Cross-modal sensory signaling shapes vestibulo-motor circuit specificity

Inauguraldissertation

Zur

Erlangung der Würde eines Doktors der Philosophie
vorgelegt der
Philosophisch-Naturwissenschaftlichen Fakultät
der Universität Basel

von

Emanuela Basaldella
aus Aviano, Italy

Basel, 2016

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakult¨at

auf Antrag von

Prof. Dr. Silvia Arber

(Fakultätsverantwortliche)

Prof. Dr. Peter Scheiffele

(Korreferat)

Basel, den 08.12.15

Prof. Dr. Jörg Schibler

(Dekan)

To my parents

Table of Contents

Chapter 1. Introduction

From invertebrates to mammals, the capability to perform movement is a critical aspect for the survival of the individual. It is essential for securing food, escaping from predators or situations of danger, for communicating and establishing social relations and for ensuring reproduction. In performing these actions, the central nervous system (CNS) plays a crucial role in controlling the production of coordinate and purposeful movement of the body in space. To understand how the CNS orchestrates movement, we need to break down the problem into many fundamental questions of motor control. Some of these questions are the following: how is the multiplicity of muscles and joints operated synchronously in a purposeful and dynamic manner? How is sensory information, reporting outside environment and inner body condition, used to influence motor behavior? How and where in the CNS are these complex computational problems solved?

1.1 History of Motor Control Theories

In the old times these kind of questions had nothing to do with science: Greek philosophers like Plato and Aristotele have been the first ones trying to explain movement. It is only from the beginning of the 20^{th} century with the development of science and technology that these questions were addressed in scientific terms. Charles Scott Sherrington and Ivan Pavlov were pioneer scientists that gave a substantial contribution in understanding how movement is generated and controlled.

Their work influenced the future decades of neuroscience research. In 1895, Sherrington observed that monkeys with a de-afferented arm were not using that limb anymore despite the presence of intact motor descending innervation from the brain. Based on this observation, he formulate the conclusion that sensory reflexes are basic essential elements required for the articulation of more complex motor behaviors (Mott and Sherrington 1894, Burke 2007). Also the Russian physiologist Pavlov explained movement as a combination of two types of reflexes, the inborn and conditioned ones (Green 1997). For both of these two neurophysiologists and their initiated schools, the activation of the reflex arches by sensory stimuli represent the building blocks for the production of any more complex movement. The limitation of Sherrington and Pavlov's theories is that not all the movements can be explained by simple reflex action as argued by Graham Brown and Nikolay Bernstein, who put their emphasis on the role of endogenous networks on movement control. Brown was the first one to observe that locomotion can be induced in cats without reflexes by local spinal circuits which are able to produce the basic rhythmic patterns of flexor-extensor alternation and between left and right limbs, required for sustaining locomotion (Brown 1914). This discovery led in the following years to the advancement of the concept of central pattern generators: networks of neurons whose activity oscillates between two states and can be self-maintained without the need of sensory feedback.

Bernstein approached the question of how our CNS could control all the degrees of freedom that a movement can have by developing a hierarchical view of the CNS based on the principle that complex computational tasks are resolved by

neuronal networks at the lowest possible hierarchical level (Bernstein 1947). In his view, the brain would select among the stored general programs of motor activity, so called ‘engrams’, the ones which are necessary to perform a certain motor behavior. Subsequently, lower spinal cord networks would solve the problem of which muscles are recruited and to which extent for generating the motor output that would match the selected motor program.

1.2 Current Thoughts on Motor Command Theories

The work of Bernstein has influenced our more recent understanding of generalized motor programs. A program, like an engram, indicates an abstract representation of the parameters required for guiding muscle activation in order to perform a precise motor action (Schmidt 1980). A motor program is stored by our brain and retrieved every time that specific motor plan is generated and can be adjusted by sensory feedback in a continuous manner to stabilize body posture and allow the interaction with the outside environment (Figure 1.1). In order to produce a voluntary movement, an action plan, which entails a series of motor commands, is retrieved by our brain. We hypothesize that execution is achieved by activation of parallel, dedicated descending pathways each responsible for selective modules of movement. In this model, the modular structure of these descending pathways would allow an animal to exploit a series or different motor actions that are species-specific. In Figure 1.1 we give few examples of motor behaviors characteristic for a rodent. Since animals are embedded in a very dynamic environment, our motor performance

needs to be constantly updated with information on the internal status of our body parts, on the external environmental conditions and on the relationship between the two. For this purpose, animals are equipped with a series of sensory systems (Figure 1.1 right-side) that ensure smooth and efficient motor execution by constantly updating the ongoing motor commands with information about the external and internal body condition. What is still missing to validate our model is precise anatomical, genetic and physiological characterization of the players involved. In other words, we would need to “know who does what” and how are the motor programs generated? How are they retrieved? How are the motor commands organized? Only recently, work from our lab started addressing this last question by describing a circuit module involved in skilled motor behavior (Esposito, Capelli et al. 2014). In the work described in this PhD thesis, we will focus on the role of proprioception and the vestibular sensory system in maintaining body balance. Interestingly, despite the importance of maintaining posture and balance in every situation and for any type of movement, the neuronal circuits supporting these functions and the way they integrate with the ongoing motor programs is still under intense investigation.

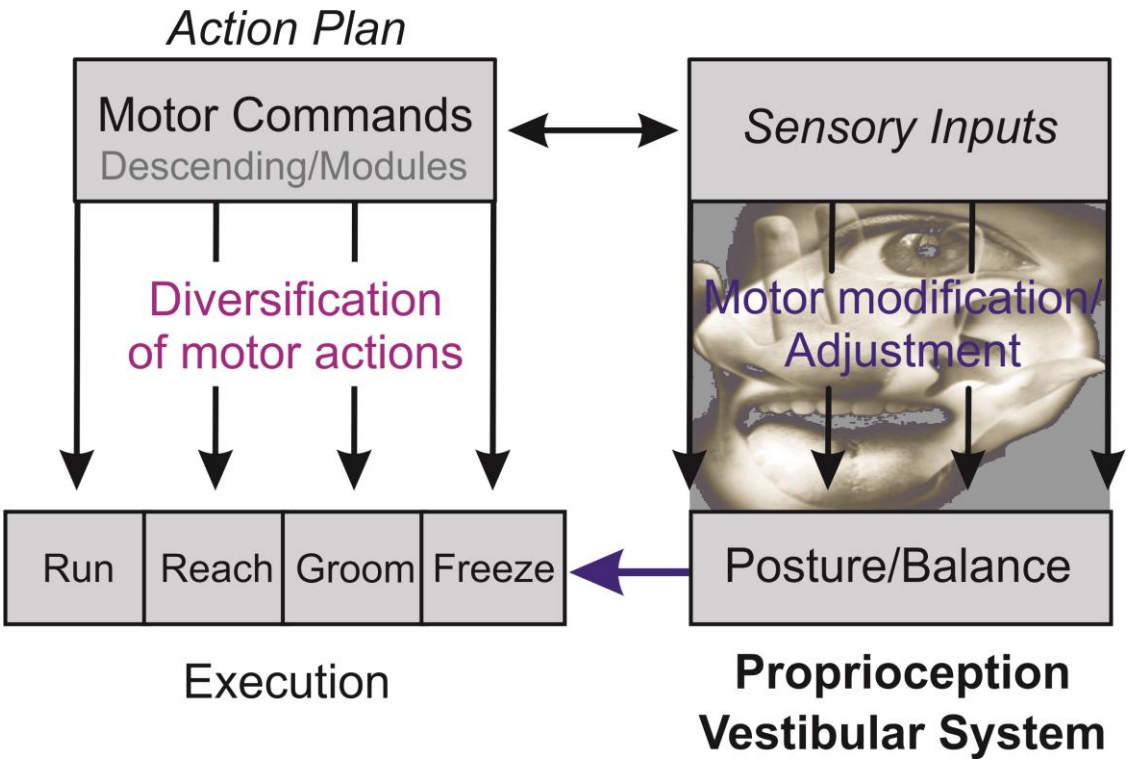


Figure 1.1 Simplified scheme illustrating our most recent view of motor control theories. See text for details.

1.3 Circuits for Maintaining Body Equilibrium

Before entering into the details of neuronal control of body balance and equilibrium, let's first clarify the meaning of these terms. Following the Collins English Dictionary an inanimate object is in an equilibrium state when the sum of all vector forces acting on it, results in a null vector. The same principle can be translated to any living organism from reptiles to fish, from birds to quadrupedal animals and humans. In every situation, when standing steady or when performing a dynamic change of position, all the body parts need to be controlled in a way that the center of mass of the individual falls constantly within the supporting surface. When this

doesn’t happen, the equilibrium is disrupted and any intended superimposed movement cannot be performed. Given the importance of maintaining the static and dynamic equilibrium in parallel with the execution of any intended motor plan, which are the dedicated, neuronal circuits that act in a continuous manner to ensure smooth motor execution? This is not an easy question to answer because those neuronal substrates act subconsciously and this fact makes it more difficult to identify the input-output sensory motor correlates for steering body balance.

In human, the Romberg test can be used for this purpose to unmask some of these mechanisms. A subject is asked to stand for 30 seconds with both feet together, eyes open and arm rested down; subsequently, he has to maintain the same position for 1 minute but with both eyes closed. A normal subject will probably show a subtitle degree of body sway when keeping both eyes closed, but a patient with a vestibular or proprioceptive disorder will feel an increased body instability, leading most of the times to an inability to maintain upright position. What we can deduce from this test is that body balance relies on the concomitant action of three sensory systems: visual, vestibular and proprioceptive. When vision is excluded but the other two sensory modalities are present, like in the eye-closed condition, the person can still maintain a fear control over its body equilibrium condition. On the contrary, when also vestibular or proprioceptive systems are compromised, their function is unmasked in a subtractive manner since the brain is not able to compute the necessary corrective movements for stabilizing the position of the body. In this PhD thesis, we aimed to understand how vestibular sensory information interacts with the final motor output system by describing the anatomical fingerprint of vestibular

input at the level of single spinal motor neurons. We also studied the mechanisms underlying the development of this connectivity matrix and revealed the existence of vestibular-proprioceptive synaptic crosstalk at level of motor neurons. Despite the fact that these two sensory systems cooperate for the common purpose of maintaining body posture and balance, they differ in terms of sensory information processed, circuit architecture and developmental mechanisms required for circuit assembly. In the next two chapters, the key features of these two systems will be introduced.

1.4 Vestibular System

1.4.1 Vestibular Receptors and Vestibular Sensory Stimulus

The vestibular system (VS) detects the head linear and angular acceleration in space and utilizes this information to control different motor functions including gaze stabilization and maintenance of body posture. Vestibular sensory information is also important for creating the perception of head-to-body position and body orientation in space with respect to the gravity force.

The vestibular sensory end organs are located in the labyrinth of the inner ear, buried inside the temporal bone. They consist of two otolithic organs, the utricle and saccule and three semicircular canals (Figure 1.2).

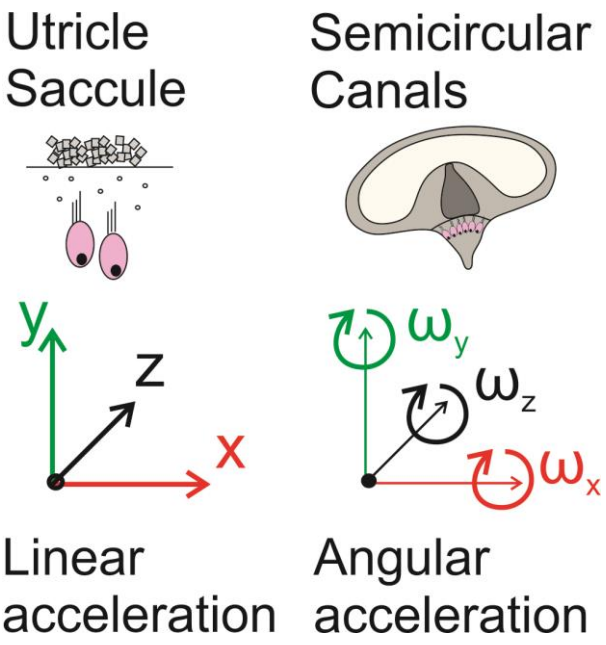


Figure 1.2 Illustration of the two types of vestibular receptor structures. Utricle and saccule, also generally called 'otolithic organs' from the presence of the otolithes, little calcium carbonate stones embedded into the viscous endolymphatic on top of the stereocilia of the vestibular endothelium. Both these organs are detecting linear head acceleration along the vertical (Saccule) and horizontal (Utricle) planes. The illustration on the right side shows a schematic view of one semicircular canal sectioned along its main axis. There are three semicircular canals, which are all interconnected to each other and their little tubular cavities are filled with endolymphatic fluid. No otolithes are present here, since the electric signal is generated from the endothelial receptor cells from the displacement of the endolymph. Each of these three structures is oriented parallel to one of the three space directions. The semicircular canals respond mostly to the angular head acceleration around the pitch, roll, or yaw axes.

The first ones detect the head displacement along the horizontal and vertical axis and derive their name from the presence of the otoconia. These are little crystals of calcium carbonate are embedded in the otolithic membrane any lay on top of the vestibular sensory epithelium, so called 'macula', surrounded by endolymph.

Head motion causes a displacement of the otolithic membrane which causes a deflection of the hear cell bundles of the epithelium, leading to the generation of a receptor potential into the hear cells that will be transmitted to the central processing

centers via the axons of the neurons in the vestibular branch of the cranial VIII nerve. The receptor cells have mirroring morphology in respect of a central line of reversal polarity called striola. Following this type of organization each given space orientation is represented twice by two population of cells with opposite polarities distributed across the line of polarity reversal. Receptor cells in the saccular macula are oriented vertically. This is why they detect head movements along the gravity force vector, while the receptors cells in the utricular macula are placed perpendicularly to them and are sensible to horizontal head movements.

While the otolithic organs sense translations and head accelerations with respect of the gravity force, the semicircular canals detect both voluntary and passive rotational movements along the pitch, roll and yaw axis. Each of the semicircular ducts is filled with endolymph and has at its base an enlargement called ampulla which contains the sensory epithelium or ‘crista’ which function as a motion sensor. Similar to what happens for the otolithic organs, an occurring head angular motion will induce a shift in the position of the hear receptor cells with respect to the endolymph, that will show a certain inertia in responding to the movement. The consequent bending of the cell receptor cilia will be transduced into an electric signal and will be used by the brain for computing the directionality of the movement.

1.4.2 Central Vestibular Afferents

The vestibular receptors cells are innervated by axons coming from bipolar sensory neurons residing in the vestibular ganglion also known as ganglion of Scarpa. These neurons send one branch to contact the hair cells of the otolithic and

semicircular canal end organs and the other branch directly to the vestibular nuclei of the brainstem and to the cerebellum. Despite the fact that otolithic and semicircular canal information is transmitted in separate channels in the periphery, the terminations of the axons are extensively overlapping centrally at the level of the vestibular nuclei (Maklad A. 2010). The vestibular sensory information is then used for computing head position in space and to regulate three main types of reflex arches: the vestibulo-ocular (VOR), vestibulo-collic (VOC) and vestibulo-spinal (VS) (Uchino and Kushiro 2011). VOR and VRC stabilize respectively the eye and the neck position when the head is in motion, to guaranty the correct perception of the additional sensory stimuli coming from the visual and auditory systems. The importance of these reflex pathways is often underestimated because their action is subconscious and difficult to uncover, but people in which the vestibular function is compromised report severe impairments of the quality of life (Atkin and Bender 1968).

A patient with bilateral vestibular dysfunction is unable to read street signs while walking, or riding a bike because the oscillations of the head induced by the swing of the body will move the visual flow in and out from the fovea causing a dramatic loss in visual acuity (Brown 1972, Falkenberg, Rubin et al. 2007). In a normal individual with a functional vestibular system, the combined action of VOR and VCR stabilizes the head as well as the eye maintaining the fixation point straight in the fovea. The circuits underlying the VOR and VCR reflexes have been under intense investigation over the last century (Lorente De Nó 1933, Dieterich and Brandt 1995, Wilson 1995, Uchino and Kushiro 2011). In contrast, much less attention has been dedicated to the elucidation of the key organizational structures in the vestibulo-

spinal pathways that influence the postural reflexes. Before entering into the details of the descending vestibular projections pathway, let's first clarify what we anatomically define as vestibular nuclear complex in the brainstem.

1.4.3 Vestibular Nuclei Anatomical Boundaries

The vestibular nuclear complex, as the name suggests, represents a group of subnuclei whose borders have been first anatomically described by Clarke in 1861 (Clarke 1861). Since the beginning of the 20th century, the description and anatomical definition of the different vestibular subnuclei did not change dramatically from the one proposed in 1957 by Brodal and Pompeiano (Pompeiano 1957).

At present, literature still defines 4 major sub-nuclear divisions according to Paxinos' atlas (Paxinos and Franklin 2004): the superior (SuVe), lateral (LVe), medial (MVe) and spinal vestibular (SpVe) nuclei.

The SuVe nucleus is located dorsomedially with respect to LVe and it is bordered rostrally by the parabrachial nucleus, caudally by the group Y, ventro-medially by the MVe and dorso-laterally by the superior cerebella peduncle (scp).

The LVe nucleus can be recognized by the presence of bigger neurons with respect to cells present in the other subnuclei. Its position is defined laterally by the inferior cerebellar peduncle (icp), dorsally by the SuVe, medially by the MVe, caudally by the floor of the IV ventricle and ventrally by the SpVe.

The SpVe is characterized by small-sized cells bordered laterally by the icp, ventrally by trigeminal nucleus (Sp5), solitary tract and solitary nucleus. Medially it confines

with the MVe, dorsally is continues into the LVe nucleus and caudally it ends in the cuneate nucleus.

The MVe spans along the entire rostro-caudal extension of the vestibular nuclear complex, and it is delimited medially by the IV ventricle and laterally by the SpVe, LVe and SuVe. A magnocellular and a parvicellular division of the nucleus can be made based on the cell size distribution.

1.4.4 Primary Vestibular Afferent Input Distribution

The VN complex represents a very important center for sensory-motor integration. They in fact use the information about head position and acceleration in space to extract motor commands required to stabilize head movements as well as for controlling body posture.

The linear and angular acceleration signals detected by the utricle, saccule and the three semicircular canals are conveyed by the vestibular nerve afferents to the ipsilateral vestibular nuclei in the brainstem as well as to the cerebellum where they terminate mainly on the granule cell layer of lobules IX (uvula) and X (nodulus) constituting the so called ‘vestibulo-cerebellum’, a region which has an important role in adaptation after vestibular damage (Barmack, Baughman et al. 1993). Cerebellar and brainstem axonal projections originate from two different sets of primary vestibular neurons each of them innervating one of the two sets of vestibular receptor hair cells that have opposite directionality across the line of polarity reversal (Maklad A. 2010). The functional role of this parallel circuit architecture organization is still

unknown. Concerning the specific topologic distribution of axonal terminations within the vestibular territory, electrophysiological studies done in frog have shown how semicircular canals and otolithic organs signals converge at level of a single secondary vestibular neurons and they are broadly distributed across all vestibular subnuclei (Straka, Holler et al. 2002). Interestingly, electrophysiological studies done in cat show that 44% of vestibular cells receiving saccular inputs also receive inputs from the posterior semicircular canals. About half of these converging cells project to the spinal cord and are located mainly in the lateral, spinal and medial vestibular subnuclei (Sato H. 2000). In the same study, Sato and coworkers hypothesize a functional role of this pathway in the vestibulo-collic reflex to produce those compensatory neck movements required to stabilize an imposed head rotation around the roll axis. Indeed, such kind of acceleration produces an activation of both the semicircular canals and the otolithes that will respond to the linear vector component of the force generating the rotational movement. The same group showed also that sensory information coming from posterior semicircular canals and from the saccule can reach as far as L3 lumbar spinal cord level to influence the limb and trunk motor neuron recruitment (Kushiro, Bai et al. 2008). This pathway will be more extensively described in the next chapter. For a detailed review on the differences between otolithic and canal-activated pathways, refer to (Uchino and Kushiro 2011).

1.4.5 Other Input Sources Converging on Vestibular Nuclei

The vestibular nuclear complex is not only a sensory relay center but represents a computational center for sensory-motor integration (Figure 1.3).

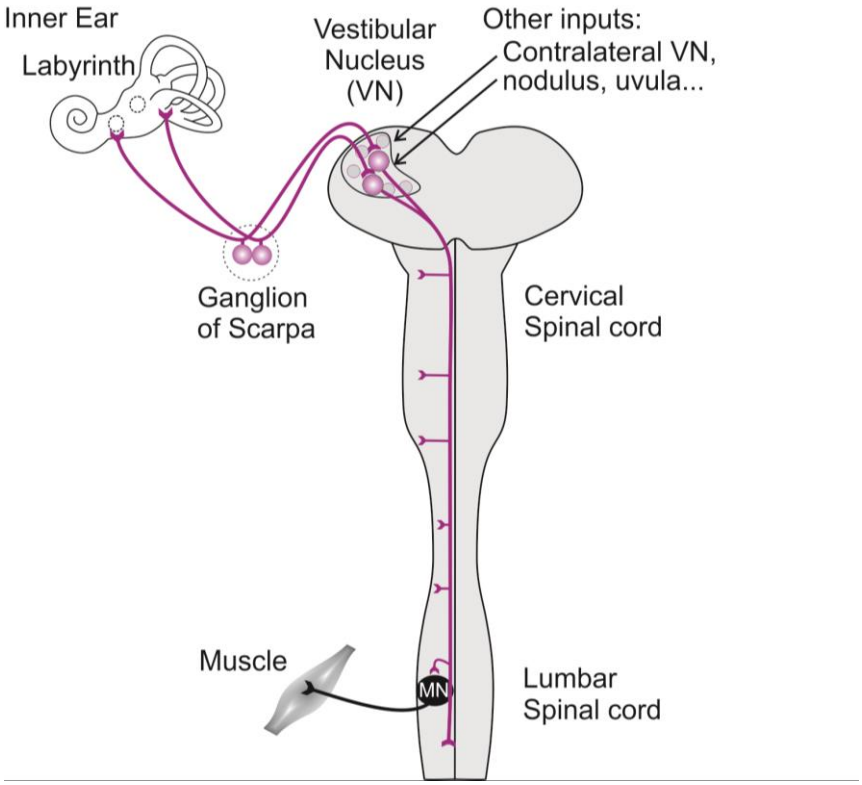


Figure 1.3 The architecture of vestibulo-spinal pathway and its main input sources. The secondary vestibular neurons project to the spinal cord (here highlighted in purple) for controlling motor neurons innervating limb and trunk muscles. They do not simply relay vestibular sensory information arriving from the inner ear through the vestibular sensory neurons sitting in the Ganglion of Scarpa, but they compute the motor output signal by integrating information coming from different input sources and predominantly from contralateral vestibular nuclei and deep cerebella nuclei.

Apart from the direct vestibular sensory input and the cerebellar innervation, the secondary vestibular neurons, with the exception of those residing in the LVe, make

reciprocal connections with other vestibular neurons residing in the other subnuclei of the ipsi- and contralateral side and this feature is preserved from amphibians to mammals (Epema, Gerrits et al. 1988, Malinvaud, Vassias et al. 2010). This commissural pathway has a glutamatergic component which represents about 30% of all the commissural vestibular fibers and a GABAergic one which account for the other 70%. (Holler and Straka 2001, Malinvaud, Vassias et al. 2010). This reciprocal innervation between symmetric vestibular structures serves to maintain a balanced discharge of vestibular neurons at rest (Graham and Dutia 2001), to recalibrate the input unbalance when a unilateral vestibular damage occurs (Curthoys 2000) and to increase the gain sensitivity for angular head motion (Markham, Yagi et al. 1977).

Another important partner for vestibular information processing is the cerebellum. As already mentioned in the previous chapter, different cerebellar regions including nodulus, uvula, flocculus, paraflcculus, vermis and deep cerebellar nuclei receive either primary or secondary vestibular information. The nodulus an uvula are reciprocally interconnected to MVe and SuVe (Balaban and Porter 1998, Barmack 2003) and they contribute to spatial dynamics of the VOR, as shown in monkey (Wearne, Raphan et al. 1998). Purkinje cells located in flocculus and paraflocculos (Krauzlis and Lisberger 1994) instead participate to the VOR by coding the eye positional information during target fixation and they velocity information during small pursuit movements (Noda and Suzuki 1979). The anterior region of the cerebellar vermis is required for mediating the vestibulo-spinal reflexes triggered by the incoming neck proprioceptive input signaling head-to-body displacement (Manzoni,

Pompeiano et al. 1998, Barresi, Grasso et al. 2012), we will talk more about this cyrcuit in Chapeter 4.1.4.

Purkinje cells of the vermis project to the fastigial nucleus, inhibiting through GABAergic synapses its output. The rostral fastigial nucleus, one of the deep cerebellar nuclei, cooperates for the body posture stabilization by encoding vestibular information more in both a body- than head-centered reference frame obtained by integrating proprioceptive and vestibular input (Kleine, Guan et al. 2004). This last evidence indicates an important role of this nucleus in motor programs related to posture and gait control and in producing postural responses and orienting behaviors. An important efferent excitatory connection, decussates in the cerebellum, then travels via the uncinated fasciculus and reaches the contralateral LVe and SpVe. A smaller fraction of fibers doesn't decussate and reach the same structures within the ipsilateral vestibular nuclear complex (Asanuma, Thach et al. 1983). Nodulus and flocculus apart from receiving direct vestibular sensory input, project to the MVe and SpVe and are involved in coordinating the oculo-motor responses (Asanuma, Thach et al. 1983).

Despite the primitive nature of the vestibular sensory information, vestibular nuclei contribute to higher intellectual functions providing information related to the perception of self motion, spatial navigation and internal models of gravity. Several thalamic nuclei integrate input from secondary vestibular nuclei with proprioceptive and visual information and are involved in the previously mentioned functions including the ventroposterior complex, the ventroanterior–ventrolateral complex, the intralaminal nuclei and the posterior nuclear group. The multisensory neurons

residing in the thalamus, project further up to the vestibular cortex: in non-human primates, the parieto-insular vestibular cortex (PIVC) has been proposed as the core vestibular region, see (Lopez and Blanke 2011) for a detailed review on vestibulo-thalamic connections and (Carleton and Carpenter 1983) for a more general overview of the afferent and efferent connections of each vestibular subnucleus.

1.4.6 Descending Control of Spinal Cord Motor Neurons

Among the vestibular pathways involved in motor control, the vestibulo-spinal connection is probably the most fascinating one because its effect is distributed along all levels of the spinal cord to influence broadly motor neuron activity and ultimately the precision of muscle recruitment. Despite its importance, more effort has always been dedicated to study vestibulo-ocular and vestibulo-collic reflex arches than to understand the vestibulo-spinal projections. Three main descending tracts originate from the vestibular nuclei territory and descend to innervate different spinal cord levels. Following the hodological nomenclature, based on axon trajectories, we can identify a medial vestibulo-spinal tract, descending on both ipsi (iMVST) and contralateral side (cMVST) and a lateral vestibulo-spinal tract, descending exclusively on the ipsilateral side (LVST). The LVST is the only vestibular pathway of vestibular origin reaching as far as lumbar spinal cord levels in cat (Grillner and Hongo 1972), while both iMVST and cMVST do not reach further than mid thoracic levels (Figure 1.4) (Nyberg-Hansen 1964). Neurons belonging to any of these three pathways

projecting down to the spinal cord give rise to several collaterals at different spinal cord levels (Shinoda, Ohgaki et al. 1989).

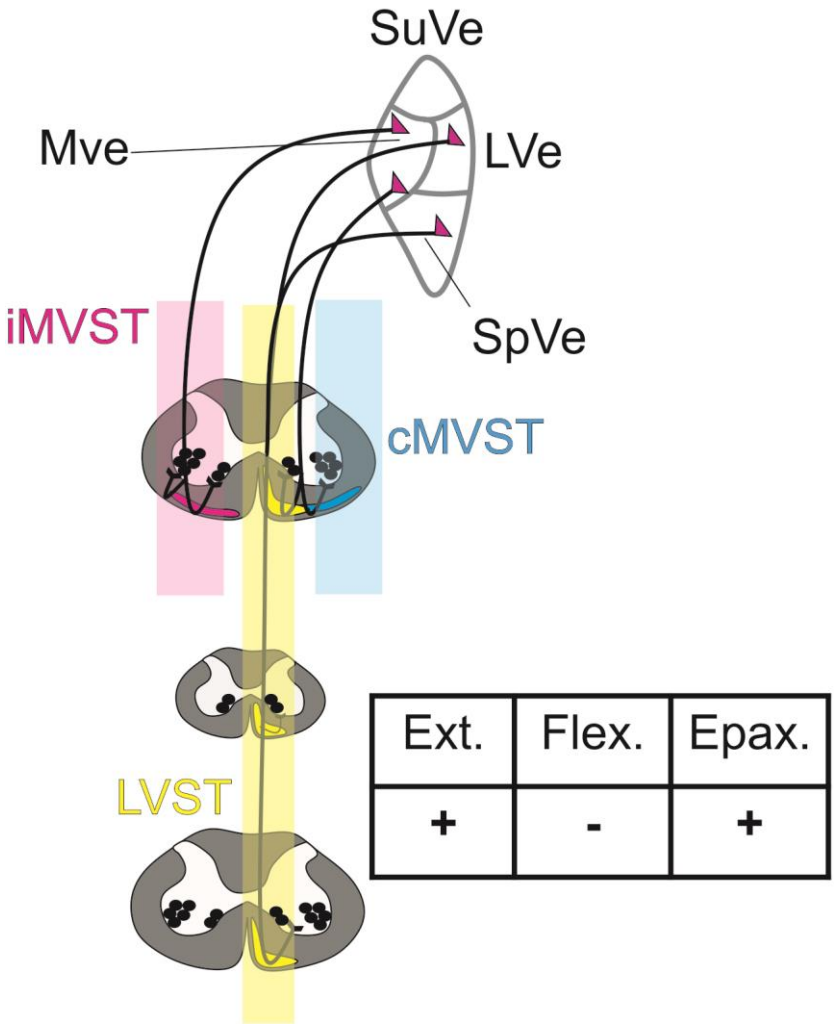


Figure 1.4 The three main vestibulo-spinal cord pathways. The LVST (highlighted in yellow) originates mainly from LVe and SpVe and is the only one reaching the lumbar spinal cord and exerting an excitatory action over the extensor or epaxial motor neurons and an inhibitory one over the flexor motor neurons (see table on the right side). MVST has an ipsilateral descending branch (here in magenta) and a contralateral one (in cyan). The axons projecting in these subgroups stop at thoracic levels and the cells bodies of these two projection streams mainly reside in MVe.

Moreover, these projection patterns are evolutionarily highly conserved in different species from amphibians to birds and mammals (Shinoda, Ohgaki et al. 1989, Díaz,

Puelles et al. 1998, Straka, Holler et al. 2002). However, the relationship between traditional anatomical nomenclature and the hodological one is controversial (Díaz, Glover et al. 2003, Pasqualetti, Díaz et al. 2007). Anatomical studies done in cat in 1977 suggest that LVe with a minor contribution from the SpVe are the main sources of axons projecting into the LVST, while the MVe, part of the LVe and a minor fraction of the SpVe are projecting into the MVST (Figure 1.4) (Rapoport, Susswein et al. 1977). While the MVST innervates neck and oculo-motor motor pools (Uchino, Isu et al. 1988), the LVST provides excitatory input to the extensor motor neurons of the lumbar spinal cord both in a direct monosynaptic manner or through intermediate neurons (Grillner and Hongo 1972). The same pathway exerts an inhibitory action over flexor motor neurons of the same spinal cord region, exciting intermediate inhibitory neurons. The functional outcome of this fine connectivity matrix is to contract the extensor muscles when it required to counteract the gravity force for example each time our foot touches the ground at the beginning of the stance phase of the step cycle (Orlovsky 1972), when postural adjustments need to be done or when counteracting an unexpected perturbation. The question would then be how and to which extent the vestibular system interacts with other sensory modalities for the execution of the selected motor program or for adopting the right corrective measures in response to an involuntary displacements.

For the control of the correct hindlimb movement and the lower trunk position, proprioception plays a key role by constantly updating the spinal cord as well as the higher brain region on the relative position of the different body parts in space and on the status of muscle contraction. Despite the final behavioral output elicited by each

one of these two pathways seems to converge toward the common goal of stabilizing body position in space, the level at which these two pathways converge and how they interact is still largely unknown. In the next chapter, I will introduce briefly the concept of proprioception before reviewing the most important evidence of vestibulo-proprioceptive interactions in the nervous system.

1.5 Proprioception

As we mentioned already in the first chapter, vestibular and proprioceptive systems cooperate to continuously adjust motor commands and ensure smooth movement execution. But what exactly does proprioception mean and which are its anatomical correlates?

Proprioception is a term introduced for the first time by Sherrington in his pioneering work published in 1907 in the context of reflex action in motor control theory (Chapter 1.1). Today, we call proprioception the sensation of relative position of the different body parts and their movement in space. This information is conveyed by mainly two classes of proprioceptive sensory end organs: the Golgi tendon organs and the muscle spindles. The first ones are free nerve endings and transmit mechanical information centrally about the joint movement through Ib fibers; the second ones are a widespread net of sensory receptors embedded in almost every muscle of our body (e.g. spindles are almost absent from diaphragm muscles (Corda, Von Euler et al. 1965)) that monitor the contraction status of the muscle fibers and convey this information centrally, through type Ia and II afferents fibers, whose cell

bodies reside in the dorsal root ganglia (DRG). Feedback from proprioceptors in crucial for accurate execution of movement and can act at different stages of information processing within the CNS. It can directly modulate the activity of motor neurons, the final stage before the motor output, via a monosynaptic reflex arch (producing the inverse myotatic reflex and the knee-jerk reflex) (Figure 1.5).

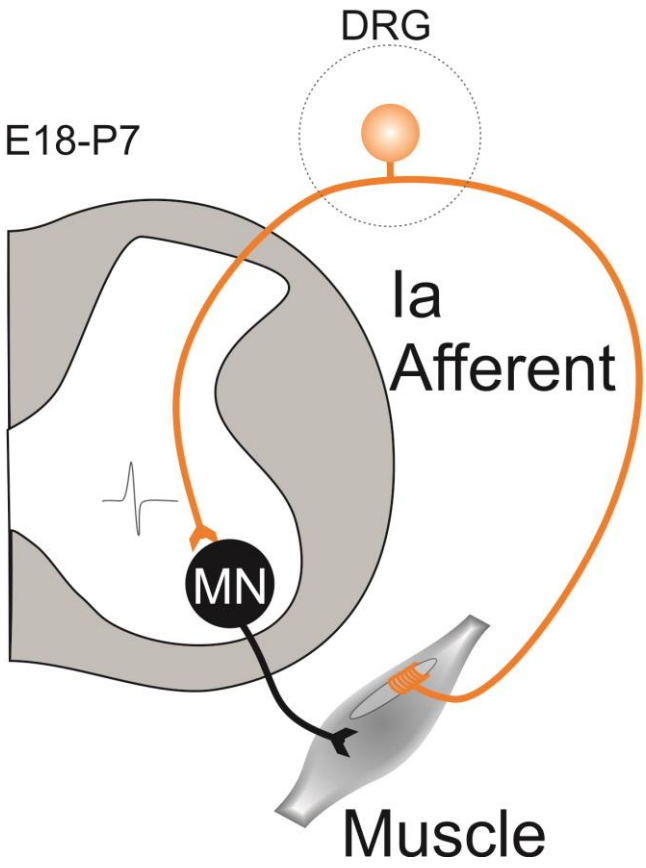


Figure 1.5 Elements of the monosynaptic reflex arch. Ia afferent fibers are the bipolar axons of the sensory neurons (here in orange) residing in the dorsal root ganglion (DRG). One axon end extends in the periphery and innervates the muscle spindle (represented ad grey oval structure within the muscle). This mechanoreceptor detects the status of muscle contraction and sends this information centrally through the second branch of the Ia axon, which enters the spinal cord, sends collaterals to interneurons and finally synapses back to the alfa-motor neuron innervating the extrafusal muscle fibers of the homonymous muscle, creating the 'Ia reflex arch' circuit (for simplicity only the monosynaptic connections have been drawn). The specificity of connections in this circuit is already established as early as E18 in mice, and it becomes fully functional within the first postnatal week (Mears and Frank 1997).

But proprioceptive sensory information acts also through polysynaptic pathways to modify ongoing motor programs in a continuous feedback loop for ensuring accurate movement execution (Pearson 2004, Ausborn, Stein et al. 2007). Input from cutaneous sensory afferents and local propriospinal networks are participating in this process as well and are integrated with proprioceptive information at different stages of motor execution (Rossignol, Barriere et al. 2008). In particular, for controlling body posture and balance, vestibular information represents a key sensory information channel complementing proprioception with crucial information about head acceleration and alignment with respect to the gravity force and about the head-to-body relative position. Having multiple sensory modalities converging on the same integration center allows retrieving the desired information from different channels when one of them is not available. Moreover, multisensory interactions play an essential role in higher-level functions such as spatial navigation and solution of sensory ambiguity. Because of their intrinsic complexity, these very intriguing cross-modal vestibular functions have just begun to be explored. In the next chapter, we will provide some examples of such interactions at the circuit level.

1.6 Cross-Modal Sensory Interaction Involves Vestibular and Proprioceptive Systems

The ability to control posture requires a precise computation system for extracting at each moment in time the position of head, and body-to-head in space. While the vestibular organs reliably solve the first task, no dedicated set of sensors

detect whole-body motion, thereby a coordinate transformation needs to be done utilizing vestibular and proprioceptive systems. As already described in Chapter 1.4.5, one place where this computation happens is the cerebellar fastigial nucleus (FN). Within the rostral part of the fastigial nucleus two classes of neurons can be found: unimodal neurons responding only to vestibular stimulation, and bimodal neurons responding to vestibular and neck proprioceptive afferents stimulation (Brooks and Cullen 2009). Bimodal neurons are likely to be the ones described to encode vestibular information in a body-centered reference frame (Kleine, Guan et al. 2004). The exact computational mechanism behind this coordinate transformation is still not understood. Additionally, the rostral FN projects to the postero-lateral ventral nucleus of the thalamus (Asanuma, Thach et al. 1983), probably contributing to perception of self-motion in space. Interestingly, it is possible to find vestibulo-proprioceptive interactions already one step down in the computational ladder of vestibular information processing, at the level of secondary vestibular neurons.

Recently, the advance of recording technologies allowed experimenters to perform single unit recordings from vestibular nuclei of actively moving monkeys and these findings have changed the way we understand the contribution of vestibular information to active and passive movement control. In alert rhesus monkeys, when a passive whole-body translation is applied to stimulate vestibular sensory receptors, both the primary vestibular afferents and the secondary vestibular neurons reliably encode head direction in space (Sadeghi, Minor et al. 2007). When however the vestibular receptors are stimulated through an active head-to-body movement, the primary vestibular afferents still reliably detect the occurring translation, as for the

passive situation but the firing of the secondary vestibular neurons is suppressed. Interestingly, when only proprioception stimulation was triggered by a body-to-head movement, no effect was recorded at the level of secondary vestibular neurons. These experiments demonstrated that the motor output of the secondary vestibular neuron is strongly attenuated during active movements when proprioceptive feedback matches the efference copy of the intended movement (Roy and Cullen 2001).

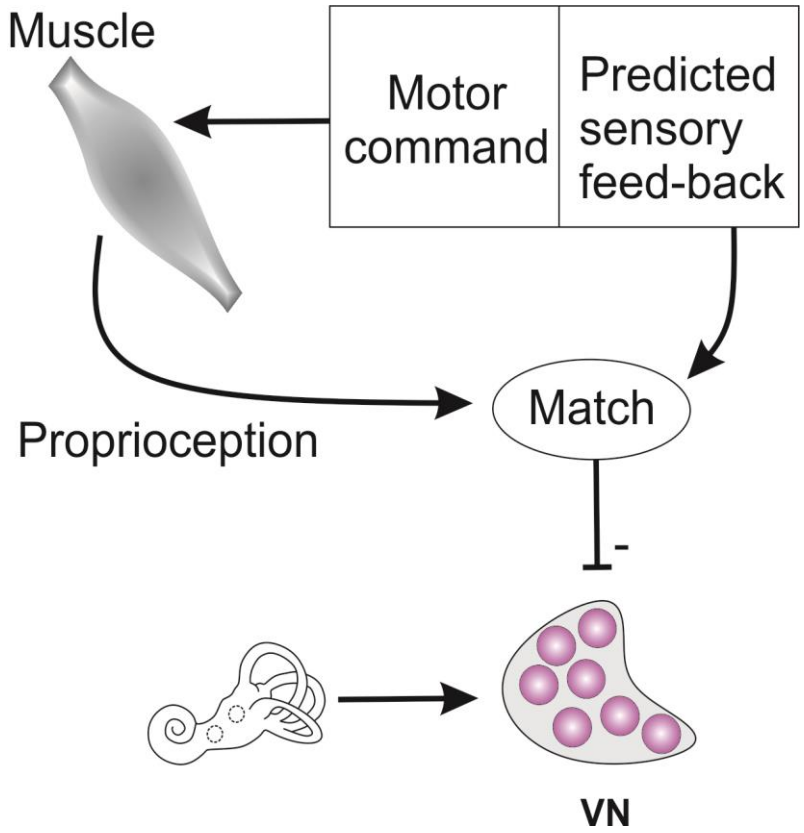


Figure 1.6 One example of cross-modal sensory interaction between vestibular and proprioceptive system at the level of vestibular nuclei. When the intended motor command is chosen to be implemented, an expectation of the consequent sensory feedback is generated. This expectation is further compared with the actual proprioceptive signal consequent to the motor command execution; when the two matching the vestibular signal output is suppressed. This mechanism may be responsible for suppressing the vestibular action on posture correction when the movement generated is voluntary **(Adapted from Roi and Cullen 2001)**

The so far reviewed effects of vestibulo-proprioceptive sensory integration are mainly addressing the problem of extrapolating a body-centered reference coordinate frame. More basic mechanisms of interaction emerge by moving towards the final stage of the motor output: the motor neuron.

At the level of spinal local circuits, vestibular and proprioceptive inputs converge at level of the ‘Ia inhibitory interneurons’ which receive Ia excitatory input from extensor limb muscles and inhibits the flexor motor neurons on the same side. Single pulse electrical stimulation of the LVe axons ipsilaterally projecting, does not produce any inhibitory response on motor neurons in the lumbar spinal cord, but concomitant stimulation of Ia fibers from knee extensor quadriceps leads to a disynaptic IPSP on the knee flexor motor neurons. (Grillner and Hongo 1972). The described mechanism provides clear evidence for a vestibular-proprioceptor interaction at the level of the last order interneurons before the motor output is produced.

In this PhD thesis, we will present another interesting mechanism for vestibulo-proprioceptive interaction occurring at the spinal motor neuron level just before the motor output is sent to regulate muscle contractions.

Chapter 2. Multisensory Signaling Shapes Vestibulo-Motor Circuit Specificity

Multisensory Signaling Shapes

Vestibulo-Motor Circuit Specificity

Emanuela Basaldella[1, 2], Aya Takeoka[1, 2], Markus Sigrist[1, 2] and Silvia Arber[1, 2]

[1] Biozentrum,

Department of Cell Biology, University of Basel,

4056 Basel, Switzerland

[2] Friedrich Miescher Institute for Biomedical Research

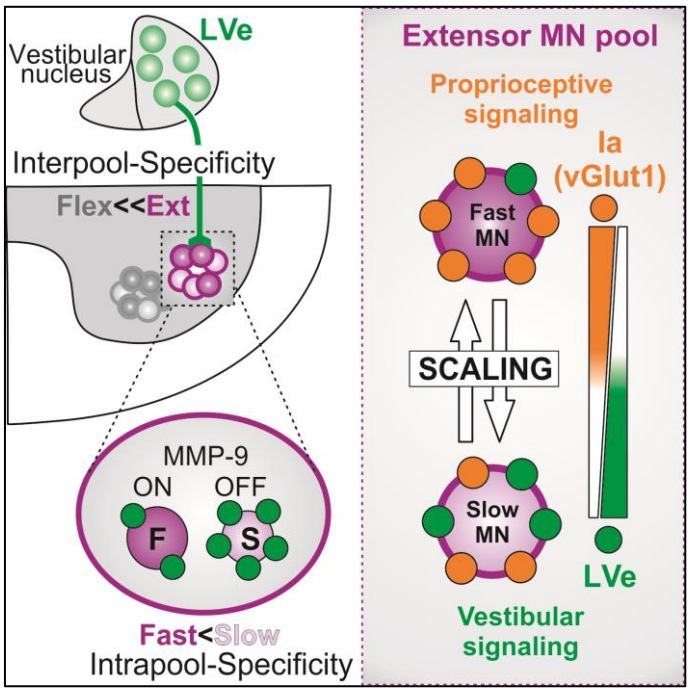
4058 Basel, Switzerland

Correspondence to S.A.

silvia.arber@unibas.ch

2.1 Summary

The ability to continuously adjust posture and balance is necessary for reliable motor behavior. Vestibular and proprioceptive systems influence postural adjustments during movement by signaling functionally complementary sensory information. Using viral tracing and mouse genetics, we reveal two patterns of synaptic specificity between brainstem vestibular neurons and spinal motor neurons, established through distinct mechanisms. First, vestibular input targets preferentially extensor over flexor motor pools, a pattern established by developmental refinement in part controlled by vestibular signaling. Second, vestibular input targets slow-twitch over fast motor neuron subtypes within extensor pools, while proprioceptors exhibit inversely correlated connectivity profiles. Genetic manipulations affecting the functionality of proprioceptive feedback circuits lead to adjustments in vestibular input to motor neuron subtypes counterbalancing the imposed changes, without changing the sparse vestibular input to flexor pools. Thus, two sensory signaling systems interact to establish complementary synaptic input patterns to the final site of motor output processing.



Graphical Abstract

2.2 Introduction

Descending motor control pathways are essential to regulate spinal circuits involved in movement (Lundberg 1975, Grillner and Dubuc 1988). Specificity of synaptic connections between upper motor control centers and the spinal output system provides the anatomical substrate to implement movement variety and precision. As animals grow up, they engage in progressively more diverse and refined motor behaviors, paralleling the establishment of functionally mature descending input to spinal circuits. Despite the importance of this descending connection matrix, the organization of its key components and especially the elucidation of developmental mechanisms involved in its establishment are still under intense investigation.

The ability to continuously adjust posture and balance during movement matures at postnatal stages in mammals (Brown 1981, Geisler, Westerga et al. 1993) and is essential to guarantee body stability. Due to the importance of these adaptive mechanisms for the execution of highly diverse motor programs, circuits steering body stabilization must exhibit a high degree of tuning flexibility. Two parallel and functionally complementary sensory signaling systems play key roles in this process. In the vestibular system, one central sensory organ in the inner ear monitors linear and rotational acceleration and provides input to the vestibular nucleus of the brainstem (Brodal and Pompeiano 1957, Angelaki and Cullen 2008). Descending vestibulo-spinal projection neurons transmit this information to spinal circuits to provide postural stability (Lund and Pompeiano 1968, Wilson and Yoshida 1968, Grillner, Hongo et al. 1970, Shinoda, Ohgaki et al. 1988). The somatosensory system represents a complementary signaling system in which sense organs are distributed throughout the entire body (Brown 1981, Matthews 1981, Abraira and Ginty 2013).

Within this system, proprioceptive sensory neurons located in dorsal root ganglia (DRG) monitor self-generated action and extrinsic perturbations in the periphery. Of these, muscle spindle afferents report the state of muscle contractions from specific sites in the periphery directly to spinal motor neurons through monosynaptic reflex arcs (Eccles, Eccles et al. 1957, Brown 1981, Windhorst 2007).

Revealing the organization of synaptic connections to spinal motor neurons is crucial to understand how vestibular and proprioceptive information influences motor output. Studies in the adult cat provide the first evidence that vestibular neurons preferentially target extensor motor neuron pools (Grillner, Hongo et al. 1970). In contrast, proprioceptors contact motor neurons of most pools in the spinal cord. A motor pool receives direct synaptic input from muscle spindle afferents supplying the same or synergistic muscles, but not from afferents innervating antagonistic muscles (Eccles, Eccles et al. 1957, Mears and Frank 1997). Thus, both extensor and flexor motor neuron pools get direct proprioceptive input but in highly specific configurations, whereas direct vestibular input seems to be preferentially targeted to extensor motor neurons in line with its body-stabilizing and anti-gravitational function. Beyond their connectivity profiles, vestibular and proprioceptive systems also interact functionally with each other and can contribute to both enhancement or depression of responses in motor neurons (Grillner, Hongo et al. 1970).

Less is known about the mechanisms guiding developmental assembly of these two sensory systems. Specific connectivity between proprioceptors and motor neurons in the same reflex arc is already present at early postnatal developmental stages in mice (Mears and Frank 1997) and activity-independent in frogs (Frank 1990). In the vestibular system, transient developmental perturbations affect motor behavior in several species (Geisler and Gramsbergen 1998, Moorman, Cordova et

al. 2002, Walton, Harding et al. 2005, Van Cleave and Shall 2006), raising the possibility that the assembly of the vestibular system might be plastic. Together, these observations provide first hints that even though proprioceptive and vestibular systems both functionally converge on motor neurons, their organization and developmental assembly mechanisms might be distinct. Moreover, whether and how they influence each other to establish mature functionality is unknown.

In this study, we exploit intersectional viral tracing technology and mouse genetics to reveal that vestibulo-spinal projection neurons in the brainstem exhibit connection specificity to motor neurons. We demonstrate that they do not only target extensor over flexor motor neuron pools, but that within extensor pools, they preferentially connect to slow over fast motor neurons. We find that connectivity profiles arise gradually at postnatal developmental stages, paralleling postural maturation. Genetic perturbation of vestibular signaling leads to interpool connectivity defects, whereas proprioceptive feedback circuit alterations induce specific connectivity shifts in synaptic scaling of vestibular input to motor neuron subtypes. These findings support a model in which two major sensory signaling systems interact at the final motor output step to establish specific connectivity profiles by complementary cross-modal signaling.

2.2 Results

2.2.1 Spatial Organization of Spinal Projection Neurons in the Vestibular Nucleus

To delineate the position of vestibular neurons with spinal projections, we performed unilateral intraspinal injections of G-protein deficient rabies viruses encoding fluorescent marker proteins (FP) (Rab-FP) (Wickersham, Lyon et al. 2007) (Figure 2.1A, B). We found that in a three-dimensional digital brainstem model (Figure 2.1C), vestibular neurons with lumbar projections were preferentially located ipsilaterally, with dominant residence within the Lateral vestibular (LVe) nucleus and with a clear spatial segregation to a caudal cluster of non-LVe neurons that were bilaterally distributed (Figure 2.1D; including Spinal Vestibular neurons, SpVe). Vestibular neurons projecting to cervical spinal levels also showed clear, albeit less pronounced ipsilateral residence within LVe, but occupied the vestibular nucleus continuously into caudal non-LVe territory (Figure 2.1D). In summary, for both lumbar and cervical vestibular projection neurons, LVe neurons exhibited a strong ipsilateral bias (Figure 2.1D). These findings confirm and extend findings that subgroups of mouse vestibular neurons exhibit differential projection trajectories to interact with local circuits in the spinal cord (Liang, Bacskai et al. 2014, Liang, Bacskai et al. 2015).

To determine the identity of vestibular neurons exerting the most direct influence on spinal motor neurons, we next assessed abundance and position of vestibular neurons with direct synaptic connections to motor neurons. We used a transsynaptic rabies virus based approach with monosynaptic restriction (Wickersham, Lyon et al. 2007) (Figure 2.1E). The majority of neurons with direct

connections to lumbar motor neurons resided in the ipsilateral LVe nucleus, with the highest density peak more dorsal to neurons with connections to cervical motor neurons (Figure 2.1F).

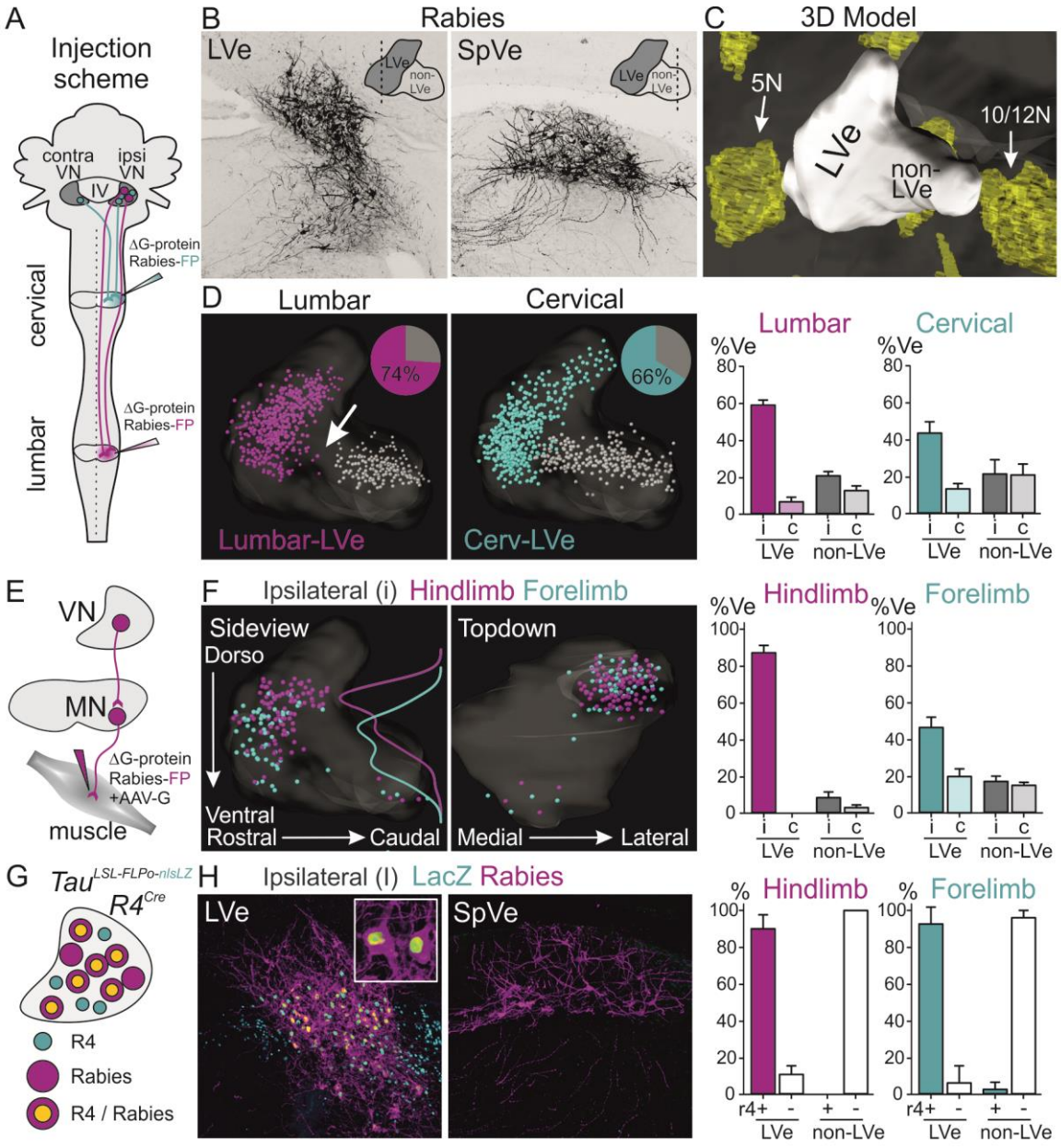


Figure 2.1. Spatial Distribution of Vestibular Neurons Regulating Spinal Motor Neurons
(A) Unilateral injections of G-protein deleted Rabies viruses encoding fluorescent proteins (FP) into cervical and lumbar spinal cord to assess the position of vestibular neurons in the brainstem.
(B) Representative coronal sections at the level of the lateral vestibular nucleus (LVe; left) and spinal vestibular nucleus (SpVe; right) ipsilateral to injection.
(C) Three-dimensional model of the vestibular nucleus used for digital reconstructions, surrounded by cranial motor nuclei 5N and 10/12N.
(D) Ipsilateral side view of digital 3D vestibular nucleus reconstructions derived from lumbar (left) and cervical (middle) spinal injections (colored neurons reside in, grey neurons outside LVe). Pie charts in upper right corners show percentages of LVe neurons. (Right) Quantification of lumbar

and cervical projection neuron composition in Ve nucleus, stratified by ipsi- and contralateral as well as LVe and non-LVe residence.
(E) Strategy for monosynaptic rabies tracing experiments to determine connectivity between vestibular and motor neurons.
(F) Side- (left) and top-down (middle) view of vestibular nucleus ipsilateral to muscle injection, depicting the position of vestibular neurons connected to FL- (cyan) and HL- (purple) innervating motor neurons. Density curves for HL and FL premotor neuron distributions along the dorso-ventral axis superimposed to the ipsilateral side-view panel. (Right) Quantification of positional distribution as in (D).
(G) Genetic strategy to mark LVe neurons by developmental origin. *R4::Cre* mice are crossed to *Tau*-reporter mice for conditional expression of nls-LacZ and FLPo expression to assess the percentage of premotor vestibular neurons marked by R4-origin.
(H) Most HL- or FL- premotor (Rabies^{ON}) neurons in LVe are marked by *R4::Cre* induced LacZ, whereas premotor neurons resident in SpVe do not carry this tag (left, middle: exemplary images; right: quantification).

To gain genetic access to neurons in the LVe nucleus, we applied a lineage tracing approach for neurons developmentally derived from different rhombomeric (R) origin (Figure 2.1G). To permanently mark R4-derived neurons, we used intersectional breeding of *R4::Cre* mice (Di Bonito, Narita et al. 2013) and the conditional neuronal reporter strain *Tau*$^{lox\text{-}STOP\text{-}lox\text{-}Flp\text{-}INLA}$ (Pivetta, Esposito et al. 2014). This strategy labeled the majority of lumbar-projecting LVe neurons, but the R4-marker was entirely excluded from non-LVe neurons (Figure 2.1H), which are derived from more caudal rhombomeres (data not shown). Together, the existence of the clearly delineated ipsilateral cluster of vestibular neurons in the LVe nucleus and the access to specific targeting approaches allowed us to next dissect projection trajectory and connection specificity of these neurons to lumbar spinal circuits with precision.

2.2.2 Lateral Vestibular Synaptic Input Is Biased to Extensor Motor Neurons

To reveal the descending projection trajectory and the synaptic arborization pattern of LVe neurons to the lumbar spinal cord, we performed focal injections of adeno-associated viruses (AAV) into the LVe nucleus. We used AAVs expressing tdTomato for axonal tracing and/or a fusion protein between Synaptophysin and GFP or Myc (Syn-Tag) for synaptic reconstructions (Figure S2.1A-C).

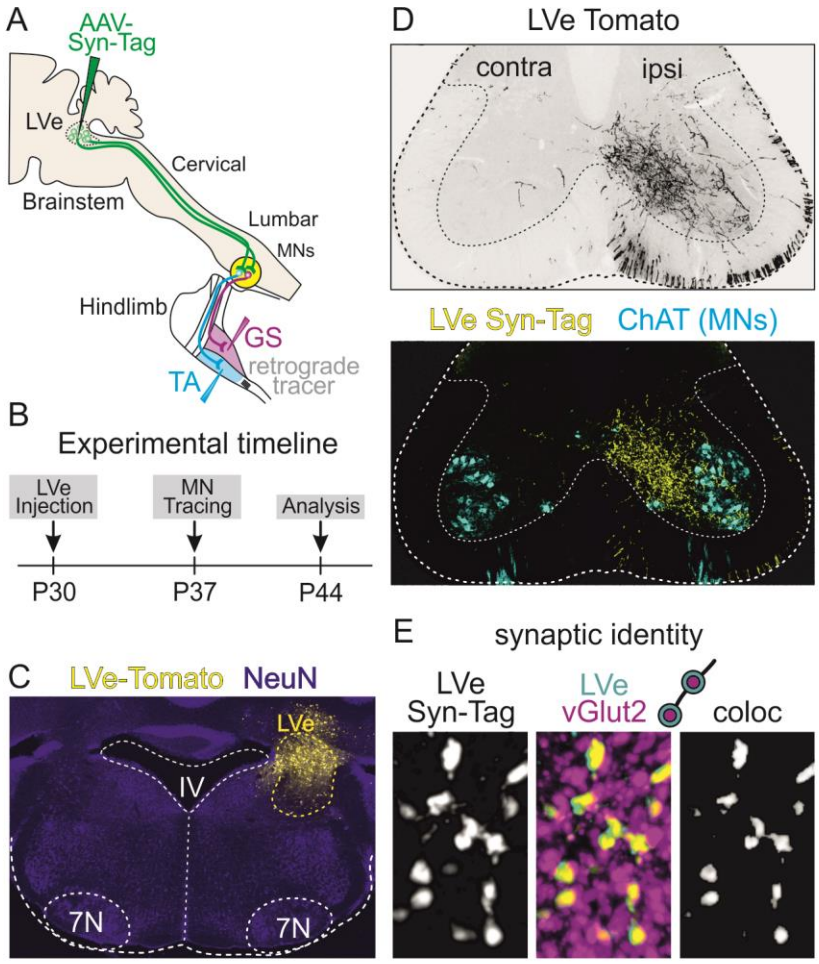


Figure S2.1. Anterograde Mapping of Vestibulo-spinal Connections, Related to Fig. 2.2
(A, B) Schematic diagram illustrating experimental approach and timeline. AAV-Syn-Tag is injected into LVe of P30 mice, followed by retrograde marking of GS or TA motor neurons by muscular tracer injections at P37, and analysis of synaptic input at P44.
(C) Coronal section of brainstem at the level of LVe to determine AAV injection specificity.
(D) Transverse spinal cord section at lumbar level L5 to visualize LVe axons by Tomato, synapses by Syn-Tag accumulation, and motor neurons by ChAT expression.

(E) High resolution image of Syn-Tag LVe synaptic terminals in the lumbar spinal cord demonstrating excitatory, glutamatergic (vGlut2) identity.

We found that axons descending from the LVe nucleus to the lumbar spinal cord were confined to ipsilateral white matter tracts (Figure S2.1D), consistent with previous experiments (Liang, Bacskai et al. 2014). Analysis of Syn-Tag distribution in the lumbar spinal cord revealed the highest density of synaptic terminals in lamina VIII ipsilateral to injection (Figure S2.1D, Figure S2.2). Many Syn-Tag puncta were also detected throughout the ipsilateral ventral spinal cord below the central canal including lamina IX containing $ChAT^{ON}$ motor neurons (Figure S2.1D, Figure S2.2). A similar distribution pattern was observed upon AAV-FRT-Syn-Tag LVe injection in *R4::Cre/Tau*$^{lox\text{-}STOP\text{-}lox\text{-}Flp\text{-}INLA}$ mice (Figure S2.2). Moreover, and similar to findings in the rat (Du Beau, Shakya Shrestha et al. 2012), the majority of $Syn\text{-}Tag^{ON}$ terminals accumulate the vesicular glutamate transporter vGlut2 (74.6%; Figure S2.1E), demonstrating that LVe spinal projection neurons provide excitatory input to the lumbar spinal cord.

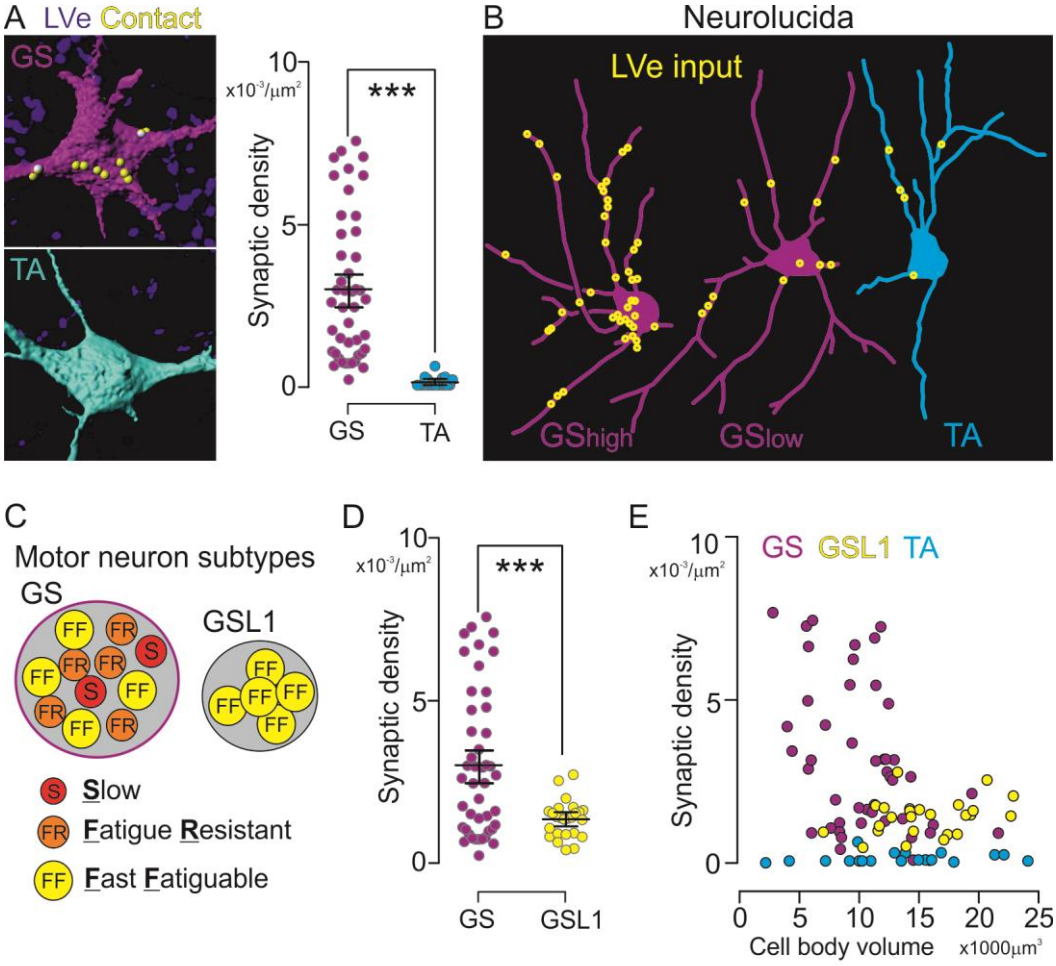


Figure 2.2 Vestibular Input Stratifies by Motor Neuron Subtype and Size
(A) Digital reconstruction and quantification of LVe synaptic input density to GS and TA motor neurons (each dot represents one motor neuron).
(B) Representative Neurolucida reconstructions of GS/TA motor neurons and LVe synaptic input (yellow). GS examples with high and low input density are shown.
(C) Motor neuron subtype composition of GS and GSL1 motor pool stratified into Slow (S), FR (Fatigue resistant), and FF (Fast Fatiguable) subtypes.
(D) Quantification of synaptic density of LVe input to GS and GSL1 motor neurons (each dot represents one motor neuron).
(E) Synaptic density of LVe input to analyzed motor neurons plotted against cell body volumes. See also Figure S2.1 and Figure S2.2.

We next assessed whether LVe input to the lumbar spinal cord exhibits synaptic specificity with respect to the identity of contacted motor neurons. We combined LVe AAV-Syn-Tag injections with retrograde tracing of motor neurons from identified hindlimb muscles (Figure S2.1A, 2.2A). We analyzed LVe input to motor neurons pools innervating the ankle extensor Gastrocnemius (GS) and the ankle flexor Tibialis Anterior (TA), due to their functional antagonism as well as previous evidence for GS-

biased vestibular synaptic input in the cat (Grillner, Hongo et al. 1970). Vestibular input was also strongly biased toward the GS compared to the TA motor neuron pool in mice, a bias detected irrespective of cell body or dendritic analysis of reconstructed GS/TA motor neurons (Figure 2.2A, B).

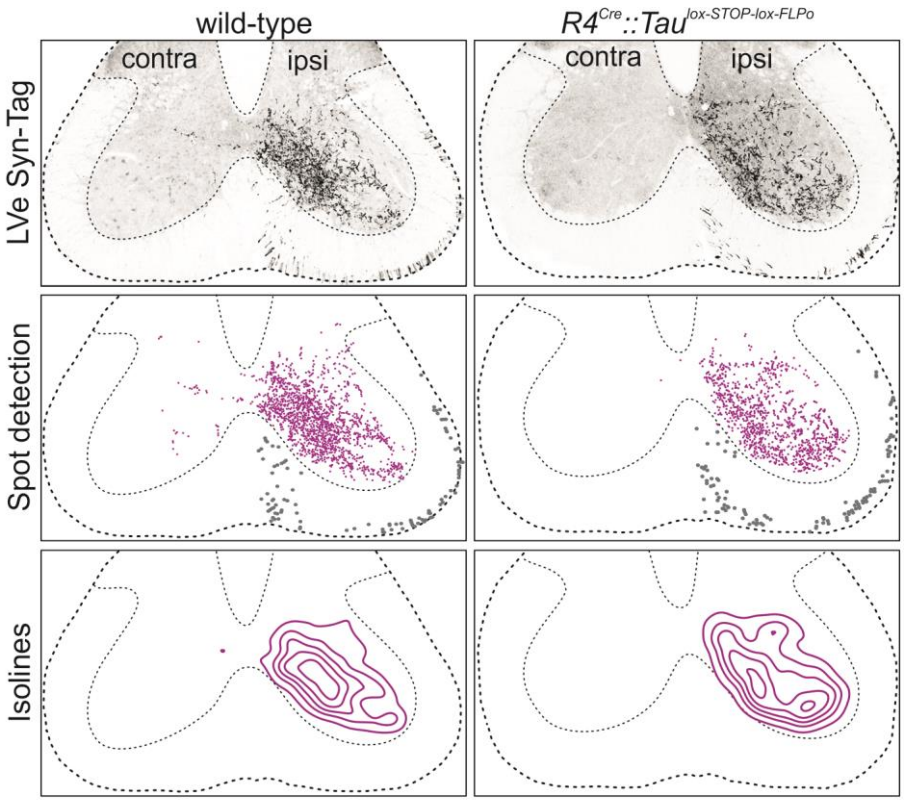


Figure S2.2. LVe Synaptic Terminal Distribution in the Lumbar Spinal Cord, Related to Figure 2.2
Reconstruction of LVe Syn-Tag marked terminals in the lumbar spinal cord of wild-type (left) and *R4^{Cre} :: Tau$^{lox\text{-}Stop\text{-}lox\text{-}FLPo}$* (right) mice reveals similar distribution of descending axonal tracts (grey in middle panels) and synapses (purple in middle panels, isolines in bottom panels).

2.2.3 Lateral Vestibular Input Avoids GS-L1 Motor Neuron Subtypes

Despite this striking difference in overall input between GS and TA motor neurons, we noted that LVe synaptic input to individual GS motor neurons was highly variable.

While some GS motor neurons received low (GS-low) LVe input, others were targeted by high-density (GS-high) LVe input (Figure 2.2A, B). These findings suggest that not all GS motor neurons are equally favored targets for vestibular input and raise the question of the underlying reason for this variability.

Most skeletal muscles are composed of a mixture of different fiber types innervated by three functionally matched alpha motor neuron subpopulations. These motor neuron subtypes are differentially recruited during movement and include fast fatiguable (FF), fatigue resistant (FR) and slow motor units (Burke 1967, Kanning, Kaplan et al. 2010). In the mouse, the most lateral subcompartment of the lateral GS muscle (GS-L1) is a very valuable exception to this rule in that it is innervated exclusively by FF motor neurons (Pun, Santos et al. 2006). This property allowed us to assess LVe input specifically to FF motor neurons within the GS motor pool (Figure 2.2C). We found that GS-L1 FF motor neurons received only low-density LVe input, and notably significantly less than the entire GS motor pool (Figure 2.2C, D). In addition, cell body volume values of motor neurons innervating the GS-L1 compartment have a tendency to accumulate in the upper two-thirds of the distribution spectrum (Figure 2.2E). Nonetheless, and consistent with previous observations (Burke, Dum et al. 1982), such size range classifications are not sufficient to unambiguously assign motor neuron subtype identity. In summary, GS-L1 FF motor neurons receive low-density LVe input, raising the possibility that this input is preferentially targeted to specific motor neuron subtypes within extensor pools.

2.2.4 LVe Input Prefers Molecularly Defined Slow Motor Neurons in Extensor Pools

We next aimed to generalize our finding that LVe inputs might prefer slow motor neuron subtypes. Recent observations demonstrate that chondrolectin (Chodl) and matrix metalloprotease-9 (MMP-9) are expressed by fast motor neurons (Enjin, Rabe et al. 2010, Kaplan, Spiller et al. 2014, Leroy, Lamotte d'Incamps et al. 2014). In mice expressing the membrane marker protein placental alkaline phosphatase (PLAP) from the *Chodl* locus (*Chodl*^{PLAP}) (Sakurai, Akiyama et al. 2013), a large majority of Chodl^{ON} lumbar ChAT^{ON} motor neurons in the lateral motor column (LMC) coexpressed MMP-9 (92%), and all GS-L1 FF motor neurons were PLAP^{ON}/MMP-9^{ON} (Figure 2.3A-C). We first quantified LVe synaptic input density to lumbar LMC motor neurons overall in *Chodl*^{PLAP} mice. Stratification of LVe input density by PLAP^{ON} and PLAP^{OFF} status of targeted LMC motor neurons revealed significantly lower input density to PLAP^{ON} than putative alpha PLAP^{OFF} motor neurons (Figure 2.3D, E). Furthermore, there was a significant inverse correlation between LVe synaptic input density and motor neuron cell body volume (Figure 2.3E).

To determine whether the uncovered LVe synaptic input rule based on Chodl/MMP-9 stratification also applies to motor neuron subtypes within a given extensor motor pool other than GS (Figure 2.2C-E), we analyzed two more motor pools innervating extensor muscles. The ankle extensor muscle Soleus (Sol) is innervated by an approximately equal number of slow and FR motor neurons in mice, but does not contain any FF motor neurons (Pun, Santos et al. 2006, Kaplan, Spiller et al. 2014). Analysis of LVe input density to Sol motor neurons stratified by MMP-9 status revealed significantly lower values for MMP-9^{ON} than MMP-9^{OFF} Sol motor neurons (Figure 2.3F).

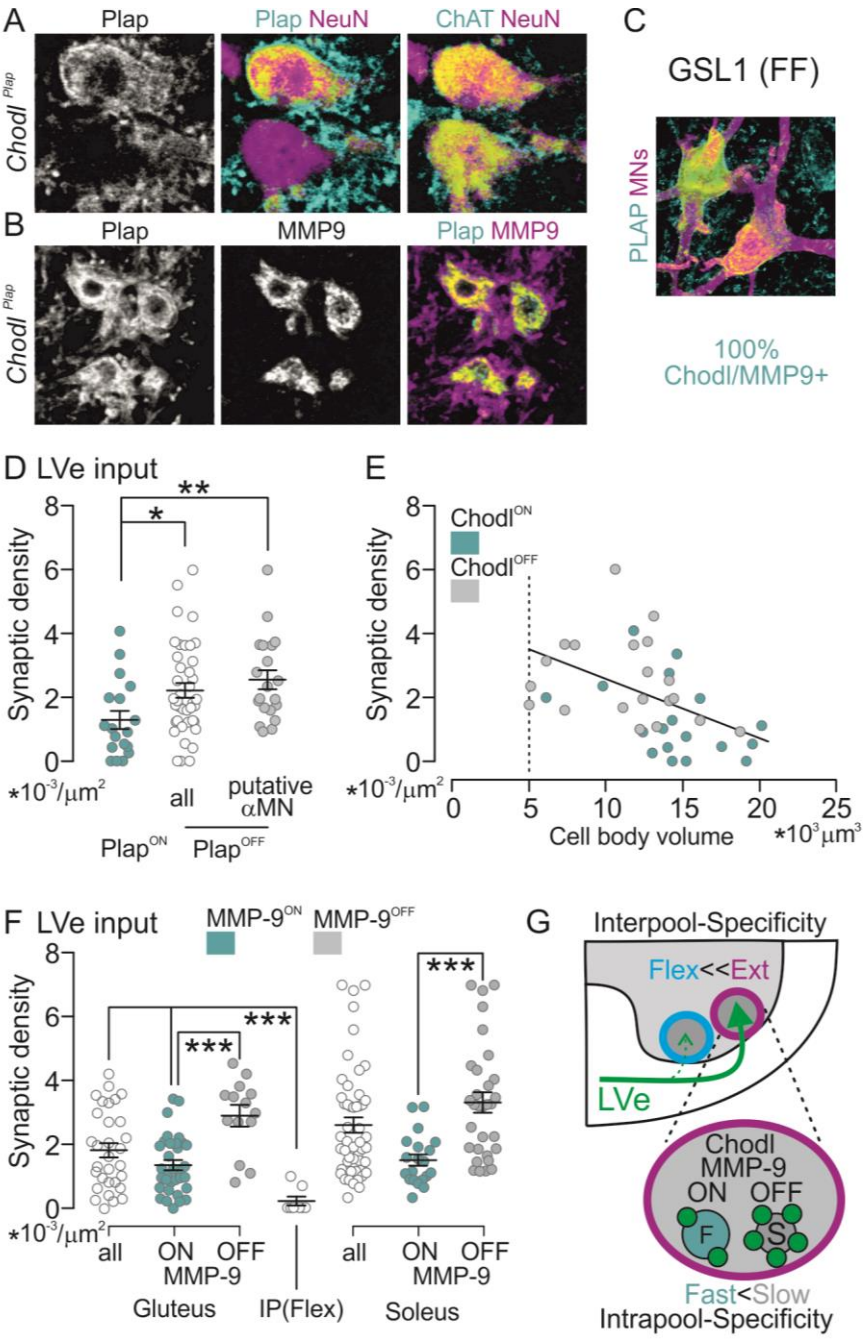


Figure 2.3. Vestibular Input Preferentially Targets Putative Slow over Fast Motor Neurons

(A, B) ChAT^{ON}/NeuN^{ON} alpha motor neurons in the lumbar spinal cord of *Chodl*^{PLAP} mice fractionate into Chodl^{ON} and Chodl^{OFF} population, of which the Chodl^{ON} neurons also express MMP-9.

(C) Retrogradely marked GSL1 FF motor neurons express Chodl.

(D) Density of LVe synaptic input to PLAP^{ON}, PLAP^{OFF} (all, or excluding putative gamma motor neurons using a size cut-off criterion of 5000 □m^3) motor neurons.

(E) Synaptic density of LVe input to PLAP^{ON} and putative alpha PLAP^{OFF} motor neurons analyzed in (D) plotted against cell body volumes (r= -0.548, P=0.0005).

(F) Analysis of LVe input density for Gluteus and Soleus motor neurons stratified by MMP-9 expression status (cyan: MMP-9^{ON}; grey: MMP-9^{OFF}). Input to antagonistic hip flexor muscle iliopsoas (IP) is also shown.

(G) Summary diagram of synaptic specificity between LVe and motor neurons. LVe preferentially targets extensor over flexor motor pools (top; interpool specificity) and within extensor pools preferentially slow over fast motor neuron subtypes (bottom; intrapool specificity, green dots represent synapses).

Additionally, since the Sol motor pool does not contain any FF motor neurons (Pun, Santos et al. 2006), these findings indicate that slow motor neurons are not only a preferred LVe target over FF, but also over FR motor neurons. We next assessed LVe input to MMP-9 stratified motor neurons innervating the hip extensor gluteus (GL) and found that also for this pool, MMP-9^{ON} populations received significantly lower input than the MMP-9^{OFF} cohort (Figure 2.3F). Moreover, the corresponding functionally antagonistic hip flexor (iliopsoas) motor pool showed LVe input density values similarly low as to TA flexor motor neurons (Figure 2.3F), thus generalizing our findings to other motor neuron pools.

Together, our experiments support a model in which synaptic input specificity of LVe neurons to lumbar LMC motor neurons is organized at different levels (Figure 2.3G). First, LVe axons seek out extensor over flexor motor pools as preferred synaptic targets in agreement with previous work (Grillner, Hongo et al. 1970). Second, LVe synaptic contacts preferentially target slow over fast motor neuron subtypes within an extensor pool. These findings raise the question of how this synaptic specificity arises during development and what may be factors regulating its establishment.

2.2.5 Developmental Refinement of Vestibular Synaptic Input Specificity to Motor Neurons

To assess synaptic input specificity of LVe neurons to lumbar motor neurons during development, we carried out spatially confined injections of AAV-Syn-Tag into

the LVe nucleus early postnatally, and retrogradely labeled GS or TA motor neurons (Figure 2.4A). The earliest time point for which it was technically possible to achieve consistent high-level Syn-Tag accumulation from LVe neurons in the lumbar spinal cord was P7. GS motor neurons at P7 receive synaptic input at densities similar to adult (Figure 2.4B). However, while the difference in input density between GS and TA motor neurons was already established at P7, LVe terminals frequently contacted TA motor neurons at an overall significantly higher input density than in the adult (Figure 2.4B). To assess during which time window the transition to mature connectivity profiles emerges, we carried out synaptic input mapping at progressively later developmental time points (Figure 2.4A). We found that LVe neurons still contact TA motor neurons at P11, but that developmental refinement was complete by P17 (Figure 2.4B).

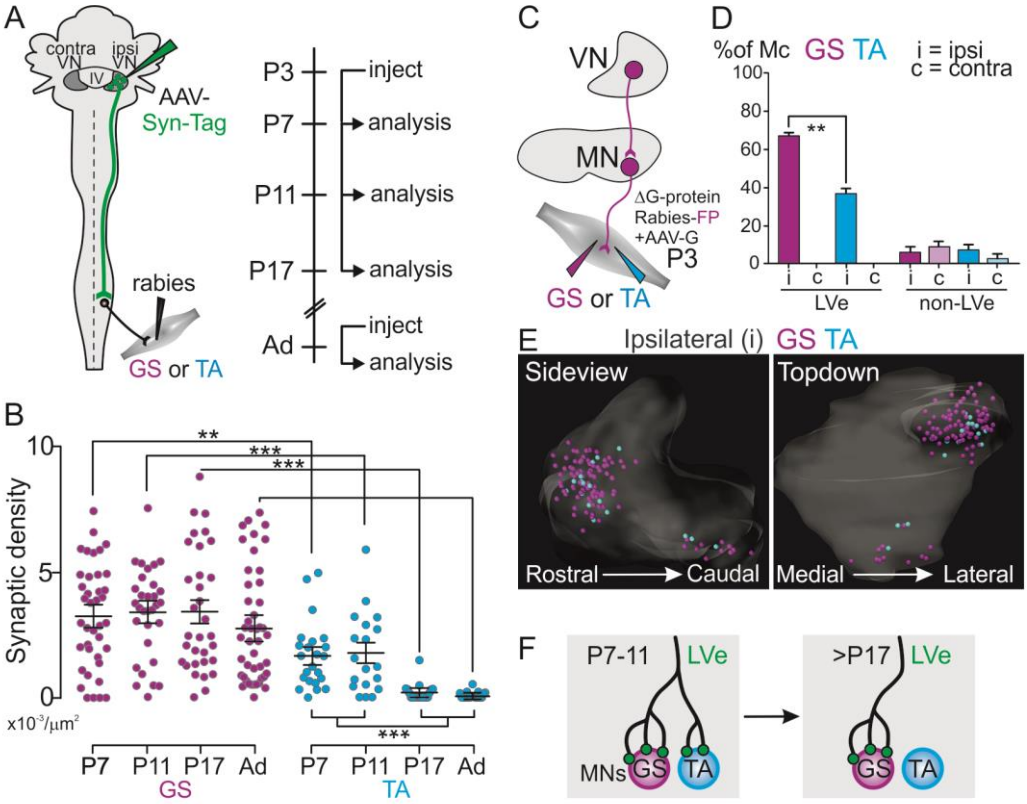


Figure 2.4. Developmental Refinement of Vestibular Input to Lumbar Motor Neurons

(A) Experimental approach used and timeline. AAV-Syn-Tag is injected into LVe of P3 (or adult) mice, followed by retrograde marking of GS or TA motor neurons by muscular tracer injections.
(B) Synaptic density of LVe input to GS and TA motor neurons at P7, P11, P17 and adult stages.
(C-E) Monosynaptic rabies tracing experiment at P3. Quantification of marked neurons in LVe and non-LVe territory (Figure 2.1), both ipsi- (i) and contra-(c) lateral to muscle injection (normalized to Rabies neuron number in Magnocellular nucleus). Side- and top-down view of ipsilateral vestibular reconstruction depicting GS (purple) and TA (cyan) vestibular neurons shown in (E). Neurons connected to GS or TA motor neurons were intermingled and no spatial segregation was discernable (Figure 2.4E).
(F) Summary diagram illustrating developmental refinement process of LVe input to GS and TA motor neurons.

To determine whether LVe contacts to TA motor neurons at early postnatal stages represent synaptic contacts, we applied monosynaptic rabies viruses to muscles innervated by GS and TA motor neurons (Figure 2.4C). We found that LVe neurons connect to both GS and TA motor pools at these stages, but significantly more LVe neurons were labeled after GS than TA muscle injections, at a ratio comparable to the anterograde synaptic density measurements at P11 (Figure 2.4D, E). Together, these data confirm our anterograde tracing results, demonstrating that initial developmental synaptic contacts to TA motor neurons are eliminated between P11 and P17, when they reach a mature connectivity profile (Figure 2.4F).

2.2.6 Perturbing Vestibular Signaling Affects Establishment of Interpool Synaptic Specificity

To elucidate the mechanisms by which selectivity of vestibular input to spinal motor neurons is established, we used two different genetic models in the mouse exhibiting altered vestibular neuron signaling. We asked how these perturbations

influence the establishment of mature connectivity profiles between vestibular neurons and spinal motor neurons.

We first analyzed *NADPH oxidase 3* (*Nox3*) mutant mice (Figure 2.5A). These mice lack mineralized particles called otoconia in the inner ear's utricle and saccule, leading to selective defects in perception of gravity and linear acceleration, but they exhibit intact semicircular canal vestibular as well as auditory sensory inputs (Paffenholz, Bergstrom et al. 2004). Of the five known vestibular input channels, predominantly utricular or posterior semicircular canal nerve activation influences lumbar spinal circuits through the lateral vestibular tract (Uchino and Kushiro 2011). *Nox3* mutant mice therefore exhibit congenitally altered LVe input to the lumbar spinal cord, lacking information derived from the utricular sensory input channel but not from semicircular canals.

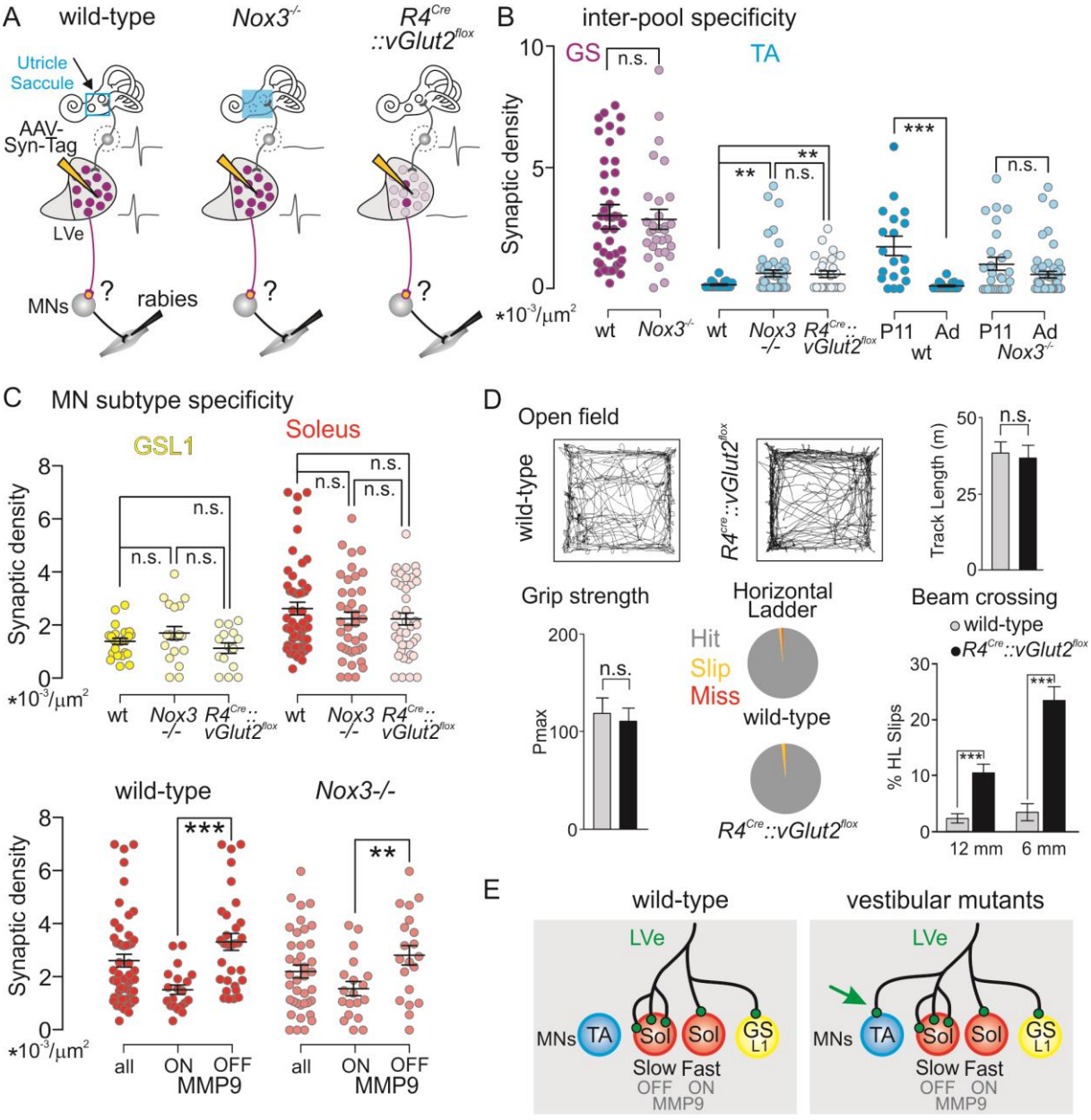


Figure 2.5. Perturbation of Vestibular Input Channel Results in Connectivity Defects to Motor Neurons

(A) Cellular phenotypes and analysis of wild-type, *Nox3*$^{-/-}$ and *R4*^{Cre}*::vGlut2*^{flox} mice. *Nox3*$^{-/-}$ mice exhibit defects in otolith-organ derived vestibular sensory input to brainstem vestibular neurons, whereas *R4*^{Cre}*::vGlut2*^{flox} mice lack functional output from vestibular neurons to the spinal cord. AAV-Syn-Tag injections are performed to quantify synaptic input density to motor neuron subpopulations.

(B) Synaptic density of LVe input to GS and TA motor neurons at adult (GS, TA) and P11 (TA) stages for wild-type and *Nox3* mutant mice. TA motor neurons were analyzed in adult *R4*^{Cre}*::vGlut2*^{flox} mice.

(C) Synaptic density of LVe input to GSL1 and Sol motor neurons in wild-type, *Nox3* mutant, and *R4*^{Cre}*::vGlut2*^{flox} mice (top row). Data for Sol motor neurons in wild-type and *Nox3* mutant mice displayed stratified by MMP-9 expression status (bottom row).

(D) Behavioral analysis of wild-type and *R4*^{Cre}*::vGlut2*^{flox} mice in open field arena (tracks of individual mice; quantification of track length moved in 10 minutes), grip strength, horizontal ladder precision (hit, slip and miss categories displayed in pie chart), and beam crossing on 12mm and 6mm thick beam.

(E) Summary diagram of synaptic input analyzed between LVe and motor neuron subtypes in wild-type mice and vestibular mutants. Note ectopic synaptic input to TA motor neurons in vestibular mutants. See also Figure S2.3.

We first determined whether *Nox3* mutation affects the establishment of LVe synaptic inputs to the functionally antagonistic motor neuron pools GS and TA. We found that there was no difference in LVe input density to GS motor neurons between wild-type and *Nox3* mutant mice, but that TA motor neurons received LVe input at a significantly higher density in *Nox3* mutant than wild-type mice (Figure 2.5B). When we compared LVe synaptic input density at P11, a time point before mature connectivity profiles are reached in wild-type mice, LVe input to TA motor neurons was not different between wild-type and *Nox3* mutant mice (Figure 2.5B). Moreover, between P11 and adult stages in *Nox3* mutant mice, no significant refinement of LVe input to TA motor neurons occurred (Figure 2.5B). Together, these findings demonstrate that LVe neurons maintain aberrant synaptic input to flexor motor neurons when otolithic vestibular signaling is non-functional

We next asked whether utricular vestibular signaling also influences LVe connectivity profiles to fast and slow motor neuron subtypes. There was no significant difference in LVe synaptic input to FF GSL1 motor neurons between wild-type and *Nox3* mutant mice (Figure 2.5C). We also analyzed LVe input density to Sol motor neurons stratified by MMP-9 status to distinguish between fast (MMP-9^{ON}) and slow (MMP-9^{OFF}) motor neuron subtypes. While *Nox3* mutant mice still exhibited clear intrapool differences to these motor neuron subtypes, the connectivity stratification was less pronounced than in wild-type mice (Figure 2.5C). Together, these findings suggest that *Nox3* mutants exhibit defects in interpool but no major intrapool LVe synaptic connectivity.

We next analyzed an intersectional mouse mutant in which the synaptic output of most LVe neurons is functionally muted from the earliest developmental stages. This genetic strategy is based on our observations that most LVe neurons projecting to lumbar spinal levels are of developmental rhombomeric origin R4 and express the glutamate transporter vGlut2. In agreement, genetic elimination of *vGlut2* from R4-derived LVe neurons (*R4^{Cre}::vGlut2^{flox}* mice) abolishes vGlut2 protein from the vast majority of spinal synapses derived from LVe neurons (Figure 2.5A, Figure S2.3).

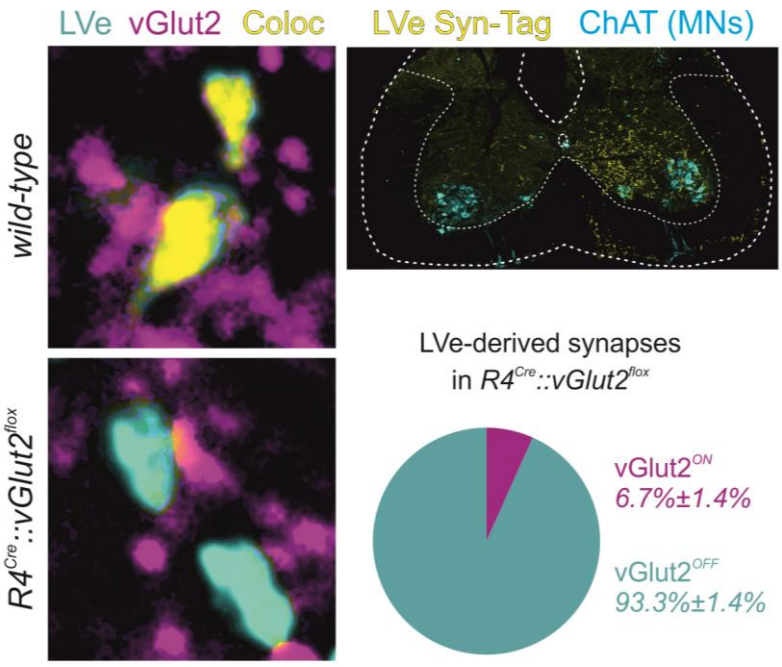


Figure S2.3. Elimination of vGlut2 from Lateral Vestibular Nucleus Projection Neurons, Related to Figure 2.5
LVe Syn-Tag marked terminals accumulate vGlut2 protein in the lumbar spinal cord of wild-type but not in *R4^{Cre}::vGlut2^{flox}* mice, demonstrating successful elimination of vGlut2 from these terminals with the applied genetic strategy (low resolution overview of synaptic terminal distribution and quantification shown to the right).

Since *R4^{Cre}::vGlut2^{flox}* mice have not been characterized before, we determined whether they exhibit motor behavioral deficiencies compatible with impaired LVe function. *R4^{Cre}::vGlut2^{flox}* mice executed open field navigation, grip strength and horizontal ladder tasks similar to wild-type mice (Figure 2.5D). In contrast, they

exhibited defects in tasks predicted to profoundly engage the vestibular system. *R4^{Cre}::vGlut2^{flox}* mice walking on a narrow beam showed significantly more slips than wild-type mice, and this phenotype was particularly pronounced on 6mm over 12mm wide beams (Figure 2.5D). These behavioral experiments suggest that elimination of *vGlut2* from R4-derived LVe neurons affects vestibular function and leads to motor defects attributable to such perturbations.

We next assessed synaptic input to TA motor neurons in these mice and found a significantly higher synaptic input density compared to wild-type (Figure 2.5B), similar to the phenotype in *Nox3* mutant mice. Lastly, also similar to our observations in *Nox3* mutant mice, we found no differences in LVe synaptic input to GSL1 and Sol motor pools in *R4^{Cre}::vGlut2^{flox}* compared to wild-type mice (Figure 2.5C).

In summary, genetic perturbation of selective vestibular input channels or muting synaptic output of vestibular neurons result in similar connectivity defects between LVe neurons and flexor motor neurons (Figure 2.5E). Our observations also reveal that additional factors must play important roles in scaling vestibular input specificity to motor neuron subtypes. Considering the established roles of vestibular and proprioceptive systems in posture and balance, an interesting hypothesis to test is whether these two systems influence each other in establishing their respective connection specificities to motor neurons.

2.2.7 Proprioceptive Signaling Influences Vestibular Synaptic Density to Motor Neurons

Given the striking LVe synaptic input variation to different motor neuron subtypes, we first determined the organization of direct synaptic input by

proprioceptive afferents to motor neuron subtypes. Of proprioceptors, only muscle spindle afferents connect directly to motor neurons and their synaptic terminals accumulate the vesicular glutamate transporter vGlut1 (Oliveira, Hydling et al. 2003, Pecho-Vrieseling, Sigrist et al. 2009).

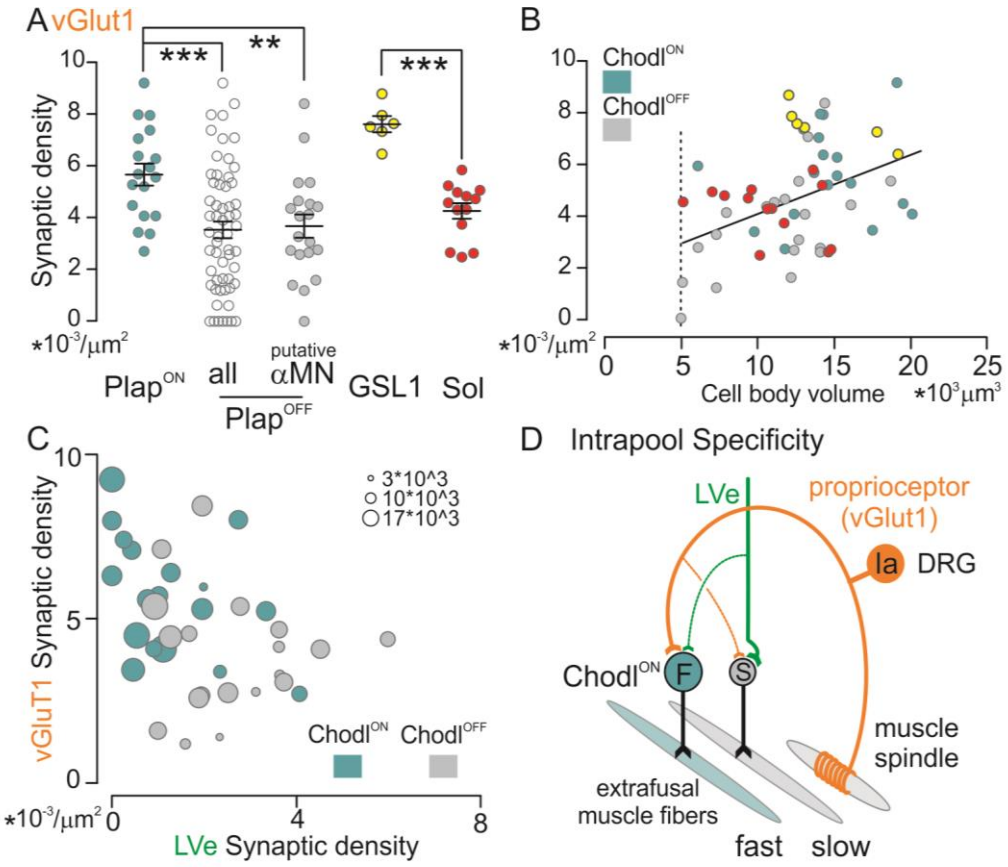


Figure 2.6. Vestibular and Proprioceptive Input Anti-correlated by Motor Neuron Subtype

(A) Density of vGlut1 synaptic input to PLAP^{ON}, PLAP^{OFF} (all, or excluding putative gamma motor neurons using a size cut-off criterion of 5000 □m^3), GSL1 and Soleus motor neurons.

(B) Synaptic density of vGlut1 input to PLAP^{ON} and putative alpha PLAP^{OFF} motor neurons analyzed in (A) plotted against cell body volumes (r= 0.448, P=0.0054). GSL1 and Sol motor neurons are also displayed in this plot but not included in the correlation analysis.

(C) Plot of vGlut1 vs LVe synaptic input density to PLAP^{ON} and putative alpha PLAP^{OFF} motor neurons in relation to cell body volume illustrated by diameter of plotted circles.

(D) Intrapool stratification of LVe and Ia proprioceptive vGlut1 input to fast (F; Chodl^{ON}) and slow (S; Chodl^{OFF}) alpha motor neurons, revealing anti-correlated synaptic input densities.

Analogous to our analysis of LVe input to motor neuron subtypes (Figure 2.3), we quantified vGlut1 input density to Chodl^{ON} putative fast motor neurons, Chodl^{OFF} putative slow motor neurons, as well as to identified GSL1 (exclusively FF) and Sol (many slow) motor neurons.

We found that vGlut1 input density was higher for GSL1 and Chodl^{ON} motor neurons than for Sol and Chodl^{OFF} putative alpha motor neurons (Figure 2.6A), a finding opposite to our analysis of input densities derived from the LVe nucleus (Figure 2.3D, E). Moreover, cell body volumes and vGlut1 synaptic input density were positively correlated to each other (Figure 2.6B), further supporting the notion that fast motor neurons with relatively large cell bodies receive a higher density of vGlut1 inputs than smaller, Chodl^{OFF} alpha motor neurons. Analysis of both LVe and vGlut1 input to the same cohort of motor neurons stratified by Chodl-expression status and cell size confirmed this conclusion (Figure 2.6C, D).

To determine whether the status of proprioceptive input to a motor neuron influences the organization of LVe input to the same motor neuron, we analyzed two mouse mutants with opposite proprioceptive synaptic phenotypes to motor neurons (Figure 2.7A). *Egr3* mutant mice exhibit early postnatal degeneration of muscle spindles, leading to non-functional muscle spindle afferents (Tourtellotte and Milbrandt 1998, Chen, Tourtellotte et al. 2002). In contrast, *Mlc::NT3* mice overexpress NT3 from skeletal muscle fibers, resulting in survival of superfluous proprioceptive afferents with aberrant and more synaptic connections to central synaptic partners (Wang, Li et al. 2007).

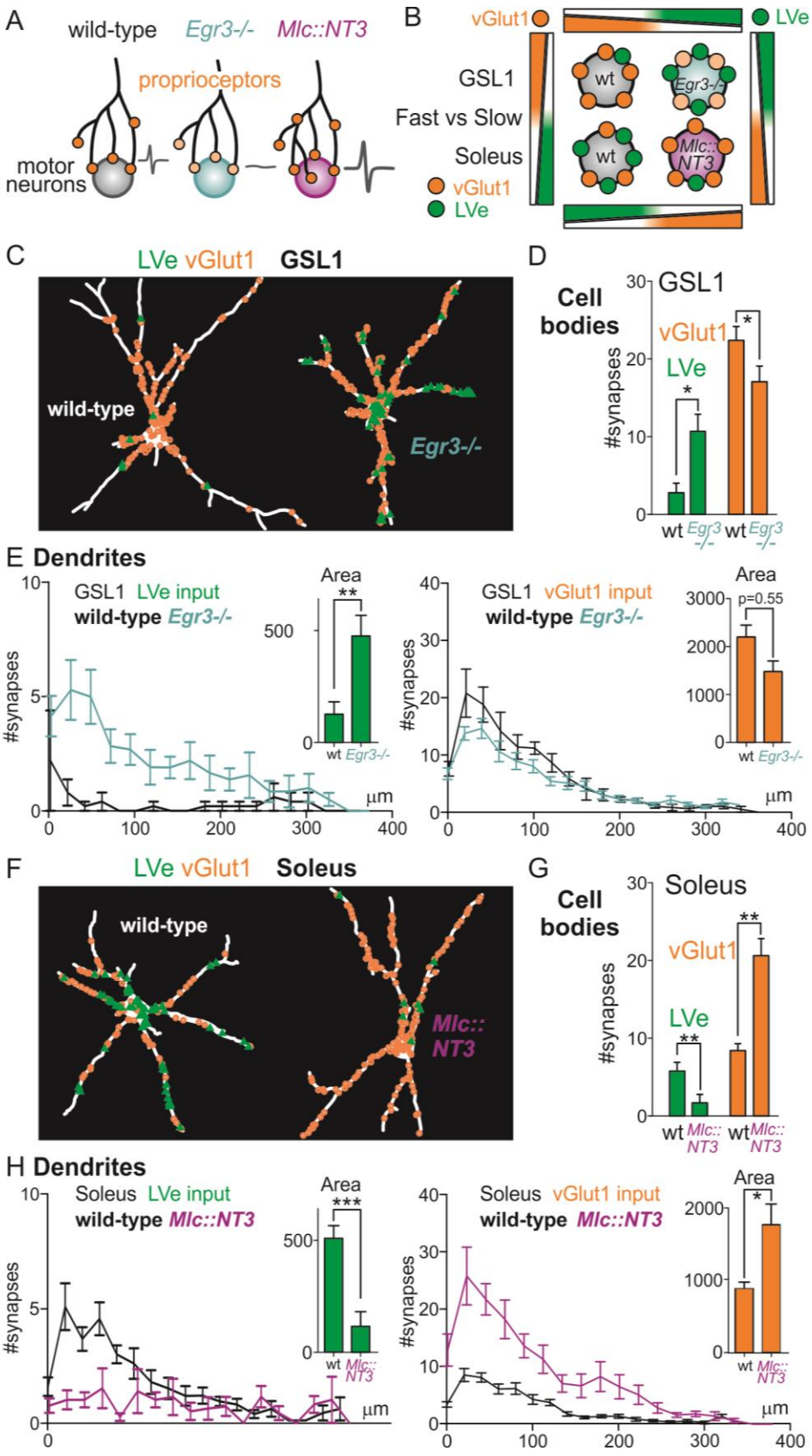


Figure 2.7. Muscle Spindle Signaling Influences Input to Motor Neuron Subtypes
(A) Synaptic input status of proprioceptors to alpha motor neurons in wild-type, *Egr3*$^{-/-}$ and *MLC::NT3* mice. *Egr3*$^{-/-}$ proprioceptive terminals are physically present but non-functional, whereas they show over-proliferation and aberrant connections in *MLC::NT3* mice.

(B) Summary diagram illustrating main findings. LVe inputs to motor neuron subtypes of extensor pools are affected by genetic manipulation of proprioceptor input function.
(C-H) Neurolucida reconstruction (C, F) and quantification (D, E, G, H) of LVe and vGlut1 synaptic input to GSL1 and Soleus motor neurons in wild-type, compared to *Egr3*$^{-/-}$ (for GSL1) and *MLC::NT3* (for Soleus) mice both at the cell body (D, G) and dendrite (D, H) level. In (E, H) area under curves are quantified and shown in bar graphs. See also Figure S2.4, Figure S2.5, and Figure S2.6.

To assess LVe input to motor neurons in these two mutant mouse strains compared to wild-type mice, we quantified synaptic input to motor neuron cell bodies and dendrites (Figure 2.7A, B).

In *Egr3* mutant mice, we analyzed LVe and vGlut1 input to GSL1 motor neurons, normally exhibiting high-vGlut1 and low-LVe input (Figure 2.7C-E). In these mice, vGlut1 contacts to GSL1 motor neurons are present (Figure 2.7D, E) despite their non-functionality (Tourtellotte and Milbrandt 1998, Chen, Tourtellotte et al. 2002). However, LVe input to GSL1 motor neurons is significantly increased in *Egr3* mutant mice (Figure 2.7D, E). Conversely, in *Mlc::NT3* mice, we analyzed LVe and vGlut1 input to Sol motor neurons that normally receive relatively low-vGlut1 and high-LVe input (Figure 2.7F-H). As expected, Sol motor neurons received significantly more vGluT1 input in *MLC::NT3* than wild-type mice, but LVe input density was strongly reduced (Figure 2.7G, H).

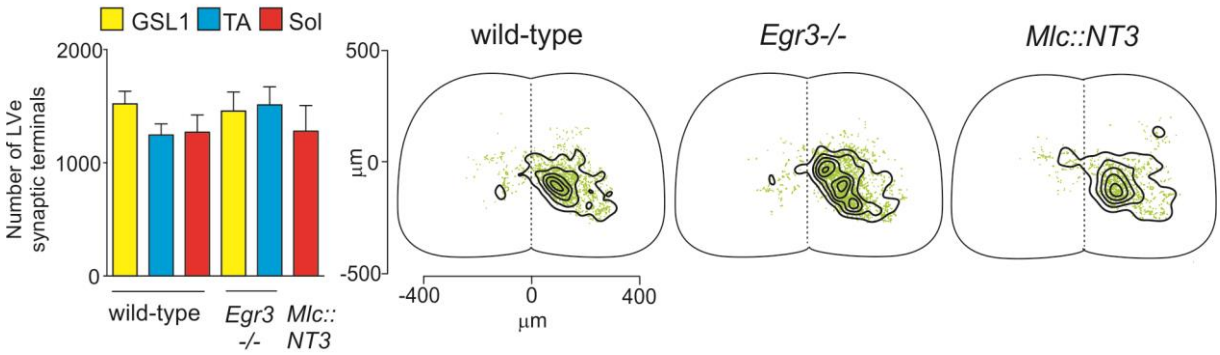


Figure S2.4. LVe Synaptic Terminal Distribution Across Genotypes and Injections, Related to Figure 2.7

Quantification of synaptic terminals in the lumbar spinal cord of mice ipsilateral to LVe injection (left) and contour plots of synaptic density distributions in wild-type, *Egr3* mutant and *MLC::NT3* mice.

Despite these differences in LVe connectivity to motor neuron subtypes however, overall LVe synaptic patterns in the spinal cord were not perturbed across genotypes and injection conditions (Figure S2.4).

To determine whether the lack of direct functional proprioceptive input to motor neurons in *Egr3* mutant mice also influences LVe input to flexor motor neurons, we next compared input to TA motor neurons between wild-type and *Egr3* mutant mice. We found that there was no significant difference between genotypes (Figure S2.5A-D). These results demonstrate that altered proprioceptive signaling to flexor motor neurons cannot overrule the scarcity of LVe input to these neurons. Thus, the assembly of LVe inputs at the motor pool and motor neuron subtype level employs distinct developmental mechanisms.

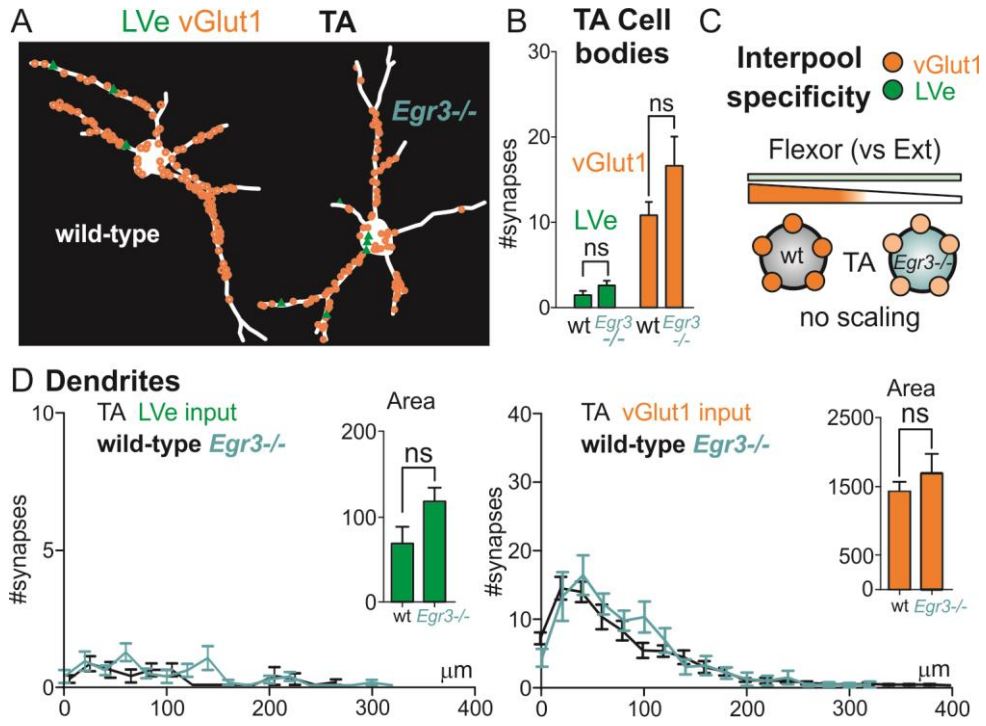


Figure S2.5. Muscle Spindle Signaling Deficiency Does not Affect Scarcity of Vestibular Input to Flexor Motor Neurons, Related to Figure 2.7

Neurolucida reconstruction (A), quantification (B, D) and summary diagram (C) of LVe and vGlut1 synaptic input to TA motor neurons in wild-type compared to *Egr3*$^{-/-}$ mice both at the cell body (B) and dendrite (D) level. In (D), area under curves are quantified and shown in bar graphs. Note that interpool specificity of LVe input to extensor and flexor motor neurons is not affected by genetic manipulation of proprioceptor input function (C).

Lastly, to test whether the synaptic scaling of these two complementary sensory systems operates bidirectionally, i.e., whether altered LVe input scales vGlut1 input to motor neurons, we analyzed vGlut1 input to Sol motor neurons in *Nox3* mutant mice. We detected a striking increase in vGlut1 terminals to Sol motor neurons in these mutants compared to wild-type mice (Figure S2.6A, B). This finding suggests that LVe signaling influences the scaling of proprioceptive inputs to motor neurons.

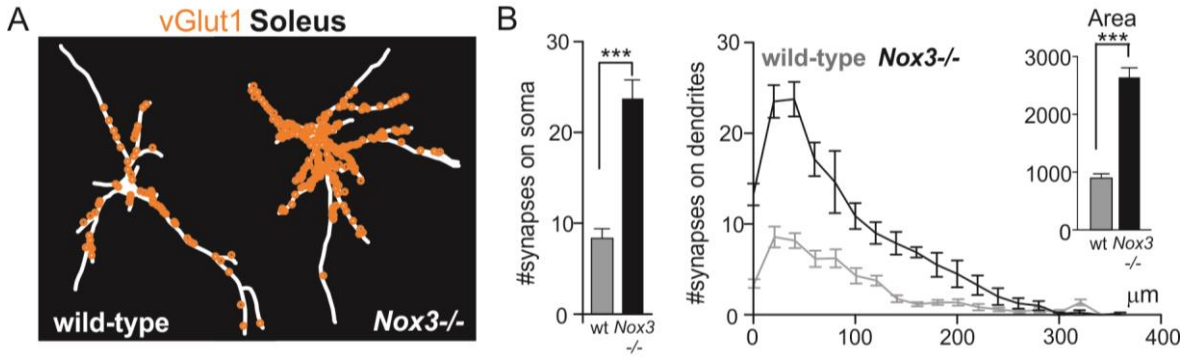


Figure S2.6. Vestibular Signaling Scales Proprioceptive Input to Soleus Motor Neurons, Related to Figure 2.7
(A, B) Neurolucida reconstruction (A) and quantification (B) of LVe and vGlut1 synaptic input to Soleus motor neurons in wild-type compared to *Nox3* mutants both at the cell body and dendritic level. Area under curves are quantified and shown in bar graphs.

2.3 Discussion

The control of posture and balance is essential for motor performance. The vestibular system plays an important role in this process through its ability to stabilize and adjust body position during movement (Wilson and Yoshida 1968, Grillner, Hongo et

al. 1970, Angelaki and Cullen 2008). Using genetic perturbation experiments, we demonstrate that signaling interactions between the proprioceptive and vestibular system play a key role in shaping connection specificity between vestibular neurons in the brainstem and spinal motor neurons. We discuss how these findings advance our understanding of vestibular system function, especially in the context of connectivity refinement and functional interaction with proprioceptive circuitry to ensure smooth motor behavior.

2.3.1 Motor Neuron Subtype Identity Aligns with Synaptic Input Specificity

Work on the cat lumbar spinal cord demonstrates that select lumbar extensor motor pools are favored direct synaptic targets for LVe input compared to flexor counterparts (Grillner, Hongo et al. 1970), a profile we find to be conserved in mice. A key insight of our work is that the observed extensor-flexor interpool specificity pattern is supplemented by a preference of LVe input to target slow over fast motor neuron subtypes within each extensor pool analyzed, and notably, this bias is even detectable at the level of a general lumbar LMC motor neuron analysis.

What may be the functional reasons behind the identified vestibulo-motor connectivity profile to preferentially target slow over fast motor neurons within extensor pools? Vestibular input enhances the activation of motor neurons innervating extensor muscles exhibiting antigravitational function, and can produce large motoneuronal depolarizations through temporal summation (Grillner, Hongo et al. 1970). This is physiologically relevant since vestibular neurons fire at high frequencies (Angelaki and Cullen 2008), also detected in awake behaving mice

(Beraneck and Cullen 2007), demonstrating that the vestibular system has the capability to contribute to motoneuronal recruitment. Our work shows that the vestibular system contributes to this process by preferential targeting of slow extensor motor neuron subtypes selectively recruited during endurance and postural tasks and with the ability to support contractions without fatigue (Burke 1967, Kanning, Kaplan et al. 2010). In contrast, fast motor neurons receive sparse direct vestibular input, in line with these motor neurons being recruited during fast and powerful muscle contractions but to fatigue quickly (Burke 1967, Kanning, Kaplan et al. 2010).

Our work is focused on synaptic input specificity directly to motor neurons, but vestibular signaling also acts through indirect pathways via spinal interneurons, and these pathways also employ specific connectivity rules following motor pool specific patterns (Grillner, Hongo et al. 1970). Even though flexor motor pools do not receive direct excitatory LVe inputs, disynaptic pathways can specifically inhibit them, and thereby further enhance the differential functional impact that LVe signaling exhibits on extensor and flexor motor pools. Electrophysiological studies on the organization of peripheral and rubrospinal inputs to motor neurons demonstrate that indirect inputs can also exhibit fiber-type specific functional connectivity profiles (Burke, Jankowska et al. 1970). Whether indirect inputs to motor neurons in the vestibular system also follow the intrapool motor unit twitch-type organizational principle as direct ones do will be an interesting question to pursue.

2.3.2 Multisensory Integration in the Motor Output System

The functionality of the motor system depends heavily on continuous integration of sensory information of different modalities. Multisensory inputs influence many neuronal elements along motor output pathways, a general organizational principle that is evolutionarily conserved even to circuits regulating *Drosophila* larvae behavior (Ohyama, Schneider-Mizell et al. 2015). Focusing on the last synapse of motor output pathways affecting movement, we found that vestibular and proprioceptive inputs converge on slow and fast motor neuron subtypes with an inverse anatomical synaptic scaling profile. While we favor the view that functional complementarity plays a role in synaptic scaling, whether similar scaling processes can also occur between functionally non-complementary inputs remains to be determined.

Our findings raise the question of how and where proprioceptive and vestibular systems interact functionally. Most relevant for our study, the vestibular system can enhance proprioceptive inputs in a synergistic manner (Grillner, Hongo et al. 1970). Moreover, vestibular input to motor neurons inherently carries multisensory information. Vestibular neurons are secondary neurons in the chain of sensory input processing, receiving primary vestibular sensory input as well as indirect feedback from the proprioceptive and visual system (Angelaki and Cullen 2008). In particular, somatosensory feedback circuits activated by passive hindlimb movement regulate vestibular neuron activity (Arshian, Hobson et al. 2014). Thus direct vestibular input to motor neurons combines multiple sensory streams of different degrees of integration and we found that these inputs are organized into precise patterns and are complementary to direct proprioceptive inputs.

2.3.3 Developmental Mechanisms Guiding the Assembly of Inputs to Motor Neurons

The precise developmental assembly of synaptic inputs to motor neurons is a prerequisite for the functionality of the mature motor system. Despite its importance however, mechanistic insight exists for only a limited number of functionally defined neuronal subpopulations with synaptic access to motor neurons. The wiring specificity between proprioceptors and motor neuron pools within the same reflex arc is established early and through mechanisms independent of neuronal activity (Frank 1990, Mears and Frank 1997). Combinatorial action of neuronal and retrograde molecular factors as well as positional cues play important roles in instructing sensory-motor connectivity (Wenner and Frank 1995, Arber 2012). Yet sensory connectivity to synergistic motor pools refines at postnatal stages, a process influenced by proprioceptor neuron activity (Mendelsohn, Simon et al. 2015).

Here, we have assessed time course and mechanisms of vestibular input assembly and refinement to motor neurons. We found that while significant input differences between extensor (GS) and flexor (TA) motor neurons are already established at early postnatal stages, a likely activity-dependent postnatal synaptic refinement process abolishes vestibular input to TA motor neurons. We revealed that this process is driven at least in part by vestibular signaling itself. The time window during which refinement occurs (P11-P17) matches the emergence of posture and weight bearing in rodents (Geisler, Westerga et al. 1993), raising the possibility that maturation of synaptic input may be linked to the emergence of postural behavioral abilities.

The second level of synaptic input scaling to motor neuron subtypes is shaped by bidirectional sensory signaling. Genetic manipulations affecting either the functionality of muscle spindle feedback or vestibular signaling resulted in adjustments of the other channel counterbalancing the genetically imposed changes. How could such input adjustment to motor neurons be regulated? We found that in mice, proprioceptive connections exhibit higher proximal synaptic input with gradually decreasing input on distal dendrites, in agreement with recent input reconstructions to rat motor neurons (Rotterman, Nardelli et al. 2014). Interestingly, compensatory LVe input distribution to GSL1 FF motor neurons in *Egr3* mutant mice scales accordingly. Since these muscle spindle afferent synapses are present but non-functional (Chen, Tourtellotte et al. 2002), it is likely that the observed adjustment of synaptic input to motor neurons is not merely a competition for synaptic space. A plausible mechanism instead might be that synaptic input to motor neurons is regulated locally through retrograde and homeostatic mechanisms involving postsynaptic feedback from motor neurons. In this context, it is interesting to consider that individual group Ia afferents connect to almost all motor neurons supplying the same muscle (Mendell and Henneman 1968). Ia input density scaling therefore likely occurs at the level of individual motor neurons according to subtype identity. Moreover, proprioceptor-driven vestibular synaptic scaling only operates on motor neuron pools to which LVe input has direct functional impact, as we observed no input scaling to TA motor neurons that receive proprioceptive but are devoid of LVe input. Thus, the two studied sensory channels differentially influence the refinement and scaling process of inputs to motor neurons, further supporting the idea that multiple independent layers regulate input specificity to motor neuron subtypes.

Bidirectional synaptic compensation may also explain at least part of the relatively minor locomotor phenotypes observed in *Egr3* mutants (Takeoka, Vollenweider et al.

2014) and *R4^{Cre}::vGlut2^{flox}* mice analyzed here, both of which exhibit signaling defects in the respective sensory system starting during development. Interestingly, cross-modal sensory regulation during development also appears to operate in humans. Patients with infant-onset vestibular system dysfunction show limited behavioral abnormalities likely due to somatosensory compensatory mechanism, whereas compensation following adult injury to the vestibular system is restricted (Horak, Shupert et al. 1994). These observations suggest that there might be a developmentally-defined critical period for cross-modal sensory regulation to adjust circuitry to motor neurons needed for posture and balance. Together, our work uncovers how sensory inputs of functionally complementary modality converge and influence each other at the final output step controlling movement, providing an important contribution to understanding specificity and function of the motor system.

2.4 Acknowledgements

We are grateful to M. Mielich for expert technical help, M. Tripodi for help and guidance during initial stages of the project, F. Roselli for insights into motor neuron subtypes and muscle subcompartments, M. Studer for sharing *R4::Cre* mice with us, S. Bourke, C. Genoud and L. Gelman from the FMI imaging facility, N. Ehrenfurchter from the Biozentrum Imaging facility, and M. Stadler from the FMI Bioinformatics Platform for help and advice with image acquisition and analysis, and to P. Caroni and B. Roska for discussions and comments on the manuscript. E. B. was supported by a fellowship of the Werner Siemens Foundation, A.T. by an International Foundation for Research in Paraplegia (IRP) fellowship. All authors were supported by an ERC Advanced Grant, the Swiss National Science Foundation, the Kanton Basel-Stadt and the Novartis Research Foundation.

2.5 Experimental Procedures

2.5.1 Mouse Genetics

Tau$^{lox\text{-}STOP\text{-}lox\text{-}Flp\text{-}INLA}$ (Pivetta, Esposito et al. 2014), *Chodl*^{PLAP} (Sakurai, Akiyama et al. 2013), *NADPH oxidase 3* (*Nox3*) mutant (Paffenholz, Bergstrom et al. 2004), *vGlut2*^{flox} (Jax Mice Strain #007583), *Egr3* mutant (Tourtellotte and Milbrandt 1998), *R4*^{Cre} (Di Bonito, Narita et al. 2013) and *Mlc::NT3* (Wang, Li et al. 2007) mouse strains were maintained on a mixed genetic background (129/C57Bl6). Housing, surgery, behavioral experiments and euthanasia were performed in compliance with the Swiss Veterinary Law guidelines.

2.5.2 Virus Production and Injections

Rabies viruses (Rabies-mCherry and Rabies-GFP: Rabies-FP) used were amplified and purified from local viral stocks following established protocols (Wickersham, Lyon et al. 2007, Stepien, Tripodi et al. 2010). All AAVs used in this study were described previously (Esposito, Capelli et al. 2014, Pivetta, Esposito et al. 2014, Takeoka, Vollenweider et al. 2014) and of genomic titers >1x10e13. Additional information on anterograde and retrograde viral tracing, immunohistochemistry, imaging and anatomical quantification are found in Extended Experimental Procedures.

2.5.3 Anterograde AAV Tracing Experiments

For LVe targeted viral delivery, we performed stereotaxic injections using high precision instruments (David Kopf) under isofluorane anesthesia. A small hole was drilled and a pulled calibrated glass pipette (Drummond Scientific) was used for local infusion of ~100nl virus by multiple short pulses (5msec, 0.5Hz) using a picospritzer.

The glass pipette was retracted after a 5-minute pause. Coordinates used for targeting LVe in adult were 0.24mm antero-posterior, 0.134mm medio-lateral and 0.355mm dorso-ventral from lambda. Coordinates at early postnatal stages were 0.17mm antero-posterior, 0.11mm medio-lateral, 0.15mm dorso-ventral from lambda. Mice were sacrificed 4-7 days post-injection for early postnatal experiments and two weeks for adult injection experiments. For retrograde marking of motor neurons, we injected rabies-FP or fluorescent dextran into specific muscles and perfused mice 4 or 7 days thereafter respectively. Muscle identity was assigned according to (Greene 1935) and GSL1 was defined as the L1 subcompartment of the lateral GS as described before (Pun, Santos et al. 2006).

2.5.4 Retrograde Rabies Tracing Experiments

Intraspinal injections: Intraspinal injections were performed as previously described (Pivetta, Esposito et al. 2014, Takeoka, Vollenweider et al. 2014). Briefly, upon laminectomy, we locally (C2-5 or L2-5 spinal cord) and unilaterally applied ~100nl virus by multiple short pulses (3msec, 0.5Hz) using a picospritzer (Parker). To verify injection precision and efficiency of infection, all mice were co-injected with AAV-expressing nuclear tags (Takeoka, Vollenweider et al. 2014). 4 days post-virus transduction, mice were sacrificed and unilaterality of injections was confirmed by immunohistochemistry.

Monosynaptic retrograde tracing: To visualize neurons with monosynaptic connections to forelimb (FL) or hindlimb (HL) innervating motor neurons, we injected AAV-G-protein and transsynaptic rabies (Rab-FP) viruses into either FL or HL muscles at postnatal day (P) 3-4 as described before (Stepien, Tripodi et al. 2010, Tripodi, Stepien et al. 2011, Esposito, Capelli et al. 2014, Pivetta, Esposito et al. 2014). Mice were sacrificed 8 (FL) or 10 (HL) days following injection. For broad

muscle injections, many proximal and distal limb muscles were targeted with multiple injections. Muscle injection specificities were confirmed using a fluorescent dissection microscope subsequent to perfusion.

2.5.5 Immunohistochemistry, Imaging and Analysis

Immunohistochemistry: All mice were perfused with 4% paraformaldehyde. All tissue was cryoprotected in 30% sucrose/PBS and cut on a cryostat (brain: 40-80□m coronal slices; spinal cord: 40-60□m transverse sections). Antibodies used in this study were: chicken anti-GFP (Invitrogen), chicken anti-LacZ (Chemicon), guinea pig anti-vGlut1 (Chemicon), guinea pig anti-vGlut2 (Chemicon), goat anti-ChAT (Chemicon), goat anti-LacZ (Biogenesis), goat anti-MMP-9 (Sigma-Aldrich), mouse anti-Alkaline phosphatase (Sigma-Aldrich), mouse anti-Myc (ATCC), mouse anti-NeuN (Chemicon), rabbit anti MMP-9 (Abcam) and rabbit anti-RFP (Rockland). Fluorophore-coupled secondary antibodies were from Jackson or Invitrogen. Floating tissue sections were incubated with antibodies in individual wells and mounted for imaging in sequential order.

3D brainstem reconstructions: Images were acquired using a MacroFluoZ6 (Leica; 5x objective). All pictures were aligned manually using Amira software (Visualization Science Group) as previously described (Esposito, Capelli et al. 2014). Rabies labeled premotor neurons were assigned manually using Imaris spot detection (Bitplane), and color-coded according to location based on Paxino's mouse brain atlas. For monosynaptic premotor tracing, datasets from n=6 for Gastrocnemius, n=4 for Tibialis Anterior, n=5 for broad HL, n=5 for broad FL injections were used. For reconstruction of brainstems upon intraspinal injections, we used data from n=5 for lumbar (L2-L5) and n=4 for cervical (C2-C5) injections. Kernel density estimates in

Figure 1F were calculated in R using the function 'density' as described (Tripodi, Stepien et al. 2011).

Synaptic density analysis on motor neuron cell bodies: Images were acquired using a custom-made dual spinning-disk microscope (60xobjective; Life Imaging Services GmbH, Basel, Switzerland) using a step size of 0.2µm for 60µm thick sections. Motor neuron cell bodies and proximal dendrites (up to 70µm from soma) were reconstructed using Imaris. Surface and volume were calculated using the Imaris statistics module after contours of labeled neurons were marked on every 5th plane of the z-stack. LVe synaptic appositions on the motor neuron cell surface were manually identified using Imaris spot detection function. Synaptic density was determined by dividing the number of appositions by the calculated surface for each reconstructed motor neuron. For assessment of LVe input to motor neurons, the number of reconstructed motor neurons used for analysis was as follows: Wild-type (P7: GS n=39, TA n=23; P11: GS n=31, TA n=19; P17: GS n=32, TA n=19; P44: GS n=45, TA n=19, GSL1 n=25, Soleus n=38, Gluteus n=45, Iliopsoas n=9; LMC motor neurons in *Chodl*^{PLAP} mice n=37), *Nox3*$^{-/-}$ (P11: GS n=26, TA n=28; P44: GS n=30, TA n=31, GSL1 n=19, Soleus n=35) and *R4*^{cre}*::vGlut2*^{flox} (P44: Soleus n= 41, GSL1 n=15, TA=27) derived from n=2-4 mice per data point.

Neurolucida reconstructions and analyses: A custom-made dual spinning-disk microscope (60x objective) and custom developed scripts were used to stitch image tiles using Fiji for cell body and dendrite reconstructions. Dendrites of labeled motor neurons were traced using Neurolucida (v10.0, Microbrightfield). Contours of cell body and dendritic origins were identified manually. Position of Syn-Tag (LVe) and/or vGlut1^{ON} synapses contacting motor neurons was identified in relation to distance from motor neuron cell body. Traced neurons and synaptic positions were exported

to Neuroexplorer (v10.0, Microbrightfield) and dendrograms were constructed for each dendrite. Distance from the cell body was calculated for each identified synaptic terminal contacting a motor neuron. Wild-type (Sol n=17, GSL1 n=6, TA n=9), *Egr3* mutants (GSL1 n=13, TA n=9), *Mlc::NT3* (Sol n=13), *Nox3*$^{-/-}$ (Sol n=15) derived from n=3-4 mice for each data point analyzed.

2.5.6 Behavioral Analysis

Open field, grip strength test, horizontal ladder locomotion and beam tests were performed as previously described (Esposito et al., 2014; Takeoka et al., 2014; Carter et al., 2001). Open field task: To assess basic locomotor activity, we measured the total path length and locomotion speed during 10 minutes exploration of a square arena (50x50cm). The arena was placed inside a noise-isolated chamber and video tracking was performed under dim light to reduce anxiety levels. Acquired data was analyzed using Viewer2 software (Biobserve, Bonn, Germany) every 30 seconds.

Grip strength analysis: FL and HL grip strength of mice was measured using a grip strength meter (TSE Systems) as previously described (Esposito, Capelli et al. 2014). Each mouse was tested on 4-5 consecutive trials and average force was calculated and expressed as g (1g = 9.8×10^{-3}N) using the grasping grip 4-Paw-Measurement module.

Ladder locomotion: Mice were food deprived and trained daily for four days to walk on a ladder (1m long, 2cm rung interspace) to get a pellet reward placed at the end. Each training session consisted of 10 runs per day. Quantification of hit, slip or miss paw placement for HL was determined from slow motion videos acquired at 100Hz during the last day of the training period (approximately 50 steps/mouse analyzed).

Beam crossings: A balance-beam apparatus was constructed as described (Carter, Morton et al. 2001). Mice were handled for 2-3 days before the training session to reduce manipulation-induced stress. On the first day, each mouse was trained to cross the large circular beam (12mm diameter) from an open platform to a sheltered box on the opposite side five times with 5min inter-trial interval. On the second day, the same protocol was repeated followed by a session using a narrower, 6mm beam. Trials were recorded with a high-speed camera at 100Hz. The percentage of HL slips (defined as steps with HL misplacement combined with the caudal half of torso touching the beam/all steps analyzed) was calculated. Note that conditional elimination of *vGlut2* from neurons using Cre-lox technology has previously been shown to significantly reduce glutamatergic functional neuronal output (Koch, Dela Cruz et al. 2011).

2.5.7 Statistics

All statistical analysis, plots and linear regression lines were made using GraphPad PRISM (v6.0). Column bar graphs and dot plots represent the average value ± SEM. The means of different data distributions were compared using an unpaired Student's t test (Figures 2.1D, 2.1F, 2.1H, 2.2A, 2.2D, 2.3D, 2.3F, 2.4B, 2.4D, 2.5B, 2.5C, 2.5D, 2.6A, 2.7D, 2.7G, S2.3, S2.4, S2.5B, S2.6A). Correlation analysis was used for Figures 3E and 6B. A one-way ANOVA for independent measurements was used for comparing multiple TA data sets in Figure 2.4B. The area under the frequency-distribution curves in Figures 2.7I, 2.7H, S2.5, S2.6B was used as a measure of synaptic input on dendrites. The correlogram plot shown in Figure 2.6C was obtained in R using the library ggplot. Significance level is defined as follows for all analyses performed: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Chapter 3. LVe-Cell Ablation Induces Limited Proprioceptive Rescaling in the Adult

3.1 Introduction

The previous chapter described a mechanism of cross-modal sensory interaction between vestibular and proprioceptive systems. We found that these two systems rescale their level of motor neuron innervation in a reciprocal manner. A question that arises is whether this phenomenon can be induced also in the adult animal by selective ablation of LVe excitatory neurons and what is its functional motor correlate.

3.1.1 Introduction to Neuronal Plasticity

With the term ‘plasticity’, we here refer to a spectrum of different types of reorganizations possible in the CNS in order to better respond to perceived sensory stimuli. Reorganizations can involve remodeling of circuit architecture by synapse consolidation or elimination (Lamprecht and LeDoux 2004) or modulation of the molecular or electrophysiological properties of single neurons (Lamprecht and LeDoux 2004). The advantage of a plastic CNS is reflected in the ability to produce

different behavioral outputs optimized for dynamically changing external conditions. The ability of adapting quickly and purposefully to an environment has probably been a key factor in providing a reproductive advantage to the human species and favoring its evolution.

Nevertheless, neuronal plasticity is not always associated with positive outcomes. There are instances in which an abnormal level of synaptic turnover is causes very severe cognitive impairments (Cruz-Martín, Crespo et al. 2010, Faludi and Mirnics 2011). The CNS needs to find the right balance between the possibility to change and adapt to the outside world and the necessity to consolidate the neuronal configuration more suitable to sustain the successful behavior. For this reason, the degree of CNS plasticity is modulated, in all animal species, in an age-dependent manner. At early developmental stages, most of our sensory systems have time windows during which incoming sensory information can extensively reorganize their central circuit connections, and this has most prominently been studied in the visual system (Hubel and Wiesel 1970, Hensch 2004, de Villers-Sidani, Chang et al. 2007). When this permissive or critical period closes, neuronal plasticity dramatically decreases and circuit architecture is stabilized. To which extent vestibular circuits can undergo plastic remodeling once reaching mature configuration is an open question. We will prepare the ground for answering this question in the next chapters.

3.1.2 Vestibular System Plasticity

The existence of a critical period, in which the vestibular sensory input is required for proper development and maturation of the system, is debated due to the

difficulty of establishing vestibular sensory deprivation studies on earth, where the gravity force is a premise (Jamon 2014).

What seems to be clear is that vestibular system connections are plastic and that vestibular sensory alterations can impact on motor behavior development in an age-dependent manner. Rats matured in space orbit, thereby in absence of gravity, inherit a permanent inability to swim and an impaired ability to perform the surface righting reflex (Walton, Benavides et al. 2005, Walton, Harding et al. 2005). Moreover, in the same species, removal of vestibular organs before P5 causes head bobbing at least until P40 (the study was stopped at this developmental time point) (Geisler and Gramsbergen 1998).

3.1.3 Effects of Vestibular Nerve Lesions: A Lesson From Amphibians

In adult animals, unilateral removal of labyrinthine organs causes a series of deficits on postural and ocular reflex aches. These defects disappear over time due to a mechanism called 'vestibular compensation'. This mechanism has been widely investigated since the beginning of the 19th century and it became the most common experimental paradigm utilized for studying brain plasticity.

In the next paragraphs, we will explain which behavioral symptoms the vestibular deprivation entails and what sort of plastic circuit rearrangements subtend the vestibular functional compensation in the adult. The behavioral defects derived from vestibular endorgan ablation were described for the first time by Ewald, who

observed a postural body and limb asymmetry induced by unilateral labyrinthectomy in frog (Ewald 1892). Striking was the observation that the entire body was tilted around the roll axis towards the lesioned side, with an extension of the fore- and hindlimb (HL) away from the center of body mass, as if trying to counteract an artificial external force. The same postural changes were also associated with electric lesions of the vestibular nuclei of the brainstem in rats (Sprague and Chambers 1953). Moreover, postural defects were mostly attributed to disruption of otolithic signaling pathways, since they could be largely recapitulated by lesioning only the utricular nerve branch in frogs (MacNaughtan 1946). This very last observation is particularly interesting to us because it links, once again, the otolithic organs with the control of spinal reflexes.

3.1.4 Labyrinthectomy in Mammals Induces Vestibular Compensation

In light of the evolutionarily old origin of the vestibular system and its conserved structure across different species, amphibians like frogs, represent a convenient experimental model used still today in the field of vestibular research (Branoner and Straka 2015). Nevertheless, in the last 50 years with the exponential growth of mouse genetics (van der Weyden, White et al. 2011), new tools broadened the spectrum of questions that could be addressed in circuit neuroscience, and the future horizon is even more promising (Hsu, Lander et al.). For this reasons, bringing

the vestibular compensation paradigm into mouse became a need more than an option.

In mammals the effects of unilateral labyrinthectomy can be divided into two categories with respect to the head motion: static and dynamic symptoms. The first ones include spontaneous nystagmus plus head and body tilt along the roll axis (Fetter and Zee 1988, Beraneck, Hachemaoui et al. 2003) while the second ones are represented by defects in the spatial and temporal aspects of vestibulo-ocular and vestibulo-spinal reflexes (Borel, Harlay et al. 2004). The static symptoms are recovered during a variable time interval, that goes from a few days in mouse and guinea pig (Gliddon, C. et al. 2004, Aleisa, A. et al. 2007), to a few weeks in cat or monkey (Smith and Curthoys 1989), while the dynamic ones recover later and not completely (Allum 2012). These examples of functional behavioral recovery show that the phenomenon of vestibular compensation can be induced in frogs as well as in mice but it only accounts for static vestibular symptoms. Circuit rearrangements leading to VOR compensation have been widely described, while the ones involved in compensation of postural and vestibulo-spinal reflexes are still not understood.

Before dissecting the details of the aforementioned vestibular compensation mechanisms, it is worth spending few words on the experimental attempts of augmenting, instead of cancelling, vestibular stimulation during development. This condition is generally obtained by exposing animals to prolonged periods of centrifugation. In mice, the published results appear to some extent contradictory. Jamon and colleagues found that mice undergoing centrifugation between P10 to P30 (Jamon and Serradj 2009) had permanent motor deficits while Beraneck and

coworkers reported only transitory ones (Beraneck, M et al. 2012). The controversy is probably attributable to the fact that with centrifugation, vestibular information is only altered but not suppressed, as is the case for space flights or labyrinthectomy.

3.1.5 Elements of Vestibulo-Spinal Plasticity

Relevant studies performed in cat yield new insights with respect to the physiological correlates of vestibular compensation. Precht and colleague (Fau, H. et al. 1966) were the first neuroscientists trying to explain the behavioral vestibular compensation phenomenon in terms of neuronal circuit rearrangement. In cat, immediately after unilateral labyrinthectomy, cells in the ipsilesional VN are silenced because of the lack of vestibular nerve input. Four to six weeks later, the functional recovery of the static symptoms of vestibular labyrinthectomy is paralleled by the reappearance of the signature electrophysiological properties of the VN cells on the ipsilesional side, despite the lack of activity in vestibular primary afferent fibers. The reasons for reemergence of vestibular neuron activity are thought to involve gain modulation of commissural inhibitory connections from the contralateral vestibular nuclei (Chapter 1.4.5) (Galiana, Flohr et al. 1984) mediated by metabotropic GABA type B receptors expressed in all nuclear subdivision (Johnston, Him et al. 2001).

Other studies report anatomical changes with induction of synaptogenesis in ascending dorsal root fibers (Dieringer, Künzle et al. 1984) and vestibular commissural fibers in frog (Will, Kortmann et al. 1988). In this anatomical study, the authors found a 50% increase of collaterals from ipsilesional vestibular nuclei to the contralesional ones compared to the non-treated condition. No change was found for

the vestibular commissural connections projecting in the opposite direction. A limitation of the study seems to be the very broad and unspecific diffusion of the dye used for labelling commissural axons.

As already mentioned, functional recovery after unilateral labyrinthectomy does not involve primary sensory neurons residing in the ganglion of Scarpa, thereby any compensation must occur at the level of secondary vestibular neurons or locally in the spinal cord on circuits controlling motor neuron regulation. While gain control and reactive synaptogenesis act both at the level of the brainstem, we wondered whether additional changes at the cellular or circuit level can happen in parallel in the spinal cord, at lumbar levels.

3.1.6 Experimental Question

By using mouse mutants in which proprioceptive (*Egr3*, *mlc::NT3*) or vestibular (*Nox3*) functionality is genetically altered, we uncovered a mechanism of synaptic rescaling acting in a bidirectional and reciprocal manner. The next point we wanted to address is related to the functional meaning of this very interesting mechanism. We imagined that postural stabilization would be achieved by combining vestibular and proprioceptive information in a complementary homeostatic manner. The reciprocal weight of the two channels would be increasing or decreased, in function of the reliability of their input sources at any moment in time. Following this hypothesis, the loss of vestibular function would leads to decreased reliability of this input source with respect of the proprioceptive one during motor control execution, in a homeostatic manner. This multisensory cross-talk could be participating in the process of

vestibular compensation (Chapter 3.1.4) and could be happening at multiple different stages in the motor control pathway (Chapter 1.4.5).

In our previous experiments (Chapter 2.2), vestibular and proprioceptive alterations are induced by genetic means during embryonic or early postnatal developmental stages, when vestibular circuits undergo plastic changes before reaching the mature state (Chapter 3.1). Is this sensory cross modal interaction an exclusive developmental property or can it be induced also in the adult when the vestibular system is matured and the CNS plasticity is overall decreased? In other words, could our cross-modal synaptic rescaling be part of a homeostatic process subtending the adult vestibular compensation?

In the next section, we started addressing these problems by testing whether a selective loss of excitatory LVe neurons in the adult mouse could induce a compensatory response from the proprioceptive system at level of spinal motor neurons.

3.2 Results

To address the previously introduced question, we generated a mouse model of unilateral vestibular loss of function, by killing at adult stages (P65), the LVe glutamatergic neurons. After a recovery period of 7 weeks, the presence of an eventual cross-modal proprioceptive compensation was assessed by measuring the relative change in the number of vGlut1 terminals onto Sol motor neurons, compared to the non-treated condition. As a reminder, we found before that Sol motor neurons

are characterized by input from a relatively low number of proprioceptive terminals but relatively high levels of vestibular input (Chapter 2.2.6).

3.2.1 Anatomical Evidence for Efficient DTR-Mediated Neuron Loss

In order to target vestibular excitatory neurons in LVe, we performed unilateral stereotactic injections with a cocktail of CRE-dependent AAV vectors, delivered unilaterally in the LVe nucleus of a *vGlut2*^{CRE} mouse line (n=11). The virus mix was composed of 3 different AAVs in addition to blue beads for checking injection specificity. The selective cell killing was mediated by conditional expression of human Diphtheria-Toxin Receptor (DTR) and subsequent Diphtheria Toxin (DT) ligand administration (Buch, Heppner et al. 2005). To quantify the amount of neuron loss, we co-injected an AAV-TVA-NLS-GFP and an AAV-Syn-Myc to indirectly measure the *vGlut2*^{CRE} cells left in LVe and their synaptic terminals in the lumbar spinal cord. From the post-mortem analysis of the injection sites, we confirmed the efficient and selective cell loss in vestibular territory based on the following observations: 1) only a very sparse nuclear GFP signal was left in the vestibular territory (Figure 3.1A left side). 2) Beads location was in the LVe (Figure 3.1A left side). 3) Expression of the neuronal marker NeuN (Mullen, Buck et al. 1992) in the ipsilateral LVe region was decreased compared to the contralateral, non-injected side (Figure 3.1A right side). 4) Absence of detectable Myc signal from the lumbar spinal cord (data not shown).

3.2.2 Behavioral Effects associated with LVe Excitatory Neuron Loss

Two weeks after AAV delivery, we divided the animals into two groups: an experimental one received an intraperitoneal injection of (DT) (n=6) and a control group which received only PBS (n=5). To have a read-out of the behavioral effect of the DTR-mediated cell killing, we compared the performance of the two groups of animals on the beam crossing assay. We have shown in our previous work how this powerful behavioral assay can unmask specific motor impairments resulting from vestibular deficits (Chapter 2.2.7).

In agreement with previous results from our group (Esposito, Capelli et al. 2014), already 4 days after administration of the drug, we observed behavioral defects attributable to DTR-selective neuronal loss. By simply looking at the experimental animals in their home cage, no striking postural or motor alteration was visible. Only when challenged on the beam crossing assay, we observed a net increase in the average number of HL slips for the experimental in respect to the control group. The same trend is maintained 2 days later, with a slight decrease of their statistical significance (Unpaired Student t-test p=0.0029 and p=0.0181, respectively 4 and 6 days post DT injection) (Figure 3.1B). This observation are similar to the phenotype detected in $R4^{Cre}$::$vGlut2^{flox}$ mice (see Chapter 2.2.6 for a more extended discussion) and gave us evidence for an effective vestibular loss of function through selective cell ablation in LVe.

To assess the presence on an eventual Ia synaptic rescaling on Sol motor neurons, we waited a time interval of 7 weeks to largely accommodate the time required for complete vestibular compensation (Tighilet, mourre et al. 2014) and for matching the plateau phase of functional recovery after spinal cord lesion (Takeoka, Vollenweider et al. 2014).

3.2.3 Loss of LVe Cells in Adult Induces Limited Proprioceptive Synaptic Compensation

Four days before termination of the experiment (Figure 3.1C), Sol motor neurons were retrogradely labelled by injections of G-deleted rabies RFP in Sol muscles of both legs. We only processed spinal cords of experimental animals precisely injected in the LVe (assessed by beads location). A total of 5 over 6 brains met this criteria, one had the beads outside the anatomical LVe borders thereby it has been excluded also from the beam test quantification (Figure 3.1B). Muscle injection specificity was also assessed post-mortem.

The spinal cords of the best three animals injected were cut and stained for: RFP (expressed by the rabies infected Sol motor neurons), vGlut1 (filling the muscle spindle proprioceptive afferents) and Myc (accumulated in the synaptic terminals of spared LVe glutamatergic neurons).

In all the three sectioned spinal cords, the Myc signal was almost absent from the spinal grey matter, a sign of complete axon degeneration after DTR-mediate cell ablation. For this reason, we focused only on vGlut1 synaptic input on cell body and

dendritic tree of motor neurons from the ipsi- and contralateral side to the injected LVe. One example of a reconstructed Sol motor neuron from each group is shown in Figure 3.1D. Looking at these two representative cell reconstructions, no appreciable difference in the number of vGlut1 terminals stands out. In fact, focusing on soma, no statistical difference can be detected when comparing ipsi- and contra-lesional side to a wild-type non-treated control group (Figure 3.1E). Nevertheless, a small but significant increase in the number of vGlut1 appositions emerged in the ipsilateral Sol motor neurons at level of the proximal dendrites up to around 150 μm from the soma (Figure 3.1F), when compared to the contralateral Sol or to the control groups.

In summary, performing focal injections of CRE-dependent AAV-DTR into the LVe of a *vGlu2*^{CRE} line, we were able to induce a selective loss of vestibular function in the adult. Interestingly, the loss of vestibular function was associated with an only mild increase of the vGlut1 input on the proximal dendrites of Sol motor neurons of the ipsilesional side. We will discuss these results in the next chapters.

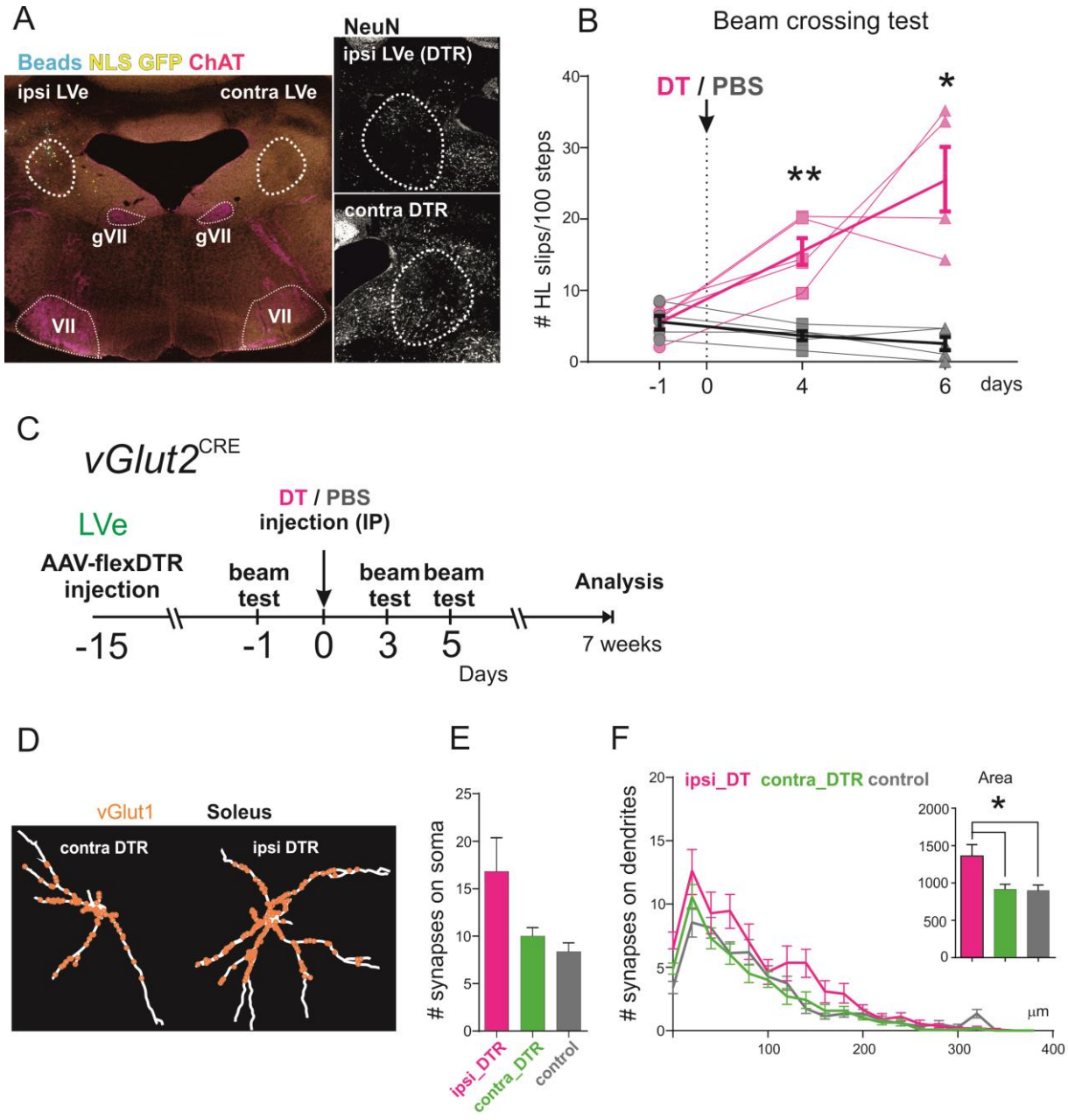


Figure 3.1 Induction of proprioceptive rescaling in the adult by selective vestibular cell ablation.

(A) Example of an LVe injection site in *vGlut2*^{CRE} mouse, beads in cyan, AAV-NLS-GFP in yellow and ChAT in magenta. The picture was taken after DT-mediated killing of the LVe neurons, for this reason very few cells are expressing NLS-GFP and the NeuN staining is more sparse that on the contralateral LVe, which is intact (side panels).

(B) Effect of LVe cell killing, 4 and 6 days after DT injection. In pink the group with DT administration and in grey the control one treated with PBS. Thicker lines indicate the average of each of the two groups DT or PBS injected.

(C) Experimental time line.

(D-F) Neurolucida reconstruction of exemplary Sol motor neurons in ipsi and contralateral side to the DTR injection (D). Quantification of vGlut1 synaptic input on Sol motor neurons soma (E) and dendrites (F).

3.3 Methods

DTR-mediated killing of LVe cells: A CRE-dependent AAV carrying a flexed version of the human diphtheria toxin receptor gene (DTR) (Buch, Heppner et al. 2005) was injected into the LVe as described in Chapter 2.5.2. To increase the specificity of our targeting to the glutamatergic cell population projecting to the spinal cord, we performed the AAV injections into a *vGlut2*^{Cre} (Vong, Ye et al. 2011) background. Together with the AAV DTR we injected and AVV-flex-Syn-Myc and a flex-TVA-NLS-GFP, respectively in a 2:1:1 ratio, blue beads were also included in the mix to localize the exact center of injection. A total of 11 females of age comprised between 10 and 12 weeks was used for this experiment. We waited 15 days for full expression of the construct, the animals were then divided in two groups: the first received one dose of DT (Sigma D0564) intraperitoneally equal to 100 ng of DT per g of body weight (n=6); the second received the same volume of PBS (n=5). Four days before sacrifice, soleus motor neurons were labelled bilaterally delivering G-deleted Rabies-RFP intramuscularly. Seven weeks after the DT injection, animals were scarified and tissue prepared for immunohistochemistry. Synaptic density analysis was performed with Neurolucida as previously described (Chapter 2.5.5) on 11 motor neurons of the ipsilateral side, 12 of the contralateral one from a total of 3 animals.

In Figure 3.1E-F the control Sol motor neurons refers to the group of cells from the wild types animals used already in Figure S2.6.

Beam crossing: Procedure and analysis performed as previously described in Chapter 2.5.6. The number of HL slips was compared before and after LVe killing to test for the emergence of a vestibular defect induced by DTR killing.

Immunohistochemistry: Animals were perfused and tissue processed as described in Chapter 2.5.5. Antibodies used in this part of the study were: chicken anti-GFP (Invitrogen), guinea pig anti-vGlut1 (Chemicon), goat anti-ChAT (Chemicon), mouse anti-Myc (ATCC), mouse anti-NeuN (Chemicon), and rabbit anti-RFP (Rockland). The secondary antibodies used were donkey anti mouse Cy3, donkey anti mouse Alexa 488 donkey anti guinea pig Cy5, donkey anti chicken Alexa 488, donkey anti goat Cy5 (secondary antibodies were purchased from Jackson or Invitrogen). Lumbar spinal cord portions were cut at 60μm, sectons were incubated with antibodies in individual wells and mounted for imaging onto glass slides.

3.4 Discussion

The evolutionarily old origin of vestibular system would advocate in favor of a genetically engraved code of molecular cues able to guide the axons to their correct postsynaptic targets. Nevertheless, loss of vestibular sensory stimulation during development induces long-lasting visual and balance dysfunctions in the adult (Chapter 3.1.2-3.1.3). The system retains a certain degree of plasticity also in the adult, when a series of homeostatic changes of the anatomical and

electrophysiological properties of the vestibular nucleus neurons compensate the damage induced by the unilateral loss of labyrinthine end organs (Galiana, Flohr et al. 1984, Will, Kortmann et al. 1988).

Along the same line, recent studies (Mendelsohn, Simon et al. 2015) provide evidence that the thought to be hard-wired proprioceptive-motor neuron connection (Mears and Frank 1997) is also influenced to some extent by sensory input to guide connectivity refinement in the heteronymous reflex arc. The pattern that emerges so far is that vestibular and proprioceptive systems possess an intrinsic degree of circuit plasticity that can be triggered by specific sensory input deprivation paradigms, as well as by cross-modal signaling (Chapter 2.2.7). We wanted to ask whether and to which extent vestibular deprivation in the adult, can induce a proprioceptive input rescaling at the level of the soleus motor neurons.

3.4.1 The Experimental Set Up

In frog, it was shown that vestibular imbalance due to unilateral labyrinthectomy can induce sprouting of sensory fibers ascending from the spinal cord in the dorsal funiculus and terminating at the level of the vestibular nuclei (Dieringer, Künzle et al. 1984, Neuhuber and Zenker 1989). Other functional studies in guinea pig demonstrated the involvement of the spinal cord in vestibular compensation after unilateral labyrinthectomy. Indeed, most of the postural and motor deficits that are compensated at the chronic stages reappear after spinal cord transection at thoracic levels (Jensen 1979). The authors attribute this effect to the

facilitatory action of the ascending inputs on the ipsilesional vestibular cell activity (Azzena, Mameli et al. 1977).

Despite the fact that the reviewed literature argues in favor of a series of plastic changes happening centrally at level of vestibular nuclei, our experimental observations on vestibular and proprioceptive mutants described in the previous chapter shows that the motor neurons in lumbar spinal cord represent an additional place for cross-modal sensory interaction. Knowing that central vestibular nuclear lesions produce similar effects to the peripheral neurectomy (Barale, Corvaja et al. 1971), we tested whether killing the LVe excitatory neurons would trigger a synaptic input rescaling from proprioceptive afferents at the motor neuron level in the frame of vestibular compensation. The choice of focusing on Sol motor pool draws its basis from the previous observation that cross-modal rescaling can be induced only on specific motor pools (Chapter 2.2.7; FigureS2.5).

The intersectional approach we used (AAV-flex-DTR injected in LVe of *vGluT2*^{CRE}) for targeting LVe excitatory cells has been previously shown to produce efficient and selective neuronal loss in combination with DT administration (Esposito, Capelli et al. 2014).

3.4.2 DTR-Mediated Killing of LVe Cells Causes Aspecific Vestibulo-Motor Defect

In our previously characterized intersectional genetic mouse model of vestibular-neuron silencing (*R4^{Cre}::vGlut2^{flox}* mice), general motor performance was comparable to the wild type. Even in the challenging tasks involving precise paw placement, like for instance the ladder crossing assays, the two groups performed equally well (Chapter 2.2.6). Nevertheless, when we challenged the ability of the mice to balance on a narrow beam suspended between two elevated platforms, the mutants struggled to cross its entire length (Figure 2.5). Because of its efficacy in unmasking vestibular loss of function, we used the beam crossing test also for the experiments described in this chapter.

We quantified the proficiency in the task by looking at the number of HL slips, since the same parameter was selectively affected by blocking the neurotransmitter release from LVe excitatory cells. It is noteworthy that HL motor neurons receive exclusive vestibular innervation through the LVe nucleus.

All animals had comparable baseline conditions: 2 weeks after AAV injection, they crossed the beam with high accuracy in HL placement, indicating a complete recovery from surgery and no side effects derived from the sole expression of the AAV-flex-DTR. The following day, the animals were divined in two groups 6 animals received intraperitoneal DT injection, while the other 5 and only PBS.

Already four days after the administration of DT and PBS, the treated group showed a significant increase in the number of HL slips with respect to the control group that

instead tended to improve the accuracy of the hind limb placement. Since all of the experimental animals used for this plot here have been checked postmortem for injection specificity, we can link the behavioral consequence to the loss in LVe excitatory cells, even if we cannot exclude a minor contamination from the other vestibular subnuclei. A major limitation of this loss of function model is exactly this: the impossibility to quantify and localize precisely the amount of cell loss. It would be convenient, in the future, to switch to a method that permanently silences the cell output and allows tagging of the affected cell. One possibility would be to use an AAV-mediated conditional expression of the Tetanus Toxin (Baines, Robinson et al. 1999) in the *vGlut2*^{CRE} line. This method would allow a direct identification and quantification of the silenced cells. Another important point to consider, would be the addition of one more control group treated with DT but lacking the AAV-flex-DTR. In this way, we would be able to exclude any unspecific side effects induced by DT itself.

We did not observe any obvious postural or more general behavioral defect in the experimental group with respect to the control one and this could have different explanations. First, we know that LVe neurons are the almost (a minor contribution comes from the SpVe) exclusive vestibular projection to the lumbar spinal cord (Grillner, Hongo et al. 1970), and their contribution to VOR reflex control is minor (Uchino and Kushiro 2011). Moreover, any defect at the level of muscles innervated by motor neurons residing at cervico-thoracic levels could have been compensated by other vestibular nuclei projecting along the MVST (Chapter 1.4.6).

An alternative explanation could derive from the presence of different mechanisms of vestibular compensation acting at the brainstem level that are compensating the static defects in posture caused by vestibular cell loss (Gliddon, C. et al. 2004, Aleisa, A. et al. 2007). Interestingly, both models of vestibular dysfunction, the *r4::vGlut2*^{flox} and the DTR-mediated cell ablation showed similar and milder effects in comparison to the *Nox3* mutant (Paffenholz, Bergstrom et al. 2004). This difference could derive from the wide distribution of the otolithic input to the cerebellum and all the other vestibular subnuclei (Chapter 1.4.2) (Barmack, Baughman et al. 1993, Maklad and Fritzsch 2003). For these reasons, a complete lack of the otolithic information channel will have an umbrella effect on several vestibular reflexes like vestibulo-ocular, vestibulo-spinal as well as on higher cognitive processes involving cortex and cerebellar circuits (Chapter 1.4.5).

3.4.2 Limited Proprioceptive Rescaling and Vestibular Compensation in Adulthood

Our previous experiments in *Nox3* mutants (Fig. S2.5) demonstrated that, vestibular-proprioceptive cross-talk can be bidirectional. In fact, congenic absence of otolith and consequent lack of linear acceleration information causes an increase of approximately 1.5 times in the number of vGlut1 synapses on the Sol motor neurons (Figure S2.5). A similar compensatory response from the proprioceptive afferents, can be observed also in a chronic mouse model of spinal cord hemisection that eliminates LVe input to ipsilateral lumbar spinal cord along with other descending

circuit components (data not shown). Surprisingly, loss of vestibular cells in the adult was sufficient to induce a statistical significant increase in the number of vGlut1 inputs upon Sol motor neurons but the magnitude of the response was dramatically lower than the effect seen in *Nox3* mutants.

From this result, we cannot derive strong conclusions, but we can make few considerations. There are clearly different compensatory dynamics for static and dynamic vestibular symptoms: the first ones recover immediately and the second ones recover slowly if at all (Chapter 3.1.4). In light of this observation, we can assume that if proprioceptive rescaling plays a role in vestibular compensation, it must be associated with the recovery of the dynamic vestibular symptoms. It is fair to assume that walking on the beam would be powerful to unmask the dynamic symptoms associated with timing and precision of vestibulo-spinal reflex (Allum 2012). Unfortunately, we did not behaviorally test our animals before termination of the experiments, but our expectation would be to observe still a higher number of HL slips compared to the pre-DT injection condition. If this were the case, we could conclude that the lack of proprioceptive rescaling might be associated with the lack of recovery of the dynamic vestibular symptoms.

Inverting the problem, could we start from a situation in which proprioceptive rescaling is observed, and correlate it to the state of a vestibular symptom recovery? We tried to address this question in *Nox3* mutant mice that show the strong proprioceptive upscaling phenotype. Unfortunately, it was not possible to train these mice on the beam crossing assay since they were unable to stay on the starting platform, probably because of defects to other central vestibular and cerebellar

pathways depending upon proper otolithic input integration (see for review (Buttner-Ennever 1999, Uchino and Kushiro 2011)).

3.4.3 General Considerations

What emerges clearly from our experiments is that killing of LVe excitatory neurons in the adult does not produce the same sensory cross-modal interactions induced by genetic means during development. The observed condition of limited plasticity could be due to two main reasons.

First, a low efficiency of the DTR-mediated cell killing could spare some neurons that would be enough to still convey vestibular input to lumbar motor neurons. It is difficult to quantify the efficiency of the infection because DTR expressing cells are ablated at the end of the experiment. As already mention in the previous chapter, the expression of a Tetanus Toxin in a *vGlut2*^{CRE} mouse would solve this problem (Baines, Robinson et al. 1999).

A second possible reason might be that the lack of LVe-only could be not sufficient to induce a strong compensatory response from Ia afferents, since other descending brainstem/cerebellar pathways carrying the vestibular information to the spinal cord could compensate for it. For example, neurons in the medial reticular formation mediate the transmission of vestibular input to the diaphragm and abdominal motor neurons (Mori, Bergsman et al. 2001). Moreover, neurons in the peduncolo pontine nucleus of the brainstem (PPN) receive vestibular input (Horowitz, Blanchard et al. 2005) and mediate muscle atonia acting through local spinal cord

networks (Takakusaki, Kohyama et al. 2003). This view would be in line with our observation of a stronger proprioceptive anatomical response induced by spinal cord hemisection compared to the LVe neuron ablation experiment described here. Hemisection injury interrupts many other sources of descending pathways and this may also include vestibular-related input to the spinal cord.

A third hypothesis would assume that cross-modal synaptic rescaling between vestibular and proprioceptive stimuli would happen only in a restricted developmental time window that is no longer open in the adult. Nevertheless, high levels of proprioceptive innervation, comparable to the ones seen in *mlc::NT3* mouse mutants, can be induced by performing thoracic hemisection (data not shown). This observation would lead us back to the second hypothesis, indicating that the adult CNS retains the ability of undergoing plastic remodeling in the adult, but in a stimulus-dependent manner. The fact that we failed to observe the same response in the DTR-mediated LVe killing experiment may indicate that this is not the appropriate stimulus for inducing proprioceptive plasticity in the adult.

3.5 Conclusions

Our experiments address the question of whether loss of excitatory vestibular cell input in the adult would be sufficient for inducing a proprioceptive input rescaling on lumbar Sol motor neurons, a preferred vestibular target, and how this correlates with vestibular symptom compensation.

Our results suggest that proprioceptive rescaling is severely limited in the model of adult vestibular loss of function and it correlates with the long time course of recovery observed for the dynamic symptoms of vestibular defect.

More points remain still to be studied. These include some of the following questions: Which are the molecular substrates guiding synapses formation or elimination? How does the motor neuron regulate its level of direct monosynaptic input in a specific manner? What is the functional outcome of synaptic rescaling at the level of the last integration center before motor output? Which are the triggers for disclosing synaptic plasticity in the adult?

Chapter 4. Final Discussion

4.1 Functional Correlates of Vestibulo-Spinal Connections

Understanding brain function can be compared to the problem of deciphering an ancient language whose meaning has been lost in time. Anatomy alone would be instrumental for breaking down the words into morphemes and infer the structure of the language; functional experiments would allow us to associate content and context to the word and creating sematic rules. Following this analogy, it becomes clear that only combining both anatomy and functional studies it would be possible to fully understand the mechanism of action of neuronal circuits regulating the modules of motor control.

For this reason, clarifying how vestibular circuit function influences body posture maintenance or more generally motor behavior performance is important. The most common approach used in motor control for linking neuronal circuit organization to its functional correlates is represented by loss-of-function studies. Hereafter, we will review the most interesting findings of our study and we will try to put them in the context of other work to extract their meaning and significance from a broader perspective.

4.1.1 The Vestibulo-Spinal Pathway Increases Extensor Muscle Tone

Since the beginning of the 20th century, Fulton and colleagues correlated the presence of an intact vestibulo-spinal circuit with body muscle rigidity, induced after decerebration. In this series of experiments, considered today 'classics' of vestibular research, Fulton noticed that muscle tension can be decreased ipsilaterally to a vestibular lesion performed after decerebration (Fulton, Liddell et al. 1930). Bach and Magoun, with a series of focal lesion experiments, have further restricted the region responsible for decerebrate rigidity to the LVe territory (Bach L. 1947). In more recent studies, lesions have been performed at each of the three stages of the vestibulo-spinal pathway (Chapter 1.4): from the labyrinthine organs (Stapley, Ting Lh et al. 2006), the vestibular nuclei (Yu and Eidelberg 1981) and the ventromedial funiculus (Brustein and Rossignol 1998). From all the aforementioned evidence, we can extrapolate that the anatomical bias observed in the distribution of vestibular connections to extensor over flexor motor neurons implies a clear functional role, not only in the decerebrated cat condition but also in the intact animal. Built on top of this evidence, we can ask for which behavioral state or motor action the vestibular activation on extensor muscles is required.

4.1.2 Vestibular Action During Static and Dynamic Equilibrium Maintenance

Recollecting the effects of vestibular labyrinthectomy in mammals presented in Chapter 3.1.4, we can deduce that: (1) the extensor muscle recruitment by vestibular descending pathways is required more during dynamic than static equilibrium tasks; (2) maintenance of quiet standing position doesn't depend exclusively upon vestibular function. In fact, postural defects initially observed after vestibular nucleus lesions can be fully compensated by other sensory systems in cat (Thomson, Inglis et al. 1991) as well as in humans (Birren 1945). Among the other sensory systems accounting for vestibular compensation in quiet stance maintenance, a major role is played by the proprioceptive system. For example, combination of vestibular and proprioceptive signals contribute respectively, to the ankle and hip strategies in maintaining body posture in human (Allum, Bloem et al. 1998)

One major limitation of the experiments presented so far, resides in the methods used. In fact, lesions are often unspecific because it is difficult to avoid passing-by fibers or neighboring nuclei and this would make the final behavioral perturbation more complicated to interpret. To circumvent this problem, we performed a pilot optogenetic experiment where we selectively targeted the LVe excitatory cells. Our results, even if preliminary, further refine the role of LVe neurons in maintaining extensor muscle tone in a lateralized manner. Indeed light stimulation of LVe neurons, while the mouse was walking in an open field box, induced a shift of the body axes toward the stimulated side, as if the mouse was trying to balance on a tilted surface. The stimulation-induced

vestibular phenotype observed, is coherent with our anatomical evidence of a monosynaptic excitatory projection originating from LVe and terminating ipsilaterally on extensor motor neuron pools. This is also in line with the electrophysiological profile of the mono- and polysynaptic vestibulo-spinal connections described in cat (Grillner, Hongo et al. 1971). Interestingly, analogous postural changes, but mirroring affecting the contralateral side, can be induced by vestibular nerve lesions in frogs (Ewald 1892). This observation reflects the importance of the inhibitory commissural connections in modulating the motor output of vestibulo-spinal neurons (Chapter 1.4.5). Moreover, the same commissural connections, responsible for the fast recovery of the static symptoms (including postural ones) induced by vestibular labyrinthectomy (Chapter 3.1.4), are likely to mediate also the quick quenching of behavioral responses observed upon consecutive LVe stimulations. Finally, vestibular stimulation does not 'per se' produce a movement, and this is different from other brainstem regions (Garcia-Rill, Skinner et al. 1985, Esposito, Capelli et al. 2014), but it is able to trigger postural adjustments effective in shifting the center of body mass to help executing the ongoing motor programs (Chapter 1.2).

4.1.3 Vestibular Action in Equilibrium Maintenance is Modulated by Neck Proprioceptor Signaling

Going back to our initial question presented in Chapter 4.1.1, it still not clear which particular motor actions require a direct recruitment of extensor muscles from the

vestibular nucleus. We discussed in the previous chapter the quiet stance maintenance, but what about the condition of quiet stance restoration after equilibrium perturbation?

This question has been addressed experimentally in cat and the results indicate a differential engagement of vestibulo-spinal responses depending of the type of perturbation. For instance, intact cats had no problem in maintaining their stance position while performing voluntary high-amplitude head movements, but after a bilateral labyrinthectomy, the same head movement led to a posture destabilization and consequent fall (Stapley, Ting Lh et al. 2006). On the other hand, when the perturbation was applied to the standing surface, animals with bilateral labyrinthectomy could still succeed in maintaining their stance equilibrium, despite the presence of a transient overresponse (Macpherson and Inglis 1993).

These data suggest that vestibular control of the extensor muscle tone is important for posture control, especially when a head-to-body movement is actively performed. This observation reconnects with a model from (Roy and Cullen 2001) presented in Chapter 1.6, where the activity of vestibulo-spinal neurons is proposed to be modulated by the concomitant action of proprioceptive and motor efferent copy signal.

4.1.4 A Circuit Model Hypothesis for Gating Vestibulo-Spinal Action

The model presented by Roy and Cullen is very intuitive even if we do not know where the required computations take place in the brain. Anyway, collecting published

evidence, we can draw a plausible circuit model that will help us designing and interpreting future functional experiments to perform. In our scheme (Figure 4.1), the anterior vermis and the LVe are the key computational centers for modulating vestibulo-spinal motor output. The primary vestibular sensory input would reach in parallel the LVe nucleus as well as to the deep cerebellar nuclei uvula, nodulus (Chapter 1.4.4) as well as the anterior vermis (Gerrits, A. et al. 1989). The anterior vermis is a good candidate location for hosting multimodal sensory integration and motor efferent copy comparison. In fact, in addition to the vestibular sensory input, this nucleus receives proprioceptive information from the periphery of the body, partially mediated through the LRN (Precht, R. et al. 1977). The anterior vermis is innervated also from different areas of the motor cortex (Coffman, Dum et al. 2011), probably carrying the efference copy of the motor signal. The inhibitory output of the anterior vermis is mediated by Purkinje cells, which directly inhibit or indirectly disinhibit LVe neurons (Andersson and Oscarsson 1978) (not shown in Figure 4.1). We do not know yet whether LVe neurons, which receive inhibition from the vermis, are the same that are directly excited by primary vestibular afferents but the convergence of these two signal processing pathways might sustain the computational operations required for modulating the vestibular increase of extensor muscle tone. One problem of this model is related to the timing of input convergence at the level of the LVe neurons. In fact, primary vestibular neurons make monosynaptic connections with LVe cells, while input processing thought the vermis involves a polysynaptic loop. The solution might be found in the membrane properties (Straka, Vibert et al. 2005) of the LVe neurons themselves, that could be able to compensate for the time discrepancy. All these hypothesis need to be tested

experimentally. Moreover it would be worth investigating the role of rostral fastigial nucleus (Chapter 1.4.5) in cross-modal sensory interaction and in the computation of the body coordinate system, since it represents another important center for multimodal integration.

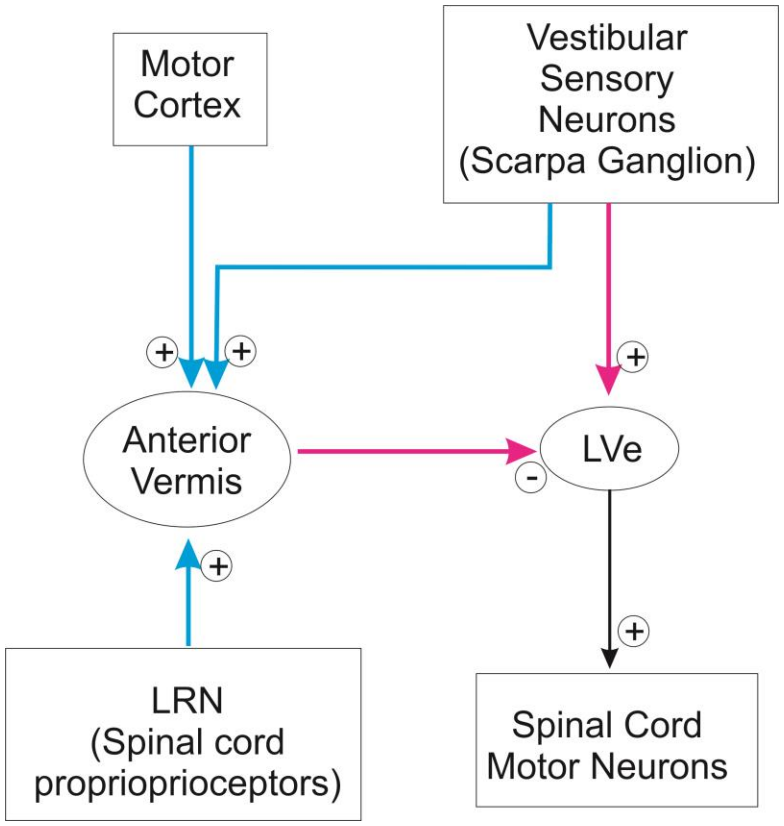


Figure 4.1 Possible circuit connection scheme modulating vestibulo-spinal reflex. The anterior vermis and the LVe are presented as major signal integration centers. In cyan the three different sources of input impinging on to the anterior vermis: motor cortex (Coffman, Dum et al. 2011), primary vestibular afferences (Gerrits, A. et al. 1989) and proprioceptors via LRN (Precht, R. et al. 1977). In magenta the two input channels converging to the LVe and probably modulating its output: primary vestibular afferences, and Purkinje cell output from the anterior vermis (Andersson and Oscarsson 1978). The signs + and - indicates respectively an overall excitatory or inhibitory connection, no information on the mono- or polysynaptic nature of the connection is given here.

4.1.5 Vestibular Contribution to Locomotion

Locomotion for almost all mammalian species indicates the concatenation of series of steps, one after the other, efficient in producing body translation in space. Every single step entails of a stance and swing phase. During the swing phase, the leg is unloaded from the body weight and the foot is lifted up from the ground by a concomitant activation of the flexor muscles. The stance begins when the heal touches the ground and it is associated with extensor muscle activation, required for bearing body weight. During locomotion, many steps are performed in a smooth and repetitive manner, and the extensor muscles tone needs to be rhythmically increased at the beginning of each stance phase to allow body weight support. Is the vestibular nucleus activity required for performing this motor action?

Again the answer comes from vestibular-lesion studies. In cat, a bilateral destruction of the LVe induces ataxia that progressively recovers but with a marked reduction of the extensor tone during the stance phase (Yu and Eidelberg 1981). Moreover, the LVe cells, recoded during locomotion, show a rhythmic firing pattern with a peak of activity that correlates with the beginning of each stance phase. This observation is further supported by evidence that an electrical stimulation of LVe neurons is effective in increasing the amplitude of LVe cell firing only if it occurs within their period of activity. A stimulation in phase with the swing phase of locomotion has little impact on the step cycle (Orlovsky 1972). Interestingly, from our preliminary experiments, when we activated optogenetically the excitatory neurons in LVe, we induced an increased extensor muscular tone independently of the step cycle phase or the behavioral condition. This difference is probably due mainly to the higher stimulation

efficiency obtained with our method with respect to electrical stimulation used for the cat experiments. Interestingly, vestibular rhythmic modulation during walking could be abolished by cerebellar ablation (Orlovsky 1972). This indicates a crucial role of the deep cerebellar nuclei in vestibular information processing and integration with the upcoming proprioceptive input. The same bias of vestibular input to the extensors, is also observed at level of the forelimbs muscle (Marlinsky 1992).

It would be interesting to investigate further, which other structures in the brain or brainstem are driving vestibulo-spinal input during the entire locomotor burst, or are modulating its activity across different locomotor speeds (Mori, Matsuyama et al. 1988). It is, for instance, well know that stimulation of a brainstem region called Mesencephalic Locomotor Region (MLR) (Skinner and Garcia-Rill 1984) can induce locomotion, but it is not clear yet which circuit hierarchy links MLR to vestibular or other brainstem nuclei involved in locomotion.

4.2 Vestibulo-Spinal Connection Specificity: Can It Be Explained Only By Motor Neuron Type Diversity?

The deductive reasoning which led us to formulate the question expressed in the title derives from three main observations: (1). Vestibulo-spinal neurons connect preferentially to slow motor neurons ($MMP9^{OFF}$) of extensor pools and avoids flexor counterparts (Figure 2.3). (2). This specific observation can be generalized to LMC motor neurons in the lumbar spinal cord, using molecular markers (Figure. 2.3) (3). Each given muscle has a specific signature for the ratio of fast/slow motor units. This

ratio is thought to be set to match the mechanical and metabolic contraction properties required to operate its needed function (Ariano, Armstrong et al. 1973, Bloemberg and Quadrilatero 2012).

Based on these observations, we asked whether flexor muscles would be mainly made up of fast motor units. If this were the case, we would be able to combine the two rules of vestibulo-spinal connection specificity into one, dependent only on the motor neuron type identity.

We tried to address this point experimentally stratifying, different motor neuron pools based on MMP9 expression. Six motor neuron pools were back-labelled from the corresponding anatomically identified muscles. We chose two pure flexors (biceps femoris, iliopsoas), two pure extensors (vastus lateralis, soleus) and one pair operating in a bifunctional flexor-extensor manner at the level of two different joints (rectus femoris, semitendinosus). From preliminary results, we were not able to detect an enrichment in $MMP9^{ON}$ (fast) motor neurons with respect to the extensor pools. Moreover from Figure 2.3F, we can appreciate a statistically significant difference between the fraction of $MMP9^{ON}$ motor neurons belonging to the extensor pool and any other motor neuron belonging to a flexor pool. The first one still receives a minor fraction of LVe terminals while the second one is completely avoided. These observations would support our idea of a double-layered rule guiding LVe connections specificity with respect to the lumbar motor neuron population. Our work just started to address this point, but the strategic importance played by the metabolic motor neuronal properties in setting the vestibulo-spinal connectivity rules remains still unclear. Following in this direction, it will be interesting to test whether perturbing the characteristic fast-to-slow

ratio of a pool would induce a rescaling of vestibular and proprioceptive inputs accordingly to the model suggested in the Graphical Abstract of Chapeter 2. The existence of two distinct rules determining the specificity of the vestibulo-motor neuron connectivity raises the question of how they are established during development. We already found that vestibular input and cross-modal sensory interaction can play a role in this process but it is quite reasonable to assume that the gross connectivity matrix organization determined by genetic and molecular factors, for reasons we will discuss in the next section.

4.3 Genetic and Environmental Factors influence Vestibular System Development

In light of our developmental-refinement finding and the evolutionary old origin of the vestibular system, it would be reasonable to assume a two-stage model for circuit maturation, combining genetically determined rules and sensory-derived information.

4.3.1 Developmental Mechanism of the Primary Afferent Projections

Differently from for other sensory systems, a lack of information exists on the actual mechanism guiding the synaptic specificity of the vestibular system. The primary vestibular neurons sitting in the ganglion of Scarpa do not show a precise pattern of segregation: neurons innervating different vestibular endorgans are only loosely

clustered with representation of different sensory information largely overlapping (Maklad and Fritzsch 1999). This type of organization would favor a model in which the peripheral target recognition from the sensory neuron happens before or independently of molecular gradients locally present in the Scarpa ganglion, in a cell autonomous manner. This type of organization is similar to the one present in the DRG where sensory neurons signaling different sensory modalities are distributed in a salt-and-pepper manner (Lee, Friese et al. 2012). Even this sensory-motor connection specificity, for long time thought to be exclusively genetically determined (Mears and Frank 1997), has been recently shown to undergo a phase of activity-dependent refinement. In fact, genetic blockade of neurotransmitter release from the primary sensory neuron, leads to an increased number of synapses on the heteronymous motoneuronal pools (Mendelsohn, Simon et al. 2015). Vestibular sensory neurons innervate the peripheral receptor organs in between E18 and P7 (Van De Water, Wersall et al. 1978) and send central projections from E17 to P10 in mouse (Desmadryl and Sans 1990). The central projection of primary sensory neurons innervating different vestibular endorgans are largely overlapping as explained in Chapter 1.4.2 to allow a fast integration of multiple inputs. Again, the information available on the development mechanisms of such connections is very scarce. It will be important in the future to discover the rules of synaptic specificity at different stages of vestibular information processing, to favor the understanding of vestibulo-spinal system.

4.3.2 Developmental Mechanism of the Secondary Vestibulo-Spinal Projections

Altman and Bayer report a time frame for VN neuron development that spans from E11 to E15 in rat, with a peak of LVe post-mitotic cells at E12 (Altman and Bayer 1980).The LVST is one of the first brainstem projections reaching the lumbar spinal cord already at E16.5 (data not shown), and the axon trajectory is determined by the genetic identity of the LVe cells (Chen, Takano-Maruyama et al. 2012). Despite the electrophysiological properties of the LVe cells mature only in first two postnatal weeks (Dutia and Johnston 1998), stimulation of the tract is able to elicit action potentials at all spinal cord levels already at P0 (Kasumacic, Glover et al. 2010). Because of its early development, it is reasonable to assume that genetic cues guide the establishment of the gross connectivity matrix that will refine later to accommodate different sensory-motor transformations, but experimental evidences of this process are currently missing. In the second part of our study, we addressed this point reporting the presence of transient connections from LVe to the flexor motor neurons innervating the TA muscle that disappear around the second postnatal week. This refinement process involves at least partially vestibular sensory input mediated by vestibular or cerebellar (at this point we cannot exclude it cell activity. Even if the gravity force is a constant stimulus, the second order vestibular neurons are also premotor centers and their change in connectivity might be reflecting the development of a more complex motor repertoire from the animal. Following this logic, it appears reasonable to ask whether TA transient connections are functional. It is possible to address this point with an in-vitro assay

stimulating axons of LVe neurons projecting to the spinal cord at early postnatal stages and recording the elicited responses from the TA motor neurons.

4.3.2 Analogies with Visual System Development

Evidence exists that also vestibular sensory signaling during the first two weeks can impact the on the electrophysiological signatures of the VN cells in mouse (Eugène, Deforges et al. 2009). The first two postnatal weeks seem to be critical for activity-dependent refinement of other sensory and motor systems. For example, the visual system undergoes extensive changes in cell and circuit properties following a biphasic model leading to the formation of a retinotopic map of axonal projections in the visual cortex. In an initial phase the thalamo-cortical axons are guided to form a gross connectivity pattern (Molnár, Garel et al. 2012). In a second stage spontaneous activity in the projection axons will refine the coarse patter to a highly tuned one (Desai, Cudmore et al. 2002). At this point, we cannot conclude that this model would be valid for describing the developmental mechanism of all mammalian sensory systems. In fact, variations of the model are quite common and reviewed by (Hensch 2004). To know whether vestibular system development would follow such two-stage logic, it would be mandatory to find the molecular code responsible for setting up the initial coarse connectivity phase. A hint could derive again from the visual system, where the Eph receptor family is involved in formation of the retinotopic maps through the interaction with and their ligands (Tessier-Lavigne and Goodman 1996). Interestingly, the receptor EphA4 is detected in the vestibular hair cells (Bianchi and Liu 1999) and other classes

of receptors could expressed by the sensory vestibular neurons. For instance, mice mutants for *EphB2* show circling behavior due to a central defect of endolymph production at the level of the vestibular endogens (Cowan, Yokoyama et al. 2000). To discover the molecular code behind the initial phase of vestibulo-spinal connection specificity, it would sound reasonable to consider also the Eph receptor-ligand expression at level of functionally or metabolically different motor neurons in the spinal cord.

4.4 Final Considerations on Cross-Modal Sensory Signaling

Finally, from our last findings it seems that proprioceptive and vestibular systems can interact reciprocally by rescaling their level of motor neuron innervation in a complementary manner. This finding opens up a new stage for vestibular function compensation that takes place at the motor neuron level, the last station before motor output. The exact function that is being compensated is difficult to explain in terms of behavior because it is most probably related to motor neuron excitability or modulation of their membrane properties. Moreover, it would be very interesting to investigate the molecular code involved in vestibular or proprioceptor input rescaling. On one hand, we can gain insights into how sensory stimuli can impact on synapse formation and elimination, and this may help us to understand the process of developmental refinement in vestibulo-spinal connections. On the other hand, it will open up new possibilities for intervention when one of the sensory channels is compromised. The

recovery process could be favored by triggering a cross-modal rescaling involving proprioceptors, vestibular or more broadly other descending brainstem projections.

Chapter 5. Aknowlegments

At first, I would like to thank my PhD advisor Silvia Arber for providing ideas and guidance especially through the initial difficult parts of my project and for supporting, also financially, in the last 3 years. Being in this lab, I had the opportunity to growth from a scientific and personal point of view.

A special thanks to Aya Takeoka a great colleague and scientist. Working with her I learned a lot and enjoyed all the steps from the experiments to the data analysis and writing. Among the others I learned what it means to strive for high ends.

Thanks to Markus Sigrist for his precious help not only in processing a lot of spinal cord tissue, but only for essential contribution in the viral vector production and lab organization.

Marco Tripodi helped me in getting started in the lab and he inspired me to start this project. Thanks to him we could get in contact with Prof. Michelle Studer that I thank again for providing us with the r4::CRE mouse line.

Francesco Roselli contributed to the development of the all idea of fast and slow connectivity bias. I thank him for the inspiring and fruitful discussions.

I am thankful to Peter Scheiffele and Botond Roska having been part of my PhD Committee. They understood my project and contributed with stimulating discussion to its evolution.

I thank all the people in the lab for the precious inputs and great work environment. In particular: Ludwig for introducing me and helping a lot during the optogenetic recordings; Soledad for teach me to use her 3D brain-reconstruction; Chiara for explaining me how to use the automatized spinning disk acquisition method; Daisuke for the very sharp observations and thoughtful discussions; Keith for exchanging ideas and correcting my English.

Federico, for introducing the beauty of statistics and for having been always there, also in the most difficult times.

My parents to be always with me.

Chapter 6. Bibliography

Abraira, V. E. and D. D. Ginty (2013). "The sensory neurons of touch." Neuron **79**(4): 618-639.

Aleisa, M., Z. A. and K. E. Cullen (2007). "Vestibular compensation after unilateral labyrinthectomy: normal versus cerebellar dysfunctional mice." (0707-7270 (Electronic)).

Allum, J. (2012). "Recovery of vestibular ocular reflex function and balance control after a unilateral peripheral vestibular deficit." Frontiers in Neurology **3**.

Allum, J. H. J., B. R. Bloem, M. G. Carpenter, M. Hulliger and M. Hadders-Algra (1998). "Proprioceptive control of posture: a review of new concepts." Gait & Posture **8**(3): 214-242.

Altman, J. and S. A. Bayer (1980). "Development of the brain stem in the rat. III. Thymidine-radiographic study of the time of origin of neurons of the vestibular and auditory nuclei of the upper medulla." J Comp Neurol **194**(4): 877-904.

Andersson, G. and O. Oscarsson (1978). "Climbing fiber microzones in cerebellar vermis and their projection to different groups of cells in the lateral vestibular nucleus." Experimental Brain Research **32**(4): 565-579.

Angelaki, D. E. and K. E. Cullen (2008). "Vestibular system: the many facets of a multimodal sense." Annu Rev Neurosci **31**: 125-150.

Arber, S. (2012). "Motor circuits in action: specification, connectivity, and function." Neuron **74**(6): 975-989.

Ariano, M. A., R. B. Armstrong and V. R. Edgerton (1973). "Hindlimb muscle fiber populations of five mammals." J Histochem Cytochem **21**(1): 51-55.

Arshian, M. S., C. E. Hobson, M. F. Catanzaro, D. J. Miller, S. R. Puterbaugh, L. A. Cotter, B. J. Yates and A. A. McCall (2014). "Vestibular nucleus neurons respond to hindlimb movement in the decerebrate cat." J Neurophysiol **111**(12): 2423-2432.

Asanuma, C., W. T. Thach and E. G. Jones (1983). "Brainstem and spinal projections of the deep cerebellar nuclei in the monkey, with observations on the brainstem projections of the dorsal column nuclei." Brain Res **286**(3): 299-322.

Asanuma, C., W. T. Thach and E. G. Jones (1983). "Distribution of cerebellar terminations and their relation to other afferent terminations in the ventral lateral thalamic region of the monkey." Brain Research Reviews **5**(3): 237-265.

Atkin, A. and M. B. Bender (1968). "Ocular stabilization during oscillatory head movements: Vestibular system dysfunction and the relation between head and eye velocities." Archives of Neurology **19**(6): 559-566.

Ausborn, J., W. Stein and H. Wolf (2007). "Frequency control of motor patterning by negative sensory feedback." J Neurosci **27**(35): 9319-9328.

Azzena, G. B., O. Mameli and E. Tolu (1977). "Vestibular units during decompensation." Experientia **33**(2): 234-236.

Bach L., M., H. W. (1947). "The vestibular nuclei as an excitatory mechanism for the cord." (0014-9446 (Print)).

Baines, R. A., S. G. Robinson, M. Fujioka, J. B. Jaynes and M. Bate (1999). "Postsynaptic expression of tetanus toxin light chain blocks synaptogenesis in Drosophila." Current Biology **9**(21): 1267-S1261.

Balaban, C. D. and J. D. Porter (1998). "Neuroanatomic substrates for vestibulo-autonomic interactions." J Vestib Res **8**(1): 7-16.

Barale, F., N. Corvaja and O. Pompeiano (1971). "Vestibular influences on postural activity in frog." Archives Italiennes de Biologie **109**(1): 27-36.

Barmack, N. H. (2003). "Central vestibular system: vestibular nuclei and posterior cerebellum." Brain Research Bulletin **60**(5–6): 511-541.

Barmack, N. H., R. W. Baughman, P. Errico and H. Shojaku (1993). "Vestibular primary afferent projection to the cerebellum of the rabbit." The Journal of Comparative Neurology **327**(4): 521-534.

Barresi, M., C. Grasso, L. Bruschini, S. Berrettini and D. Manzoni (2012). "Effects of trunk-to-head rotation on the labyrinthine responses of rat reticular neurons." Neuroscience **224**: 48-62.

Beraneck, M. and K. E. Cullen (2007). "Activity of vestibular nuclei neurons during vestibular and optokinetic stimulation in the alert mouse." J Neurophysiol **98**(3): 1549-1565.

Beraneck, M., M. Hachemaoui, E. Idoux, L. Ris, A. Uno, E. Godaux, P.-P. Vidal, L. E. Moore and N. Vibert (2003). "Long-Term Plasticity of Ipsilesional Medial Vestibular Nucleus Neurons After Unilateral Labyrinthectomy." Journal of Neurophysiology **90**(1): 184-203.

Beraneck, M., M. B. M, A. L. Se´ac'h, M. Jamon and P. P. Vidal (2012). "Ontogeny of mouse vestibulo-ocular feflex following genetic or environmental alteration of gravity sensing." PLoS ONE **7(7)**: 1-13.

Bernstein, N. A. (1947). On the Construction of Movements. Moscow.

Bianchi, L. M. and H. Liu (1999). "Comparison of Ephrin-A ligand and EphA receptor distribution in the developing inner ear." The Anatomical Record **254**(1): 127-134.

Birren, J. E. (1945). "Static equilibrium and vestibular function." Journal of Experimental Psychology **35**(2): 127-133.

Bloemberg, D. and J. Quadrilatero (2012). "Rapid determination of myosin heavy chain expression in rat, mouse, and human skeletal muscle using multicolor immunofluorescence analysis." PLoS One **7**(4): e35273.

Borel, L., F. Harlay, C. Lopez, J. Magnan, A. Chays and M. Lacour (2004). "Walking performance of vestibular-defective patients before and after unilateral vestibular neurotomy." Behavioural Brain Research **150**(1–2): 191-200.

Branoner, F. and H. Straka (2015). "Semicircular canal-dependent developmental tuning of translational vestibulo-ocular reflexes in Xenopus laevis." Dev Neurobiol **75**(10): 1051-1067.

Brodal, A. and O. Pompeiano (1957). "The vestibular nuclei in cat." J Anat **91**(4): 438-454.

Brooks, J. X. and K. E. Cullen (2009). "Multimodal Integration in Rostral Fastigial Nucleus Provides an Estimate of Body Movement." The Journal of Neuroscience **29**(34): 10499-10511.

Brown, A. G. (1981). Organization of the spinal cord. New York, Springer Verlag.

Brown, B. (1972). "Resolution thresholds for moving targets at the fovea and in the peripheral retina." (0042-6989 (Print)).

Brown, T. G. (1914). "On the nature of the fundamental activity of the nervous centres; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system." The Journal of Physiology **48**(1): 18-46.

Brustein, E. and S. Rossignol (1998). "Recovery of Locomotion After Ventral and Ventrolateral Spinal Lesions in the Cat. I. Deficits and Adaptive Mechanisms." Journal of Neurophysiology **80**(3): 1245-1267.

Buch, T., F. L. Heppner, C. Tertilt, T. J. A. J. Heinen, M. Kremer, F. T. Wunderlich, S. Jung and A. Waisman (2005). "A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration." Nat Meth **2**(6): 419-426.

Burke, R. E. (1967). "Motor unit types of cat triceps surae muscle." J Physiol **193**(1): 141-160.

Burke, R. E. (2007). Sir Charles Sherrington's The integrative action of the nervous system: a centenary appreciation.

Burke, R. E., R. P. Dum, J. W. Fleshman, L. L. Glenn, A. Lev-Tov, M. J. O'Donovan and M. J. Pinter (1982). "A HRP study of the relation between cell size and motor unit type in cat ankle extensor motoneurons." J Comp Neurol **209**(1): 17-28.

Burke, R. E., E. Jankowska and G. ten Bruggencate (1970). "A comparison of peripheral and rubrospinal synaptic input to slow and fast twitch motor units of triceps surae." J Physiol **207**(3): 709-732.

Buttner-Ennever, J. A. (1999). "A review of otolith pathways to brainstem and cerebellum." Ann N Y Acad Sci **871**: 51-64.

Carleton, S. C. and M. B. Carpenter (1983). "Afferent and Efferent Connections of the Medial, Inferior and Lateral Vestibular Nuclei in the Cat and Monkey." Brain Res. **278**: 29-51.

Carter, R. J., J. Morton and S. B. Dunnett (2001). "Motor coordination and balance in rodents." Curr Protoc Neurosci **Chapter 8**: Unit 8 12.

Chen, H. H., W. G. Tourtellotte and E. Frank (2002). "Muscle spindle-derived neurotrophin 3 regulates synaptic connectivity between muscle sensory and motor neurons." J Neurosci **22**(9): 3512-3519.

Chen, Y., M. Takano-Maruyama, B. Fritzsch and G. O. Gaufo (2012). "Hoxb1 Controls Anteroposterior Identity of Vestibular Projection Neurons." PLoS ONE **7**(4): e34762.

Clarke, L. (1861). London.

Coffman, K. A., R. P. Dum and P. L. Strick (2011). "Cerebellar vermis is a target of projections from the motor areas in the cerebral cortex." Proceedings of the National Academy of Sciences **108**(38): 16068-16073.

Corda, M., C. Von Euler and G. Lennerstrand (1965). "Proprioceptive innervation of the diaphragm." The Journal of Physiology **178**(1): 161-177.

Cowan, C. A., N. Yokoyama, L. M. Bianchi, M. Henkemeyer and B. Fritzsch (2000). "EphB2 guides axons at the midline and is necessary for normal vestibular function." Neuron **26**(2): 417-430.

Cruz-Martín, A., M. Crespo and C. Portera-Cailliau (2010). "Delayed Stabilization of Dendritic Spines in Fragile X Mice." The Journal of Neuroscience **30**(23): 7793-7803.

Curthoys, I. S. (2000). "Vestibular compensation and substitution." Curr Opin Neurol **13**(1): 27-30.

de Villers-Sidani, E., E. F. Chang, S. Bao and M. M. Merzenich (2007). "Critical period window for spectral tuning defined in the primary auditory cortex (A1) in the rat." J Neurosci **27**(1): 180-189.

Desai, N. S., R. H. Cudmore, S. B. Nelson and G. G. Turrigiano (2002). "Critical periods for experience-dependent synaptic scaling in visual cortex." Nat Neurosci **5**(8): 783-789.

Desmadryl, G. and A. Sans (1990). "Afferent innervation patterns in crista ampullaris of the mouse during ontogenesis." Brain Res Dev Brain Res **52**(1-2): 183-189.

Di Bonito, M., Y. Narita, B. Avallone, L. Sequino, M. Mancuso, G. Andolfi, A. M. Franze, L. Puelles, F. M. Rijli and M. Studer (2013). "Assembly of the auditory circuitry by a Hox genetic network in the mouse brainstem." PLoS Genet **9**(2): e1003249.

Díaz, C., J. C. Glover, L. Puelles and J. G. Bjaalie (2003). "The relationship between hodological and cytoarchitectonic organization in the vestibular complex of the 11-day chicken embryo." The Journal of Comparative Neurology **457**(1): 87-105.

Díaz, C., L. Puelles, F. Marín and J. C. Glover (1998). "The Relationship between Rhombomeres and Vestibular Neuron Populations as Assessed in Quail–Chicken Chimeras." Developmental Biology **202**(1): 14-28.

Dieringer, N., H. Künzle and W. Precht (1984). "Increased projection of ascending dorsal root fibers to vestibular nuclei after hemilabyrinthectomy in the frog." Experimental Brain Research **55**(3): 574-578.

Dieterich, M. and T. Brandt (1995). "Vestibulo-ocular reflex." Current Opinion in Neurology **8**(1): 83-88.

Du Beau, A., S. Shakya Shrestha, B. A. Bannatyne, S. M. Jalicy, S. Linnen and D. J. Maxwell (2012). "Neurotransmitter phenotypes of descending systems in the rat lumbar spinal cord." Neuroscience **227**: 67-79.

Dutia, M. B. and A. R. Johnston (1998). "Development of action potentials and apamin-sensitive after-potentials in mouse vestibular nucleus neurones." Experimental Brain Research **118**(2): 148-154.

Eccles, J. C., R. M. Eccles and A. Lundberg (1957). "The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones." J Physiol **137**(1): 22-50.

Enjin, A., N. Rabe, S. T. Nakanishi, A. Vallstedt, H. Gezelius, F. Memic, M. Lind, T. Hjalt, W. G. Tourtellotte, C. Bruder, G. Eichele, P. J. Whelan and K. Kullander (2010). "Identification of novel spinal

cholinergic genetic subtypes disclose Chodl and Pitx2 as markers for fast motor neurons and partition cells." J Comp Neurol **518**(12): 2284-2304.

Epema, A. H., N. M. Gerrits and J. Voogd (1988). "Commissural and intrinsic connections of the vestibular nuclei in the rabbit: a retrograde labeling study." Experimental Brain Research **71**(1): 129-146.

Esposito, M. S., P. Capelli and S. Arber (2014). "Brainstem nucleus MdV mediates skilled forelimb motor tasks." Nature **508**(7496): 351-356.

Esposito, M. S., P. Capelli and S. Arber (2014). "Brainstem nucleus MdV mediates skilled forelimb motor tasks." Nature **508**(7496): 351-356.

Eugène, D., S. Deforges, N. Vibert and P. P. Vidal (2009). "Vestibular critical period, maturation of central vestibular neurons, and locomotor control." Ann N Y Acad Sci. **1164**: 180-187.

Ewald, J. R. (1892). "Physiologische Untersuchungen iiber das Endorgan des N. Oktavus. ." Bergmann: Wiesbaden. .

Falkenberg, H. K., G. S. Rubin and P. J. Bex (2007). "Acuity, crowding, reading and fixation stability." Vision Research **47**(1): 126-135.

Faludi, G. and K. Mirnics (2011). "Synaptic changes in the brain of subjects with schizophrenia." International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience **29**(3): 305-309.

Fau, P. W., S. H. and C. H. Markham (1966). "A mechanism of central compensation of vestibular function following hemilabyrinthectomy." (0022-3077 (Print)).

Fetter, M. and D. S. Zee (1988). "Recovery from unilateral labyrinthectomy in rhesus monkey." Journal of Neurophysiology **59**(2): 370-393.

Frank, E. (1990). "The formation of specific synaptic connections between muscle sensory and motor neurons in the absence of coordinated patterns of muscle activity." J Neurosci **10**(7): 2250-2260.

Fulton, J. F., E. G. T. Liddell and R. MCK. (1930). "The influence of unilateral destruction of the vestibular nuclei upon posture and the knee-jerk." Brain **53**(3): 327-343.

Galiana, H. L., H. Flohr and G. M. Jones (1984). "A reevaluation of intervestibular nuclear coupling: its role in vestibular compensation." Journal of Neurophysiology **51**(2): 242-259.

Garcia-Rill, E., R. D. Skinner and J. A. Fitzgerald (1985). "Chemical activation of the mesecephalic locomotor region." Brain Research **330**(1): 43-54.

Geisler, H. C. and A. Gramsbergen (1998). "Motor development after vestibular deprivation in rats." Neurosci Biobehav Rev **22**(4): 565-569.

Geisler, H. C., J. Westerga and A. Gramsbergen (1993). "Development of posture in the rat." Acta Neurobiol Exp (Wars) **53**(4): 517-523.

Gerrits, N. M., E. A., v. L. A. . and E. Dalm (1989). "The primary vestibulocerebellar projection in the rabbit: absence of primary afferents in the flocculus." (0304-3940 (Print)).

Gliddon, C. M., D. C. and P. F. Smith (2004). "Rapid vestibular compensation in guinea pig even with prolonged anesthesia." (0304-3940 (Print)).

Graham, B. P. and M. B. Dutia (2001). "Cellular basis of vestibular compensation: analysis and modelling of the role of the commissural inhibitory system." Experimental Brain Research **137**(3-4): 387-396.

Green, C. D. (1997). Classics in the History of Psychology,By Ivan P. Pavlov (1927). York University, Toronto, Ontario. **2**.

Greene, E. C. (1935). Anatomy of the rat. New York, Hafner Press.

Grillner, S. and R. Dubuc (1988). "Control of locomotion in vertebrates: spinal and supraspinal mechanisms." Adv Neurol **47**: 425-453.

Grillner, S. and T. Hongo (1972). "Vestibulospinal effects on motoneurones and interneurones in the lumbosacral cord." Prog Brain Res **37**: 243-262.

Grillner, S., T. Hongo and S. Lund (1970). "The vestibulospinal tract. Effects on alpha-motoneurones in the lumbosacral spinal cord in the cat." Exp Brain Res **10**(1): 94-120.

Grillner, S., T. Hongo and S. Lund (1971). "Convergent effects on alpha motoneurones from the vestibulospinal tract and a pathway descending in the medial longitudinal fasciculus." Exp Brain Res. **12(5)**: 457-479.

Hensch, T. K. (2004). "CRITICAL PERIOD REGULATION." Annual Review of Neuroscience **27**(1): 549-579.

Holler, S. and H. Straka (2001). "Plane-specific brainstem commissural inhibition in frog second-order semicircular canal neurons." Experimental Brain Research **137**(2): 190-196.

Horak, F. B., C. L. Shupert, V. Dietz and G. Horstmann (1994). "Vestibular and somatosensory contributions to responses to head and body displacements in stance." Exp Brain Res **100**(1): 93-106.

Horowitz, S. S., J. Blanchard and L. P. Morin (2005). "Medial vestibular connections with the hypocretin (orexin) system." The Journal of Comparative Neurology **487**(2): 127-146.

Hsu, Patrick D., Eric S. Lander and F. Zhang (2014). "Development and Applications of CRISPR-Cas9 for Genome Engineering." Cell **157**(6): 1262-1278.

Hubel, D. H. and T. N. Wiesel (1970). "The period of susceptibility to the physiological effects of unilateral eye closure in kittens." The Journal of Physiology **206**(2): 419-436.

Jamon, M. (2014). "The development of vestibular system and related function in mammals: Impact of gravity." Frontiers in Integrative Neuroscience **8**.

Jamon, M. and N. Serradj (2009). "Ground-Based Researches on the Effects of Altered Gravity on Mice Development." Microgravity Science and Technology **21**(4): 327-337.

Jensen, D. W. (1979). "Vestibular compensation: Tonic spinal influence upon spontaneous descending vestibular nuclear activity." Neuroscience **4**(8): 1075-1084.

Johnston, A. R., A. Him and M. B. Dutia (2001). "Differential regulation of GABA(A) and GABA(B) receptors during vestibular compensation." Neuroreport **12**(3): 597-600.

Kanning, K. C., A. Kaplan and C. E. Henderson (2010). "Motor neuron diversity in development and disease." Annu Rev Neurosci **33**: 409-440.

Kaplan, A., K. J. Spiller, C. Towne, K. C. Kanning, G. T. Choe, A. Geber, T. Akay, P. Aebischer and C. E. Henderson (2014). "Neuronal matrix metalloproteinase-9 is a determinant of selective neurodegeneration." Neuron **81**(2): 333-348.

Kasumacic, N., J. C. Glover and M. C. Perreault (2010). "Segmental patterns of vestibular-mediated synaptic inputs to axial and limb motoneurons in the neonatal mouse assessed by optical recording." J Physiol. **588(Pt 24)**: 4905-4925.

Kleine, J. F., Y. Guan, E. Kipiani, L. Glonti, M. Hoshi and U. Büttner (2004). "Trunk Position Influences Vestibular Responses of Fastigial Nucleus Neurons in the Alert Monkey." Journal of Neurophysiology **91**(5): 2090-2100.

Koch, S. M., C. G. Dela Cruz, T. S. Hnasko, R. H. Edwards, A. D. Huberman and E. M. Ullian (2011). "Pathway-specific genetic attenuation of glutamate release alters select features of competition-based visual circuit refinement." Neuron **71**(2): 235-242.

Krauzlis, R. J. and S. G. Lisberger (1994). "Simple spike responses of gaze velocity Purkinje cells in the floccular lobe of the monkey during the onset and offset of pursuit eye movements." J Neurophysiol **72**(4): 2045-2050.

Kushiro, K., R. Bai, N. Kitajima, A. Sugita-Kitajima and Y. Uchino (2008). "Properties and axonal trajectories of posterior semicircular canal nerve-activated vestibulospinal neurons." Experimental Brain Research **191**(3): 257-264.

Lamprecht, R. and J. LeDoux (2004). "Structural plasticity and memory." Nat Rev Neurosci **5**(1): 45-54.

Lee, J., A. Friese, M. Mielich, M. Sigrist and S. Arber (2012). "Scaling Proprioceptor Gene Transcription by Retrograde NT3 Signaling." PLoS ONE **7**(9): e45551.

Leroy, F., B. Lamotte d'Incamps, R. D. Imhoff-Manuel and D. Zytnicki (2014). "Early intrinsic hyperexcitability does not contribute to motoneuron degeneration in amyotrophic lateral sclerosis." eLife **3**.

Liang, H., T. Bacskai and G. Paxinos (2015). "Termination of vestibulospinal fibers arising from the spinal vestibular nucleus in the mouse spinal cord." Neuroscience **294**: 206-214.

Liang, H., T. Bacskai, C. Watson and G. Paxinos (2014). "Projections from the lateral vestibular nucleus to the spinal cord in the mouse." Brain Struct Funct **219**(3): 805-815.

Lopez, C. and O. Blanke (2011). "The thalamocortical vestibular system in animals and humans." Brain Research Reviews **67**(1–2): 119-146.

Lorente De Nó, R. (1933). "Vestibulo-ocular reflex arc." Archives of Neurology & Psychiatry **30**: 245-291.

Lund, S. and O. Pompeiano (1968). "Monosynaptic excitation of alpha motoneurones from supraspinal structures in the cat." Acta Physiol Scand **73**(1): 1-21.

Lundberg, A. (1975). Control of spinal mechanisms from the brain. New York, Raven Press.

MacNaughtan, I. P. J. a. M., W. J. (1946). " Some experiments which indicate that the frog's lagena has an equilibrial function." J. Lao,ng. Otol. **61**: 204-221.

Macpherson, J. M. and J. T. Inglis (1993). "Stance and balance following bilateral labyrinthectomy." Progress in brain research **97**: 219-228.

Maklad, A. and B. Fritzsch (1999). "Incomplete segregation of endorgan-specific vestibular ganglion cells in mice and rats." J Vestib Res **9**(6): 387-399.

Maklad, A. and B. Fritzsch (2003). "Development of vestibular afferent projections into the hindbrain and their central targets." Brain Res Bull. **60(5-6)**: 497-510.

Maklad A., K. S., Wong E Fau, Bernd Fritzsch, (2010). "Development and organization of polarity-specific segregation of primary vestibular afferent fibers in mice." (1432-0878 (Electronic)).

Malinvaud, D., I. Vassias, I. Reichenberger, C. Rossert and H. Straka (2010). "Functional organization of vestibular commissural connections in frog." J Neurosci **30**(9): 3310-3325.

Manzoni, D., O. Pompeiano and P. Andre (1998). "Neck influences on the spatial properties of vestibulospinal reflexes in decerebrate cats: Role of the cerebellar anterior vermis." Journal of Vestibular Research: Equilibrium and Orientation **8**(4): 283-297.

Markham, C. H., T. Yagi and I. S. Curthoys (1977). "The contribution of the contralateral labyrinth to second order vestibular neuronal activity in the cat." Brain Research **138**(1): 99-109.

Marlinsky, V. V. (1992). "Activity of lateral vestibular nucleus neurons during locomotion in the decerebrate Guinea pig." Experimental Brain Research **90**(3): 583-588.

Matthews, P. B. (1981). "Evolving views on the internal operation and functional role of the muscle spindle." J Physiol **320**: 1-30.

Mears, S. C. and E. Frank (1997). "Formation of specific monosynaptic connections between muscle spindle afferents and motoneurons in the mouse." J Neurosci **17**(9): 3128-3135.

Mendell, L. M. and E. Henneman (1968). "Terminals of single Ia fibers: distribution within a pool of 300 homonymous motor neurons." Science **160**(3823): 96-98.

Mendelsohn, A. I., C. M. Simon, L. F. Abbott, G. Z. Mentis and T. M. Jessell (2015). "Activity regulates the incidence of heteronymous sensory-motor connections." Neuron **87**: 111-123.

Mendelsohn, A. I., C. M. Simon, L. F. Abbott, G. Z. Mentis and T. M. Jessell (2015). "Activity Regulates the Incidence of Heteronymous Sensory-Motor Connections." Neuron **87**(1): 111-123.

Molnár, Z., S. Garel, G. López-Bendito, P. Maness and D. J. Price (2012). "Mechanisms controlling the guidance of thalamocortical axons through the embryonic forebrain." The European journal of neuroscience **35**(10): 1573-1585.

Moorman, S. J., R. Cordova and S. A. Davies (2002). "A critical period for functional vestibular development in zebrafish." Dev Dyn **223**(2): 285-291.

Mori, R. L., A. E. Bergsman, M. J. Holmes and B. J. Yates (2001). "Role of the medial medullary reticular formation in relaying vestibular signals to the diaphragm and abdominal muscles." Brain Research **902**(1): 82-91.

Mori, S., K. Matsuyama, K. Takakusaki and T. Kanaya (1988). Chapter 18 The behaviour of lateral vestibular neurons during walk, trot and gallop in acute precollicular decerebrate cats. Progress in Brain Research. O. Pompeiano and J. H. J. Allum, Elsevier. **Volume 76:** 211-220.

Mott, F. W. and C. Sherrington (1894). "Experiments upon the influence of sensory nerves upon movement and nutrition of the limbs. Preliminary communication." Proceedings of the Royal Society of London **57**(340-346): 481-488.

Mullen, R. J., C. R. Buck and A. M. Smith (1992). "NeuN, a neuronal specific nuclear protein in vertebrates." Development **116**(1): 201-211.

Neuhuber, W. L. and W. Zenker (1989). "Central distribution of cervical primary afferents in the rat, with emphasis on proprioceptive projections to vestibular, perihypoglossal, and upper thoracic spinal nuclei." The Journal of Comparative Neurology **280**(2): 231-253.

Noda, H. and D. A. Suzuki (1979). "Processing of eye movement signals in the flocculus of the monkey." J Physiol **294**: 349-364.

Nyberg-Hansen, R. (1964). "Origin and termination of fibers from the vestibular nuclei descending in the medial longitudinal fasciculus. An experimental study with silver impregnation methods in the cat." The Journal of Comparative Neurology **122**(3): 355-367.

Ohyama, T., C. M. Schneider-Mizell, R. D. Fetter, J. V. Aleman, R. Franconville, M. Rivera-Alba, B. D. Mensh, K. M. Branson, J. H. Simpson, J. W. Truman, A. Cardona and M. Zlatic (2015). "A multilevel multimodal circuit enhances action selection in Drosophila." Nature **520**(7549): 633-639.

Oliveira, A. L., F. Hydling, E. Olsson, T. Shi, R. H. Edwards, F. Fujiyama, T. Kaneko, T. Hokfelt, S. Cullheim and B. Meister (2003). "Cellular localization of three vesicular glutamate transporter mRNAs and proteins in rat spinal cord and dorsal root ganglia." Synapse **50**(2): 117-129.

Orlovsky, G. N. (1972). "Activity of vestibulospinal neurons during locomotion." Brain Research **46**: 85-98.

Orlovsky, G. N. (1972). "The effect of different descending systems on flexor and extensor activity during locomotion." Brain Research **40**(2): 359-372.

Paffenholz, R., R. A. Bergstrom, F. Pasutto, P. Wabnitz, R. J. Munroe, W. Jagla, U. Heinzmann, A. Marquardt, A. Bareiss, J. Laufs, A. Russ, G. Stumm, J. C. Schimenti and D. E. Bergstrom (2004). "Vestibular defects in head-tilt mice result from mutations in Nox3, encoding an NADPH oxidase." Genes Dev **18**(5): 486-491.

Paffenholz, R., R. A. Bergstrom, F. Pasutto, P. Wabnitz, R. J. Munroe, W. Jagla, U. Heinzmann, A. Marquardt, A. Bareiss, J. Laufs, A. Russ, G. Stumm, J. C. Schimenti and D. E. Bergstrom (2004). "Vestibular defects in head-tilt mice result from mutations in Nox3, encoding an NADPH oxidase." Genes Dev. **18**: 486-491.

Pasqualetti, M., C. Díaz, J.-S. Renaud, F. M. Rijli and J. C. Glover (2007). "Fate-Mapping the Mammalian Hindbrain: Segmental Origins of Vestibular Projection Neurons Assessed Using Rhombomere-Specific Hoxa2 Enhancer Elements in the Mouse Embryo." The Journal of Neuroscience **27**(36): 9670-9681.

Paxinos, G. and K. B. Franklin (2004). The mouse brain in stereotaxic coordinates, Gulf Professional Publishing.

Pearson, K. G. (2004). "Generating the walking gait: role of sensory feedback." Prog Brain Res **143**: 123-129.

Pecho-Vrieseling, E., M. Sigrist, Y. Yoshida, T. M. Jessell and S. Arber (2009). "Specificity of sensory-motor connections encoded by Sema3e-Plxnd1 recognition." Nature **459**(7248): 842-846.

Pivetta, C., M. S. Esposito, M. Sigrist and S. Arber (2014). "Motor-circuit communication matrix from spinal cord to brainstem neurons revealed by developmental origin." Cell **156**(3): 537-548.

Pompeiano, A. B. a. O. (1957). "The vestibular nuclei in the cat." J. Anat. (London) **91**: 438-454.

Precht, W., V. R. and R. H. Blanks (1977). "Functional organization of the vestibular input to the anterior and posterior cerebellar vermis of cat." (0014-4819 (Print)).

Pun, S., A. F. Santos, S. Saxena, L. Xu and P. Caroni (2006). "Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF." Nat Neurosci **9**(3): 408-419.

Rapoport, S., A. Susswein, Y. Uchino and V. J. Wilson (1977). "Properties of vestibular neurones projecting to neck segments of the cat spinal cord." J Physiol **268**(2): 493-510.

Rossignol, S., G. Barriere, A. Frigon, D. Barthelemy, L. Bouyer, J. Provencher, H. Leblond and G. Bernard (2008). "Plasticity of locomotor sensorimotor interactions after peripheral and/or spinal lesions." Brain Res Rev **57**(1): 228-240.

Rotterman, T. M., P. Nardelli, T. C. Cope and F. J. Alvarez (2014). "Normal distribution of VGLUT1 synapses on spinal motoneuron dendrites and their reorganization after nerve injury." J Neurosci **34**(10): 3475-3492.

Roy, J. E. and K. E. Cullen (2001). "Selective Processing of Vestibular Reafference during Self-Generated Head Motion." The Journal of Neuroscience **21**(6): 2131-2142.

Sadeghi, S. G., L. B. Minor and K. E. Cullen (2007). "Response of vestibular-nerve afferents to active and passive rotations under normal conditions and after unilateral labyrinthectomy." J Neurophysiol **97**(2): 1503-1514.

Sakurai, K., M. Akiyama, B. Cai, A. Scott, B. X. Han, J. Takatoh, M. Sigrist, S. Arber and F. Wang (2013). "The organization of submodality-specific touch afferent inputs in the vibrissa column." Cell Rep **5**(1): 87-98.

Sato H., I. M., Kushiro K., Zakir M. Uchino Y., (2000). "Convergence of posterior semicircular canal and saccular inputs in single vestibular nuclei neurons in cats." Exp Brain, Res(0014-4819 (Print)).

Schmidt, R. A. (1980). 8 On the Theoretical Status of Time in Motor Program Representations. Advances in Psychology. E. S. George and R. Jean, North-Holland. **Volume 1:** 145-166.

Shinoda, Y., T. Ohgaki, T. Futami and Y. Sugiuchi (1988). "Vestibular projections to the spinal cord: the morphology of single vestibulospinal axons." Prog Brain Res **76**: 17-27.

Shinoda, Y., T. Ohgaki, Y. Sugiuchi and T. Futami (1989). "Comparison of the branching patterns of lateral and medial vestibulospinal tract axons in the cervical spinal cord." Prog Brain Res **80**: 137-147; discussion 127-138.

Skinner, R. D. and E. Garcia-Rill (1984). "The mesencephalic locomotor region (MLR) in the rat." Brain Research **323**(2): 385-389.

Smith, P. F. and I. S. Curthoys (1989). "Mechanisms of recovery following unilateral labyrinthectomy: a review."

Sprague, J. M. and W. W. Chambers (1953). "Regulation of posture in intact and decerebrate cat: I. Cerebellum, reticular formation, vestibular nuclei." Journal of neurophysiology **16**(5): 451-463.

Stapley, P. J., Ting Lh, Kuifu C., E. D. and J. M. Macpherson (2006). "Bilateral vestibular loss leads to active destabilization of balance during voluntary head turns in the standing cat." (0022-3077 (Print)).

Stepien, A. E., M. Tripodi and S. Arber (2010). "Monosynaptic rabies virus reveals premotor network organization and synaptic specificity of cholinergic partition cells." Neuron **68**(3): 456-472.

Straka, H., S. Holler and F. Goto (2002). "Patterns of canal and otolith afferent input convergence in frog second-order vestibular neurons." J Neurophysiol **88**(5): 2287-2301.

Straka, H., N. Vibert, P. P. Vidal, L. E. Moore and M. B. Dutia (2005). "Intrinsic membrane properties of vertebrate vestibular neurons: Function, development and plasticity." Progress in Neurobiology **76**: 349-392.

Takakusaki, K., J. Kohyama and K. Matsuyama (2003). "Medullary reticulospinal tract mediating a generalized motor inhibition in cats: iii. functional organization of spinal interneurons in the lower lumbar segments." Neuroscience **121**(3): 731-746.

Takeoka, A., I. Vollenweider, G. Courtine and S. Arber (2014). "Muscle spindle feedback directs locomotor recovery and circuit reorganization after spinal cord injury." Cell **159**(7): 1626-1639.

Tessier-Lavigne, M. and C. S. Goodman (1996). "The molecular biology of axon guidance." Science **274**(5290): 1123-1133.

Thomson, D. B., J. T. Inglis, R. H. Schor and J. M. Macpherson (1991). "Bilateral labyrinthectomy in the cat: motor behaviour and quiet stance parameters." Experimental Brain Research **85**(2): 364-372.

Tighilet, B., C. mourre and M. Lacour (2014). "Plasticity of the histamine H3 receptors after acute vestibular lesion in the adult cat." Frontiers in Integrative Neuroscience **7**.

Tourtellotte, W. G. and J. Milbrandt (1998). "Sensory ataxia and muscle spindle agenesis in mice lacking the transcription factor Egr3." Nat Genet **20**(1): 87-91.

Tripodi, M., A. E. Stepien and S. Arber (2011). "Motor antagonism exposed by spatial segregation and timing of neurogenesis." Nature **479**(7371): 61-66.

Uchino, Y., N. Isu, T. Ichikawa, S. Satoh and S. Watanabe (1988). "Properties and localization of the anterior semicircular canal-activated vestibulocollic neurons in the cat." Exp Brain Res **71**(2): 345-352.

Uchino, Y. and K. Kushiro (2011). "Differences between otolith- and semicircular canal-activated neural circuitry in the vestibular system." Neurosci Res **71**(4): 315-327.

Uchino, Y. and K. Kushiro (2011). "Differences between otolith and semicircular canal-activated neural circuitry in the vestibular system." Neuroscience Research **71**(4): 315-327.

Van Cleave, S. and M. S. Shall (2006). "A critical period for the impact of vestibular sensation on ferret motor development." J Vestib Res **16**(4-5): 179-186.

Van De Water, T. R., J. Wersall, M. Anniko and H. Nordeman (1978). "Development of the sensory receptor cells in the utricular macula." Otolaryngology **86**(2): Orl297-304.

van der Weyden, L., J. White, D. Adams and D. Logan (2011). "The mouse genetics toolkit: revealing function and mechanism." Genome Biology **12**(6): 224.

Vong, L., C. Ye, Z. Yang, B. Choi, S. Chua Jr and Bradford B. Lowell (2011). "Leptin Action on GABAergic Neurons Prevents Obesity and Reduces Inhibitory Tone to POMC Neurons." Neuron **71**(1): 142-154.

Walton, K. D., L. Benavides, N. Singh and N. Hatoum (2005). "Long-term effects of microgravity on the swimming behaviour of young rats." (0022-3751 (Print)).

Walton, K. D., S. Harding, D. Anschel, Y. e. T. Harris and R. Llinás (2005). "The effects of microgravity on the development of surface righting in rats." The Journal of Physiology **565**(Pt 2): 593-608.

Walton, K. D., S. Harding, D. Anschel, Y. T. Harris and R. Llinas (2005). "The effects of microgravity on the development of surface righting in rats." J Physiol **565**(Pt 2): 593-608.

Wang, Z., L. Y. Li, M. D. Taylor, D. E. Wright and E. Frank (2007). "Prenatal exposure to elevated NT3 disrupts synaptic selectivity in the spinal cord." J Neurosci **27**(14): 3686-3694.

Wearne, S., T. Raphan and B. Cohen (1998). "Control of Spatial Orientation of the Angular Vestibuloocular Reflex by the Nodulus and Uvula." Journal of Neurophysiology **79**(5): 2690-2715.

Wenner, P. and E. Frank (1995). "Peripheral target specification of synaptic connectivity of muscle spindle sensory neurons with spinal motoneurons." J Neurosci **15**(12): 8191-8198.

Wickersham, I. R., D. C. Lyon, R. J. Barnard, T. Mori, S. Finke, K. K. Conzelmann, J. A. Young and E. M. Callaway (2007). "Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons." Neuron **53**(5): 639-647.

Will, U., H. Kortmann and H. Flohr (1988). HRP Study on Structural Changes in the Commissural Fiber System of Rana temporaria Following Labyrinthectomy. Post-Lesion Neural Plasticity. H. Flohr, Springer Berlin Heidelberg**:** 345-355.

Wilson, V. J., Boyle R, Fukushima K, Rose Pk, Shinoda Y, Sugiuchi, Y., Uchino, Y. (1995). "The vestibulocollic reflex." **5(3)**(0957-4271 (Print)): 147-170.

Wilson, V. J. and M. Yoshida (1968). "Vestibulospinal and reticulospinal effects on hindlimb, forelimb, and neck alpha motoneurons of the cat." Proc Natl Acad Sci U S A **60**(3): 836-840.

Windhorst, U. (2007). "Muscle proprioceptive feedback and spinal networks." Brain Res Bull **73**(4-6): 155-202.

Yu, J. and E. Eidelberg (1981). "Effects of vestibulospinal lesions upon locomotor function in cats." Brain Research **220**(1): 179-183.