**Invited Review**

**Development of neural correlates of linear motion in the rat vestibular nucleus**

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**Abstract:** The capability of the central vestibular system in utilizing cues arising from the inner ear determines the ability of animals to acquire the sense of head orientations in the three-dimensional space and to shape postural movements. During development, neurons in the vestibular nucleus (VN) show significant changes in their electrophysiological properties. An age-dependent enhancement of membrane excitability is accompanied by a progressive increase in firing rate and discharge regularity. The coding of horizontal and vertical linear motions also exhibits developmental refinement in VN neurons. Further, modification of cell surface receptors, such as glutamate receptors, of developing VN neurons are well-orchestrated in the course of maturation, thereby regulating synaptic efficacy and spatial coding capacity of these neurons in local circuits. Taken together, these characteristic features of VN neurons contribute to developmental establishment of space-centered coordinates within the brain.

**Key words:** spatial recognition; vestibular nucleus; otolith organs; linear acceleration; development

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**1 Introduction**

Receiving signals from the vestibular afferent, the vestibular nucleus (VN), which is the first relay station within the vestibular pathway, plays a central role in coding orientation of the head in space and in coordinating postural movement [1, 2]. Interaction between the VN on either side of the brainstem also plays a cardinal role in the processing of sensory cues [3–6]. Information encoded by central vestibular neurons is useful for a variety of physiological functions. Generation of reflexive bodily responses toward unbalanced states is...
mediated by the vestibulospinal and vestibulocollic tracts that are involved in the control of limb muscles (for keeping balance) and neck muscles (for support of the head) [7, 8]. Control of compensatory movements of the eyes for stabilization of visual gaze [9, 10] is mediated by the vestibulo-ocular tract. Also, communications between the VN and cerebellum contribute significantly to the maintenance of equilibrium and stabilization of gaze [11]. Acquisition of the sense of head position [12–14] as well as construction of our perception about self-motion [15, 16] are mediated via ascending projections to the cerebral cortex [17]. In addition, VN neurons send projections to the nucleus of the solitary tract, dorsal motor nucleus of the vagus nerve, and other brainstem regions involved in autonomic responses associated with motion sickness [18, 19].

In this review, the capacity of the central vestibular system in processing spatial information during postnatal development will be discussed, focusing on developmental changes in the electrophysiological properties of VN neurons as well as developmental coding of linear motions in the three-dimensional space. In addition, it becomes evident that cell surface neurotransmitter/neuromodulator receptors play an important role in regulating the properties of VN neurons. In particular, we will discuss the functional roles of ionotropic and metabotropic glutamate receptors in developmental fine tuning of the vestibular circuitry for spatial detection.

2 Developmental changes in electrophysiological properties of central vestibular neurons involved in coding orientation

2.1 Membrane properties
In mice, most neurons in the medial VN displayed immature membrane excitability and low spontaneous discharge rate at P5 [20, 21]. Pacemaker conductance for the generation of a steady spontaneous discharge and overshooting sodium spikes were not observed until P10. The rate of spontaneous discharge increased to the adult level by P30. Medial VN neurons also exhibited a rostro-caudal gradient in the time course of the maturation. During postnatal development, cells located in the rostral region acquired high firing rates and mature shapes of action potential earlier than those in the caudal region.

Neurons in the medial VN can be classified into two major functional subtypes (viz. type A and type B neurons) by their action potential profiles, which are different in terms of the shape of their spikes and after-hyperpolarizations (AHP) [22–24]. Type A cells having a single deep AHP correspond to the tonic, regular vestibular neurons (with more stable spontaneous activity), whereas type B cells having an early fast and a delayed slow AHP correspond to the phasic, irregular vestibular neurons (with more irregular spontaneous activity) [25]. The ratio of type A to type B neurons is different between species (such as rodent, guinea pig and chicken), probably due to their specific signal processing requirements of the vestibular system [26]. Furthermore, a third class of neurons, which are non-homogeneous cells that could not be fitted into either one of the two main classes, is known as type C neurons. These are only observed in the medial VN, but not other vestibular subnuclei [27].

During the first two weeks of postnatal development in rats, the width of the action potentials generated in the medial VN decreased as the firing rate increased. Before P10, a significant decline in firing rate was observed when sustained depolarization was applied. From P15 onwards, the neurons showed a firing rate higher than 100 spikes per second, and exhibited only little adaptation toward sustained depolarization [28]. These changes in the properties of spike generation roughly corresponded to the alteration of the expression of Kv3.1 potassium channel [29]. Kv3.1 potassium channel, first detectable in the rat VN at E17, underwent a progressive increase that persisted into the second postnatal week, during which an increase in firing rate was observed. Consistently, most of the neurons that expressed Kv3.1 potassium channel had narrow action potentials and exhibited high-frequency firing rates with little or no spike adaptation [29].

In mice with knock-out of the potassium channel gene (KCNE1−/−), hair cells in the inner ear were completely degenerated after birth [30], resulting in rapid bilateral circling during locomotion. In these mutant mice, VN neurons showed a low expression of calcium-binding proteins (such as calbindin and calretinin) and a delay in maturation of membrane properties (in terms of membrane potential, spike features and AHP). Notably, potassium channels in the VN have recently been shown to be involved in the expression of vestibular behaviors in adult rats [31].

2.2 Response characteristics to linear acceleration
Translational movement on the horizontal plane is
essentially sensed by the utricles while that on the vertical plane is sensed by the saccules of the otolith organs [32–34]. These signals are then relayed to the VN via the primary vestibular afferents [35].

The dynamic properties of otolith-related neurons in the VN were first revealed in canal-plugged cats subjected to wobble rotations that presumably activated only the otolith end organs in the inner ear [36]. With increase in the frequency of stimulation, some neurons were characterized by stable phase lead (otolith-afferent-like pattern) and others by progressive phase lag (otolith-forelimb reflex-like pattern). The latter group of otolith-related VN neurons has been implicated to be involved in otolith-spinal reflexes during head positional changes in space [37], thereby mediating motor balance.

With sinusoidal linear acceleration at different directions on the horizontal plane, central otolith neurons are characterized by two response vectors of different spatial and temporal quadratures [38, 39] rather than the traditional single polarization vector [40, 41]. Based on their spatiotemporal tuning properties, otolith-related VN neurons are categorized as one-dimensional (with narrowly spatiotemporal-tuned properties) or two-dimensional neurons (with broadly spatiotemporal-tuned properties). For the latter group, one vector encodes linear acceleration (i.e. the maximum response vector) whereas the second perpendicular vector encodes the time derivative of linear acceleration (i.e. the minimum response vector). These response features of otolith-related VN neurons were also manifested using off-vertical axis rotations (OVAR), which sequentially stimulate all hair cells in the utricle and in the horizontally bent regions of the saccule [42–44]. In response to OVAR in clockwise and counter-clockwise directions, a large proportion of VN neurons showed a velocity-stable ratio of symmetric bidirectional response sensitivity (i.e. symmetric response magnitudes to clockwise and counter-clockwise rotations). Conversely, some VN neurons exhibited velocity-variable and asymmetric bidirectional response sensitivities.

Although the pattern by which spatiotemporal convergence emerges in VN neurons during postnatal development remains unexplored, some features in response to natural otolith inputs have been reported. Otolith-related VN neurons can be classified into clipped or non-clipped types based on their responsiveness to OVAR. Non-clipped neurons showed a full-cycle response to OVAR whereas clipped neurons discharged only during parts of each stimulus cycle and were silenced during the other parts of the cycle [41, 44]. In P7 rats, the majority of responsive neurons were clipped and the proportion decreased progressively during postnatal development. In adult rats, only less than one-quarter of responsive neurons showed a clipped response. Although clipped neurons exhibited lower resting discharge rates than non-clipped neurons at all age groups studied, an age-dependent increase in spontaneous activity was observed in both types of neurons [44]. Both types of neurons exhibited irregular discharge at P7 but became more regular as the rats matured. With increase in the velocity of OVAR, irregular neurons of young rats exhibited phase-stable and phase-shift responses, while those of older rats showed only the phase-stable response. This distinction was however not observed amongst regular neurons. These features of otolith-related VN neurons indicated refinement of their capability in coding spatial information during postnatal maturation of the vestibular system.

The response characteristics of VN neurons to sinusoidal linear acceleration in the vertical axis, presumably activating saccules in the inner ear, were also analyzed in alert cats [45]. Most responsive neurons displayed a firing rate modulation which followed the input frequency, with a response phase close to the motion velocity. Interestingly, the remaining neurons displayed a response waveform double that of the input frequency, suggesting that this pattern could result from convergence of otolith afferents with opposite polarization vectors. Indeed, cross-striola interaction on secondary VN neurons has been documented with in vivo electrophysiological approach [46]. Furthermore, VN neurons receiving signals from both utricular and saccular inputs have been identified in cats [47], implicating that these neurons could integrate sensory information arising from horizontal and vertical linear accelerations.

### 3 Coding of horizontal and vertical linear motions in the developing VN

Using the expression of immediately early gene c-fos and its protein product Fos which have been taken as a neuronal activation marker for novel physiological input [48, 49], the distribution of central neurons responsive to specific types of vestibular stimulation has been
identified, allowing the developmental profile of functionally activated VN neurons to be mapped.

In response to OVAR or horizontal linear motion, otolith-related neurons within the VN were detected in rats as early as P7 [50, 51]. The density of Fos-immunoreactive (Fos-ir) neurons increased progressively with age, reaching the adult level by P21. In adult rats, Fos-ir neurons were clustered in the lateral edge of the spinal VN and in the medial edge of the medial VN [50, 52, 53]. In response to sinusoidal linear acceleration along the vertical axis, VN neurons, primarily in the medial and spinal VN, were detected also from P7 onwards [54]. These therefore implicate that three-dimensional spatial orientations are encoded in otolith-related VN neurons by the end of the first postnatal week.

In P7–adult rats that were subjected to sinusoidal linear acceleration along the transverse axis on the horizontal plane, Fos-ir neurons responsive to high frequency stimulation (>1.5 Hz) were clustered in the lateral region of the medial VN while those to low frequency stimulation (<1.0 Hz) were found in the medial portion of the medial VN. An age-dependent increase in the number of these neurons was also observed [51]. With high frequency vertical linear acceleration, Fos-ir neurons in the medial VN were also predominantly found in the lateral portion [54, 55], similar to the horizontal otolith system. These findings therefore provide the anatomical basis that translational movements at different frequencies are differentially encoded in specific regions within the VN.

4 Contribution of receptors in the VN

4.1 Receptors in adult vestibular neurons

A variety of neurotransmitter/neuromodulator receptors are expressed in the VN of adult animals. Examples of receptors for neurotransmitters include glutamate [55–60], γ-aminobutyric acid (GABA) [61–63], glycine [64–66], and acetylcholine [67, 68]. Examples of receptors for neuromodulators include serotonin (5-HT) [69–71], endocannabinoid [72], and orexin receptors [31, 73]. It is noteworthy that receptors of histamine which can be classified either as a neurotransmitter or neuromodulator, are also expressed in the VN [74, 75].

Glutamate transmission, mediated by ionotropic and metabotropic glutamate receptors, plays a key role in information processing within the VN. Ionotropic glutamate receptors on second-order VN neurons participate both in direct synaptic response to primary afferent stimulation and in excitatory transmission within the intrinsic circuitry of medial VN [68, 76]. Metabotropic glutamate receptors (mGluRs) contribute to vestibular afferent-induced synaptic plasticity, particularly long-term potentiation (LTP) [56, 57, 77]. Notably, most neurons in the medial VN are under the inhibitory control of presynaptic mGluR [56]. Subtypes of mGluR in the medial VN, indeed, play differential roles in synaptic plasticity. The early induction phase of LTP is inhibited by groups II and III mGluRs while the full expression of LTP depends on group I mGluR [77, 77]. Nevertheless, current knowledge on the role of ionotropic glutamate receptors in the expression of long-term synaptic plasticity in the VN remains rudimentary.

Acetylcholine has been shown to mediate the transfer of otolith information to neurons in the medial VN [66]. It is also known to increase the spontaneous firing of VN neurons [67, 78, 79].

GABAergic inhibition within the VN arises from either the Purkinje cells of the cerebellum [80–82] or the contralateral VN neurons via commissural connections [83, 84]. This commissural inhibition provides a mechanism for enhancing the sensitivity of utricle-related VN neurons to horizontal linear acceleration and lateral tilt of the head [46, 85]. It is noteworthy that commissural inhibition in the saccular system was less prominent than in the utricular system.

In addition to glutamate, glycine is also released from vestibular afferent fibers [86, 87]. Glycine receptors in the medial VN play a modulatory role in membrane properties of neurons, preferentially on type B neurons [88].

Histaminergic transmission, generally excitatory in the VN, is involved in the modulation of motor behaviors [73–75]. Anti-histaminergic drugs have been used for clinical treatment of balance disorders and symptoms, such as vertigo, nausea and motion sickness [89, 90]. The effectiveness of anti-histamine drugs against these symptoms is resulted from a reduction of the effects of excess histamine release in the medial VN [91].

VN receives heavy serotonergic fibers arising from the raphe nucleus [92]. Electrophysiological studies indicated that most neurons in the lateral VN were depolarized following 5-HT treatment [93]. Also, 5-HT modulated both glutamate-induced excitation of VN neurons [71] and vestibulospinal reflexes [94]. Different 5-HT receptor subtypes exerted diverse effects on VN neurons. For example, 5-HT2 receptor mediated excit-
atory effect while 5-HT<sub>A</sub> receptor mediated inhibitory effect in the medial and superior VN [95-97]. Notably, agonist of 5-HT<sub>A</sub> receptor inhibited the activity of otolith-related neurons in the medial VN [98].

Endocannabinoid receptor CB1R is expressed in the VN of rats [72]. Administration of CB1R agonist led to long-lasting decrease in firing rate of VN neurons [99]. Given that endocannabinoid-mediated long-term synaptic plasticity has emerged as the key and most widely distributed form of presynaptic plasticity within the central nervous system [100], it is likely that endocannabinoids also regulate synaptic plasticity in the VN via presynaptic mechanism.

Orexin, released by neurons whose cell bodies are located in the lateral hypothalamus [101], could excite VN neurons via the modulation of Na<sup>+</sup>-Ca<sup>2+</sup> exchanger and inward rectifier K<sup>+</sup> current [31]. In rats, the central orexigenic system was found to regulate vestibular-mediated motor balance.

### 4.2 Ionotropic glutamate receptors in developing vestibular neurons

The expression profiles of ionotropic glutamate receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and N-methyl-D-aspartate (NMDA) receptor, have been documented in the developing vestibular system [102, 103]. Immature synapses undergo remodeling by modifications in the composition of receptor subunits.

AMPA receptors were expressed in VN neurons since early postnatal days [104]. The expression of GluA1 receptor subunit in VN reached peak level during the second postnatal week, and then decreased in the adult. In contrast, the expression level of GluA2 and GluA3 showed a gradual increase during the first three postnatal weeks. In neonates, the functional properties of AMPA receptors are mainly determined by GluA1 and GluA4 subunits [105], leading to the occurrence of high Ca<sup>2+</sup> influx in immature central neurons. This coincided with the absence of Ca<sup>2+</sup>-impermeable GluA2 subunit in VN neurons at P7 [104]. During later periods of postnatal development, the progressive decrease in Ca<sup>2+</sup> permeability of AMPA receptor channels [106] also corresponded with the increase in expression of GluA2 subunit in VN neurons [104].

A heterogeneous expression of NMDA receptor subunits in the rat VN during postnatal development has also been described [103]. Immunohistochemical expression of GluN2A became detectable from P10 onwards, and reached the peak level at P21, followed by a decrease at the adult stage. For GluN2B, only faint expression was found at birth. Though the expression level became much higher during the first two postnatal weeks, the immunoreactivity was reduced at P21. On the contrary, the expression of GluN2D was more stable, showing a moderate level of immunoreactivity throughout postnatal development. Interestingly, VN was the only brainstem region that expressed GluN2C [103, 107] although the expression was at a low level and found only between P5 and P10.

Otolith-related VN neurons, functionally activated by OVAR or vertical linear acceleration, were found to express AMPA or NMDA receptor subunits as early as P7 [55, 59]. Such expression pattern was observed in 80%-90% of otolith-related VN neurons in adult rats. The percentage of Fos-ir neurons expressing GluA1, GluA2/3, GluN1 or GluN2A subunit showed developmental invariance, but those expressing GluA4 or GluN2B subunit decreased with maturation. Co-localization of GluN1 with each of the AMPA receptor subunit was also found in these neurons [59, 108], suggesting cross-modulation between AMPA and NMDA receptors may occur in individual VN neurons during glutamate-mediated excitatory neurotransmission, contributing to the recognition of three-dimensional spatial orientations during development. Their developmental contribution to synaptic plasticity within the VN has yet to be elucidated. The functional significance of AMPA and NMDA receptors in neural circuit refinement during postnatal development also awaits further investigation.

### 4.3 mGluRs in developing vestibular neurons

In the developing medial VN of rats, the change in the expression profile of mGluR1 and mGluR5 subunits correlated with the shift from long-term depression (LTD) to LTP [109]. During the first postnatal week, GABA<sub>A</sub>-dependent LTD was observed in the medial VN which exhibited a high density of mGluR5-ir neurons and low immunoreactivity of mGluR1<sub>α</sub>. Blockade of mGluR5 reduced the occurrence of LTD [110]. The success of inducing LTD progressively decreased with maturation and LTD could never be induced after P14. On the contrary, LTP, which was NMDA receptor-dependent, appeared from P7 onwards and reached the adult level at P21. This profile corresponded with a progressive increase in mGluR1<sub>α</sub> and a decrease in mGluR5 expression [109]. The contribution of developmental modulation in synaptic plasticity of VN neurons...
to the maturation of vestibular behaviors remains to be addressed.

5 Conclusion and perspective

Vestibular information arising from the vestibular end organs is crucial in providing sensory cues for motor coordination, spatial recognition, and self-movement perception. During postnatal development, the membrane properties and cell surface receptors of VN neurons undergo significant modifications to enable computation of vestibular cues and triggering of plasticity machinery. Also, the coding of horizontal and vertical linear motions in the VN shows developmental refinement to the maturation of vestibular behaviors remains to be addressed.

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