increased sEPSC frequency without recovery of function three days after UVG.

In conclusion, after unilateral primary vestibular deafferentation, the onset of behavioral recovery coincides with changes in the presynaptic and postsynaptic ionic conductances in vestibular nuclei neurons. Increased sEPSC frequency in the principal cells on the lesion side of uncompensated chickens could be due to increased presynaptic glutamate release at terminals which undergo decreased Kv1.2 expression. The increased spontaneous spike discharge rate in the principal cells on both sides of the compensated chick-
Fig. 5. A, sEPSC frequency in the principal cells from controls, and lesion and intact sides of compensated and uncompensated chickens three days after UVG. There is a significant increase in sEPSC frequency on the lesion side in uncompensated chickens. B–J, Kv1.2 immunolabeling in synaptotagmin-positive terminal profiles on principal cell bodies from controls (B–D), lesion (E–G) and intact sides (H–J) of uncompensated chickens three days after UVG. Kv1.2 immunolabeling decreases significantly in perisomatic terminal profiles contacting the principal cell bodies on the lesion side compared to controls ($p<0.001$) and intact side ($p<0.05$). K, Mean pixel brightness ($\pm$ SEM) for Kv1.2 in synaptotagmin-positive terminal profiles contacting the principal cell bodies from controls, sham-operated, and uncompensated chickens three days after UVG. Kv1.2 decreases significantly only in the terminal profiles contacting the principal cell bodies on the lesion side three days after UVG in the uncompensated chickens ($p<0.05$).
ens, and on the lesion side of uncompensated chickens, could be attributed to increased $I_{\text{Na}}$ amplitude of the spontaneous spike firing principal cells compared to the silent cells. The decreased excitability of principal cells on the intact side of uncompensated chickens could be due to decreased $I_{\text{Na}}$ amplitude and increased $I_{\text{DS}}$ amplitude, with the latter supported by finding increased $Kv1.1$ surface expression in the principal cells. Altogether, vestibular compensation coincides with the reacquisition of two functional properties in a single class of vestibular nuclei neurons: (1) symmetric spontaneous spike discharge rates bilaterally, and (2) restoration of symmetric sEPSC frequencies bilaterally. Indeed, the sequence of events leading to changes in presynaptic activity and postsynaptic neuron ionic conductances need to be further explored.

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References


