
Development of a virtual reality system
for studying sensorimotor control in mice
and characterisation of neural responses
to locomotion in the mouse cerebellum

Tomaso Muzzu

Department of Bioengineering
IMPERIAL COLLEGE LONDON

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ABSTRACT

The function of the cerebellum has long been known to be essential in motor control and learning. Electrophysiological studies provided evidence of its involvement in locomotion, a behaviour that requires the coordination of multiple muscles and continuous adjustments to adapt to environmental conditions. Over the last forty years, step movements have been shown to be represented at a single cell level during locomotion. Imaging studies revealed that the activity of segregated populations of cerebellar neurons increases during movement as compared to stationary periods. To date, however, little is known as to how cerebellar neural activity modulates with behavioural state, such as locomotion speed. To this end, we recorded activity of cerebellar neurons from locomotion-related areas of the mouse cerebellum and characterised it during virtual reality (VR) behaviour. Experiments were performed using a novel experimental paradigm, combining a VR behavioural task with multi-unit electrophysiological recordings.

As part of the project, a VR system for mice was developed with sufficiently low latency to measure motor behaviour with closed-loop perturbation of sensory information. Detailed protocols were established for the creation and configuration of the system. Behavioural assessment revealed that mice integrated the visual feedback to control their two-dimensional position in the VR environment. In addition, the VR system was integrated with an innovative mechanical perturbation mechanism for generating force fields capable of systematically deviating mouse trajectories in the virtual world. The combination of VR behaviour with a novel perturbation paradigm extends the range of applications of VR systems for studying sensorimotor control.

During VR tasks, recordings from populations of neurons in lobules IV-V of cerebellar vermis were performed. Activity in the majority of the isolated units was found to change with locomotion speed. Some of these units were found to be also modulated with yaw velocity and stride pattern of the mouse. Further analysis revealed that these cells did not constitute separate classes of neurons but, instead, encoded all of these quantities to a greater or lesser extent. Furthermore, locomotion speed could be reconstructed with high accuracy with a simple linear decoder of neural activity of these units.

These findings indicate that cerebellar activity is strongly modulated by kinematic parameters. Further characterisation of the cerebellar neurons via transgenic techniques and novel behavioural tasks (applicable with the VR system presented here) can help increase our understanding of how movement control is achieved through the cerebellum and probe computational theories of sensorimotor integration.

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1

INTRODUCTION

1.1 Overview

It is a remarkable feat that humans can control hundreds of muscles to generate precise and coordinated movements with minimal mental effort. An exemplary behaviour of such prowess is locomotion: a precisely timed and composite sequence of muscle activation that is continuously adjusted to compensate for ever changing environmental conditions. The ability to actively move in space is a fundamental skill that has shaped the evolutionary history of every animal species and, not surprisingly, the nervous structures involved in motor control and learning share many common features. One of these structures is the cerebellum, whose function has long been established to be essential for learning and controlling movements (Flourens, 1844; Luciani, 1891; Holmes, 1939).

Being located between high cortical centres and muscles, the cerebellum acts as a strategic relay point for sensorimotor integration. The connectivity traits and the crystalline morphology of the cerebellum (see Apps and Hawkes, 2009) inspired various computational theories of motor control based on adaptive filter models and on neural implementation of internal models (Marr, 1969; Albus, 1971; Wolpert et al., 1995a; Ito, 2006; Dean et al., 2010). While these theories were proven for ocular reflex dynamics, how the cerebellum controls voluntary movements, such as locomotion, is yet to be fully understood. Therefore, discerning how the cerebellum processes

locomotion-related information is a key *step* to verify the neural substrates of computational theories of sensorimotor integration.

Locomotion takes various shapes (walking, jumping, swimming, etc.) but its basic principles, first explored by Thomas G. Brown at the beginning of last century (Stuart and Hultborn, 2008; Jones et al., 2011), are preserved across species (Orlovsky et al., 1999). For this reason it is a convenient behaviour for studying the neural bases of motor control in animal models due to its intrinsic translational potential in the treatment of motor impairments and physical disabilities.

Observing neural activity whilst subjects are engaged in a behavioural task is now more easily achievable thanks to the adoption of virtual reality (VR) systems in experimental neuroscience. These systems offer the advantage of creating a naturalistic reproduction of the world and provide the toolkit necessary to isolate features of animal behaviour whilst allowing observation of neural dynamics. Closed-loop control, animal-specific ergonomics and sensory stimuli presentation are pivotal for ensuring the performance of VR environments in animal experiments. Despite having been accounted for in rodent and insect studies with the development of optimised hardware and software (Holscher et al., 2005; Harvey, Collman, et al., 2009; Keller et al., 2012; Schmidt-Hieber and Häusser, 2013; Lindemann et al., 2003; Takalo et al., 2012; Straw, 2008), these specifications have not been thoroughly outlined. Therefore, the characterisation of a VR system performance is relevant for the sake of psychophysical validity and reproducibility of results across laboratories.

This study looks at the activity of populations of neurons during locomotor behaviour by combining a VR task with multi-unit electrophysiological recordings from the cerebellum of mice. As part of the project, a VR apparatus for mice with short closed-loop delay and sensory perturbation system was developed. The main goal of this study is to assess the activity of multiple neighboring neurons while measuring kinematic parameters of locomotion in order to help in elucidating the role of cerebellar neural networks.

1.2 The cerebellum

The cerebellum is a structure of the central nervous system situated in the occipital part of the cranial fossa and attached to the brain stem through the cerebellar peduncles. Remarkably, it is

estimated to comprise 80 per cent of the total number of the neurons of central nervous system despite accounting for only one tenth of the mass of the human brain (Azevedo et al., 2009). The function and morphology of the cerebellum are common among many animal species (Larsell, 1970) making it a convenient system to be looked at for investigating the neural substrates of motor control.

Cerebellar circuit

Over the last century anatomical and physiological studies have described how the cerebellar neural network is characterised by a stereotyped and homogeneous circuitry across the entire cortex (see Figure 1.1). The general circuit configuration of the cerebellum was first depicted over one hundred years ago in the seminal work of Ramon y Cajal (Sotelo, 2003). To this date, progress is still being made in understanding the details of the apparently simple connectivity of the cerebellar circuit.

Mossy and climbing fibres are the two main inputs of the cerebellum. Beaded fibers originating in the hypothalamus are considered the third input pathway, although only with a modulatory function (Ito, 2009; Li et al., 2014). Mossy fibres originate from spinal and brain stem centres, and carry sensory and cortical signals. They enter the cerebellum creating synaptic contacts with granule and Golgi cells in the granule cell layer, the most internal layer of the cerebellar cortex. Granule cells are inhibited by the Golgi cells which also make inhibitory synaptic contacts onto other Golgi cells (Hull and Regehr, 2012). The axons of the granule cells, the parallel fibres, ascend to the molecular layer, the most superficial layer of the cerebellum, to create multiple contacts with the dendritic tree of the Purkinje cells and molecular layer interneurons (MLI), basket and stellate cells (Jörntell et al., 2010). Parallel fibres, in turn, do not only drive inhibition to Purkinje cells through the MLIs but they also create an excitatory loop with parallel fibers and other MLIs (Astorga et al., 2015).

Purkinje cell activity is therefore modulated by the opposing effects of parallel fibers and MLIs. Purkinje cells are also contacted by climbing fibres whose inputs cause the characteristic complex spikes. Climbing fibre activity also influences the discharge of other cerebellar neurons via extra-synaptic glutamate spillover. In fact, complex spikes were shown to excite local MLIs (Szapiro

and Barbour, 2007; Mathews et al., 2012) and to modify Golgi cell responses *in vivo* during conjunctive stimulation (Xu and Edgley, 2008; Galliano, 2013). Purkinje cell axons constitute the only output of the cerebellar cortex forming inhibitory synapses with deep cerebellar nuclei neurons (Eccles et al., 1967) as well as sending collaterals to other Purkinje cells (Witter et al., 2016). These receive excitatory collaterals by the same cerebellum afferents, namely mossy fibres and climbing fibres forming a loop between the cerebellar cortex and the deep cerebellar nuclei (Ruigrok, 1997).

The cerebellar circuitry is characterised by a high degree of feedback, feed-forward and collateral connections (Ito, 2006; Coddington et al., 2013; Rieubland et al., 2014; Mathews et al., 2012), and a distinctive divergence-convergence configuration of inputs and outputs (Napper and Harvey, 1988; Person and Raman, 2012). Recent discoveries of new mechanisms of information transmission (e.g. MLIs and Golgi cells modulation by climbing fibres) and new evidence for feedforward and feedback loops around which cerebellar signals flow are catalysing new vistas in cerebellar research (Galliano, 2013).

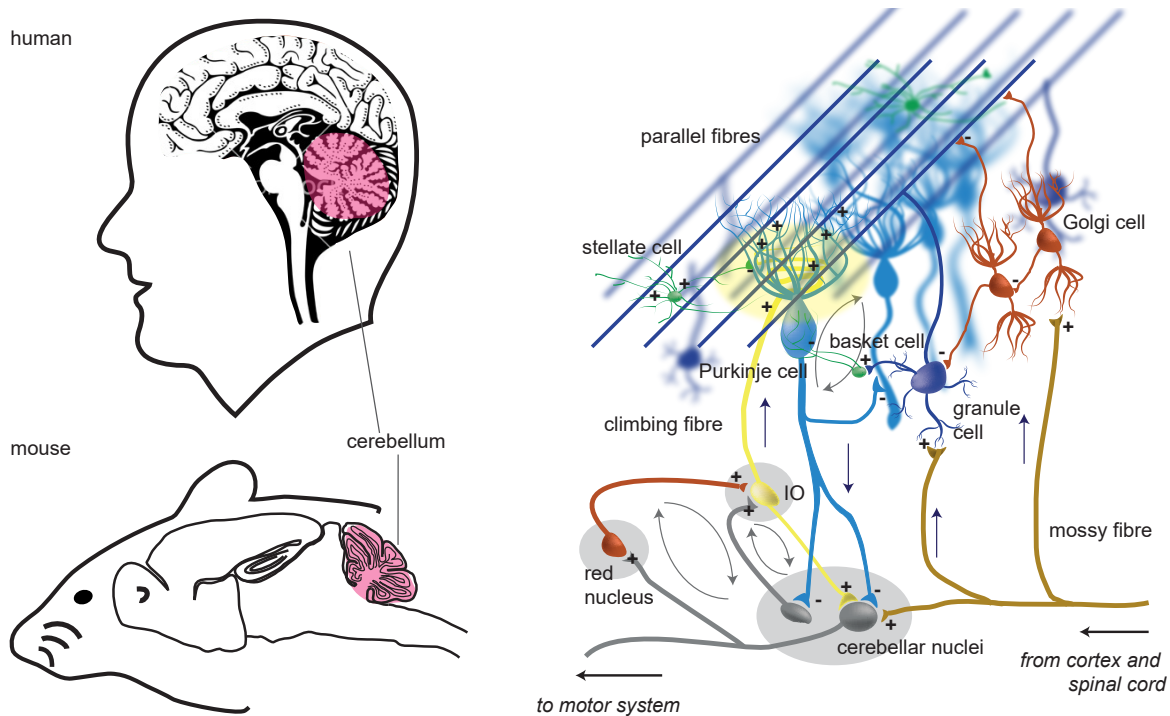


Figure 1.1: Left: human and mouse cerebellum. Right: schematic of the cerebellar circuit. IO: inferior olive.

The cerebellum is organised in ensembles of longitudinally aligned zones as defined by its chemoarchitecture and its inputs (Brochu et al., 1990; Voogd and Ruigrok, 2004; Apps and Hawkes, 2009). In fact, climbing fibre inputs originating from circumscribed areas of the inferior olive target longitudinal zones whose output Purkinje cell axons target specific regions of the cerebellar nuclei. Interestingly, electrophysiological studies in the paravermal cerebellum of cats demonstrated that climbing fibres originating within the same area of the inferior olive project to separate regions (microzones) of the longitudinal zones (Apps and Garwicz, 2000; Ekerot et al., 1991; Trott et al., 1998a; Trott et al., 1998b). The outputs of such microzones, in turn, converge to the same cerebellar nuclei defining a multi-zonal microcomplex (Oscarsson, 1979; Shambes et al., 1978; Bower, 2011; Apps and Garwicz, 2005). There is circumstantial evidence that suggest that mossy fibres carrying different information target the same multi-zonal microcomplex (parenciteHerrero2002. Because this arrangement would allow to integrate information from mossy fibres of different source with information conveyed by climbing fibres of same origin, the divergence of olivocerebellar inputs into different cerebellar microzones is thought to be at the bases of parallel sensorimotor integration.

Cerebellar function and models

The first account of cerebellar function of coordinating movements dates back to the nineteenth century thanks to the work of Jean Pierre Flourens (Flourens, 1844): ablation of the cerebellum from birds did not impair movements but only altered their coordination. Ever since, it has been reported that clinical conditions due to cerebellar damage determine symptoms such as tremor, lack of coordination (ataxia) and muscular tone (hypotonia), and impossibility of adapting movements to new conditions (Kandel, Schwartz, Jessell, et al., 2012; Martin et al., 1996). Based on these symptoms and its peculiar structure, different computational models have been proposed with the aim to explain cerebellar functions of timing, coordination of movements and motor learning.

Exclusive function of timing (Braitenberg V, 1958; Llinás and Yarom, 1986) of movements seems unlikely. In fact, features postulated by these theories lack empirical evidence, in particular the discharge periodicity of inferior olive nucleus in awake preparations and the inconsistency of

time scales between cerebellar neural activity and actual movements representation of kinematic parameters of movements (Keating and Thach, 1995).

Marr and Albus independently proposed the first computational theories of cerebellar function (Marr, 1969; Albus, 1971). They shared similar features and the same Hebbian perspective, establishing the grounds for the instruction learning theory. Briefly, they were based on the assumption that the cerebellum was the control system of motor coordination and the locus of motor learning. The parallel fibre - Purkinje cell (PF-PC) synapses were supposed to be plastic so that they were able to store association of motor command and sensory feedback upon instruction of the climbing fibre (CF) signals.

Masao Ito's work on vestibulo-ocular (VOR) and vestibulo-spinal reflexes further extended the instruction learning theory by encompassing and formalising the self-adjustment and prediction capabilities of the cerebellum (Ito, 1982; Ito, 1984). Long term depression (LTD) of PC-PF cells synapses induced by climbing fibre signals was shown to be essential for VOR adaptation. In behavioural terms, this corresponded to regulating the compensatory eye movements during VOR by adjusting the gain of the motor signal sent by the floccular Purkinje cells to adjust the eye position.

A limitation of the Marr and Albus models lies in their inability to filter signals modulated in frequency and with different lags. To overcome such drawback, Fujita modeled the cerebellum as an adaptive filter proposing that error signals carried by the CF could strengthen or weaken PF-PC synapses whether they were in phase or not with mossy fibre inputs (Fujita, 1982). The capacity of dealing with time-varying signals, such as the motor and sensory signals, self-optimization of dealing with parameters or weights via error signals (CF inputs) and the ability of increasing/decreasing such weights (two-directional plasticity of the the PF-PC synapses) are the main computational features of adaptive filters. In support of this theory a substantial body of evidence suggests that the sensorimotor information processes executed by the cerebellum as well as its microcircuitry are compatible with the adaptive filter model (see Barlow, 2002; Dean et al., 2010). Furthermore, the view of the cerebellum as an adaptive filter is compatible with the neural implementation of internal models, i.e. neural representations of the sensorimotor system.

There exist two types of internal models: - inverse model simulate the kinetic (force, torque,

etc) features of motor action, - forward model predict the sensory consequences of motor action, see (Kawato, 1999) for review. The latter is based on the principle of efference copy (Holst and Mittelstaedt, 1950). The efference copy, or reafference or corollary discharge (Sperry, 1950), is a copy of the outgoing motor signal that is used by the CNS to predict sensory feedback of the motor command in order to distinguish between self-generated sensory feedback and external feedback and check for eventual discrepancies between intended and executed movement. For example, during voluntary movements such as finger tapping, the perception of the same force applied onto the finger skin is inferior during active movements compared to resting state (Shergill et al., 2003). This mechanism allows for accurate control of grip force while holding an object. Similarly, during involuntary movements such as optokinetic responses, the stabilisation of retinal images can be explained in terms of corollary discharge: the world is still perceived stable whereas this would not be the case if the eye ball is moved with a finger.

Arguably, Ito was the first to propose the implementation of internal models in the cerebellum. In his view, voluntary movements would become more refined with practice due to the update of such internal representations so that the cerebellum, in turn, would be able to predict the future states of the motor system and anticipate them in a timely manner (Ito, 1970; Ito, 2012). Forward internal models would improve motor control by compensating for sensory feedback delays and suppressing sensory reafference during movements (Wolpert et al., 1995a; Wolpert, Miall, et al., 1998). Indeed, the implementation of forward internal models in the cerebellum has been proven to be consistent with motor behaviour in human experiments (Merfeld et al., 1999; Izawa et al., 2012) and with neural responses in the cerebellum of non-human primates during vestibular stimulation (Popa et al., 2012; Laurens et al., 2013) and in cerebellum-like structures of the electric fish (Kennedy et al., 2014; Requarth and Sawtell, 2014).

Internal models are also thought to be a strategy used by the CNS for mental processes and language inception (Ito, 2012). It is now commonly accepted that the cerebellum is also involved in cognitive functions. Evidence from tracing and functional MRI imaging studies in human and non-human primates have revealed connections of the lateral parts of the cerebellum with the prefrontal cortex as well as its involvement during cognitive tasks such as motor imagery (Ramnani, 2006; Dum and Strick, 2003; Kelly and Strick, 2003; Jeannerod and Frak,

1999). Additionally, the correlation between the cerebellum and mental disorders has been increasingly exposed by behavioural and anatomical assessment (Fatemi et al., 2012; Carper, 2000; Schmahmann, 1998).

Understanding of the neural implementation and the computational principles of the *modus operandi* of the cerebellum has evolved over the past forty years incessantly. Notably, adaptive filter models and implementation of internal models have been shown to provide the theoretical framework for accurately describing features of motor behaviour, such as VOR and voluntary movements, and the activity of cerebellar neurons. The approach of my thesis was developed in this context: by means of a VR system it would be possible to measure and alter sensory information to find neural signatures of internal models in the cerebellum, such as the efference copy of a motor command.

1.3 Role of the cerebellum in locomotion behaviour

Locomotion is a complex behaviour that requires the precise control of many muscles. The neural circuitry responsible for controlling locomotion (see figure 1.2) involves structures of the brain, the brainstem, the cerebellum and the spinal cord (Goulding, 2009). This decentralised organisation of the circuitry is preserved across species, including mice (Oscarsson, 1965; Berretta et al., 1991; Orlovsky et al., 1999; Sengul et al., 2014). Importantly, motor control of locomotion is executed by the spinal locomotor network and the cerebellum (Orlovsky et al., 1999). The former generates the stepping rhythm via the central pattern generator network whose activity is modulated by proprioceptive sensory feedback (Marder and Bucher, 2001; Grillner, 2006). The latter, instead, mediates supraspinal signals and sensory feedback modulating the activity of the central pattern generator and of the motor cortex.

Electrophysiological investigation combined with behaviour provided direct evidence for the role of the cerebellum in locomotor control and learning. In particular, the medial zone of the cerebellum, the vermis, has been identified as the area involved in controlling posture, tone, flexion and extension of limbs (Chambers and Sprague, 1955). This area, together with the intermediate part of the cerebellar cortex, is the main projection target of the spinocerebellar

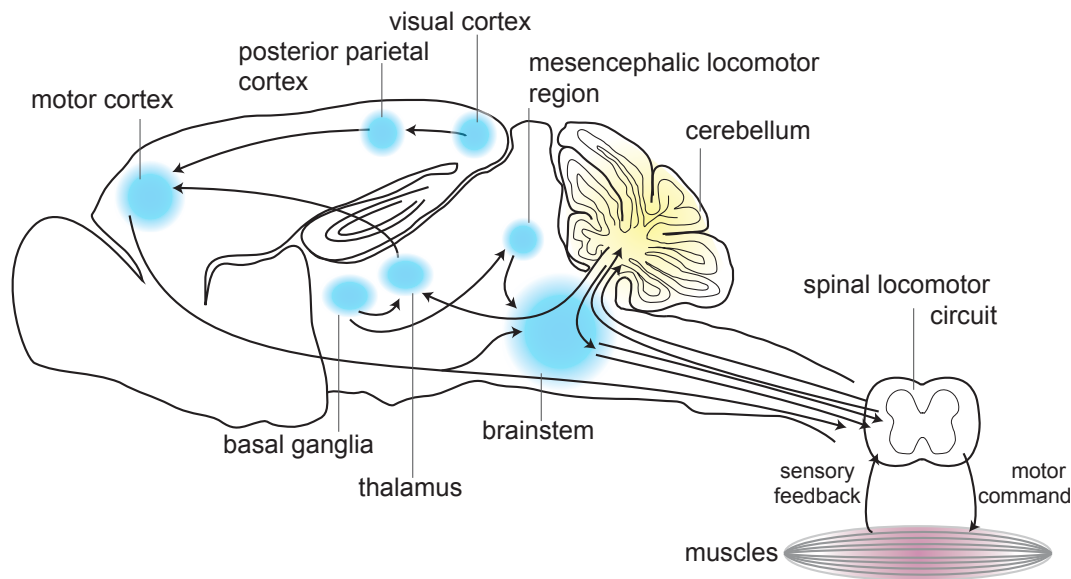


Figure 1.2: Locomotion neural circuitry. Initiation of locomotion is mediated by the mesencephalic locomotor region which receives inputs from the basal ganglia (Grillner and Robertson, 2015; Ryczko and Dubuc, 2013). This also has projections to the thalamus that, in turn, projects to the motor cortex. The cerebellum receives projections from higher cortical areas and the periphery via various brainstem nuclei (Kandel, Schwartz, and Jessell, 2000). Via thalamic relay connections, the cerebellum also projects to higher cortical areas. The motor cortex sends direct efferent connections to the muscular system and receives visual feedback via the posterior parietal cortex from the visual cortex. Adapted from Goulding, 2009 and Kiehn, 2016.

tracts. Sensory feedback and information of the spinal pattern generator are conveyed to the cerebellum via these pathways (Oscarsson, 1965; Sengul et al., 2014; Arshavsky, Gelfand, et al., 1983; Fedirchuk et al., 2013). Thanks to electrophysiological recordings of single unit activity, we know that many cerebellar neuron types carry step-related information.

The majority of these studies focussed on Purkinje cells because they are the target cells of all inputs and the sole output of the cerebellum. Indeed, these cells were found to be essential for interlimb coordination and motor adaptation (Dai Yanagihara, 1994; Ichise, 2000). Moreover, their firing rate modulates rhythmically with the step cycle in decerebrated and intact cats walking on a motorised treadmill at a constant speed (Orlovsky, 1972; Armstrong and Edgley, 1984). Although Purkinje cells of cats did not show substantial modulation of firing rate with walking speed on a treadmill (Armstrong and Edgley, 1988), it was recently observed that the rhythmical firing rate of Purkinje cells within single step cycles was correlated with behavioural parameters such as speed, acceleration and head pitch/roll (Sauerbrei et al., 2015). In a different study, Golgi cells from the intermediate zones C2 and C3 of cats, were shown to discharge rhythmically during

locomotion but no modulation with speed locomotion or muscular tension was observed (Edgley and Lidierth, 1987). On the contrary, the interneurons of the molecular layer in mice running on a spherical treadmill increased their firing rate during locomotion relatively to stationary periods (Ozden et al., 2012). In similar experimental conditions, granule cells were also shown to increase their firing rate with locomotion (Powell et al., 2015). This leaves open the question of whether and how cerebellar neurons are tuned to locomotion kinematics.

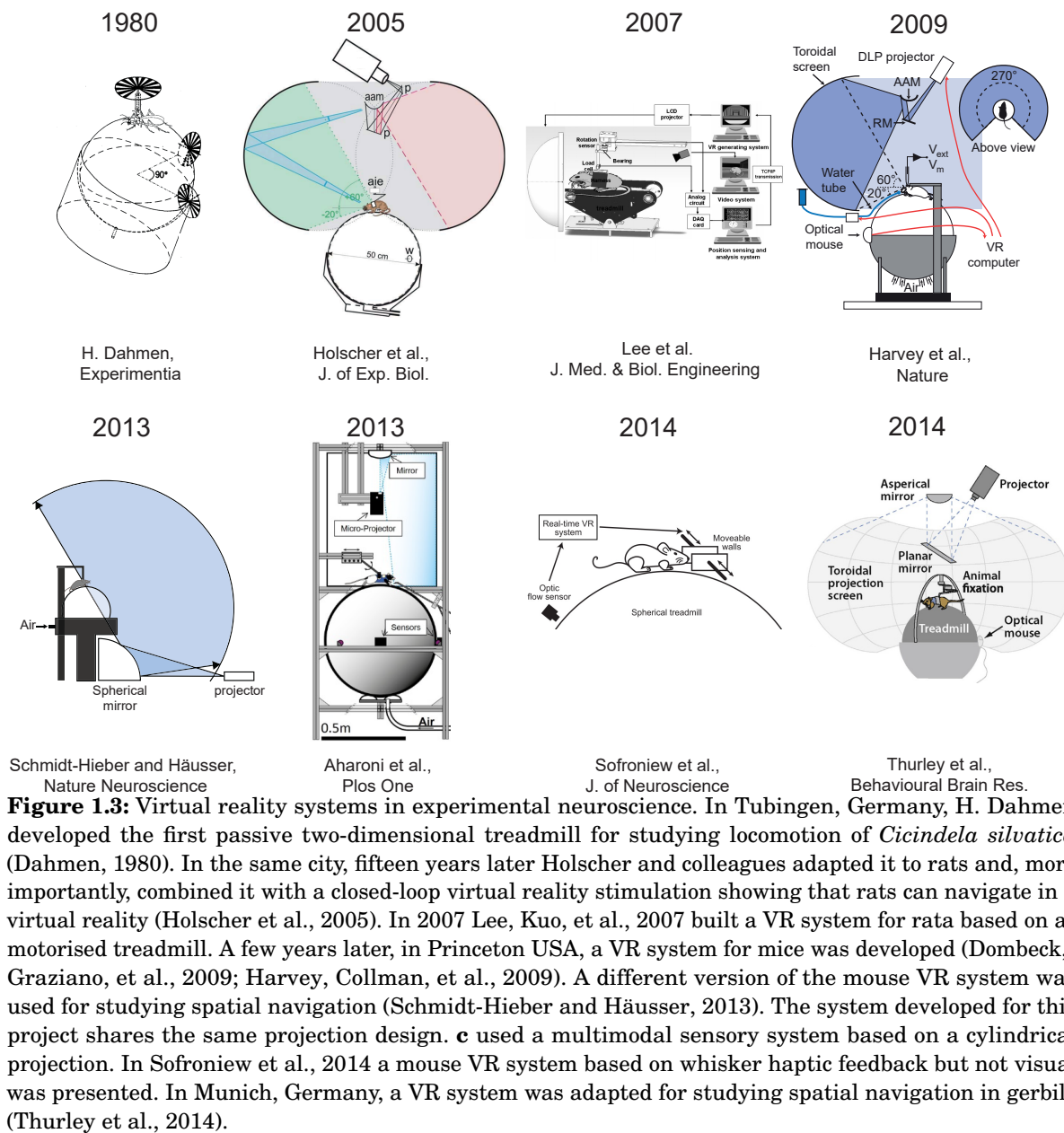
To date, little is known about how behavioural state affects the activity of cerebellar neurons and how locomotion-related information is conveyed by neighbouring neurons (possibly within a single micro-zone) of the cerebellum. Is there any central regulation of the cyclic limb movements during locomotion? Are kinematics parameters of locomotion represented at a population level? By combining a VR task and multi-unit recordings I aim to address these questions.

1.4 Virtual reality in neuroscience

Thanks to recent technological advances in computer graphics, virtual reality systems have been increasingly common in clinical and experimental research, including neuroscience (Bohil et al., 2011; Dombeck and Reiser, 2012; Minderer et al., 2016). The possibility of using mice as animal models and combining them with high-resolution recording techniques such as electrophysiology or fluorescence imaging has emerged to address research questions inherent spatial navigation, working memory, and visual information processing (Dombeck, Graziano, et al., 2009; Harvey, Collman, et al., 2009; Keller et al., 2012; Schmidt-Hieber and Häusser, 2013; Saleem et al., 2013).

Currently, the most common configuration of VR systems is based on the design used by Holscher and colleagues in 2005 in rats (see figure 1.3). A nearly frictionless air-supported spherical treadmill (or ‘treadball’) is the animal interface with the virtual world, which is projected onto a toroidal or demispherical screen that covers approximately the whole visual field of the animal. By walking or running on the ‘treadball’, animals can navigate in the VR environment and purposely move to target zones and avoid virtual walls. The animal is therefore an integral part of the closed-loop VR system as its behavioural responses determine the following state of the sensory stimulation and vice versa. Accordingly, it is crucial to adjust parameters of

the closed loop control to ensure that also the animal itself perceives such causality.



For example, the presentation of the visual stimulus should account for the low visual acuity of the mouse (Prusky et al., 2000); lags of the closed-loop - in particular the update of the VR - stimulus have to be faster than the visual feedback of the mice (Mauk and Buonomano, 2004); the gain of the virtual speed, i.e. the optic flow experienced by the mouse, has to be consistent with its locomotion speed (Saleem et al., 2013; Dombeck and Reiser, 2012). Although previous

VR systems for mice have considered some mouse-specific ergonomics criteria (e.g. inertia of the ‘treadball’), their characterisation and other performance parameters has never been addressed: configuration of the immersive visual stimulus, delay of the closed-loop VR update, acoustic noise, virtual reality layout configuration etc. Moreover, mouse VR systems, although increasingly popular in neuroscience, differ between laboratories and they are complex to build.

A key advantage of the VR systems is the potential of arbitrarily generating incongruent sensory feedback or sensory perturbations. Perturbation paradigms have already been applied to assess certain aspects of cerebellar function such as the role of climbing fibre discharge (Andersson and Armstrong, 1987), the function of inferior olive electrical junctions in motor learning (Van Der Giessen et al., 2008) or the representation of kinetic and kinematic parameters of arm movements in monkeys (Pasalar et al., 2006). However different animal models were used and results might not be necessarily valid across species.

Therefore, as part of the effort to address these issues and apply a VR behaviour paradigm to motor control research, I developed of a VR system with sufficiently low latency to measure motor behaviour, with closed-loop perturbation of sensory information.

1.5 Aim of this thesis

The aim of this thesis is to investigate the neural representation of locomotion kinematics in the cerebellum of mice and to contribute the progress of experimental neuroscience methodology.

To do so, I initially focused on the development of a VR apparatus for mice with a novel perturbation system with two complementary intents. Firstly, it is necessary to refine and characterise its function for studying the central nervous system to guarantee quality and reproducibility of the data. To this end, the characterisation of the performance and complete configuration procedures of the VR system will be described in detail in chapter 3. The second objective is to extend its application to the study of sensorimotor integration, besides spatial navigation and working memory. More specifically, the integration of a perturbation system with a mouse VR apparatus was inspired by the setups used in human and primate psychophysics studies for assessing the computational principles of sensorimotor control (Wolpert and Ghahramani,

2000; Burdet et al., 2001; Pasalar et al., 2006; Selen et al., 2009). The coupling with high resolution recording techniques such as electrophysiology or fluorescence imaging offers the great potential of proving how movements are planned, coordinated and learnt by the central nervous system.

Then, a behavioural training protocol was implemented to train mice to purposely navigate in a VR corridor. This task was also integrated with a mechanical perturbation for deviating mouse path trajectories. Besides establishing a reliable paradigm for perturbing motor behaviour in a VR task, this experimental paradigm could help exploring the neural bases of sensorimotor control adopted by the cerebellum. Mouse behaviour in the VR environment will be presented in chapter 4.

To look at whether the activity of multiple neighbouring neurons was modulated with locomotion kinematics and behavioural state, multi-unit activity recordings from the cerebellar vermis were performed while mice navigated in the VR environment. Majority of single units were found to encode kinematics parameters such as locomotion speed, acceleration and step cycle. Spatial and temporal patterns of the units were investigated to discern how the cerebellum encoded these parameters. Finally, to study the response of neural populations of different sizes, firing rates of the cerebellar units were linearly combined to decode locomotion kinematics.

The findings of the current work indicate that the activity of neurons in the cerebellar vermis of the mouse is strongly modulated by the behavioural state. These response properties might underlie mechanisms necessary for motor coordination or sensory feedback integration that could be tested via transgenic techniques and *ad hoc* behavioural tasks (applicable with the VR system presented here). This could help increase our understanding of how movement control is achieved through the cerebellum and probe computational theories of sensorimotor integration.

ELECTROPHYSIOLOGY AND BEHAVIOURAL TRAINING

Animal model of choice

Being small and highly prolific, the laboratory mouse, *mus musculus*, is one of the most used mammals in scientific research. Alongside the *drosophila*, the mouse model offers the potential of transgenic manipulation allowing for the use of optogenetic techniques to investigate single neuron functions (Rubin and Spradling, 1982; Chinwalla et al., 2002; Boyden et al., 2005). Thanks to this potential, neuroscientific studies have allowed us to better understand the contributions of spinal neurons to locomotor control (Akay et al., 2014; Bellardita and Kiehn, 2015; Kiehn, 2016) and the role of cerebellar neurons in motor and cognitive behaviours (Reeber et al., 2013; Zhou et al., 2014; Galliano and De Zeeuw, 2014; Hoogland et al., 2015).

Subjects

In this study, a total of 56 female C57BL/6 mice were used (Charles River Laboratories). Seventeen mice were used during the first stages of the project during which the first versions of the VR system and behavioural training protocol were tested. Twenty-eight animals underwent the behavioural training. Electrophysiology recordings were performed on 13 of the 28 trained animals. Because of poor quality of some recordings, only data from 10 mice are presented here. A further

11 subjects were used in awake experiments with electrophysiology without previous behavioural training (defined as naïve).

2.1 Surgery

The surgical procedures were performed when the mice were aged approximately 12 weeks. They were housed in groups of maximum five units, with a 12-hours reverse day-night cycle. Animals were kept in a designated room in our laboratory facility, adjacent to where behavioural training and electrophysiology experiments were conducted. All procedures were carried out in accordance with the animal care regulations of the United Kingdom Home Office.

Mice were anaesthetised in an induction chamber with 5% isoflurane, and then it was kept at 1.5-2% throughout the surgery. The mice head was held into place with non-rupture ear bars and a nose clamp. I used the pedal withdrawal reflexes to estimate depth of anaesthesia before injecting 0.5% lidocaine locally and 0.05% carprofen (*Rimadyl*, 1 mg/ml) subcutaneously. During the surgery, body temperature was kept at around 35 degrees and breathing was checked regularly. Eyes were moisturised with *lacri-lube* ointment at the beginning of the surgery. The fur was trimmed from the mouse head before making a sagittal incision up to the neck. The excess skin was removed and, then, the neck musculature was gently pushed caudally to reveal the interparietal and occipital bone. After that, the skull was cleaned with hydrogen peroxide and then polished from the connective tissue. To create a better grip for the attachment of the head metal plate, the skull bone surface was superficially scrapped with a surgical scalpel.

The metal plate, that serves to fix the mouse on the sphere, must guarantee mechanical stability sufficient for electrophysiology recording standards, ease animal handling and be bio-compatible. They were made of stainless steel, a relatively biologically inert alloy, light (weight of metal plate = ~0.8 grammes), and with a design to maximise the contact area with the skull and allow enough clearance for the craniotomy. The metal plates were attached to the mouse over the bregma in order to keep the occipital zone clear. For every mouse, the head plate had to be bent and curved to adapted it the skull shape and maximise contact area. It was then attached to the mouse head with a topical skin adhesive (Histoacryl, B.Braun Medical Ltd).

CHAPTER 2. ELECTROPHYSIOLOGY AND BEHAVIOURAL TRAINING

If a mouse was to be used for electrophysiological recordings, after the head metal plate had been attached, a surgical screw, serving as ground connector (M1 x 2 slot cheese machine screw, Precision Technology Supplies), was fitted into a small craniotomy on one of the parietal bones of the cranium. A mini socket (ED90265-ND, ED8250-ND, Digi-Keys electronics, USA) was attached to the screw head for connecting with the ground wires of the electrophysiology recording system. After that, a plastic well of diameter 3-4 mm was positioned in the central part of the occipital bone on the edge with the parietal bones, aligned with the sagittal suture. This was kept in place with a drop of Hystoacril. Next, all the exposed tissue, together with the base of the head metal plate, was covered with dental cement (Kemdent, Associated Dental Products Ltd), leaving only the head of the ground screw and the well above the vermis clear. This was covered with a layer of 2% agarose and Kwik-Cast sealant (WPI Ltd, UK). A final coating of multiple layers of nail varnish was painted over this area. Upon conclusion of the surgery, the mice were allowed to recover in a recovery chamber with water and food. They were then transferred to their cages to recover at least 24 hours before direct electrophysiological recording or water restriction and consequent behavioural training began.

To access the fifth and sixth lobule of the vermal part of the cerebellum a rectangular craniotomy was performed on the central part of the occipital bone (figure 2.1). This procedure was carried out during the first surgery when no behavioural training was due. Otherwise, the craniotomy was performed in a second surgery following behavioural training. This was performed at least 24 hours after the water restriction was interrupted to prevent any surgical complications. Briefly, initial procedures of the second surgery were the same as the first one. Once the animal was anaesthetised, the coatings of nail varnish were removed with acetone solution, and then the Kwik-Cast and the agarose were gently removed. The part of the occipital bone visible inside the plastic well was cleaned and moisturised before the craniotomy drilling. The dura was also removed only from the area exposed. The craniotomy was covered again with agarose, Kwik-Cast and nail varnish. Animals allowed to recover at least for 24 hours before the electrophysiology recording.

2.2 Electrophysiology

For the electrophysiological recordings I used a multi-electrode array (MEA) probe with 32 channels (Buzsaki32, NeuroNexus Technologies Inc, USA) and acquired the data via the Scout Processor amplifier from Ripple Inc, USA. Electrical potential signals from each channel were sampled at 30 kHz. The electrophysiology acquisition system also recorded a TTL pulse with frequency of 1 Hz (50% duty cycle) which was used to synchronise neural recordings with behavioural measurements. The synchronisation pulse was generated by an external pulse generator (100 kHz sampling frequency, GFG-B05G, Good Will Instruments Co, USA) whose output was also delivered to the data acquisition card controlled by the main Labview program.

The probe was mounted on a motorised micromanipulator (PatchStar model, Scientifica Ltd) by which it could be slowly inserted in the cerebellar cortex. The MEA was inserted with a vertical angle of approximately 45 degrees from the left side of the mouse (see figure 2.1). Depth measurement was zeroed once the tip of the probe touched the external surface of the cerebellum. Recordings were made at various depths. Recordings started only 5 to 10 minutes after the MEA was allowed to settle in the tissue. During the recordings, the craniotomy was kept hydrated with PBS.

2.3 Data analysis

2.3.1 Analysis of electrophysiological data

Spike sorting

Extracellular recordings were spike-sorted with the *KlustaView* software suite (Rossant et al., 2016). Briefly, after the recordings the electrophysiological data were converted to a format compatible with the *KlustaView* software ('.dat' format). The program automatically detected and sorted spikes based on pre-specified parameters, such as threshold values, filtering frequencies, time window for waveform extraction, number of features per channel, etc. These were set manually in a text file which is included in section A.6.

After the "curing" of the clusters, the spike times, their raw and filtered waveforms, shank

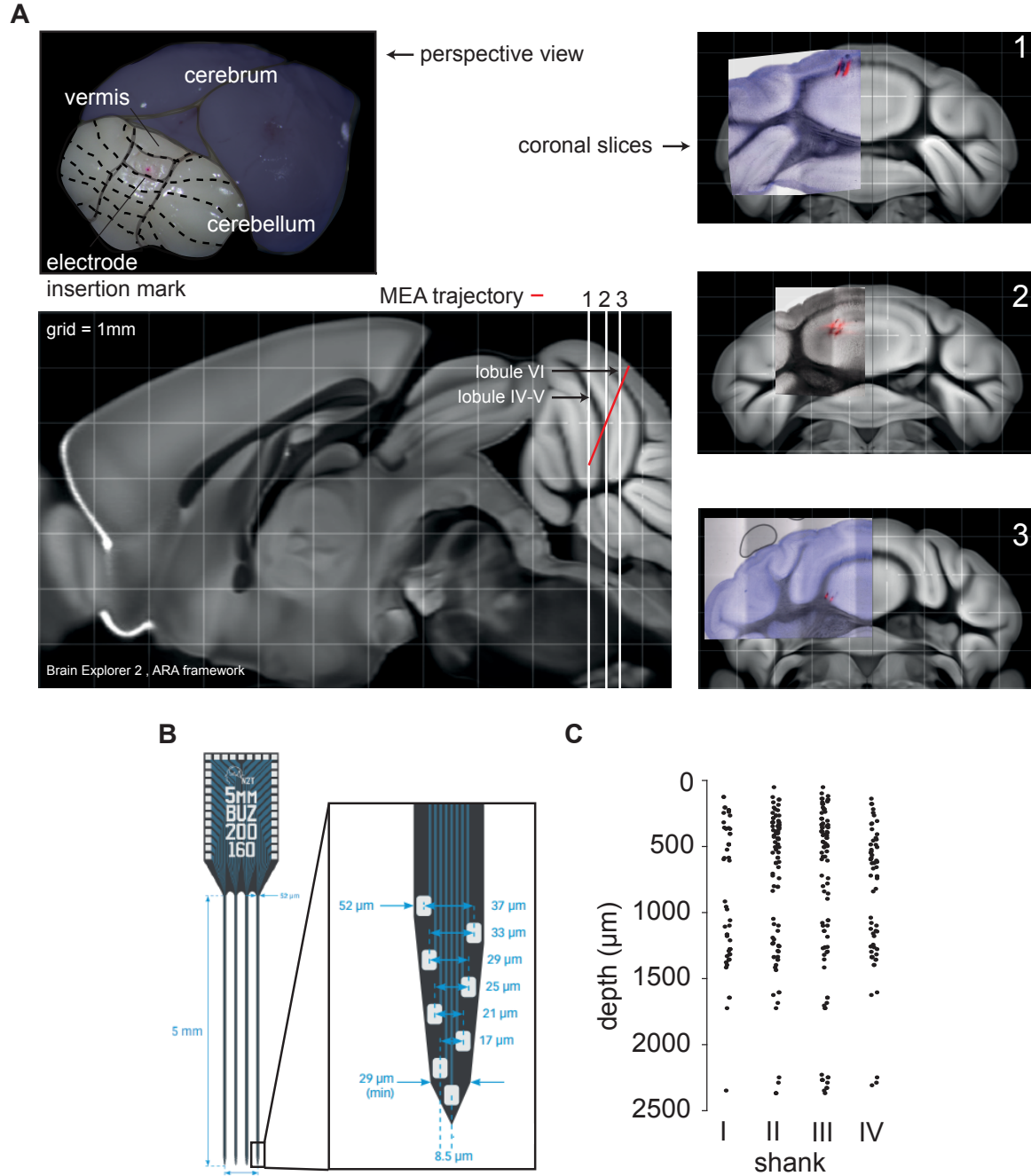


Figure 2.1: Recording location. **A)** Top left: mouse brain with red insertion mark of the electrode stained with DiI. Bottom left: sagittal plane of a mouse brain from Allen atlas (Brain Explorer 2 software) with representative MEA probe trajectory (red) and approximated position of the three coronal slices shown on the right. Right: three coronal slices with superposition of confocal images acquired from a subject used in this work. Red traces show passage of MEA probe. Only two shank lines are visible because of lack of staining of the other two. **B)** Schematic of the MEA probe used, a Buszaki 32 from NeuroNexus Technologies Inc, USA. **C)** Recording depths of the units (n=311) used in this work.

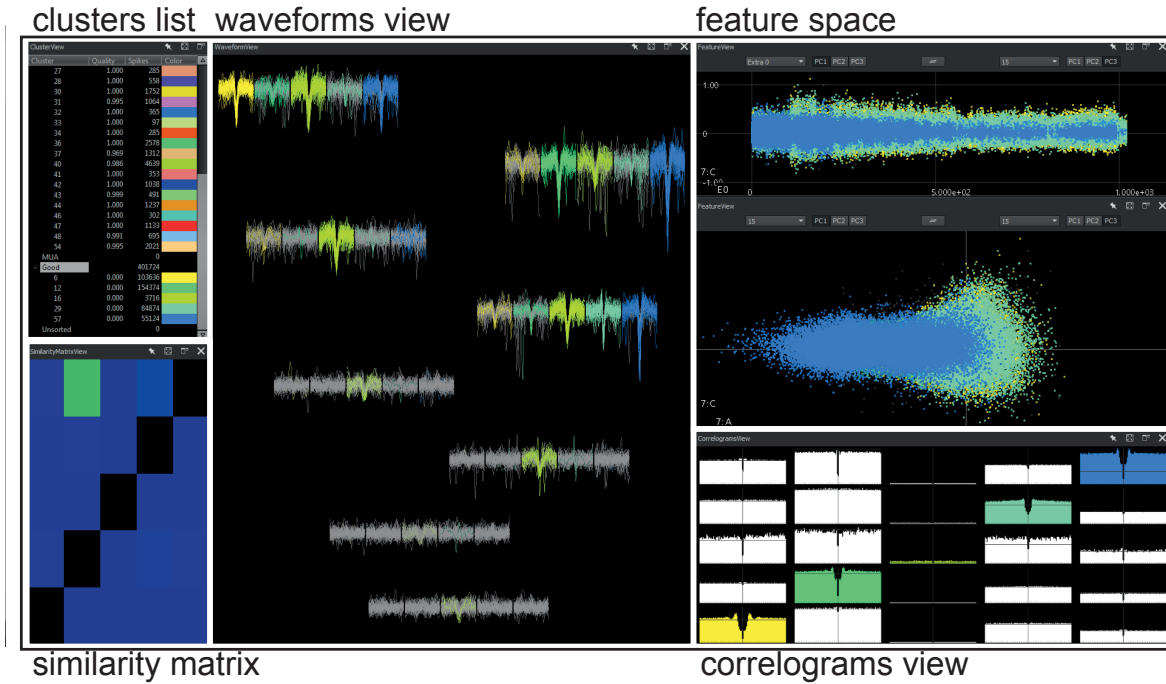


Figure 2.2: Manual curation of the automated clustering. Screenshot of klustaviewa software used for supervision of automatic clustering. Spike counts of automatically generated units are checked in the clusters list. The waveform view allows to determine the channel with the greatest amplitude. One shank at a time is shown. The similarity matrix provides a visual overview of the affinity between units. Clusters are merged and cleaned of noisy spikes by inspecting the feature space. Also spiketimes distribution over the recording duration is checked for eventual signal losses or drifts. The correlogram view shows both auto-correlograms and cross-correlograms, useful to check refractory periods of automatically generated or manually merged clusters.

and channel with strongest signal were saved to a Matlab file. The behavioural data were then synchronised with these information by aligning the sync pulses logged by the electrophysiology equipment and Labview program for behaviour (chapter 3). In this study, I did not separate complex spikes.

Cell classification

After the units were clustered, the cell identity was investigated by applying a combination of classification criteria based on two recently published algorithms (Ruigrok et al., 2011; Van Dijk et al., 2013; Hensbroek et al., 2014). Units were classified according to irregularity measures of spontaneous activity that were found to provide the highest classification accuracy, see table 2.1 for details. Units could be classified if it was possible to extract at least 60 inter-spike intervals (ISIs) from periods of spontaneous activity lasting longer than 2.5 seconds. Spontaneous activity

was regarded as such during stationary periods, i.e. when the speed of the mouse was lower than 1 cm/s. The approach used here was mainly based on the decision-tree algorithm originally proposed by Ruigrok et al., 2011 as it didn't require knowledge of the recording layer (figure 2.3). With the aim of increasing the accuracy of the approach, I included the threshold values of two parameters used by the Gaussian Process Classifier proposed by Van Dijck et al., 2013, namely mean spiking frequency and entropy of the ISI distribution. Irregularity measures were computed as shown in table 2.1.

PARAMETER	SYMBOL	FORMULA	UNITS
inter-spike interval	ISI	$Spiketimes_{j+1} - Spiketimes_j$	ms
mean spiking frequency	MSF	$\frac{Spiketimes}{Duration_{stationaryperiod}}$	Hz
entropy of ISI distribution	H	$-\sum_{j=1}^j P_{ISI} \log_2(P_{ISI})$	bit
natural log of coefficient of variation of ISI	$CVlog$	$\frac{std(\log(ISI))}{mean(\log(ISI))}$	—
median absolute difference from the median ISI	MAD_ISI	$median(ISI_{1,j} - median(ISI))$	ms
mean of 2 times the the absolute difference of successive ISIs divided by the sum of both intervals	$CV2mean$	$2\sum_{j=1}^j \frac{ISI_{j+1} - ISI_j}{ISI_{j+1} + ISI_j} / j$	—
value of the 5th percentile of ISIs	$ISI5\%$	$prctile(ISI, 5)$	ms
median of ISIs	$MedISI$	$median(ISI)$	ms

Table 2.1: Parameters for cell classification. Irregularity measures adopted to classify the cerebellar class of units. Last two formulae are Matlab functions used to compute the values.

Computation of tuning curves

Firing rate of each unit was calculated at the same frequency of the behavioural data (5-ms bins) and then smoothed with a 150-ms-wide Gaussian filter, standard deviation = 50 ms. The vectors of firing rate and speed were sorted in ascending order of speed. Data point of the tuning curves were comprised by 2000 bins for speed values greater than 1 cm/s. The data points for speed of 0 cm/s were formed by all bins of the periods when the mouse is still. The inverse of the variance of

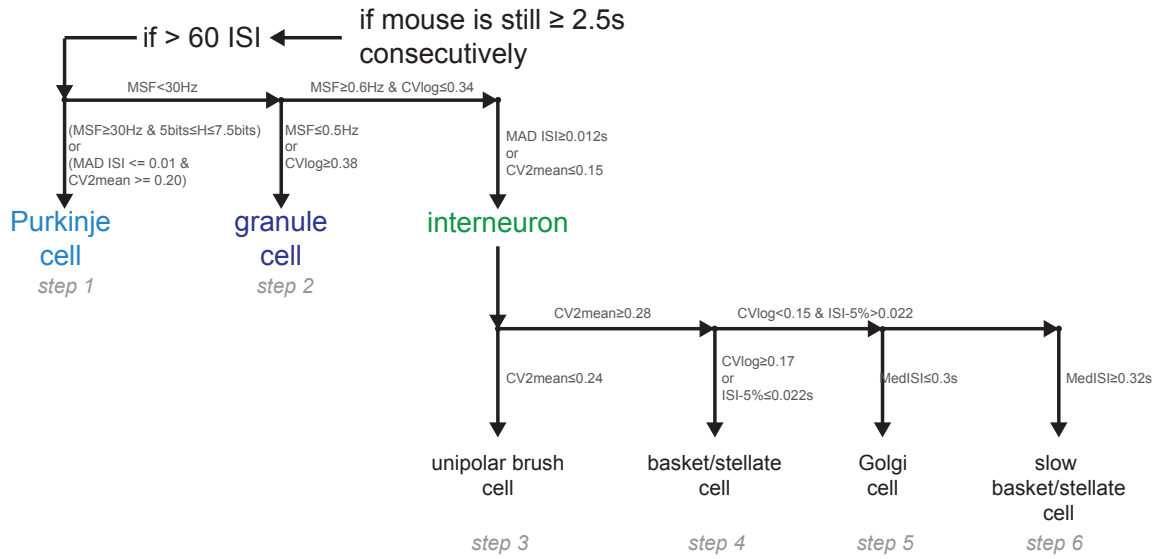


Figure 2.3: Decision tree algorithm for cell classification. Flow chart of classification steps for cerebellar units. Purkinje cells and granule cells are the first types to be recognised. The remaining units are ascribed interneuron classes. Threshold values for classification criteria are indicated for every step. Please refer to table 2.1 for acronyms and units.

each data point was used as weight for the fitting to compensate for the different bin number of data points at speed=0.

To evaluate the significance of the firing rate modulation with speed of locomotion, spike trains were randomly shifted one hundred times by at least 20 seconds and up to the duration of the recording minus 20 seconds (Lee, Hoy, et al., 2014; Kropff et al., 2015). For each iteration, firing rate was calculated and a speed tuning curve computed, and its variance measured. I then compared the variance of the original speed tuning curve with the ones from the shuffled data. If its value was greater than 99% of the variances of curves of shuffled data, then the unit was considered responsive to movement. To assess if the unit was also significantly modulated with locomotion speed, I repeated such calculation and applied the same criteria to only speeds ≥ 1 cm/s (Saleem et al., 2013).

A unit response type was defined according to the curve that best fitted the original data points. Because of the different response profiles observed with the original data, three different curves were fitted. Linear, second-degree polynomial, and double exponential fitting curves were fitted onto the original data using the built-in function of Matlab. The model that better explained the variance of the original data was kept for further analysis (Freeman et al., 2002; Saleem

et al., 2013).

The coefficients of the best fitting curve were used to determine the response type. A cell was classified as

- positively modulated if the maximum firing rate was greater than the firing rate during stationary periods and this was recorded at speeds greater than 70% of the maximal speed of the mouse;
- negatively modulated if the maximum firing rate was measured during stationary periods and the minimum firing rate was smaller than the firing rate during stationary periods and it was recorded at speeds greater than 70% of the maximal speed of the mouse;
- having a preferred speed if the maximum firing was greater than the firing rate during stationary periods and this was recorded at speeds smaller than 70% of maximal speed of the mouse.

Step cycle computation

To look at the modulation of firing rate with stepping cycle, the pitch velocity signal was high-pass filtered at 3 Hz to cancel the slow locomotion-related changes of speed. Hilbert transform was then computed and its phase was extracted as a function of time with Matlab built-in function. To ensure that pitch velocity changes were due to stepping, only putative stepping cycles longer than 50 ms and occurring only during moving periods (speed ≥ 1 cm/s) were considered. Each cycle duration was normalised with respect to time and divided in 36 equal intervals. For each interval, the instantaneous firing rate was computed as the number of spikes divided by the interval duration.

Modulation of firing rate with the phase of the stepping cycle was checked for uniformity with χ^2 test for randomness (Fisher, 1995).

Mean direction angle θ of firing rate distribution of a cell around the step cycle was computed as:

$$\theta = \arctan\left(\frac{\sum_{i=1}^n \frac{\sin \alpha_i}{n}}{\sum_{i=1}^n \frac{\cos \alpha_i}{n}}\right)$$

where numerator and denominator are the mean rectangular coordinates of the mean angle, X and Y respectively; α is the angle/phase of the resultant vector $R = \sqrt{X^2 + Y^2}$ for each cycle and n is the number of cycles or steps.

The circular standard deviation σ of a unit is a measure of the spread of the firing rate around the mean direction angle and indicates where approximately 66% of the data lie (Drew and Doucet, 1991):

$$\sigma = \sqrt{-2 \log R}$$

Phase selectivity index PSI is equivalent to the orientation selectivity index as described by Mazurek et al., 2014:

$$PSI = 1 - \frac{\sum_{i=1}^n R(\theta_n)^{2i\theta_n}}{\sum_{i=1}^n R(\theta_n)}$$

where $R(\theta)$ is the magnitude of the firing rate for any given angle $\theta = [0^\circ : 10^\circ : 360^\circ]$ for each stepping cycle.

2.3.2 Mutual information

Mutual information of firing rate and kinematics variables was computed using the continuous estimator based on the Kraskov, Stögbauer, and Grassberger (GSK) technique (Kraskov et al., 2004). I used the matlab implementation of the GSK algorithm in the JIDT toolkit (Lizier, 2014). Firing rates and kinematics variables were computed as described above and input to the GSK algorithm that returned a mutual information value for each unit.

For the step cycle calculation, the mutual information was computed between the firing rate and the phase angles of the Hilbert transform of the pitch velocity. Only periods during movement were considered and mutual information was estimated for each cycle and then averaged. Firing rate was computed every 5 ms and smoothed with a 60ms-wide Gaussian filter.

Mutual information at different time lags was computed by shifting the spike timestamps from -500 ms to +500 ms with steps of 10 ms with respect to the kinematics variables. The firing rates was re-computed and mutual information calculated for each iteration.

2.3.3 Linear decoder

The spike trains were binned in 5-ms intervals and then convoluted with a Gaussian function ($\sigma = 50$ ms, width = 150 ms) to obtain estimates of the firing rate of each neuron. The speed signal was convoluted with the same Gaussian function - I considered all speeds ≤ 1 cm/s to be stationary and thus set to 0 cm/s. Both the firing rate and the speed profile were then normalised. The decoder was trained on 70% of the data and then tested on the remaining 30% of each recording. The reconstruction was given by the following relationship

$$(2.1) \quad \hat{S} = \hat{v}f$$

where $\hat{S}$ is the reconstructed test signal, $\hat{v}$ is a matrix whose columns are the test firing rates and f is the estimator, which was given by

$$(2.2) \quad S = vf.$$

Training the decoder is equivalent to solving the equation to find the optimal value of the estimator:

$$(2.3) \quad f = (v^T v)^{-1} v^T S$$

where S is a column vector with the training speed values and v is a matrix whose columns are the training firing rates of the cells used plus a column of ones for the y intercept.

The result of this estimation was then half wave rectified as negative decoded velocities are not possible.

3

THE MUSODROME

The use of virtual reality systems in neuroscience offers the unique advantage of creating a convenient and naturalistic reproduction of the world and generating a multisensory stimulation with a high degree of experimental control and ethological validity (for review see Bohil et al., 2011; Dombeck and Reiser, 2012; Minderer et al., 2016).

The VR system presented here was developed by adapting previous designs (Dahmen, 1980; Holscher et al., 2005; Dombeck, Harvey, et al., 2010; Schmidt-Hieber and Häusser, 2013) to create a low latency, open-source experimental apparatus with integrated motor and visual perturbation features (figure 3.1). The apparatus, named Musodrome (from latin *mus musculus* for mouse and Greek *dromos* for running course), is a closed-loop virtual reality system for mice (see figure 3.1). The system was built with the purpose of creating an experimental paradigm within a highly controllable environment in terms of stimulus presentation and experimental procedures. In this chapter, I will describe the construction, characterisation, and the novel features of the MusoDrome.

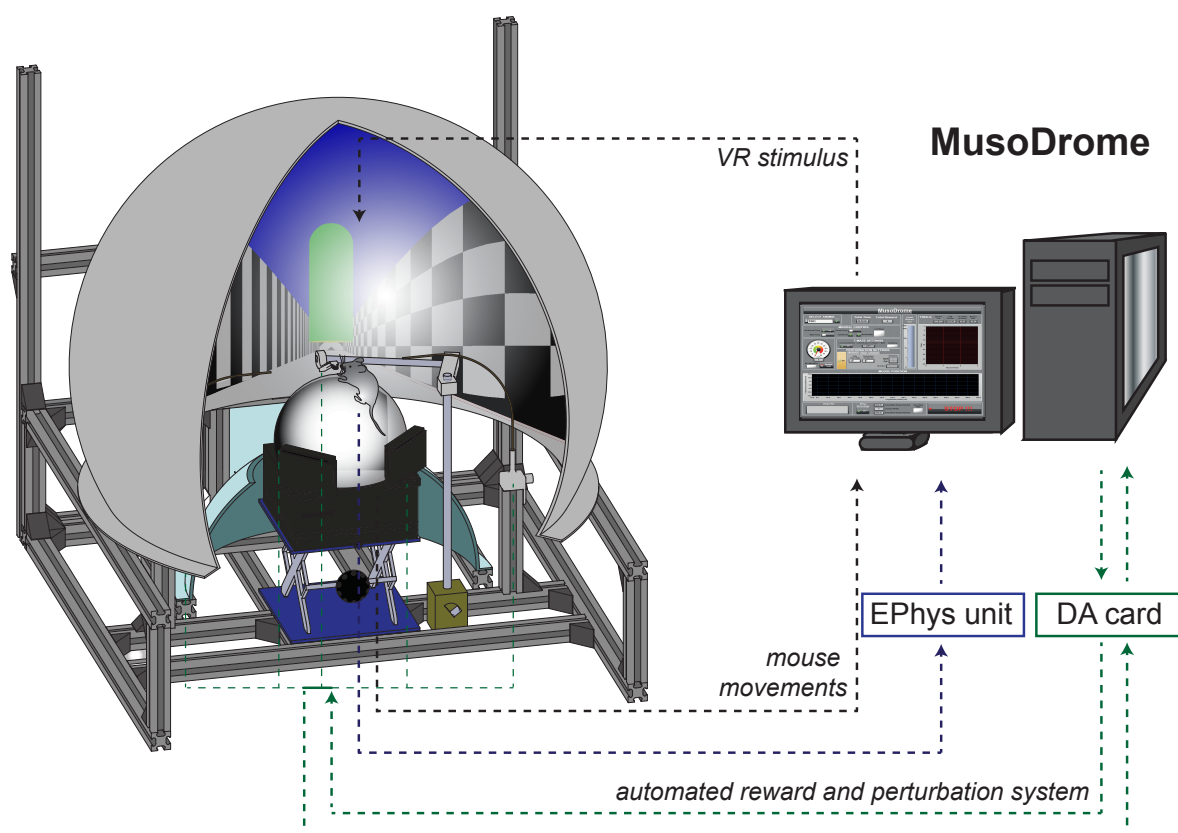


Figure 3.1: Schematic of the MusoDrome. Closed-loop control of the MusoDrome components is relayed by multiple connections to a single computer. Virtual reality stimulus is updated based on mouse movements that are transmitted via USB connection. A data acquisition (DA) card allows to control the components of the reward and perturbation system. Electrophysiological data are collected within the same machine via a electrophysiology amplifier unit. The post for head-fixing the mouse is not represented for illustration purposes.

3.1 Experimental Design

3.1.1 Main Structure

The *skeleton* of the MusoDrome is built with bars of extruded aluminium (catalogue numbers included in appendix, RS Components ltc, UK). This structure serves as a geometry reference frame for the correct placement of all the components of the projection system: the demispherical dome, the projector, the two mirrors, and the spherical treadmill (see figure 3.2). This is necessary to facilitate the subsequent visual stimulus configuration and ensure the correct positioning of the subjects during the experiments.

The projection system comprises two mirrors, one flat and one convex, whose configuration

allows to keep spacing requirements limited and costs to a minimum compared to other VR systems that use custom made mirrors (Holscher et al., 2005; Dombek, Graziano, et al., 2009; Harvey, Collman, et al., 2009; Cushman et al., 2013; Thurley et al., 2014). The flat mirror is positioned directly in front of the upright projector to deviate the light beam horizontally, circumventing the need to position the projector behind the dome (see figure 3.2 B). Analogously to another VR system (Schmidt-Hieber and Häusser, 2013), I used a low cost half-dome acrylic mirror (Signalnet Systems Ltd., UK) with a diameter of 45 cm, positioned in front of the ‘treadball’ (see figure 3.2 C). This reflects and amplifies the image onto the internal surface of the dome. It is fixed on two rails so that its angle amplification gain can be adjusted without altering the lateral alignment.

The spherical treadmill is positioned on an adjustable platform (or lab-jack, from VR&L Enterprises Ltd, UK), behind the convex mirror so that it can be regulated to accommodate the mouse more comfortably (see figure 3.2 D). In fact, subjects were fixed by their head to a rigid post so having the surface of the sphere adjustable is a necessary specification. In addition, the spherical treadmill is not fixed on the lab-jack in order to allow for horizontal re-positioning.

The demispherical dome is fabricated from an overblown acrylic truncated sphere (Talbot Design Ltd, UK) with a radius of 40 cm. The dome has a horizontal span of 270° and a vertical height of 120° , with the bottom edge at -30° from the midline (see figure 3.2 D). The visual stimulus, however, covers only 240° leaving a clearance area of 120° at the back for equipment placement and leaving access to the system. The spherical dome was positioned by aligning its vertical axis of symmetry with the centre of the aluminium base (see figure 3.2D).

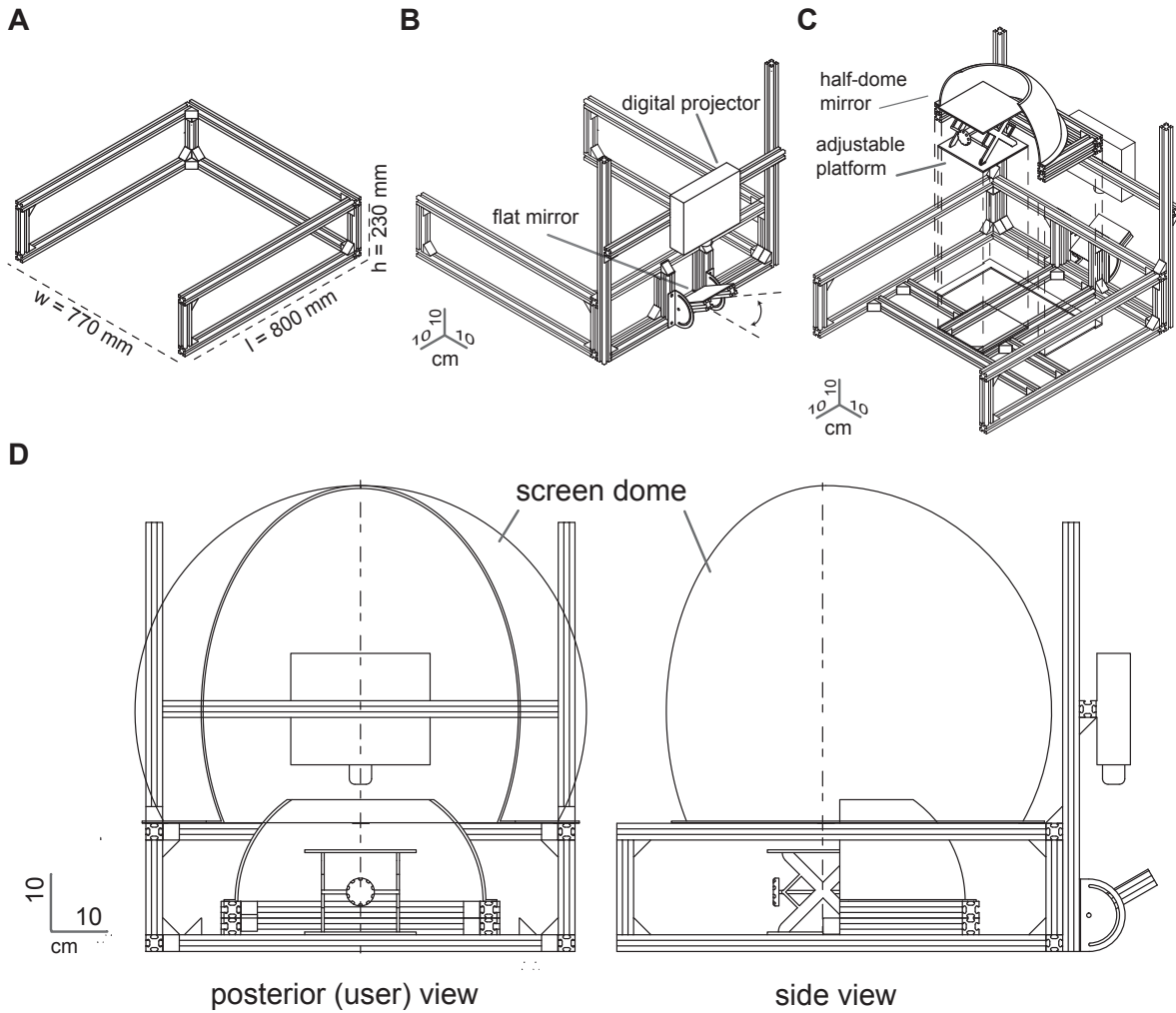


Figure 3.2: Geometry reference frame of the projection system. **A)** Base of the projection system. **B)** Lintel structure supporting the digital projector is shown in thicker lines. At the bottom, the flat mirror of the projection system is mounted on swivel joints that allow adjusting its inclination angle. **C)** Half-dome mirror is placed inside the base structure on additional aluminum bars for alignment and adjustment during the configuration of the visual stimulus. A platform, adjustable in height, is aligned with the projector-mirrors axis. The spherical treadmill is to be placed on this platform. **D)** Orthogonal projection of the posterior and lateral view of the projection system. The screen dome (rendered transparent) is aligned with the projection system and its vertical axis of symmetry is aligned with the axis of the chassis. The relative position of all the elements allows the full illumination of the internal surface of the dome.

3.1.2 Visual Stimulus Configuration

The configuration of the visual stimulus is a procedure that can be executed with different approaches depending on the chosen graphics rendering and control software. For this project, it was preferred not to use commercial game engines such as CAVE, Blender or Quake in favour of C++ with OpenGL API (application programming interface) to have full control of the virtual

physics, visual stimulus configuration, and rendering.

The computer-generated images of the visual stimulus must be adjusted to compensate for the curvature of the surface of the dome before being projected. The physical properties of the dome must be taken into account too. Normally, images are projected onto a flat surface where coordinates are linearly spaced. With spherical surfaces this is not the case so a mapping needs to be created between standard computer display coordinates and the coordinates of the screen. To this aim, I adapted a method used in planetarium projections (Bourke, 2005; Bourke, 2008) to create a conformal projection of the VR stimulus for any spherical surface. A map can be created as a grid of nodes (intersections between meridians and parallels) by tracking the polar coordinates of the screen dome into pixels units (see figure 3.3A). This ensures that the projection the images show minimal distortion and are displayed in the correct portion of the screen surface.

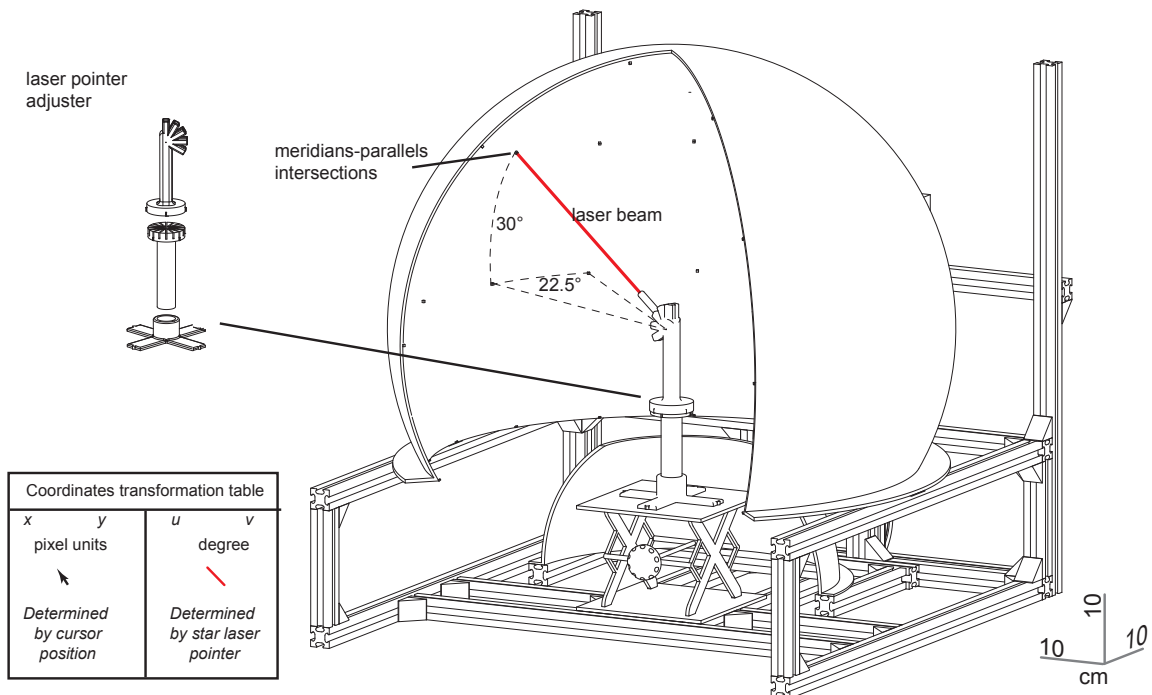
In the appendix part A.2, I show the basic steps of how to create a mapping with two LabVIEW programmes I created *ad hoc*. Briefly, the (physical) polar coordinates are saved in pixel units by simply clicking on the desired position of the screen. Once all the nodes are tracked, the map is visually checked with various gratings and corrected if necessary. At the end of the testing phase, the mesh information are saved into a header file readable by the C++ code for rendering the VR environment.

With the different gratings, it is possible to check the conformality of the projection and the actual extension of the projection (see figure 3.3B). The latter is adjusted to cover a proportion as great as possible of the mouse visual field (Drager, 1978). In figure 3.3C, the diagram shows the coverage of the stimulus around the subject which sits in the geometrical centre of the demispherical dome. Crucially, this configuration consents to illuminate only the demispherical screen keeping the mouse in the dark.

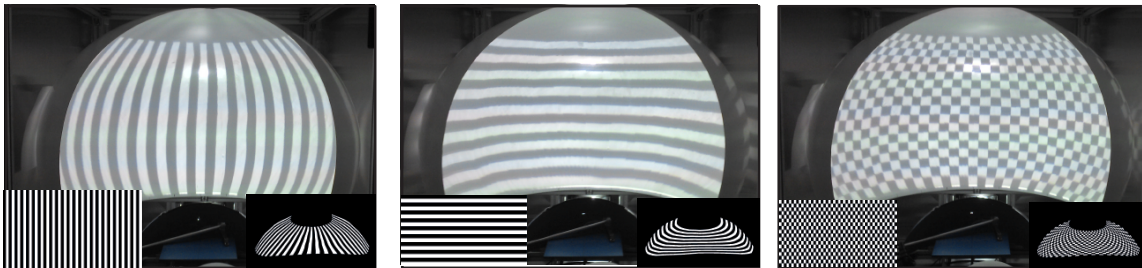
Not only does the visual stimulation have to cover the mouse visual field but the information therein also have to be delivered clearly enough for the mouse to interpret the visual images of the VR stimulus as a three-dimensional environment. For this reason, the VR landmarks, such as walls, are constructed with textures and patterns characterised by spatial frequencies within the limits of the low visual acuity of a mouse (Gianfranceschi et al., 1999; Prusky et al., 2000).

Mouse visual perception of the VR visual stimulus was simulated by low-pass filtering

A



B



C

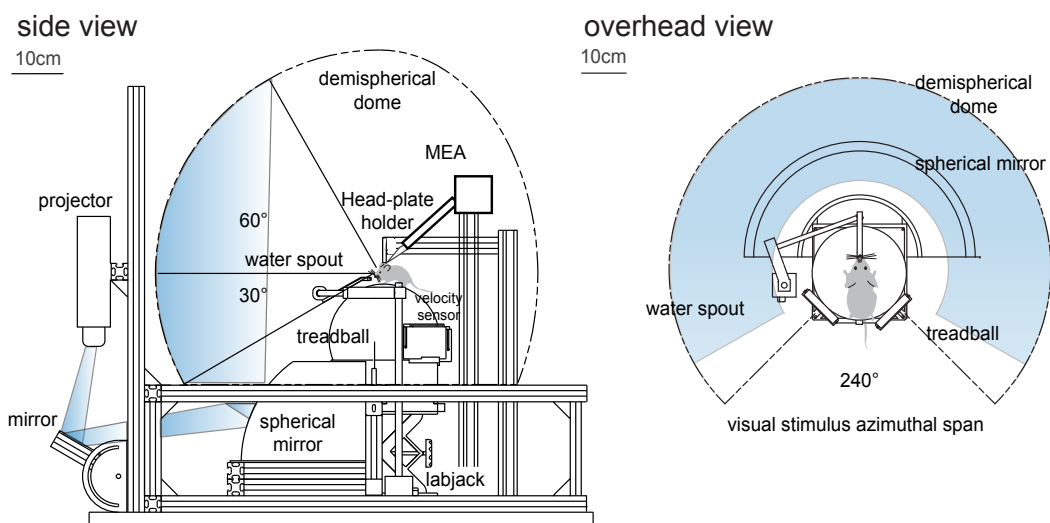


Figure 3.3: Visual stimulus configuration. **A)** Mapping of a single point node from spherical to monitor coordinates. A viable strategy to track nodes on the screen is to use a laser pointer mounted on a 3D-printed arm whose vertical and horizontal angle can be manually regulated (top left) to point to parallel-meridian intersections onto the spherical screen. The coordinates of the pixel illuminated by the laser pointer is tracked with a mouse click so a transformation table for each node can be generated (bottom left). **B)** Vertical, horizontal and check-board gratings projected onto the dome during testing. Bottom left corners show the original image and bottom right the images distorted before being projected. **C)** Lateral and above view of the projection system showing the coverage of the visual field (240° -by- 90°). Mouse drawing not to scale.

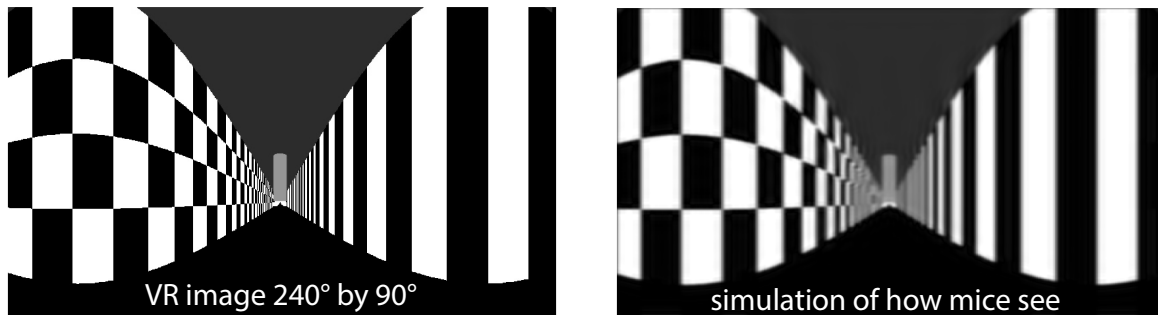


Figure 3.4: Simulation of mouse vision. A visual stimulus frame showing a VR image (240° -by- 90°) wide is low-pass filtered in the frequency domain to show only spatial frequencies within the limits of the mouse visual acuity.

a VR frame in the spatial frequency domain. The filter was based on the values of visual acuity as measured in mouse behavioural experiments in a two-alternative-forced-choice task (Gianfranceschi et al., 1999). I opted for this strategy because the results from behaviour and electrophysiology are proven to be consistent and it is a conservative approach. All frequencies above 0.7 cycles/deg are filtered out from the image (see figure 3.4). In this way, the fitness for purpose of the textures of the VR landmarks could be visually tested.

3.1.3 Spherical Treadmill

The spherical treadmill is one of the most critical parts of the system because it is where the mouse rests, walks, and is the means of control and interaction with the virtual reality system. For this reason, mouse ergonomics and comfort are paramount in its design and construction.

The spherical treadmill is composed of three main parts: the polystyrene (or styrofoam) sphere, the support cup in which the sphere can rest, and the velocity sensors. The dimensions are mainly imposed by physical requirements (dimensions-to-weight ratio) and a trade-off between costs and practicality. Polystyrene spheres are very economical and they can be easily lightened with standard techniques such as hot-wire cutting to match the inertial requirements of the mouse running dynamics. Following a preliminary testing phase of the design, the support cup was designed with a 3D rendering software (Inventor Professional 2013, Autodesk Inc. USA) and then 3D-printed (Object30 Pro, Stratasys Ltd, USA). This approach provides a frictionless and smooth rotation of the sphere, helped minimising acoustic noise and ensures accurate tracking of mouse movements. Lastly, two computer mice are used as velocity sensors and mounted on the support cup to track rotational movements of the sphere (pitch, roll and yaw).

Polystyrene sphere and lightening process

Spheres with a diameter of 20 centimetres are produced in two halves with expanded polystyrene with a specific weight of approximately 33 kg/m^3 (Poliemme SNC, Italy). This means that each sphere weighs more than 80 grammes. However, the ideal weight is 30-35 g so that their mass would be one and a half times the mass of a laboratory mouse 12-weeks old (Dahmen, 1980; Dombeck, Khabbaz, et al., 2007; Muzzu et al., 2013). This value is consistent with the weight of the animals used in this study. The new mass provides the sphere with a momentum of inertia comparable with the inertia that the mouse would experience whilst running in natural conditions. To comply with these constraints, I designed and built a hot-wire cutting device for reducing the sphere weight with precision and reproducibility, and at the same time without compromising the structure and balance of the sphere. A curved tungsten wire mounted on a wooden tablet allows to carve out undesired material from the inside of each half sphere up to the desired weight (starting weight 42.3 ± 0.6 g, reduced weight 16.4 ± 0.8 g, $\text{mean} \pm \text{s.e.m.}$, $n=20$

semi-spheres). The procedures of how to mount the hot-wire cutting device and of the lightening process are described in details in section A.3 of the appendix. Following the lightening process, the two halves are attached together, sanded to smooth irregularities, to improve the grip for the mouse, and to increase the tracking accuracy of the velocity sensors.

Spherical treadmill base

The support cup has a slightly larger diameter than the sphere to allow the creation of a cushion of air in between. The air is delivered by eight air jets evenly spaced at an angle of 45 degrees. This value is calculated to be the most efficient when considering lateral and vertical displacement of the sphere as equal forces would be applied horizontally and vertically. To make sure that the air pressures in the eight holes are the same, the base has two internal chambers to distribute the air flow evenly (see figure A.6). The air coming from a single tube goes through the bottom chamber where the flow velocity is reduced and, as a consequence, so is the acoustic noise. To further reduce the noise, the chamber is filled with packaging foam. This solution has proven to be effective at minimising the acoustic noise. In our laboratory, I recorded a background noise level¹ of about 42 dB that increased to approximately 49dB when the ‘treadball’ was operational (this values is in between the noise level of a whisper and a normal speech). The air then goes in the top chamber passing through a hole in the centre. This solution compensates for the asymmetric positioning of the single inlet to the ‘treadball’ base. The pressure necessary to keep the sphere floating is about 0.3 bar as measured by an air pressure gauge connected to the spherical treadmill with an one-metre long polyurethane tubing with a bore diameter of 5.5 mm. The vertical angular height of the support cup is 62 degrees to leave space for the velocity sensors.

Velocity sensors

The rotation of the sphere is detected by two USB computer mice (Razer Imperator, Razer Inc, USA) fixed on the treaball. The printed circuit boards (PCBs) are removed from their original cases and inserted in custom-made enclosures mounted onto the spherical treadmill base (see figure A.7). The PCBs are mounted so that their sensors (see figure 3.6) sit in front of the midline,

¹This was measured during normal working hours with a noise level meter CEL-62X, Casella Cel, UK. The computer and the projector for visual stimulus were switched on.

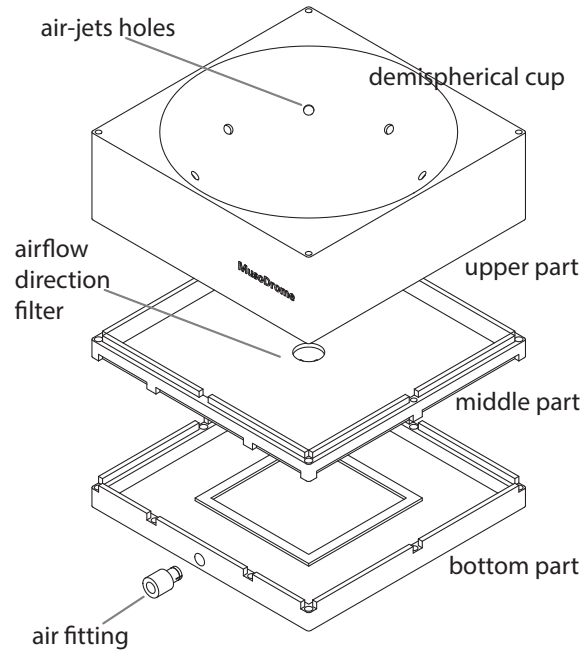


Figure 3.5: Exploded view of the spherical treadmill base. The side of the base is 19.8cm. Detailed dimensions are indicated in figure A.6.

or equator of the polystyrene sphere and ninety degrees apart. Every rotation around pitch, yaw and roll can be detected. The sensors are distant less than 2 mm from the sphere surface, so within their working range.

The two computer mice are connected via two USB ports to a computer (OS: Microsoft Windows 7). To detach the computer mice from the screen cursor and access their raw signals containing the instantaneous velocity values, NI-VISA drivers (National Instruments Corporation, USA) are substituted to the original Microsoft HID drivers. The computer mouse signals are transferred to the host computer as buffered data packets containing two perpendicular velocity values (see figure 3.6D).

To increase accuracy, the read-out rate (polling rate) of the buffer is set to 200 Hz. The velocity signals are sampled at 8 bit, i.e. -256 to 255. These units are called ‘mickeys’ and they correspond to the distance detected between two consecutive time frames. This value also depends upon the resolution of the laser sensor that is set to 1000 dot per inch (DPI) (because high-spec video-gaming mice were used, this parameter is set by adjusting the manufacturer firmware). Since the polling rate is also known, mickeys can be converted to units of velocity of the international

system SI, such as cm/s by simply multiplying these values by 2.5.

$$(3.1) \quad Velocity = Mickey \cdot 2.5 = \frac{PollingRate}{DPI} \cdot 2.5 \quad \left[\frac{cm}{s} \right].$$

In order to validate the viability of this technology for this project I calibrated (Muzzu et al., 2013) and measured the latency of the computer mice. While the spherical treadmill design is not novel, latency measures have never been performed. The latency of the computer mice is defined as the delay between the onset of the sphere movement and its information data availability to the host computer. This was measured to be within limits of wired USB connection standard (Ramadoss and Hung, 2008), being approximately 16 ms. These measurements were made in collaboration with Dr Kit Longden. They were performed on the mouse VR system described here and on a smaller version of the ‘treadball’ for fly studies (Longden et al., 2014) (see figure 3.9C).

Each sensor measures velocities along its longitudinal and transverse axes, see figure 3.6D. These are then translated into tangential velocities of the sphere along its pitch, yaw and roll direction as follows:

$$(3.2) \quad V_{pitch} = -\frac{X1 + X2}{2} \cdot \cos(45^\circ)^{-1} \quad \left[\frac{cm}{s} \right],$$

$$(3.3) \quad V_{yaw} = \frac{Y1 + Y2}{2} \quad \left[\frac{cm}{s} \right],$$

$$(3.4) \quad V_{roll} = \frac{X1 \cdot \cos(45^\circ)^{-1} + (-X2) \cdot \cos(45^\circ)^{-1}}{2} \quad \left[\frac{cm}{s} \right].$$

Velocities V_{pitch} , V_{yaw} and V_{roll} are averaged between the two computer mice. These velocity vectors are normalised by the cosine of 45° to compute the pitch and roll rotation velocities along the main reference axes.

The transformations above are implemented in a LabVIEW code that allows tracking of the sphere rotations and, hence, of the mouse movements. By integrating the velocity over the time interval between two readouts, the distance travelled can be computed.

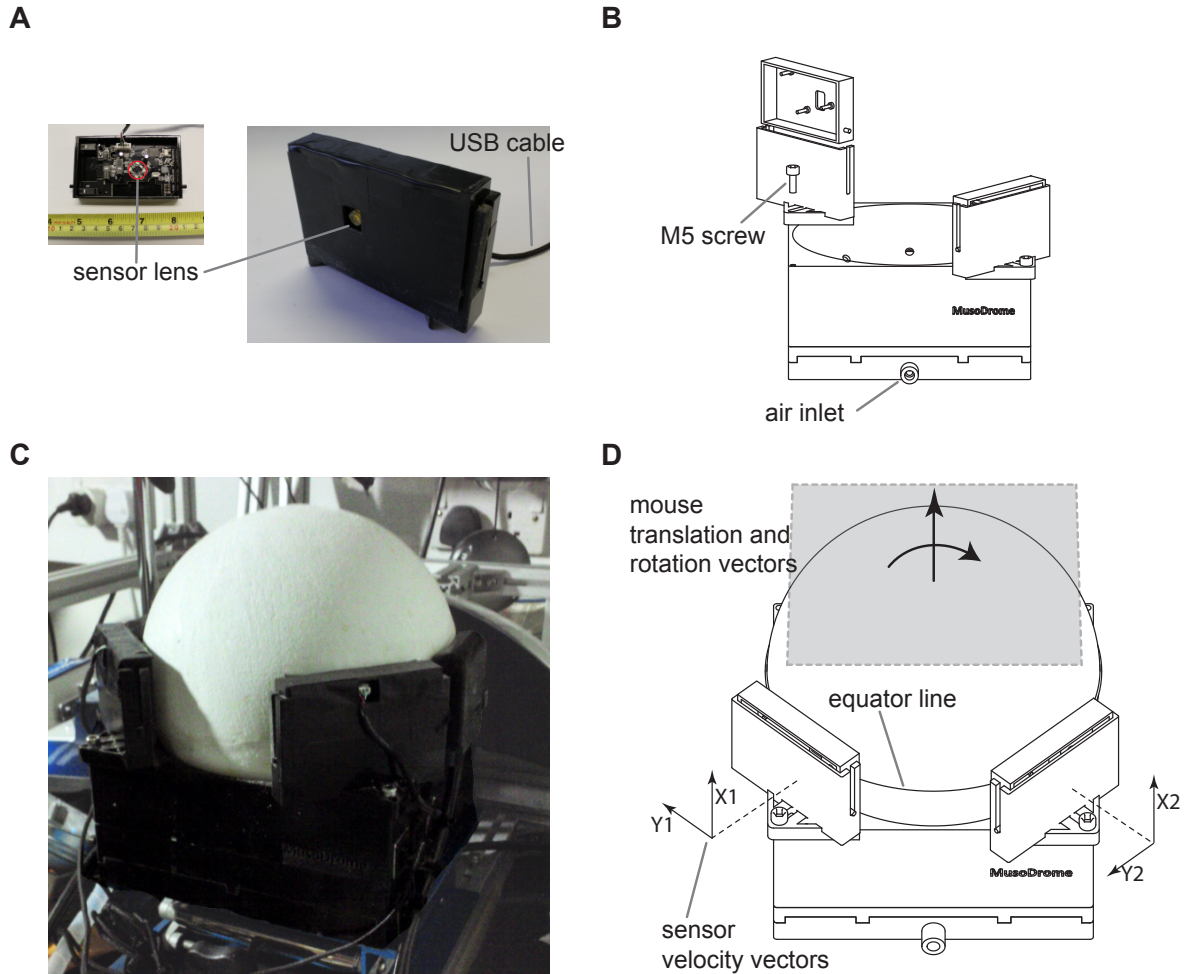


Figure 3.6: Spherical treadmill. **A)** One of the computer mouse printed circuit board (PCB) inside the 3D printed case. **B)** Positioning of the computer mouse cases on the treadmill (PCBs not shown). **C)** 3D printed spherical treadmill with velocity sensors and polystyrene sphere. **D)** 3D rendering of the *treadball* with imaginary plane of the mouse movements and velocity vectors as measured by the computer mice.

3.1.4 Automated Reward System

Subjects' participation with the VR behavioural tasks was incentivised with liquid rewards and corrective stimuli. Liquid rewards were usually given upon successful completion of behavioural tasks whereas corrective stimuli were air-puffs onto the mouse trunk that automatically engaged whenever the mouse collided with the lateral virtual walls.

Mice receive water rewards via a 3D-printed water spout with an integrated infrared (IR) beam-breaking circuit to detect lick events² (see figure 3.7). The correct positioning of the IR

²I would like to thank Dr. Matthew Brown from Chadderton's lab for providing me with the original design of the water spout.

sensor and emitter is important for the accuracy of the lick events detection to check that mice actually consumed the water given during the training sessions. The spout is mounted on a steel post and positioned near the mouse mouth. This has two conduits to deliver and remove the water. These are connected to a suction (KPV20A, Yujin Electric) and a water pump (80204-0.5, Campden Instruments Ltd) via two silicon tubings (bore 0.5 mm for the delivery tubing and 4.8 mm for the suction part, length <2 m). In order to avoid flooding of the mouth piece, the suction pump is automatically activated after six seconds from the delivery of the water. Both the pumps and the IR beam-breaking circuit are controlled in LabVIEW via an AD/DA converter or data acquisition card (NI PCIe-6321 and NI BNC-2090A, National Instruments Corporation, USA).

The air-puffers are mounted on the sides of the 'treadball' as shown in figure 3.7. They are positioned so that their outputs are distant at least 10 cm from the mouse trunk. Two solenoid valves (normally closed) regulate the air flow and are operated by two custom switch circuits (see figure 3.7). The solenoid valves are opened - so the air is puffed onto the mouse trunk - when the mouse virtual position is less than 2 mm from the virtual walls.

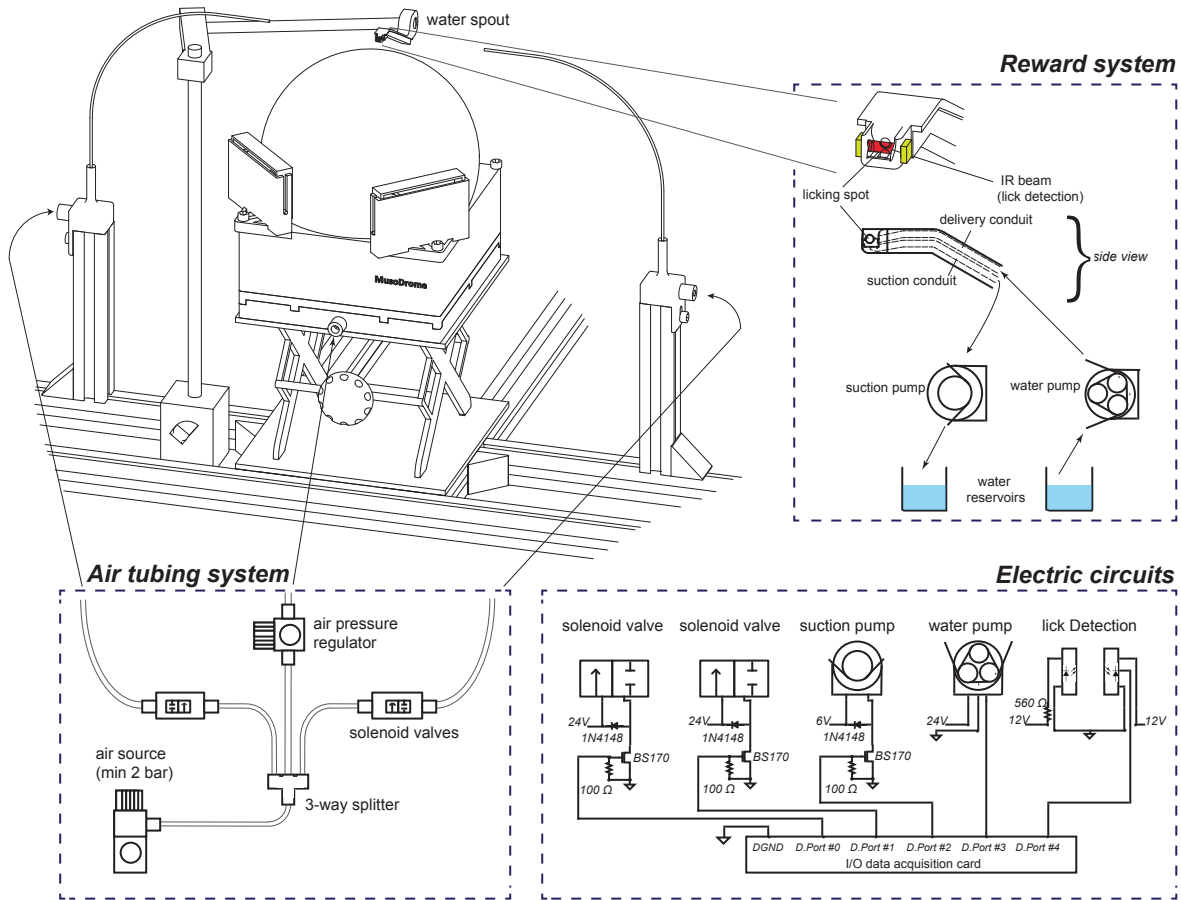


Figure 3.7: Animal interface devices. Top-left is a 3D rendering of the spherical treadmill, lateral air-puffers and water spout. Top-right, is the schematic of the water delivery system. Arrows indicated direction of water flow. Bottom-left is the schematic of the air-tubing with symbolic connections to the air-puffers. Bottom-right box shows the complete schematic of the electrical circuits with respective connections to the data acquisition card for monitoring and controlling the devices.

3.2 Control Software

To control the MusoDrome, I developed a custom programme with a modular finite state machine (FSM) architecture based in Labview and C++ with OpenGL graphics. The programme controls the VR rendering, the automated reward and the perturbation system, the data logging, and the graphical user interface. All operations are executed in parallel in different threads by single modules, or *VI*'s, organised in a 5-layered hierarchy (see figure 3.8A).

The programme updates the position of the mouse in the VR environment by integrating the velocity signals from the *treaball* every five milliseconds. These values are passed to the VR rendering task that is in charge to move the location of the mouse in the VR corridor. The gain of

the yaw velocity is set to one third to reduce the horizontal optic flow which would be otherwise too fast for the subjects to control their position.

The components of the automated reward system are controlled with a data acquisition card via digital input/output connections. The card controls the electrical circuits that regulate the function of the water pumps, the solenoid valves of the air-puffers, and the lick detection IR sensor (see figure 3.7). Only digital signals (TTL pulses) are used due to the binary nature of the events (e.g. lick - no lick, open air-puffers - close air-puffers).

A graphical user interface displays parameter toggles and behavioural information during training sessions. At the start, subject information and VR parameters are set by the user from pop-up windows (see figure A.8). General metrics such as total time of session, mouse position, speed etc. are constantly updated for the user. In addition, the console includes manual control of the animal interface devices, perturbation settings, session management, and of a tool for electrophysiology synchronisation (see figure 3.8).

At the end of each training session, data are saved onto a text file for offline processing with a sampling rate of 200 Hz. Timing of the data relies on a 100 MHz timer on the NI-DAQ card. The information saved are indicated in table 3.1.

Behavioural data		
X position [<i>cm</i>]	Y position [<i>cm</i>]	heading direction [<i>degrees</i>]
pitch velocity [<i>cm/s</i>]	yaw velocity [<i>cm/s</i>]	roll velocity [<i>cm/s</i>]
Autom. reward system		
visual stimulus ON/OFF [<i>bit</i>]	trial number [<i>numeric</i>]	reward area [<i>numeric</i>]
lick status [<i>bit</i>]	air puff left [<i>bit</i>]	air puff right [<i>bit</i>]
water pump ON/OFF [<i>bit</i>]	vacuum pump ON/OFF [<i>bit</i>]	
Perturbation system		
pressure of mechanical perturbation air jet (left) [<i>bar</i>]	pressure of mechanical perturbation air jet (right) [<i>bar</i>]	visual perturbation ON/OFF [<i>bit</i>]
Timing parameters		
Ephys trigger [<i>bit</i>]	Ephys sync pulse [<i>bit</i>]	time [<i>ms</i>]

Table 3.1: Parameters logged at the end of each training session with relative dimensions. The x and y position are computed from the velocity vector determined using only pitch and yaw as the mouse.

The creation and rendering of the virtual reality environment is entirely realised using the

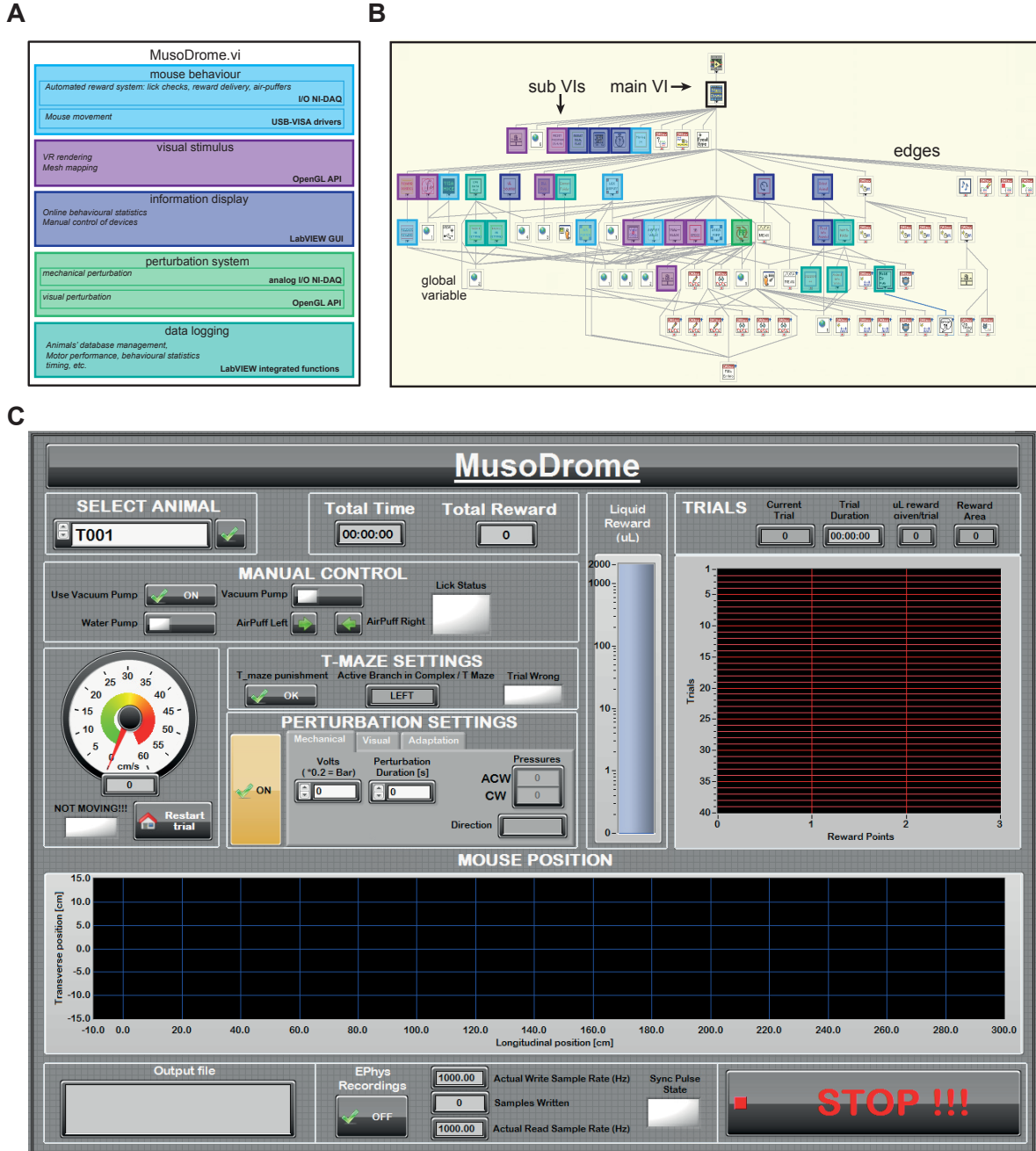


Figure 3.8: MusoDrome control programme. **A)** Main control threads of the MusoDrome software with implementation details. **B)** Diagram of the five-level hierarchy of functions or VIs. Colours of highlighted VIs indicate thread of operation. Parameters are shared through local and global variables between functions and levels. **C)** User interface of the control software. Buttons and indicators are self-explanatory. Initial sequence of parameters settings is shown in the appendix figure A.8.

OpenGL platform for C++ programming language. The C++ code that contains functions for rendering the virtual reality is interfaced with the Labview code via Microsoft's dynamic link library (DLL) protocol.

This allows for low level control of the rendering and displaying processes of the visual stimulus. The mapping of the VR frames to spherical coordinates can only be implemented via an OpenGL extension: frame buffer object architecture (FBO). This could render the digital images and apply the required distortion onto a memory buffer before the actual display onto the demispherical screen. In addition, the view of the virtual environment could be easily set to match the span of the projection screen. In fact, each frame shows a 240°-wide and 90°-high perspective view of the VR. To image such a wide span, 31 horizontally aligned virtual cameras (*glViewport*) are used.

3.2.1 Latency of the system

To characterise the latency of the MusoDrome, three separate tests were carried out. The system updates the visual stimulus upon detection of a change in the input signals following a cascade of events: 1) the computer mice detect the mouse movements by tracking the sphere motion; 2) their signals are processed by the control programme and 3) the VR stimulus is updated with the new position of the mouse in the VR environment. It is important that this process is executed within the temporal scale of the sensorimotor integration of the animal (Mauk and Buonomano, 2004) so that the mouse perceives the causality of its actions onto the VR world.

Firstly, I measured the input lag of the projector (projector model PJD6553w, Viewsonic Corporation, USA; frame rate = 120 Hz, resolution = 1280x720). This was measured to be approximately ten milliseconds³. To evaluate the latency between the movement onset and the update of the visual stimulus I used a high-speed camera (Fastcam SA5, Photron Inc., USA)⁴ to record with extremely high temporal resolution (frame rate 2000 Hz) the timing of a movement

³Data not shown here. This value was measured against a cathode ray tube (CRT) monitor (HM903DTB, Iiyama Corporation, Japan) as their display lag is extremely low. Two photo-diodes detected the changes in brightness of the projector and the CRT monitor. Since the two were mirrored, the time difference of the photo-diode signals provided with input lag information of the projector. The signals were compared by using an oscilloscope (TDS 202, Tektronix Inc., USA).

⁴I would like to thank the members of the Krapp Lab - Kit, Joseph and Ben - for kindly lending me the camera.

onset and the consequent visual stimulus update (see figure 3.9A). A motorised wheel was rotated in front of the USB velocity sensors whose signals were processed to update the visual stimulus within the MusoDrome control programme. This value was measured to be 46 ± 1.8 ms (mean $\pm$ s.e.m., $n=20$). This amount could be accounted for by considering the 10-ms lag of the projector, approximately 16 ms due to the USB velocity sensors (see figure 3.9C), and the remaining 20 ms due to computation processes of the MusoDrome programme. This time was required probably to process the double rendering of 31 views for each frame, onto the FBO first and onto the projector later. Moreover, the operating system in use - Windows 7 - is not a real-time OS therefore the interrupt signals used to acquire the velocity read-outs are buffered and might be delayed. This would explain the variance of the latency times in figure 3.9 B and C.

3.3 Perturbation System

3.3.1 Mechanical perturbation

Four air-jets are installed on the sides of the ‘treadball’, and pointed towards the polystyrene sphere surface so that the horizontal air flows tangentially to the sphere (see figure 3.10). The air-jets activate in pairs to rotate the sphere either anti-clockwise or clockwise (see figure 3.10A). Two air-jets for each direction are used to increase the drag of the air flow on the sphere surface and minimise the lateral displacement.

Two electro-pneumatic valves, or proportional pressure regulators (model VPPE-3-1-1/8-2-010-E1-T, Festo Limited, UK), one for each direction, are used to regulate the air pressure of the air jets which, in turn, determine the amount of torque applied. The torques applied to the sphere are proportional to the air pressure of the air-jets. The output pressure of the valves is proportional to an analog signal that is regulated within the Labview control software via the I/O data acquisition card.

To make sure that the forces applied for the two yaw directions are symmetrical, that linearly increase with pressure and that they vary minimally across trials, 3D printed parts and industry standard pneumatic valves are used. Preliminary tests were also carried out: air-jet activations for the two directions were applied to a still sphere at various pressure intensities (0.2:0.2:2

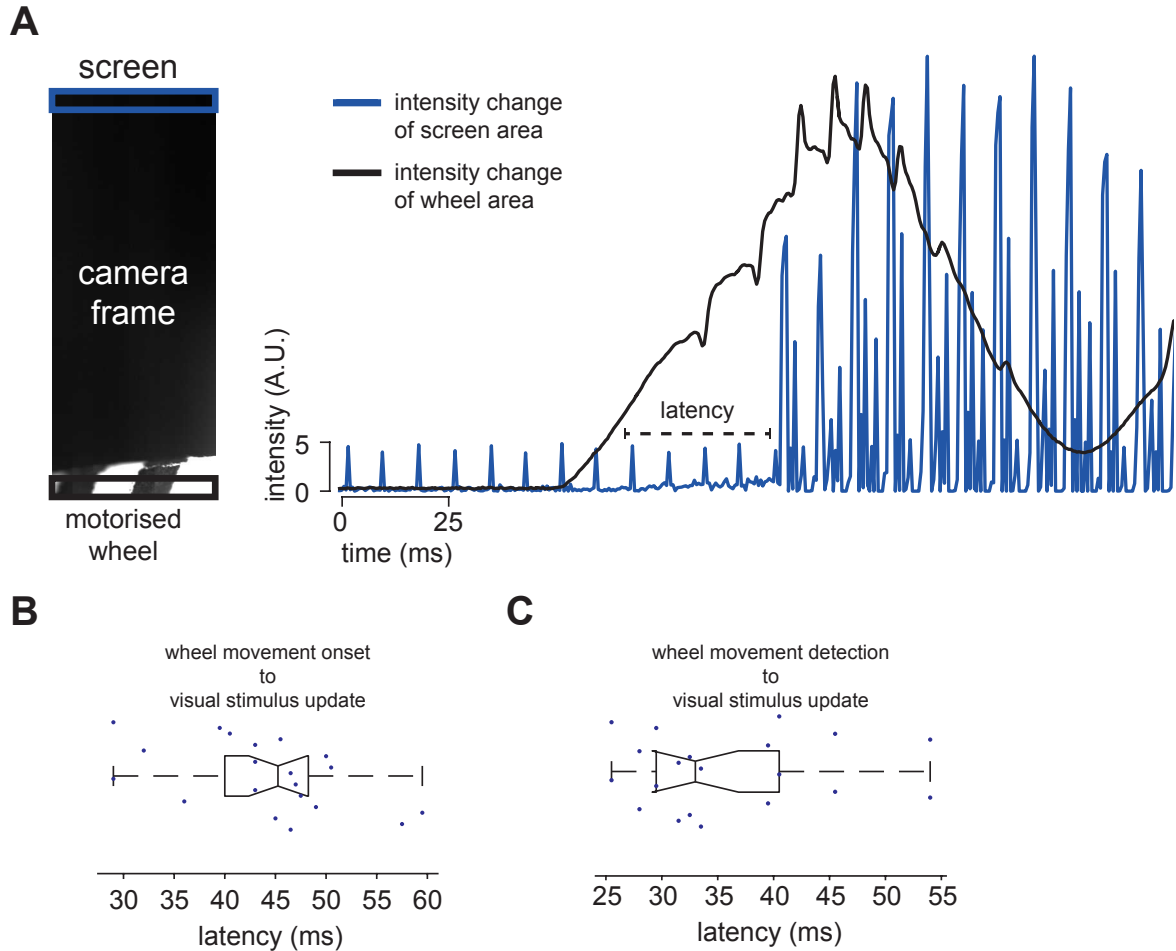


Figure 3.9: Latency of the MusoDrome. **A**) Left: frame showing field of view of the high-speed camera capturing a motorised wheel placed in front of the computer mice and the screen of the dome. Right: example of trace showing the averaged pixel intensity change of the two areas boxed in the camera frame. The regular spikes in the blue trace are due to the 120 Hz refresh of the projector. Latency is evaluated as the time difference between wheel movement onset and when screen colour changes. These are measured when their signals change by three times the standard deviations from the means preceding the wheel movement onset. **B**) Time lags of the update of the visual stimulus from the wheel movement onsets ($n=20$) as measured by the high-speed camera. **C**) Latency values from wheel movement detection to visual stimulus update ($n=20$) as measured by velocity sensors and photo-diode in front of the projector. These values are consistent with measurements performed in a similar system (same USB velocity sensors) for flies (Longden et al., 2014). Box plots show the median and quartiles in each group.

bar). These measurements confirmed that the effects of the air-jets were almost symmetrically identical for the clockwise and anti-clockwise conditions (see figure A.10 in the appendix). Once the sphere started moving, it accelerated very similarly across trials and linearly⁵ (see figure A.10 in the appendix). Furthermore, no substantial effects were observed onto the pitch and roll directions.

⁵A linear function was fitted to each velocity-pressure curve. All fits were $R^2 > 0.99$

The perturbation system was also characterised to determine the dynamics of the response and its latency. Due to the dynamics of the electro-pneumatic valves, the pressure measured by the internal sensors of the valves started rising sharply only after 0.46 ± 0.08 seconds (mean $\pm$ s.e.m., $n=126$). Consequently, the sphere started rotating at a later time: 0.83 ± 0.09 s ($n=126$) seconds from the application of the input signal (see figure A.11 in the appendix).

The force exerted on the surface of the sphere was assessed by extrapolating the acceleration from the velocity-pressure curves (figure A.11). Analytically, the force applied to the sphere is derived from the formula of the torque for a hollow sphere:

$$(3.5) \quad \tau = I\alpha = Fr = (ma)r \quad \left[\frac{N}{m} \right]$$

where I is the moment of inertia for a hollow sphere, α is the angular acceleration, m is the mass of the sphere, a is the tangential acceleration of the sphere, and r is the radius of the sphere. This transformation is possible because the sphere accelerates linearly for at least the first five seconds from the activation of the electro-pneumatic valves.

Experimental paradigms

The setting of the mechanical perturbation can be adjusted to regulate the strength and the duration of the perturbation. For every trial, the programme can be set to pseudo-randomly select whether applying the perturbation and with what probability. The direction is also chosen pseudo-randomly from a uniform distribution with 50% chances for each direction. The perturbation is automatically triggered at any point of the corridor provided that the subject is moving faster than 10 cm/s. This point is set at one third of the corridor for the data presented here (duration = 3 seconds). Alternatively, the MusoDrome programme can be configured to apply a habituation paradigm by imposing only one direction of perturbation (see figure 3.8).

3.3.2 Visual perturbation

The visual perturbation consists in shifting the horizontal angle of view by an amount specified by the user. This can be kept constantly deviated throughout the VR corridor or can be activated at specific points. The direction is selected pseudo-randomly by the programme for each trial to

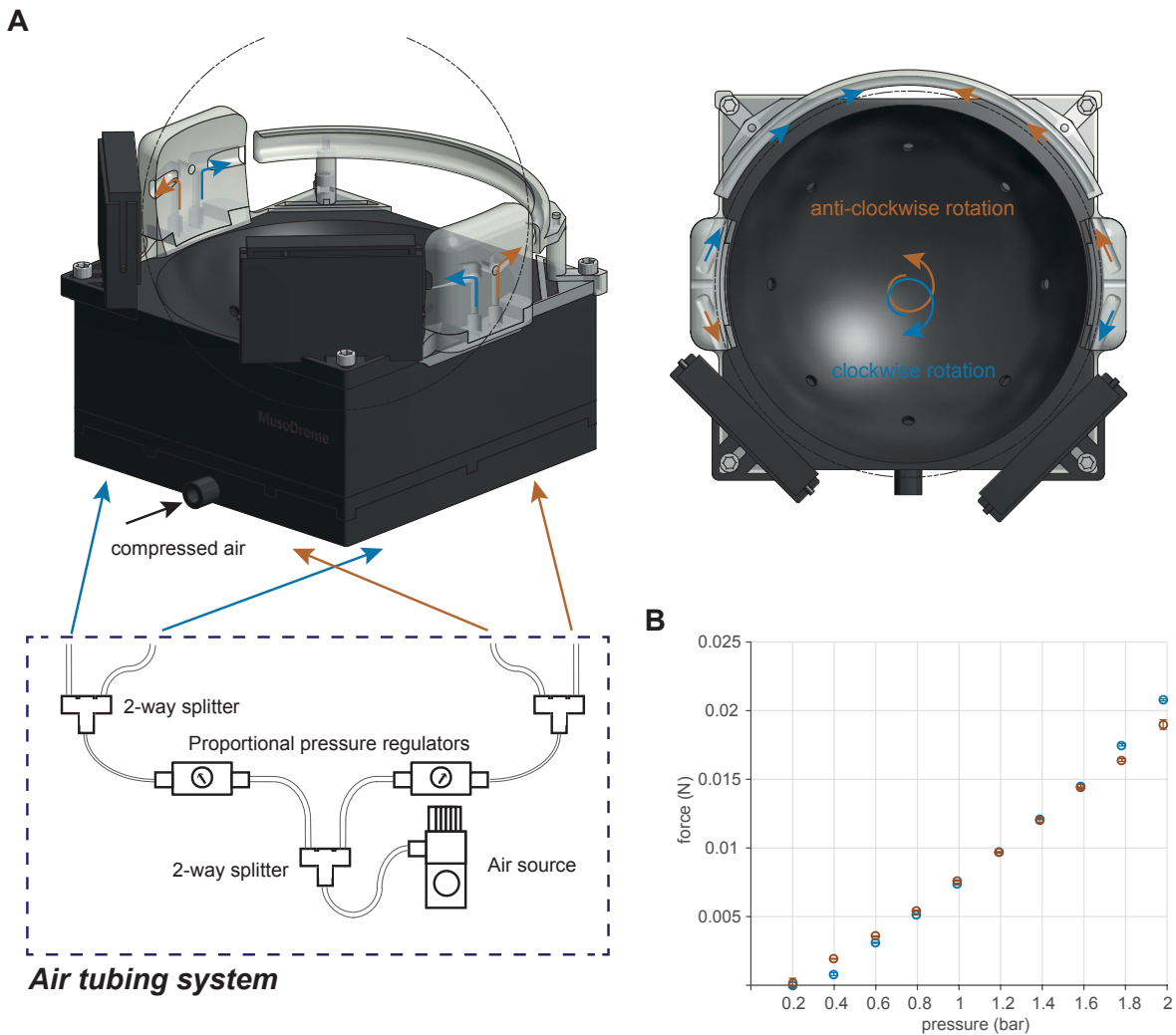


Figure 3.10: Perturbation System. **A)** Top: perspective and top view of the spherical treadmill with superimposed tangential forces acting onto the sphere. Bottom: schematic of the air-tubing with connections (arrows) to right and left air-jets. Components of the system are rendered transparent for showing the air jets. **B)** Force applied to the sphere as a function of the controlled pressure. Error bars indicate standard error of the mean, $n=7$ per data point.

avoid habituation. The rationale is that the mouse would perceive a perturbed visual feedback by seeing the world distorted by an arbitrary horizontal angle. As a consequence, the subject should adjust its gate to continue moving with a straight trajectory to which it should adjust its gate to keep moving forward. This paradigm aims to replicate the experimental conditions created in humans throwing darts through prism glasses (Martin et al., 1996). This paradigm could be useful to assess visuomotor responses within adaptation and learning frameworks.

3.4 Discussion

In this chapter I have described a VR system for mice, the MusoDrome, developed as part of this research project. It aims to extend the range of applications of this experimental tool to motor control studies. Its design and performance are validated while protocols on how to create, configure, and test the system are also provided.

The MusoDrome uses a spherical projection of the VR environment for immersive visual stimulation of mice. This is based on a simpler projection system compared to other systems. Because it is supported by a Labview software with OpenGL graphics, it brings convenient features such as straightforward configuration and manipulation of the visual stimulus attainable with the methods suggested here (see appendix). Moreover, for the first time for a VR system, the lag of the VR update has been fully outlined. Since the design of VR systems is based on USB ports for tracking mouse movements and digital projectors for visual stimulus presentation, their latency cannot be below 20-25 ms, which is fast enough for mouse behaviour. In case of insect studies, shorter lags for closed-loop paradigms have been achieved at the costs of dramatically increasing the sophistication of the hardware (Lindemann et al., 2003; Takalo et al., 2012).

Design details are provided to create an efficient and silent ‘treadball’ for mice based on the original system of Dahmen, 1980 and Dombeck, Graziano, et al., 2009. This ensures a two-dimensional control of position in virtual reality environments that would allow more visuomotor control paradigms as compared to standard treadmills (Niell and Stryker, 2010; Keller et al., 2012; Lee, Hoy, et al., 2014). A limitation of this implementation could lie in the tracking of the sphere. Since this relies on two USB computer mice connected, measures could vary between systems with different sensors, and the commercial availability of two identical mice might be limited. An effective improvement could be the control via a micro-controller (e.g. Arduino) of two image sensors such the ones used by Takalo et al., 2012 and Cushman et al., 2013. However, this approach would not yield to performance increases latency-wise because of the USB protocol constraints.

The MusoDrome control programme is developed in Labview with OpenGL graphics to effectively and rapidly execute all tasks from a single computer. It is integrated with a user-

friendly interface to easily run behavioural experiments with and without electrophysiology. The low-level management of tasks such as the three-dimensional visual stimulus, the animal interface devices, etc. allows to control all experimental variables at the same time ensuring consistency of timing and data acquisition.

Finally, I have presented a novel perturbation system to affect locomotion behaviour extending the potential of VR systems with sensory perturbation paradigms for studying visuomotor coordination while measuring neural activity. Data collected from six mice indicates that the mechanical perturbation deviates the trajectories of mice running in a VR linear track. These results will be presented in the next chapter.

4

MOUSE BEHAVIOUR IN VIRTUAL REALITY

The use of virtual reality environments in rodent experiments has been primarily aimed at investigating spatial navigation. Behavioural studies have showed that rats, mice, and gerbils can learn to navigate in VR spaces (Schnee, 2008; Youngstrom and Strowbridge, 2012; Thurley et al., 2014). In addition, electrophysiological evidence from rats and mice have demonstrated that the cellular dynamics of hippocampus and entorhinal cortex is consistent with the formation of place fields observed in freely running animals (Holscher et al., 2005; Harvey, Collman, et al., 2009; Dombeck, Harvey, et al., 2010; Schmidt-Hieber and Häusser, 2013; Ravassard et al., 2013).

The majority of these studies, however, used VR environments delimited by virtual walls that cannot be trespassed and did not provide any feedback. As a consequence, since the virtual walls did not physically affect mouse movements, subjects did not deviate from them and, as long as they applied some forward force to the sphere, their position was moved forward by "sliding" it along the walls. In fact, mice tend to rotate the sphere around the vertical axis while they walk (Dombeck and Reiser, 2012) and, although this limitation does not invalidate the results of the previous studies, from a visuomotor coordination perspective, this locomotive behaviour has little ethological validity.

To overcome this issue, I have implemented a behavioural paradigm that uses a combination of visual clues, water rewards and air-puff stimuli to train mice to navigate in a VR corridor. In

increasingly difficult training phases, mice are trained to purposely control their two-dimensional position, to reach water reward points, and avoid contact with lateral walls that trigger air-puffs directed to the mouse. The trunk was chosen as a target instead of whiskers, the normal organs used by mice for tactile sensing, to minimise distress of the animals during VR behaviour. Such auxiliary haptic feedback deviated mice from the VR walls and, consequently, was proven to be an effective means to match the visual feedback more closely to the actual locomotion pattern of the animals (Georgopoulos and Grillner, 1989).

So, when subjects move in the VR corridor, they have to rely on the visual feedback to control their two-dimensional position to avoid the lateral walls. This raises questions on what type of visual information mice use to adapt their locomotion and how they respond. To address these questions, I have devised a perturbation paradigm to rotate the spherical treadmill either clockwise or anti-clockwise while the mice run in the VR corridor. By doing so, it was possible to steer mouse trajectories and elicit behavioural responses to counteract such external force. In this chapter, I will show that mice adjust their trajectories in the VR corridor in response to the external perturbation in a way that is consistent with an anticipatory strategy.

4.1 Behavioural training protocol

4.1.1 Training phases

Following a recovery period of two days from the head-plate implant surgery, mice underwent a behavioural training. They were trained to navigate in a virtual reality environment to reach water rewards while avoiding lateral walls. Their participation was incentivised by the release of rewards and corrective stimuli depending on their virtual position. Animals were trained in cohorts of normally five units so that they could be housed in a single cage. Water bottle was removed from the cage at least 6 hours prior the first session. Concomitantly, weights of the mice were recorded to check they did not lose more than 20% of their original weight during the behavioural training.

In the two initial sessions of usually 15 minutes each, mice were habituated to the VR system. At this stage, special attention was paid towards the handling of the animals as it has been

CHAPTER 4. MOUSE BEHAVIOUR IN VIRTUAL REALITY

observed that calmer animals habituate to the head fixation more quickly. To ensure mouse comfort, I also regulated the position of the spherical treadmill in the three dimensions and check its inertia by adjusting the air pressure for each mouse (see table A.1 appendix for a list of troubleshooting solutions for animal handling issues). In addition, I placed small barriers on the mouse trunk sides to limit horizontal body rotations on the sphere and reduce the stress onto the neck muscles. The water spout was placed in front of the mouse mouth and water was released *ad libitum* to facilitate the association with the water reward system. A liquid reward provided 10 μl of water. No visual stimulus nor air-puffers were used in these habituation sessions.

From the third session, mice were presented the virtual reality projection (see figure A.12). This was the first session of behavioural training. For the first 2-3 sessions (normally one session per day), mice were placed in a virtual corridor 100-cm-long and 30-cm-wide. The duration of each session was lengthened up to 40 minutes so that the subjects could learn 1) to associate the licking of the water spout with water delivery and 2) the causal relationship between their locomotor activity and the visual feedback. To this end, mice were given free water rewards during the session and were free to navigate within the corridor. When they reached the end of the corridor, the screen was turned black for 6 seconds and the mouse virtual position reset at the beginning of the corridor.

After these preliminary sessions, the water rewards were only released upon passage of the mice underneath cylinders hanging from the ceiling, evenly spaced at each one third of the corridor. Only the cylinder with an active reward was visible. Once the mouse reached the reward area, this was turned off and the second appeared so that the mouse could unambiguously follow the correct direction to end the trial. The virtual corridor was lengthened and narrowed (200 cm by 20 cm) with the aim to rectify mouse running trajectories as much as possible for inter-trial comparison purposes. When the subjects were capable of completing at least 20 trials within 30 minutes for two consecutive sessions they were promoted to the successive phase of the training.

In the last phase of the training, lateral air puffers were pointed to each side of the subject trunk as corrective stimuli to address two behaviours. First, since the virtual walls did not physically affect mouse movements as real ones do, the mice did not deviate from the virtual walls. Second, in some instances, animals walked along one of the virtual walls for the entire

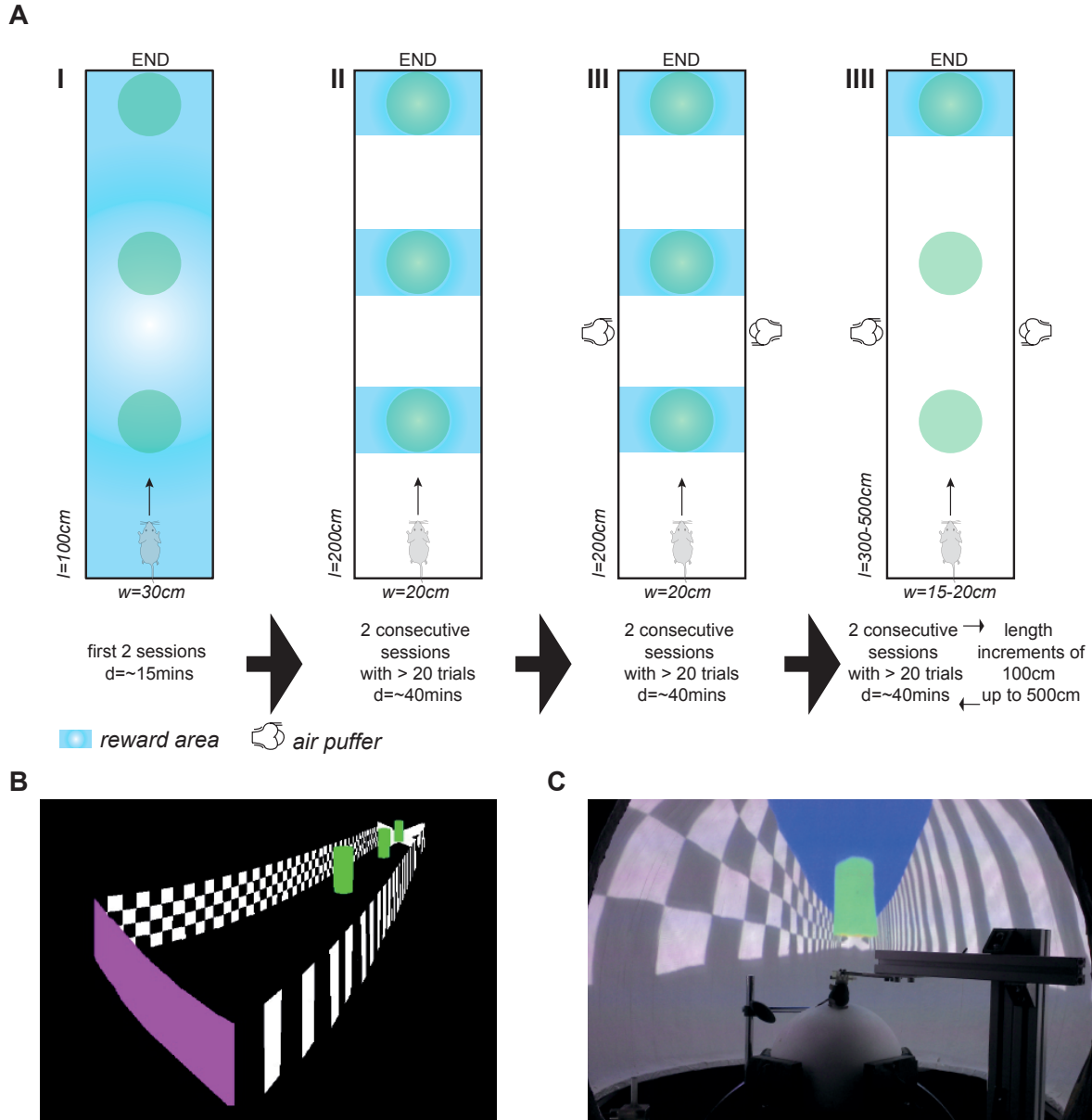


Figure 4.1: Phases of behavioural training. **A)** Diagrammatic representation of phases of behavioural training phases. Mice are trained to follow the corridor towards the end by gradually training them to habituate to the setup (I), to associate visual landmarks to water reward delivery (II), to run avoiding virtual contact with the walls by means of lateral air puffers (III), and to run towards the end without stopping (IIII). **B)** Three dimensional perspective of the virtual corridor. **C)** A mouse at the start of training protocol pointing towards the first reward point.

length of the corridor. Since they could not trespass the virtual walls, as long as they applied some forward force to the sphere, their position was moved forward by "sliding" it along the walls. Since such inconveniences would impair any assessment of the visuomotor coordination of the subjects, as soon as a mouse touched a wall the air puffer on the same side was automatically

activated, providing a haptic-like feedback to the mouse. A similar criterion for accessing the following training phase was used as before: when a subject was capable of completing at least 20 trials in 30 minutes for two consecutive sessions they were promoted to the successive phase of the training.

Corridor length was then progressively increased in steps of 100 cm depending on the mouse performance evaluated over two consecutive sessions. The width of the corridor was also narrowed up to 15 cm. The duration of the sessions was increased up to one hour to train the mice for the duration of the session with electrophysiological recordings.

Animals were trained daily, including weekends. During these days, after at least two hours from the end of a training session I gave to the mice water *ad libitum*, and then removed the water bottle again. During rest days, water bottles were placed in mouse cages for 1-2 hours.

4.1.2 Mice learn to control their 2D position in the VR environment

The behavioural performance of the mice was based on the assessment of how straight they ran along the VR corridor, how quickly they completed trials to retrieve water rewards, and whether they avoided contact from the virtual walls.

To measure how efficiently subjects could navigate in the virtual environment, the distance covered by the mouse during each trial was normalised by the length of the virtual corridor (Harvey, Collman, et al., 2009; Schmidt-Hieber and Häusser, 2013). This parameter decreased with training (see figure 4.2A) up to reaching values around one and an half for most mice (1.62 ± 0.12 , mean $\pm$ s.e.m., 21 subjects out of 28) by the fifteenth session. Details on single subjects are shown in the appendix in figures A.13 - A.18. For four of the seven mice (subjects C23, C33, C42 and C64) that did not learn to navigate in the VR environment, continued training regime (>21 sessions) did not promote any performance progress. For the other subjects (subjects C53, C61 C63), the behavioural training was interrupted within the first 14 sessions.

The participation of the subjects was quantified in trials completed per unit of time. As mice learnt to control their position and associate the release of water rewards with the completion of trials, they completed more trials per minute (see figure see figure 4.2B). On average, the subjects trained in this study completed 0.23 ± 0.07 (mean $\pm$ s.e.m., $n=21$) trials per minute on the first

session, 0.65 ± 0.09 on the tenth session and 0.77 ± 0.09 on the twenty-first session of training. The subjects that could efficiently move forward in the VR corridor also completed more trials per minute compared to the seven that could not control their position (see B panels from figures A.13 - A.18 in the appendix).

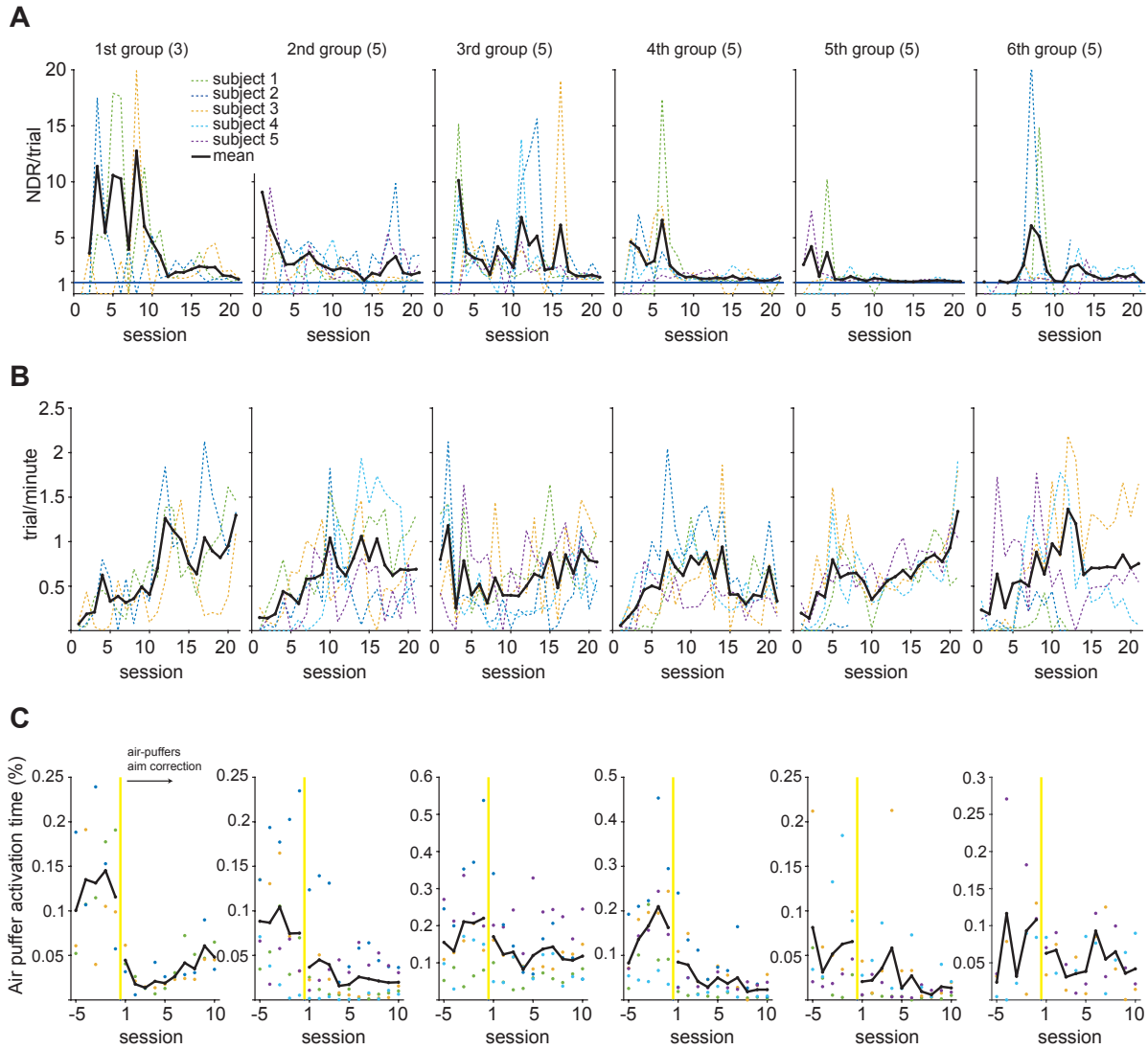


Figure 4.2: Training progress of all cohorts. **A)** Normalised distance run (NDR) per trial over the first 21 sessions. **B)** Trials completed per minute. **C)** Normalised duration of air-puffers for 5 trials before and 10 trials after air-puffers are aimed towards the mouse trunk. First cohort/group has only 3 subjects.

CHAPTER 4. MOUSE BEHAVIOUR IN VIRTUAL REALITY

	speed (cm/s)	moving time (%)	airpuff aim	water consumption	end of training
C11	20.4±9.9	91±2	10	13	15
C12	21.9±12.8	93±1	10	13	25
C13	23.6±11.7	94±1	10	12	15
C21	22±12.7	70±2	14	5	15
C22	19.4±9.5	83±3	14	3	21
C23	–	–	14	4	–
C24	17.7±9.2	65±6	14	3	19
C25	15.3±8.9	66±3	14	3	18
C31	25.6±16.3	53±1	16	5	23
C32	26.8±16.3	64±4	16	–	21
C33	–	–	11	–	–
C34	18.9±11.9	37±1	11	–	25
C35	22.9±11.9	78±4	16	5	20
C41	14.7±5.7	52±1	8	4	21
C42	–	–	8	14	–
C43	17.6±8.4	47±5	8	8	14
C44	16±7.5	49±2	7	5	13
C45	15.5±6.4	45±6	7	5	13
C51	22.9±11.8	58±4	9	3	16
C52	21.7±10.4	62±4	7	–	17
C53	–	–	–	–	–
C54	24.5±13.8	75±3	8	–	18
C55	23.6±12.6	69±5	7	7	17
C61	–	–	8	–	–
C62	22.9±11.8	76±3	7	4	16
C63	–	–	–	–	–
C64	–	–	8	8	–
C65	22±15.2	49±4	7	–	22

Table 4.1: Behavioural parameters of the subjects (ID shown in I column). II column shows mean speed of periods when mouse moves, computed over the 5 sessions following the end of the training, i.e. when the VR corridor length is set to the maximum length (mean±s.d.). III column: moving time is computed as the fraction of time spent at speed >1 cm/s of the five sessions following the end of training (mean±s.e.m., n=5). IV column indicates the session when the air puffers are aimed towards the animal's trunk. V column: session in which animals start consistently drinking water rewards are shown in the fourth column (see details in B panels from figures A.13 - A.18 in the appendix). VI column: session at which the corridor is set to the maximum length (d=500cm).

Lateral air-puffers were introduced at different stages during the stages (see table 4.1). Total time of air-puffers' activation significantly reduced following the correction of the air-puffers' aim towards the animals' trunk ($p=9.4^{-07}$, Mann-Withney U-test, comparison between five sessions before and after the application of the air-puffers for all mice). Subjects did spend less time in proximity of the walls (see C panels from figures A.13 - A.18 in the appendix) and, as shown in the following section, avoided contact with the walls even under perturbation conditions. These data suggest that mice actively control their two-dimensional position by integrating the visual

feedback.

Once subjects learn to control their position in the VR environment, they seemed to intentionally complete trials to receive water rewards (see figure 4.3B). Indeed, lick events were normally recorded following the release of water rewards. These reached frequencies up to approximately 7 licks per second and stopped after the removal of the water from the water spout (see figure 4.3F). Only eight mice did not consume the water rewards (see table 4.1). This was observed to be mainly due to subjective aversion towards the water spout.

Similar to previously reported behaviour (Harvey, Collman, et al., 2009), mice completed trials on a single run and moved at a constant speed throughout the VR corridor (see figure 4.3C and D and table 4.1 for mean values of all mice). In the example session of figure 4.3, the fraction of time spent along the transverse direction clearly shows the mouse spent majority of the session slightly off-centre towards the negative side (bottom in panel A). See C panels from figures A.13 - A.18 in the appendix for details of all subjects.

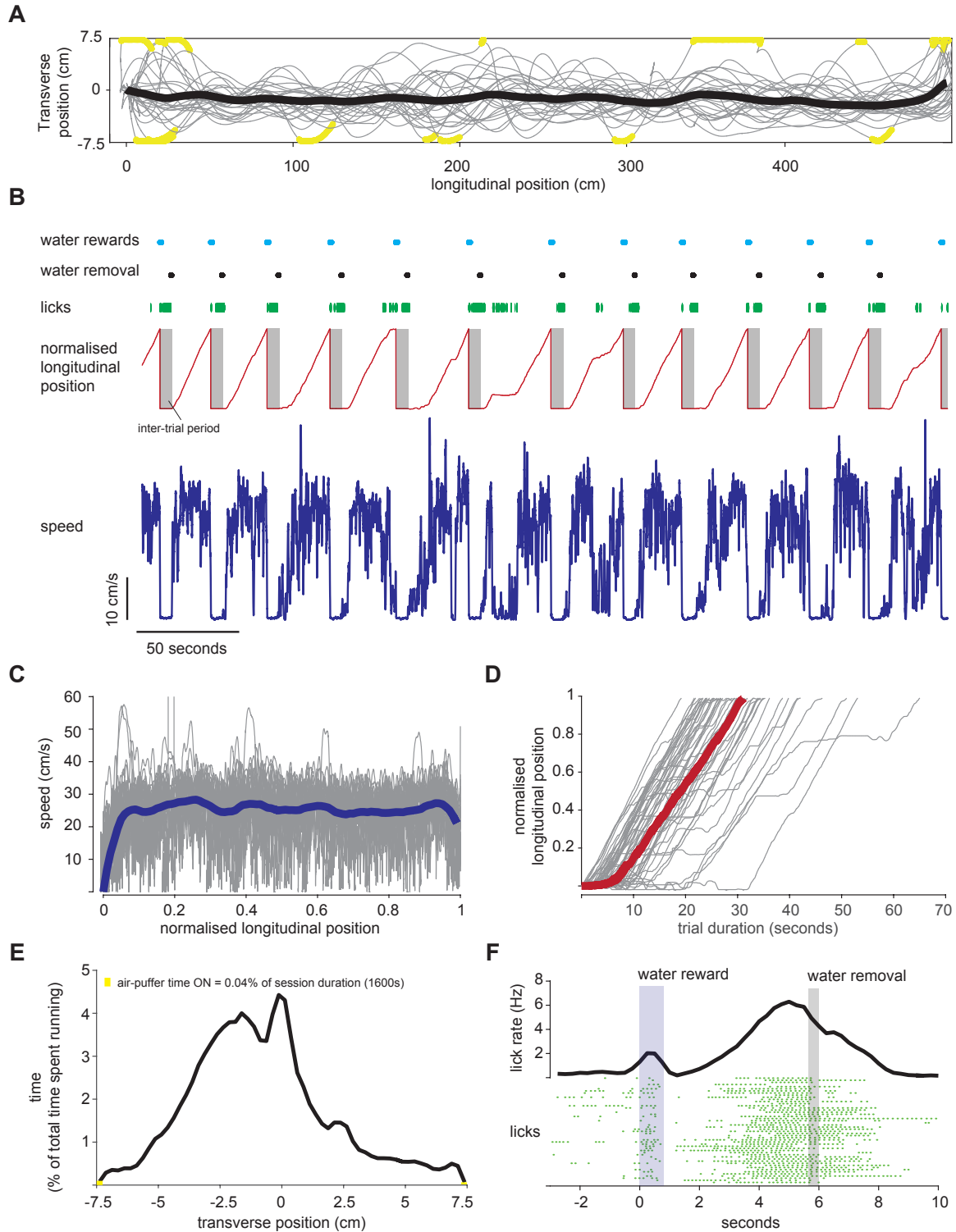


Figure 4.3: Mouse behaviour during a session. **A)** Example of running trajectories in the VR maze (subject: C15, session: 23, trials: 23). Thick line is average trajectory. Yellow marks indicate when air-puffers are triggered. **B)** Mouse moves towards the end of the VR corridor to receive the water rewards. Subset of the 13/23 trials shown in A. **C)** Mean speed of locomotion in VR maze is constant throughout its length. Thick line is average of single trials (gray). **D)** Mice complete trials in a single run. Thick line is average of single trials (gray). **E)** Proportion of time spent on the transverse direction during the entire session. **F)** Mouse licks the water reward only after its release.

4.2 Responses to mechanical perturbation

The mechanical perturbation was introduced after the end of the behavioural training protocol. The perturbation was applied to 75% of the trials and its direction (clockwise or anti-clockwise) was pseudo-randomly chosen with equal chance at the beginning of each trial. The air-jets of the mechanical perturbation were switched on when the subject surpassed the first third of the VR corridor and if it was running at speeds greater than 10 cm/s. This solution was applied to avoid activation of the perturbation while the mouse was stationary. The perturbation was activated for 3 seconds at a pressure of 0.6 bar (nominal force at sphere surface 3×10^{-3} N). This paradigm was applied to a total of nine mice from three different cohorts. The data presented here only refer to the first six mice (see table 4.2). This is due to the fact that the performance of the remaining three animals, as measured by the fraction of session time during which air-puffers were active, was poor or did not improve across sessions (see section 4.3 for details).

	sessions	clockwise	anti-clockwise	no perturbation
C11	8	144	154	99
C12	4	51	64	47
C13	5	86	103	73
C21	8	100	114	82
C22	6	79	93	51
C24	11	223	211	177

Table 4.2: Count of sessions and trials with mechanical perturbation. First columns are sessions with mechanical perturbation. Following columns show total number of trials for each of the perturbation conditions for each subject.

The application of the lateral air-jets onto the surface of the sphere causes lateral deviations of the subject trajectories consistent with the direction of perturbation (see figure 4.4A). Both conditions determine a lateral displacement of the mouse position whose direction depends on the perturbation direction: a perturbation that turns the spherical treadmill clockwise moves the mouse VR positions towards the left or the positive side of the corridor whereas an anti-clockwise perturbation determines a rightwards shift of the VR position to the right (or negative or bottom half of the corridor as it is drawn in figure 4.4B). The running direction, or heading of the mouse, was rotated symmetrically for the two perturbation conditions as compared to the period antecedent the application of the perturbation. The clockwise perturbation caused a positive rotation and the anti-clockwise a negative rotation (see figure 4.4C).

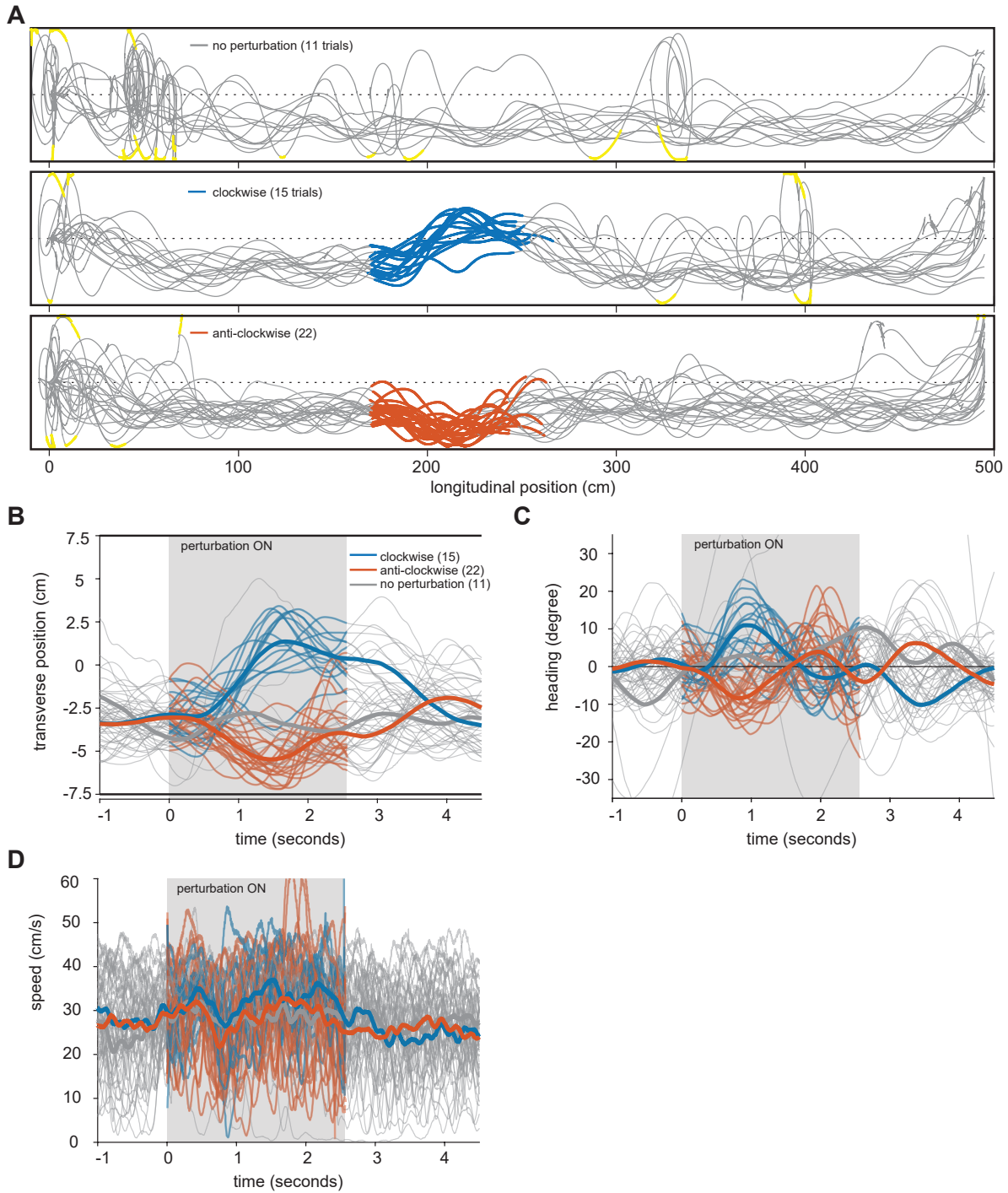


Figure 4.4: Perturbation of mouse behaviour. **A**) Trajectories in the virtual corridor (500 by 15 cm) of a sample session (48 trials) shown for different perturbation conditions. Yellow marks indicate when air-puffers are triggered. **B**) Mouse VR transverse position for the trials in A. At time $t=0$ s, pressure of the air-jets goes over 0.5 bar. Because of the latency of the air-jets (0.46 ± 0.08 s, mean $\pm$ s.e.m., $n=126$), perturbation is effectively active for approximately 2.5 seconds. Means are shown in thicker lines. **C**) Mouse VR heading during perturbation as a function of time. **D**) Speed profile during different perturbation conditions for the trials in A. Thick lines are mean speed profile for the three perturbation conditions.

4.2.1 Subjects promptly adjust their VR transverse in response to the perturbation

Before the application of the perturbation, the transverse position in the VR corridor did not differ between the different conditions, showing overlapping distributions (see figure 4.5A). The mean transverse positions measured for the trials with clockwise, anti-clockwise, and no perturbation are respectively -1.1 ± 2.6 cm (mean $\pm$ s.d., $n=683$), -1.2 ± 2.4 cm (mean $\pm$ s.d., $n=739$), and -1.3 ± 2.5 cm (mean $\pm$ s.d., $n=529$). On the contrary, after the perturbation, the VR position along the transverse direction was shifted towards the positive half of the corridor for clockwise conditions and to the negative side for anti-clockwise perturbation by approximately two centimetres for each side: the new mean transverse positions were measured to be 1 ± 2.8 cm (mean $\pm$ s.d.) for clockwise conditions and -3.1 ± 2.3 cm (mean $\pm$ s.d.) for anti-clockwise conditions (see figure 4.5B).

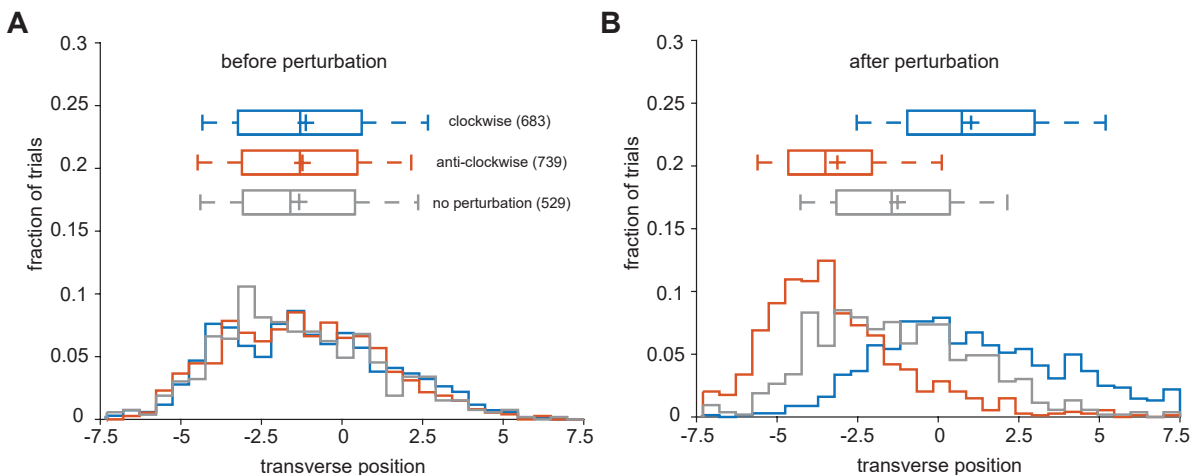


Figure 4.5: Lateral shift before (A) and after (B) the perturbation. Box plots show the median and quartiles in each group, and the cross marks the mean.

The absolute maximal lateral shift along the transverse position during the perturbation was computed to check whether mice counteract the external force by correcting their position during the application of the perturbation or they are pushed sideways for the entire duration of the perturbation (see figure 4.6). This was defined as the absolute difference between the maximal transverse position during the application of the perturbation and the position recorded at the onset of the perturbation in the same trial. The maximum lateral shift was similar in both conditions and, on average, was also greater than the difference recorded at the end of the perturbation: -3.53 ± 2.9 cm (mean $\pm$ s.d., $n=683$) for clockwise conditions and -3.25 ± 2.2 cm

(mean $\pm$ s.d., n=739) for anti-clockwise conditions. While the maximal lateral shift were recorded at the end of the perturbation in slightly more than 20% of the trials, in most cases (>70%) this was measured to be within two seconds from the onset of the perturbation: 1.67 ± 0.83 s (mean $\pm$ s.d., n=683) for clockwise conditions and 1.79 ± 0.72 s (mean $\pm$ s.d., n=739), see figure 4.6. Trails in which the shortest distance from the walls was measured at the start of the trials refer to trials in which subjects were already very close to the walls and likely heading to the opposite direction of the perturbation making this less effective.

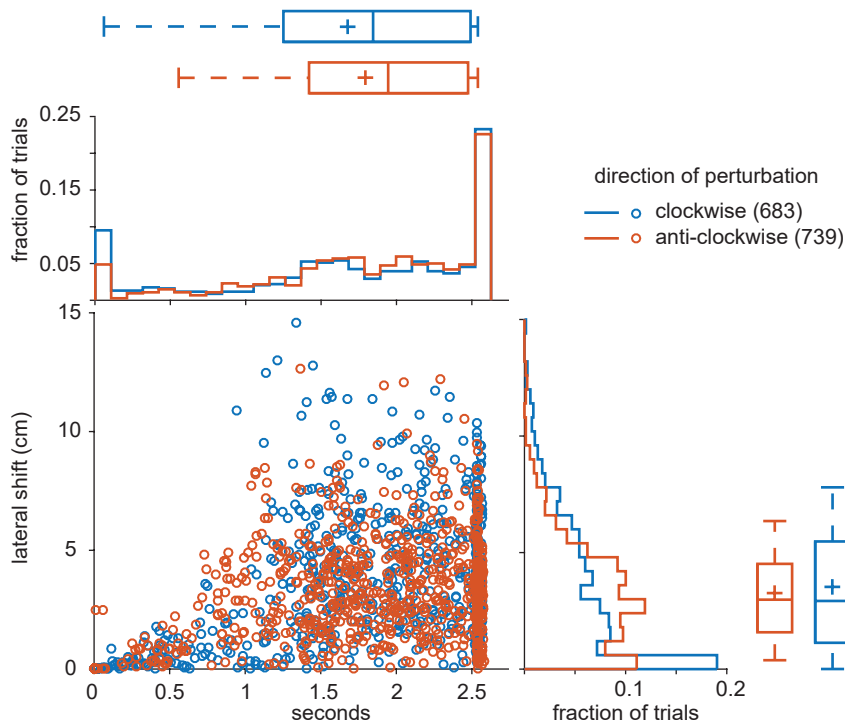


Figure 4.6: Maximal lateral shift during perturbation.

These results were not biased by the fact that subjects could not trespass the VR walls limiting the lateral shift along the transverse direction. Subjects did not touch the VR walls in 93.6% of the trials for clockwise conditions and 90.5% of the trials for anti-clockwise conditions (see figure 4.7A). The neutral heading direction (approximately nought degrees) computed when the subjects were at the closest point to the walls indicated that subjects already corrected their trajectories.

These data show that the subjects' VR position was perturbed by the external forces applied with lateral air-jets onto the spherical treadmill for the two conditions and that mice reacted to it

with a similar behavioural response within two seconds to avoid contact with the virtual walls.

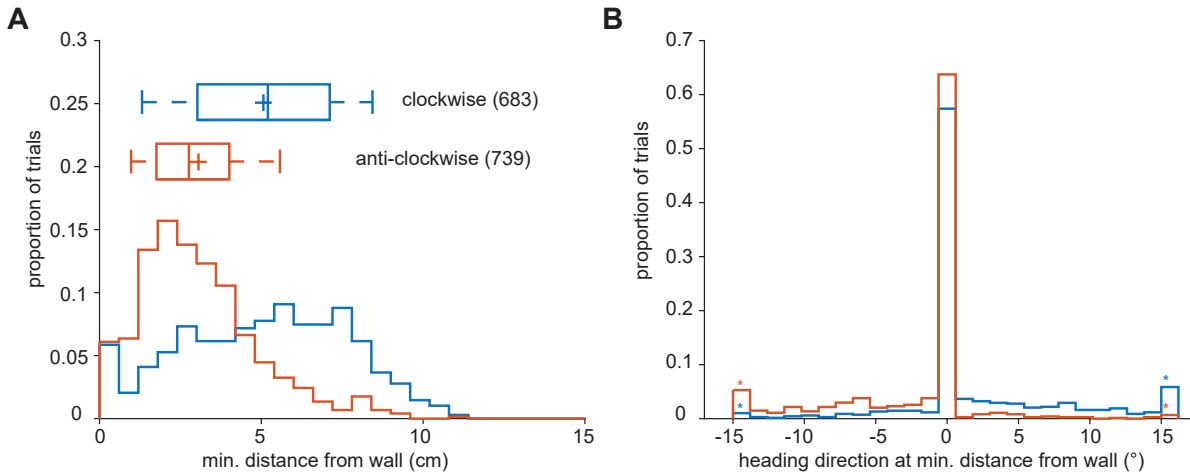


Figure 4.7: Transverse Position nearest the walls. **A)** Distance from the walls at nearest point during perturbation. **B)** Heading angle at nearest point during perturbation. Asterisks on side of histograms indicate last bar contains data points beyond limits of axis.

4.2.2 Subjects counteract the perturbation in response to heading rotation

The heading of the subjects was assessed during the application of the perturbation to qualitatively characterise and investigate the temporal evolution of the behavioural response of the mice. First, I computed the instantaneous probability of the VR heading in the positive direction (see figure 4.8). As expected, the probability of a positive heading direction in the clockwise direction stayed above chance levels ($p=0.5$) for the whole duration of the perturbation. Instead, during the anti-clockwise perturbation, it went below chance levels meaning the subjects' heading pointed to the right, in accordance with the lateral shift shown previously. The probability increased sharply up to ~ 0.8 at about one second from the perturbation onset in both conditions (for anti-clockwise conditions 1-p).

Indeed, the maximal heading angles during the application of the perturbation were recorded at similar delays from the perturbation onset: 1.24 ± 0.72 s (mean $\pm$ s.d., $n=683$) for clockwise conditions and 1.15 ± 0.70 s for anti-clockwise conditions (mean $\pm$ s.d., $n=739$), see figure 4.9. The values of the heading angles for the two conditions were measured to be symmetrically distributed with mean $15.5^\circ \pm 10.4^\circ$ (decimal degrees, mean $\pm$ s.d., $n=683$) for the clockwise perturbation and $-15.5^\circ \pm 8.1^\circ$ (decimal degrees, mean $\pm$ s.d., $n=739$) for the anti-clockwise perturbation.

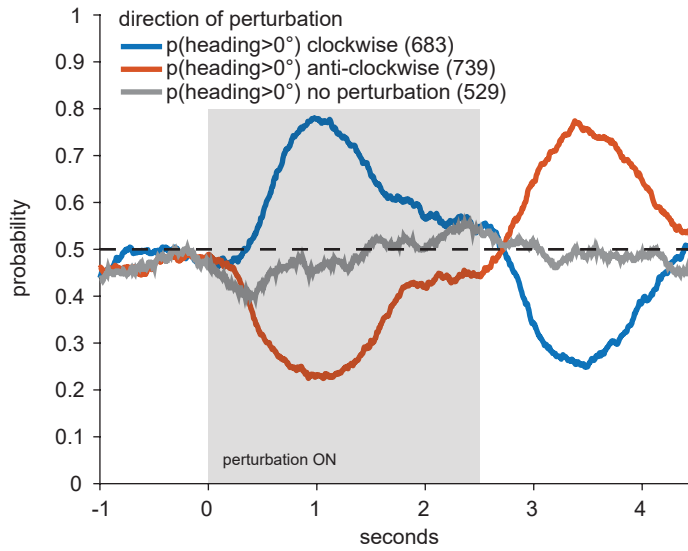


Figure 4.8: Instantaneous probability of heading direction.

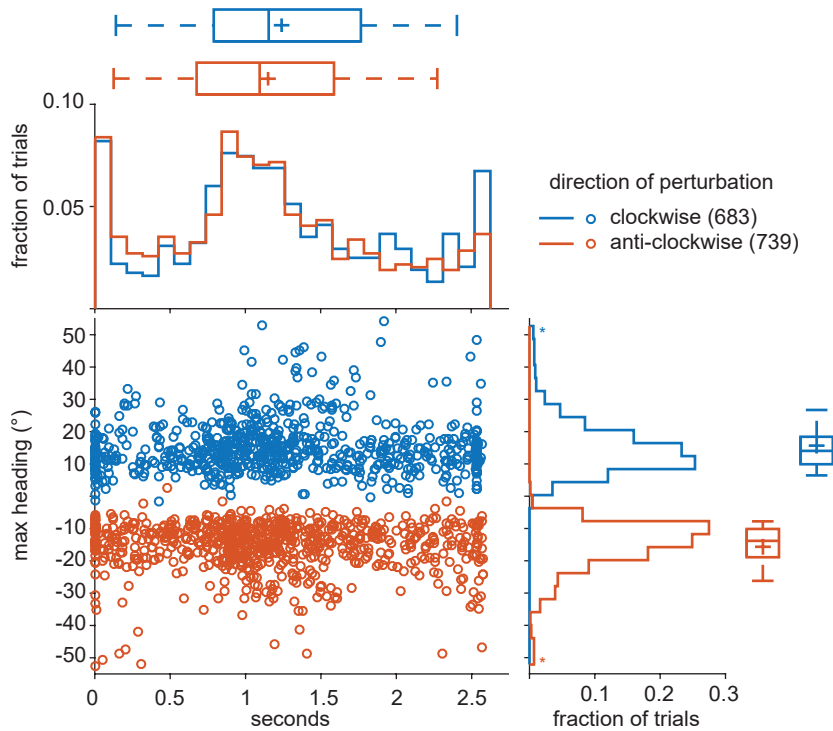


Figure 4.9: Maximal heading angle during perturbation. Asterisks on the sides of histograms indicate last bar contains data points beyond limits of the axis.

These data suggest that subjects' heading direction was principally affected during the first half of the perturbation period but they counteracted the external force in response to high heading angles (shift of the optic flow) rather than to closeness to the VR walls.

4.2.3 Subjects decrease their VR locomotion speed during the perturbation

To look at whether the subjects' response to the perturbation was accompanied by a change in locomotion speed, the locomotion speed profile was computed for all trials. On average, the subjects decrease their locomotion speed in the VR environment compared to the pre-perturbation periods (see figure 4.10). The speed profiles for the two perturbation conditions showed a decrease following the onset of the application of the perturbation. This went back to values of normal conditions after the offset of the perturbation. I reasoned that such a response could be due to a speed-vs-accuracy trade-off and/or due to an increase in muscular stiffness to counteract the external force and maintain control of the sphere while turning.

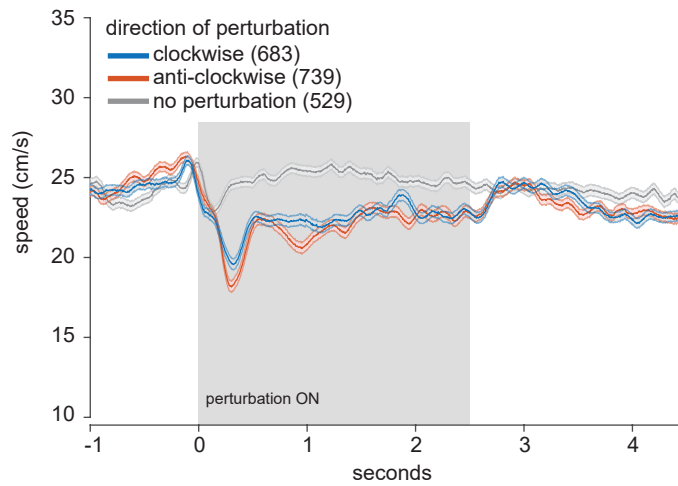


Figure 4.10: Speed profiles during perturbation conditions.

4.3 Discussion

In this chapter I have described the results of the behavioural training for a VR task and how subjects respond to a novel mechanical perturbation I integrated in the VR system outlined in the previous chapter.

Although there have already been reports of mouse behaviour in VR tasks, to the best of my knowledge, this is the first time that a VR behavioural task has been implemented within a motor control framework. The VR task requires the mouse to navigate through a corridor of variable lengths and widths to receive water rewards. To do this, mice underwent four training phases specifically aimed at: 1) increasing the familiarisation with VR system; 2) strengthening the association of the advancement in the VR corridor with the water releases; 3) developing motor skills to avoid the lateral walls; 4) increasing the throughput (number of trials) for every session. According to the measures of performance chosen, namely normalised distance run per trial, trials complete per minute, and air puffer activation time, twenty-one out of 28 mice learnt to complete the VR task within 25 sessions.

A limitation of this protocol could be the training duration. Previous studies reported a shorter training duration compared to this protocol. This is mainly for two reasons. First, mouse VR systems were developed to assess spatial navigation (Dombeck, Graziano, et al., 2009; Harvey, Collman, et al., 2009) and working memory (Harvey, Coen, et al., 2012) therefore the motor performance was not crucial nor it was reported in details. Second, in most systems, the VR stimulus was based on the Quake game engine (id Software, USA) whose physics and control of the virtual avatar are different from the the system reported here. For example, the virtual mouse body occupies a space of 8 cm (of unknown shape). Considering the virtual linear track used in those reports was only 9-cm wide, the actual width of the track was only 1 cm. In practice, the mice simply moved back and fourth in the track without actually controlling their transverse position as shown here.

Controlling a two-dimensional VR position on a air-supported sphere with three degrees of freedom is a more complex task for a head-fixed mouse. For this reason only 75% of the mice used here were able to finish the training protocol and only six out of the nine animals subjected to

the perturbation paradigm were kept for analysis. Only the training sessions with perturbation paradigm in which air-puffers were active for less than 0.01% of the session duration were used for further analysis. This threshold, that was found empirically, led to the exclusion of three subjects (a sample session of one of these is shown in figure A.19 in the appendix) and a total of 12 sessions from the data of the subjects presented here.

It would be advisable to shorten the training protocol duration by using more VR systems in parallel and running multiple sessions during one day as opposed to normally one. Schmidt-Hieber and Häusser, 2013 used an approach similar to mine for which subjects had no restricted access to water. This would definitely reduce subjects distress of the the training period.

The newly developed mechanical perturbation paradigm can influence the mouse VR trajectories systematically in either direction. The VR position of the subjects was shifted significantly following a short application of perturbation by the lateral air-jets. The results show that mice counteracted the external force within two seconds by correcting their running trajectory to the opposite direction. The subjects corrected their trajectory based on the evaluation of their heading direction rather than the proximity to the walls. Although these are preliminary results, these data suggest that mice integrated optic flow information to control their position.

Subjects could respond to heading direction shifts by integrating optic flow information. As a mouse heading deviates from a neutral heading direction, differences in optic flow between the two sides might prompt the counter-steering - essentially an optomotor stabilisation reflex. Optic flow processing is a strategy used by humans to adapt locomotion (Gibson, 1958; Warren et al., 2001; Bruggeman et al., 2007). It is important to remember that head-fixation is an unnatural condition for mice, in these conditions the vestibular inputs are largely suppressed and eye movements are different compared to freely moving animals (Wallace et al., 2013). Nonetheless, further experiments would be required to verify this hypothesis in combination with eye movement measurements to reveal what exact visual information is used during locomotion steering. Moreover, the application of only visual perturbation with similar characteristics to the one presented here might provide a useful control experiment to evaluate the contributions of the proprioceptive feedback to the behavioural responses observed. For example, such paradigm could help understanding whether the proprioceptive feedback might be driving the response

of decreasing the locomotion speed during the perturbation compared to the pre- and post-perturbation periods (see figure 4.10), whether it affects the trajectory correction delay, and if the post-perturbation heading towards the opposite direction of the perturbation period is an effect of the removal of the perturbing force in the sphere - see figure 4.8.

The application of a perturbation paradigm in VR systems brings interesting possibilities in the range of possible studies for investigating the neural bases of adaptive locomotion and probing computational theories of motor control and learning, especially in the cerebellum. Similar to what has been done in primates (Pasalar et al., 2006; Hewitt et al., 2011; Laurens et al., 2013), it would be possible to observe neural responses to various perturbation types, including application of external forces, alterations of the physical laws within the VR environment or rupture of the natural closed loop of sensations-actions. An attractive perspective would be to combine it with transgenic animals and optogenetic techniques to assess the role of single classes of cells in motor control.

CHARACTERISATION OF NEURAL RESPONSES TO LOCOMOTION IN THE MOUSE CEREBELLUM

Observation of neural activity in the cerebellum has provided conflicting evidence regarding the neural responses of cerebellar neurons to locomotion. Although Purkinje cells in the cat were not observed to have substantial firing rate modulation by walking speed on a treadmill (Armstrong and Edgley, 1988), it was recently observed that the firing rate of Purkinje cells, averaged within single steps, can be modulated both negatively and positively by locomotion speed in freely running rats (Sauerbrei et al., 2015). Golgi cells were also shown to discharge rhythmically during locomotion, however no modulation by the speed of locomotion was observed in this case (Edgley and Lidieth, 1987). In contrast, granule cells and molecular layer interneurons of mice on a spherical treadmill increase their firing rate during locomotion relative to stationary periods (Ozden et al., 2012; Powell et al., 2015), leaving open the question of whether and how cerebellar neurons are tuned to the speed of locomotion.

Electrophysiological recordings of single units in the cerebellum validated the relationship between behaviour and neural activity, but have thus far failed to account for population dynamics, providing a confined view of the cerebellar neural code. In fact, cerebellar circuitry is characterised by a high degree of feedback, feed-forward and collateral connections (Ito, 2006; Coddington et al., 2013; Mathews et al., 2012; Rieubland et al., 2014; Astorga et al., 2015), and a distinctive

divergence-convergence configuration of inputs and outputs (Napper and Harvey, 1988; Person and Raman, 2012). There are therefore important unanswered questions as to how locomotion-related information is conveyed by ensembles of cerebellar neurons, and what type of neural code is employed.

To address these questions, I recorded from movement-sensitive populations of neurons in lobules V-VI of the cerebellar vermis of mice navigating in a virtual reality (VR) environment. I characterised neurons whose activity is modulated by kinematics parameters such as locomotion speed and yaw rotation. Locomotion speed could be reconstructed with high accuracy with a simple linear decoder of neural activity of these units. These findings indicate that cerebellar activity is strongly modulated by kinematic parameters.

5.1 Subjects

Data used in this section were collected from fourteen mice - 16-24 weeks old. Following the behavioural training described in the previous chapter, multi-electrode array extracellular recordings were made from lobules V and VI of the cerebellar vermis (see figure 2.1). Four animals did not receive behavioural training, instead were allowed to run in the dark, with the virtual reality stimulus switched off, as a control group to discern the influence of visual feedback on locomotor speed encoding. Animals spent on average $57 \pm 3\%$ (mean $\pm$ s.e.m., $n=39$ recording sessions) of each recording period running (defined as speed exceeding 1 cm/s). For each animal, the recording electrode was positioned at a number of different depths (39 recordings in total, 311 units; see Figure 2.1C). Action potentials were detected and clustered to perform spike sorting (Rossant et al., 2016).

For each identified unit, cell type was determined according to recently published classification criteria (see section 2.3.1 for details). Approximately 74% of cells were ascribed to a main cerebellar cell class between Purkinje cell, granule cell or interneuron, see Figure 5.1. The classification grouped together all interneuron types, i.e. basket, stellate and Golgi cells, because of the sequential steps of the decision tree: from step 2, identified units that are not Purkinje or granule cells could only be interneurons, see Figure 2.1C. Since the entropy and logCV values (see

CHAPTER 5. CHARACTERISATION OF NEURAL RESPONSES TO LOCOMOTION IN THE MOUSE CEREBELLUM

Table 2.1 for formulae) appear to be similar for all units (5.1B and C), it is difficult to distinguish between cell classes. Since I could not rely on the presence of complex spikes for methodological reasons, the mean spiking frequency was used as main criterion to identify putative Purkinje cells. As they are known to have a spontaneous firing rate, units were considered to be putative Purkinje cells if the main spiking frequency is larger than 30 Hz regardless of the entropy figure (Van Dijck et al., 2013) raising the count of Purkinje cells to 106.

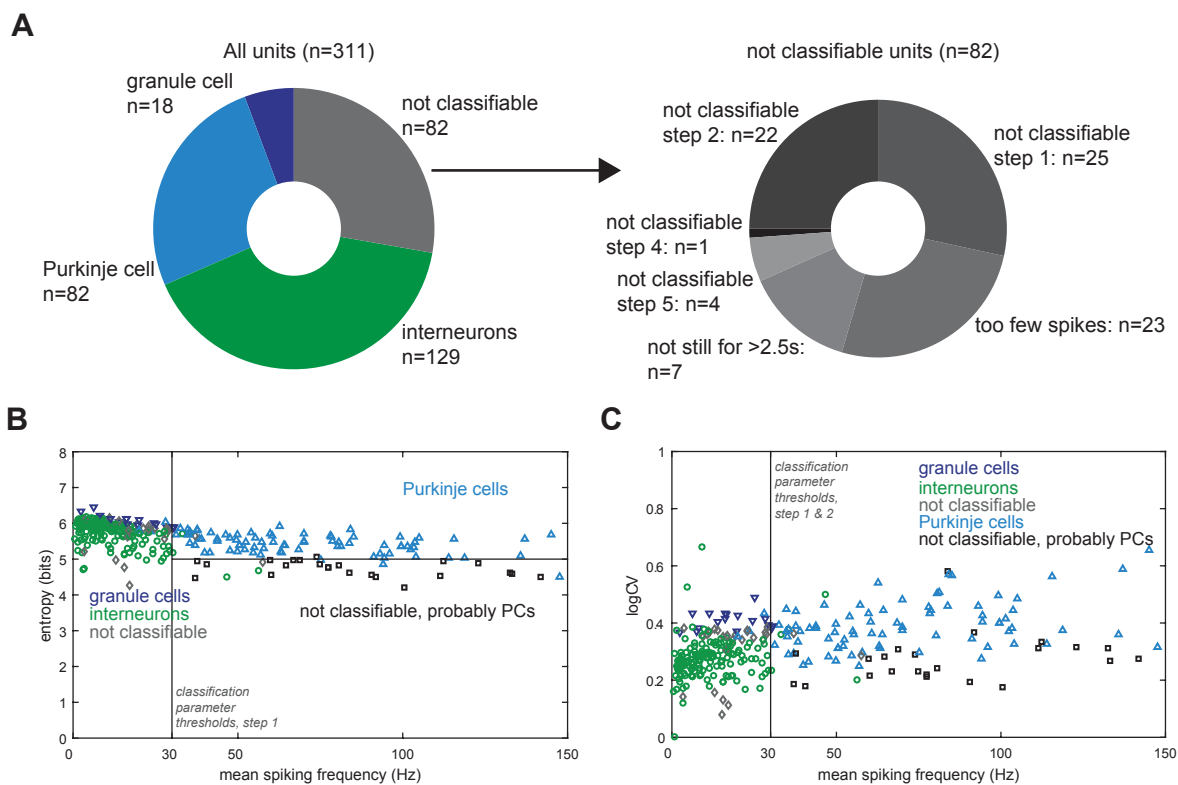


Figure 5.1: Results of cell classification. **A)** the classification algorithm ascribed a cerebellar cell class to 229 units out of 311. Right: break down of not classifiable units. **B)** Entropy and mean spontaneous spiking frequency of all units. Black lines show parameter thresholds for the first step of the classification algorithm. **C)** Logarithm of the coefficient of variation of two consecutive ISIs and mean spontaneous spiking frequency of all units. Black line shows the parameter threshold for first classification step.

5.2 Cerebellar neurons respond to speed of locomotion

The activity of many units correlated with the behavioural status of the mouse, i.e. firing rate changed with speed (Figure 5.2A-C). To determine whether neural activity correlated with speed of locomotion, I computed speed tuning curves for the firing rate of each unit (see figure 5.2D-F).

CHAPTER 5. CHARACTERISATION OF NEURAL RESPONSES TO LOCOMOTION IN THE MOUSE CEREBELLUM

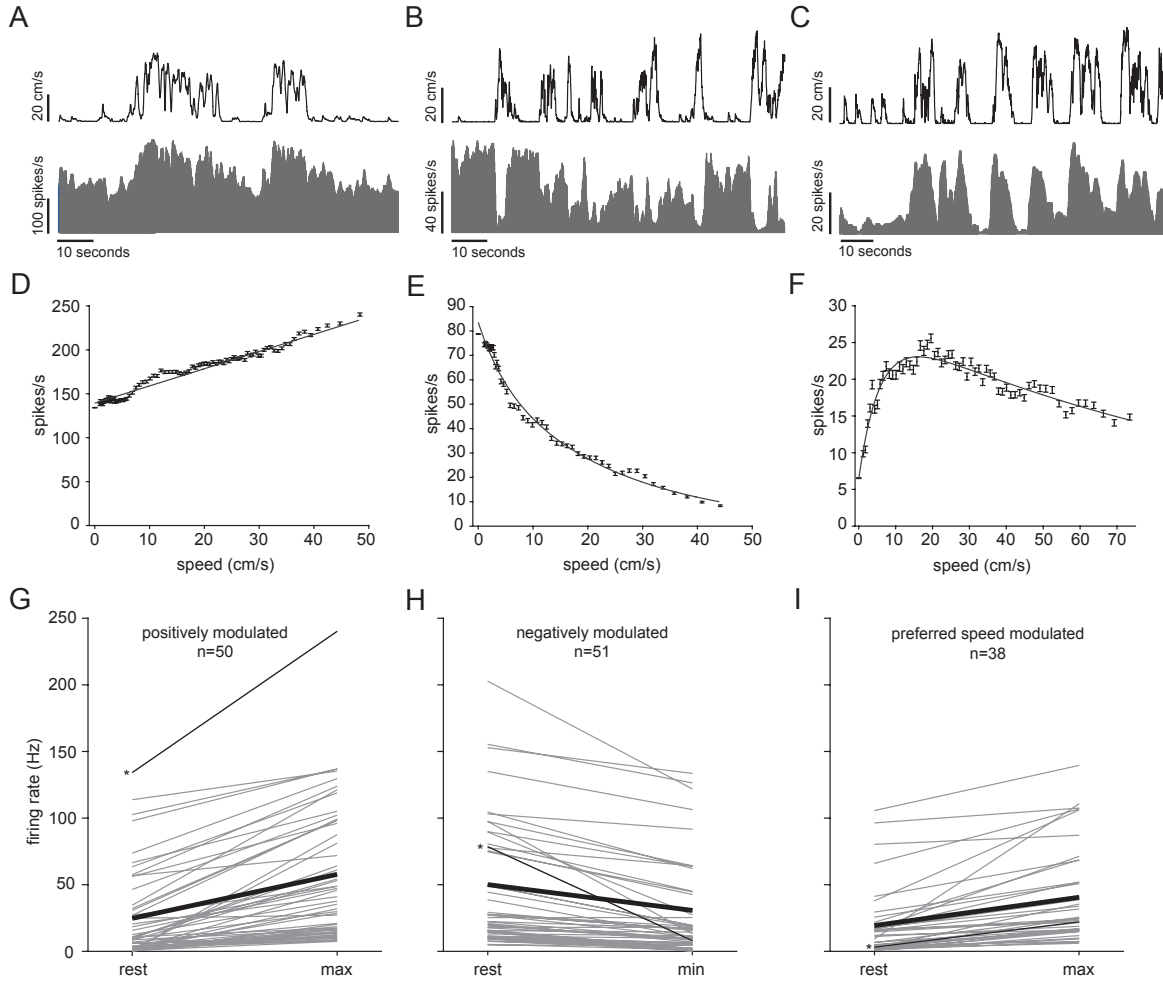


Figure 5.2: Cerebellar neurons response to locomotion speed. **A-C)** Speed and instantaneous firing rate of three example units for the three response profiles observed (bins = 0.5 seconds). **D-F)** Speed tuning curves of the examples in A-C; error bars are standard errors. **G-I)** Mean firing rate during stationary periods and speeds at which maximal firing rate is recorded. Gray lines are single units; thin black lines with asterisks on the left indicate units shown in A-C and D-F; thick black lines are average firing rates of all units within each response class.

To assess the significance of speed modulation, I shuffled the data 100 times and compared the variance of the original curve with that computed from the shuffled data. If this variance was greater than the values of at least 99 shuffled curves, the unit would be considered to be significantly speed modulated (Saleem et al., 2013; Kropff et al., 2015). I also checked whether the changes in firing rate were due only to changes in excitability between stationary and moving periods (i.e. if cells were driven by locomotion, but their activity was not modulated by speed) by repeating the above procedure but only considering speeds >1 cm/s. 159 units were found to respond to locomotion movements: 20 showed a binary response and the remaining 139 were

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modulated by speed. For these units, three classes of modulation profile (tuning class) were observed: units whose firing rate monotonically increased ($n=50$) or decreased ($n=51$) with speed, and units whose firing rate reached its maximum at a preferred speed that is $\leq 70\%$ the maximal speed achieved by the mouse ($n=38$).

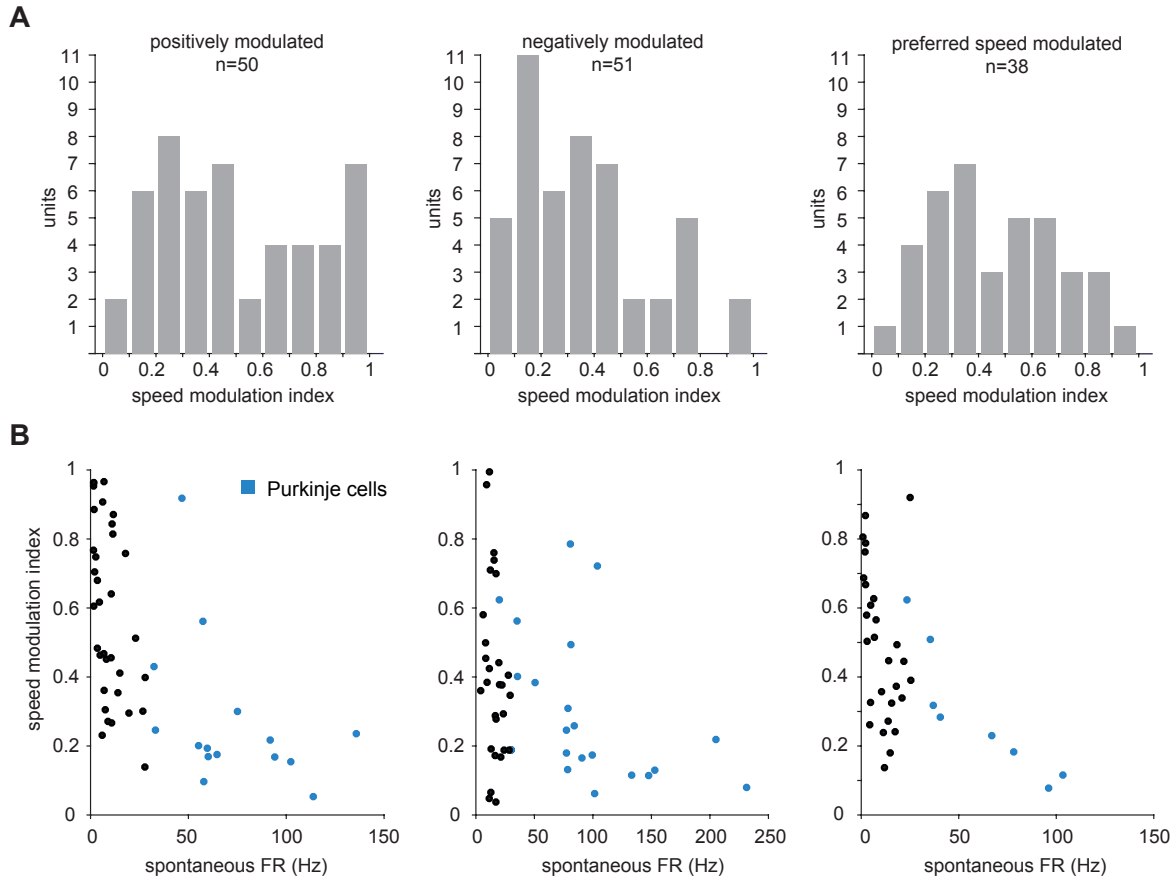


Figure 5.3: Modulation of speed tuned units. **A)** Modulation indexes for all the units significantly modulated with speed, divided by response type. **B)** Modulation index as a function of spontaneous firing rate (i.e. during stationary periods), divided by response type.

On average, positively modulated units had lower spontaneous (animal at rest) firing rates compared to those cells whose firing rate decreased with speed (Figure 5.2 G,H). Firing rate changed from 24.7 ± 4.6 Hz during resting periods to 52.2 ± 7 Hz (mean $\pm$ s.e.m., $n=50$) at maximal locomotion speed. For negatively modulated units, firing rate decreased from 50 ± 7.6 Hz under the resting condition to 30 ± 6.1 Hz (mean $\pm$ s.e.m., $n=51$) during locomotion at maximal speed. Units showing a preferred speed had on average less marked changes going from 19.3 ± 4.2 Hz at rest up to 40 ± 5.7 Hz (mean $\pm$ s.e.m., $n=38$) at the maximum speed observed (see figure 5.2I).

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I then examined whether the modulation of single cells differed within and across response tuning classes (see figure 5.3). The modulation index was defined as the ratio between the difference and the sum of the maximum and minimum firing rates measured on each tuning curve. Modulation indexes for all response types varied heterogeneously across the whole range. I found that for all response tuning classes the modulation of firing rate was negatively correlated with spontaneous firing rate. In fact, putative Purkinje cells showed lower values of modulation as compared to other cell types (see figure 5.5 B).

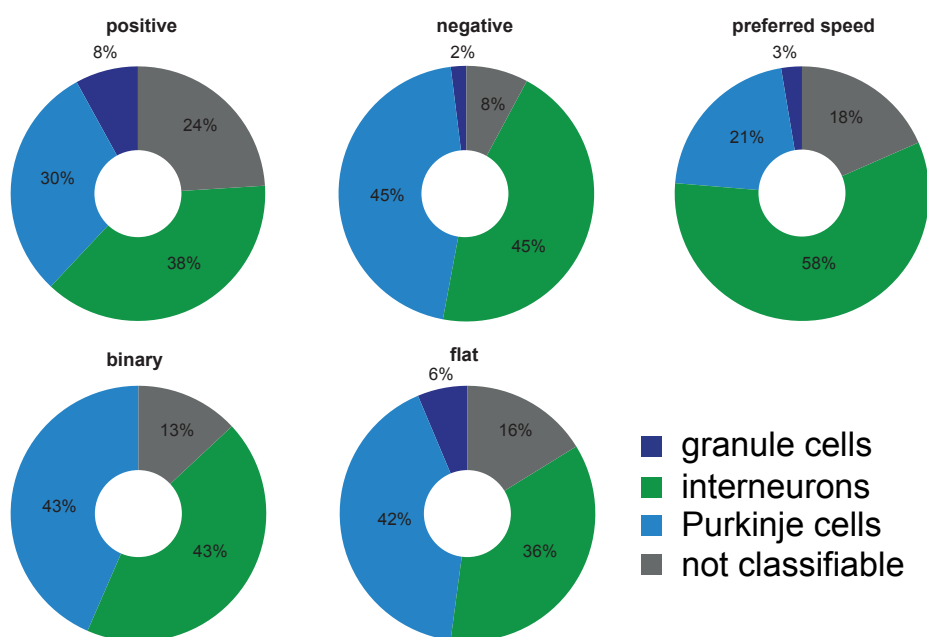


Figure 5.4: Breakdown of putative cell class for each response type.

The distribution of putative cell types with similar response profile did not show any apparent correlation with the type of response (see Figure 5.4). All cerebellar cell classes - Purkinje cells, interneurons, and granule cells - were found to respond to locomotion speed with all different tuning profiles. Units belonging to the same response tuning class were not observed to cluster spatially either: in only 8 out of 39 recordings did I find units belonging to the same response class in close proximity (i.e. in the same electrode shank - distance between electrodes $<140\ \mu\text{m}$). In all remaining recordings ($n=27$) in which I found multiple units responsive to movement in close proximity, their response type was heterogeneous - see Figure 5.5 A. Moreover, units responsive to movement were observed in all animals, with no discernible dependence on depth

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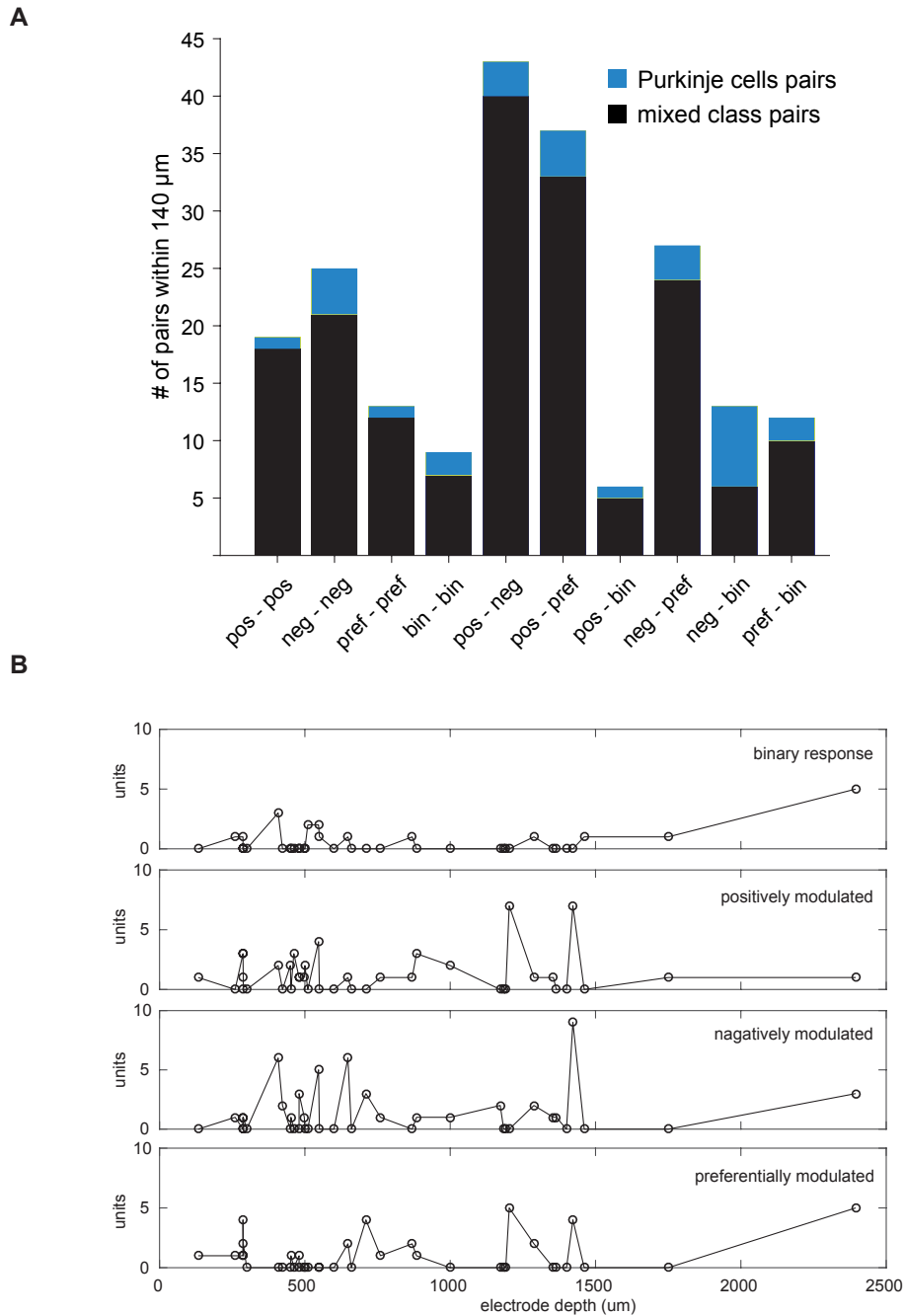


Figure 5.5: Spatial distribution of units. **A)** Number of pairs in a single shank classified per response type combination. A single shank contains eight sensing electrodes whose maximal distance from each other is 140 micrometres. Pos: positively modulated; neg: negatively modulated; pref: maximally responding at a preferred speed; bin: binary response. **B)** Poll of units responsive to movement with respect to the depth of each recording.

of recording site (see figure 5.5 B). To check whether these responses were caused by the visual feedback, recordings made in the dark from naïve (untrained) mice were compared with data

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from trained animals. Similar profile responses were observed and no differences in the level of modulation or fraction of responding units were found from these two groups. These units were therefore analysed conjointly (see figure 5.6).

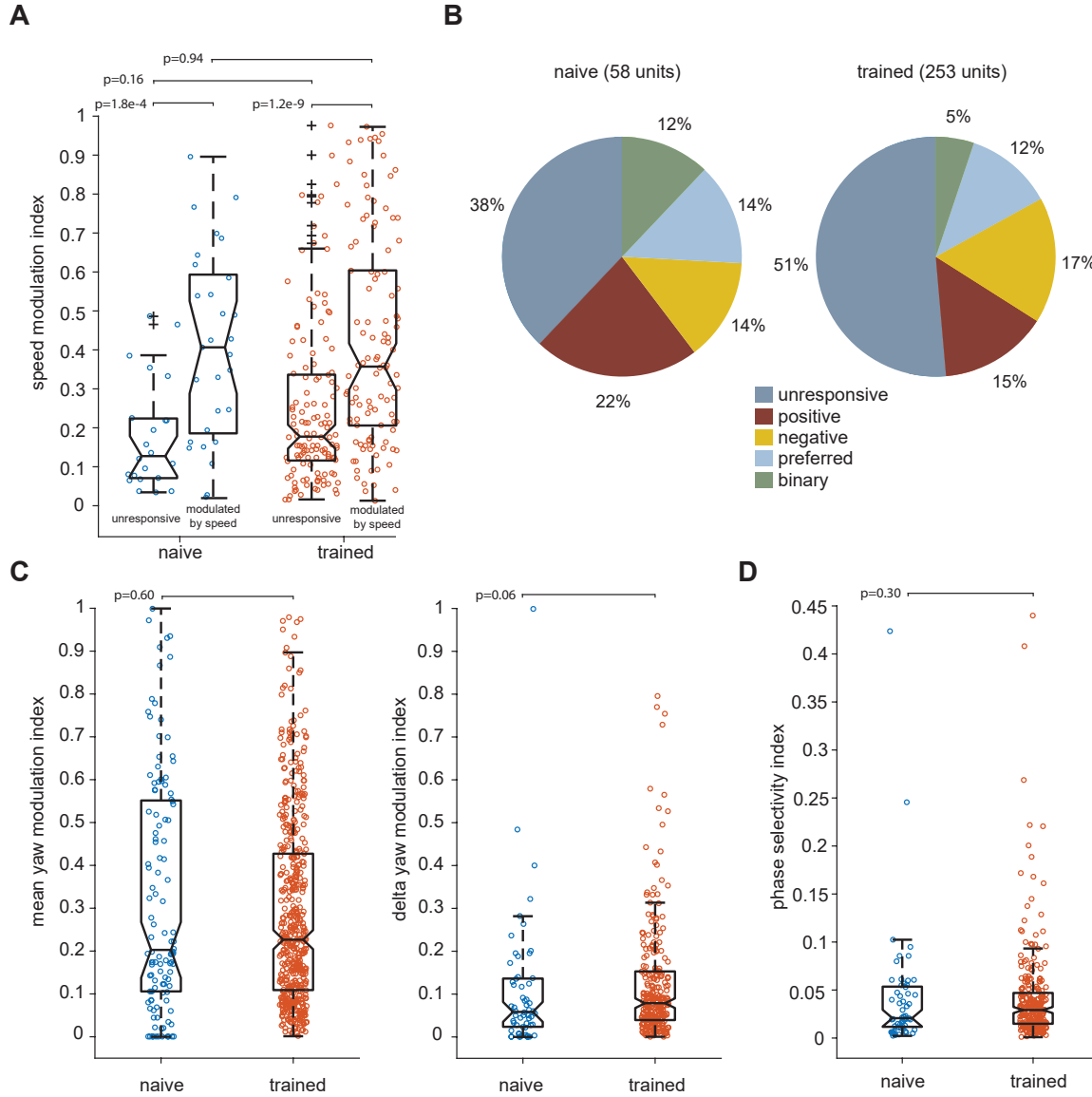


Figure 5.6: No differences in the neural populations recorded in naïve or trained animals. **(A)** The speed modulation indexes are not significantly different between naïve (total number of units=58, units modulated by speed=29) or trained animals (total number of units=253, units modulated by speed=110) for unresponsive or speed modulated units. **(B)** Classification of speed-response profiles in the naïve versus trained conditions: the percentages of each type is similar in both conditions. **(C)** Both the mean and the absolute difference between the modulation indexes between CW and CCW yaw tuning curve (delta yaw modulation index) are not significantly different. **(D)** No significant difference is found in the phase selectivity index (PSI) in the naïve versus the trained animals. P-values of Mann-Whitney U-test between the population pairs indicated by the line segments.

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Taken together, these results indicate that cerebellar neurons respond to locomotion speed by either increasing or decreasing their firing rate or responding maximally to a particular speed. Although, the classification of the cell types can not be verified, the breakdown of putative cell class for each response type suggests that all cerebellar neuron types might be modulated by locomotion speed. Furthermore, the amount of modulation might depend on the spontaneous (stationary) firing rate. Units within the same shank, i.e. in close proximity, were found to have heterogeneous response profiles indicating that there might not be a spatial clustering of units with the same tuning profile. This observation suggests that nearby cerebellar neurons, possibly belonging to the same micro-zone (Oscarsson, 1979), have opposite tuning properties and might integrate different sensorimotor information related to locomotion behaviour.

5.3 A subset of cerebellar neurons are tuned to phase of stepping cycle

Previous studies of the role of the cerebellum in locomotion control found units that are rhythmically modulated with stepping cycle (Orlovsky, 1972; Armstrong and Edgley, 1984; Armstrong and Edgley, 1988; Edgley and Lidieth, 1987; Powell et al., 2015; Sauerbrei et al., 2015). One way to produce speed tuning would be for a unit to respond by spiking at a fixed phase per step cycle, thus producing higher firing rates the more step cycles occur per second. I therefore examined whether this was the reason behind the tuning of activity to locomotion speed. Since pitch velocity is measured by motion sensors parallel to the vertical axis, with velocity sampled at a high polling rate ($f=200$ Hz), it was possible to detect the vertical oscillations caused by the mouse stepping on the sphere (see figure 5.7A). The high frequency periodicity of the signal was extracted from the original pitch velocity signal by transforming the pitch velocity with the Hilbert operator. At least 393 putative step cycles were found for each recording session (1724 ± 158 , mean $\pm$ s.e.m., $n=39$). I then measured the correlation coefficient between the firing rate (bin width = 5ms, smoothed with a 20-ms Gaussian window) and the phase of the step cycle for each unit (see figure 5.7 B) finding that only 57 units out of 311 showed a significant modulation with the stepping cycle ($p \leq 0.001$, χ^2 test for uniformity). Of these, thirty-two units were also significantly modulated by locomotion speed and only four show a binary response. Only six units were identified as Purkinje cells, two as granule cells, 38 as interneurons, and the remaining were not classified. Furthermore there was no distinct profile type of response to locomotion speed as shown in the inset pie chart in figure 5.7 B.

Mean preferred phases of the modulated units were distributed across the stepping cycle, with approximately two-thirds of the response covering on average 2.2 ± 0.06 (mean $\pm$ s.e.m, $n=57$) radians, as measured by the standard circular deviation (Drew and Doucet, 1991) (see figure 5.7B).

To quantify the step phase modulation of the response, I used an approach commonly used in the study of orientation tuning, an analogous problem (where here phase within a step cycle replaces orientation within a circular stimulus space). I calculated the normalised phase orientation vector and computed the orientation selectivity index (Mazurek et al., 2014), renamed

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here the phase selectivity index, PSI. Units significantly modulated by step phase have higher phase selectivity indexes in comparison to non-modulated units (see figure 5.7D).

The activity of 57 out of 311 cells was found to be modulated by the stepping cycle. This subset of units only partially overlaps with units modulated by speed of locomotion - 36 neurons out of 57. Moreover, their response profiles are varied suggesting that the speed modulation might not be a by-product of the rhythmic modulation of the stepping cycle as indicated in a previous study (Sauerbrei et al., 2015).

Similarly to recordings from Purkinje and granule cells (Orlovsky, 1972; Armstrong and Edgley, 1984; Armstrong and Edgley, 1988; Edgley and Lidiérth, 1987; Powell et al., 2015; Sauerbrei et al., 2015), these results suggest that also cerebellar interneurons might be modulated by the stepping cycle. The lack of validation of the cell identity, however, does not provide a definitive proof in these regards.

A

pitch velocity
20 cm/s
5 s

cm/s
80
60
40
20
0

phase
 2π
0

spikes
0.5 s

cm/s
60
40
20
0

phase
 2π
0

spikes
0.1 s

unit 2

B

units
60
50
40
30
20
10
0

cell class
nc
pc
gc
in
sn
ss
ps
pn
pp
nn
ns
ps
pn
pp
nn
ns

stepping cycle
0
 π
 2π

modulated by step phase and speed ($n=32$)
modulated by step phase with binary response ($n=4$)
modulated by step phase but not with speed ($n=21$)

speed response profile

Response Type	Count
unresponsive	21
positive	10
negative	9
preferred	13
binary	4

C

step cycle
 $1/2\pi$ 1
0.8
0.6
0.4
0.2
0

normalised FR

π

unit 2

PSI = 0.4
mean angle = 6.1 rad
angular deviation = 1.4 rad

D

PSI
0.45
0.35
0.25
0.15
0.05
0

speed modulation index
0.2 0.4 0.6 0.8 1

modulated by step phase and speed ($n=32$)
modulated by step phase with binary response ($n=4$)
modulated by step phase but NOT speed ($n=21$)
putative Purkinje cell (colour coding as above)

modulated by step phase ($n=754$)
non-modulated by step phase ($n=754$)

78

5.4 A subset of cerebellar neurons display yaw direction tuned responses

Each animal reached the end of the virtual corridor following a path that best suited them. While some drove the sphere straight along the virtual corridor, others tended to take a more curved trajectory towards the target location marking the end of each trial. As the animals exerted more strength on either one or the other side of the body when turning the sphere, I examined whether this asymmetric use of limb muscles was reflected in cerebellar neuronal activity. By extracting the yaw movement information from the motion sensors, I examined how the firing rate changed with respect to the sphere rotations around the clockwise (CW, negative yaw) and counter-clockwise (CCW, positive yaw) direction (example trace, top of figure 5.8A).

To do so, I computed tuning curves for neural activity in response to CW and CCW yaw directions, and calculated the modulation index for each cell in the two directions. For a few units, the neural response was clearly one-sided (see figure 5.8A). This was reflected in their tuning curves, and in the absolute difference between the modulation indexes of the tuning curves computed for the negative (CW) and positive (CCW) yaw directions (delta yaw modulation index - see figure 5.8B,C). Most cells, however, responded equally to either yaw direction, showing a decrease (see figure 5.8D) or increase (see figure 5.8E) in firing rate to either CW or CCW yaw movement, in an almost symmetric fashion. This is reflected in the distribution of the delta yaw modulation indexes: 63% (195 out of 311) had a difference in modulation indexes smaller than 0.1, while only 19% (58 out of 311) was greater than 0.2 (see figure 5.8F), indicating sensitivity to yaw direction. In fact many units had similar modulation indexes for both the CW and CCW yaw directions (see figure 5.8G).

I also examined whether tuning to speed influenced the yaw-direction selectivity of the 58 cells that had delta yaw modulation index greater than 0.2. Interestingly, 32 out of the 58 yaw direction sensitive units did not modulate their activity with speed (see figure 5.9A). There was also no evident relationship between the speed modulation index and delta yaw modulation index (see figure 5.9B).

These results suggest that there are units in the cerebellum that respond selectively to the

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use of one side of the body in preference to the other during locomotion steering, and that these cells are not necessarily influenced by changes in speed. It is important to remark the fact the walking on a spherical treadmill differs from natural conditions as the sphere can also move laterally (roll rotation). Therefore muscles that are not normally engaged during locomotion (e.g. adductors) might be constantly used to stabilise the sphere. Tests in freely moving animals might provide the control condition for such phenomenon.

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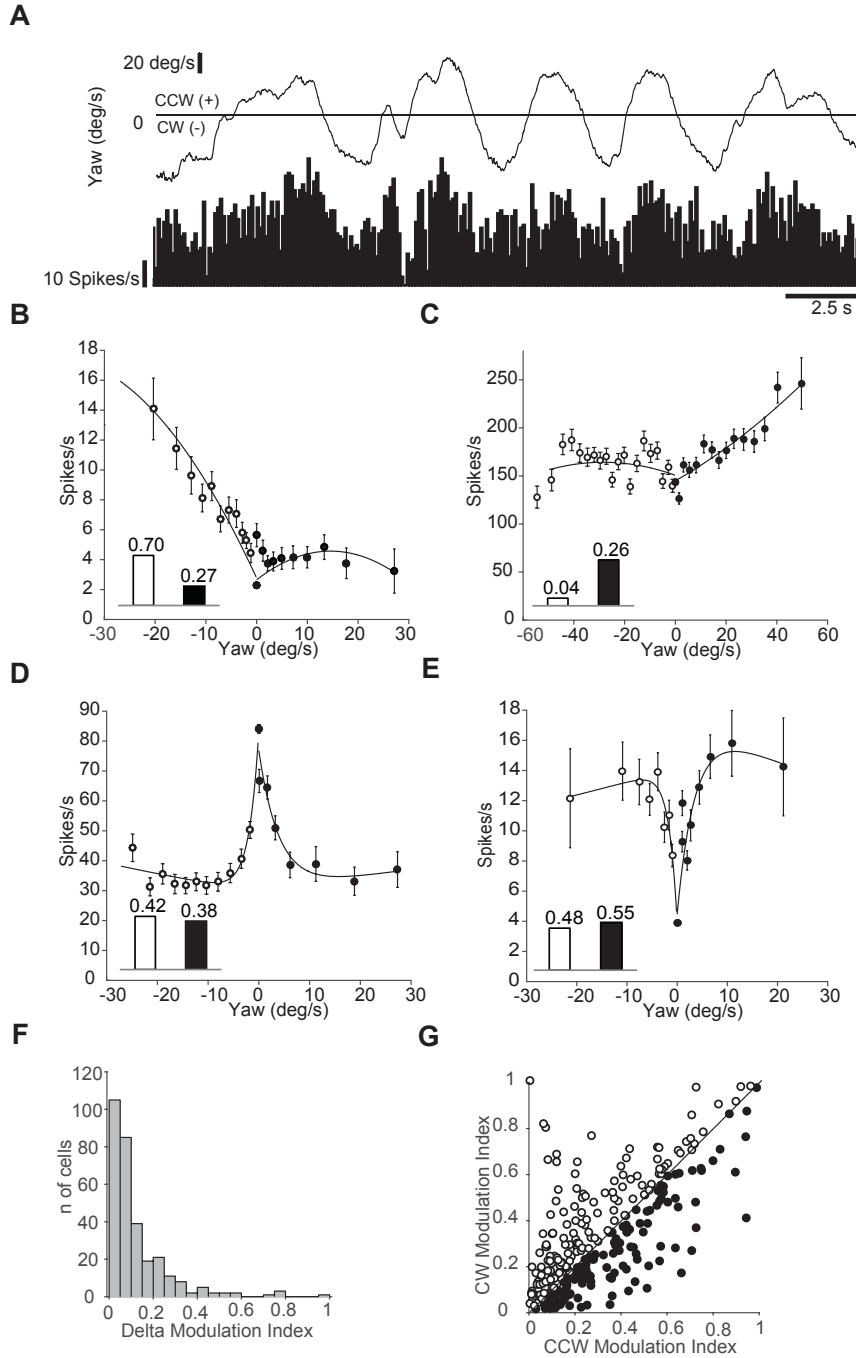


Figure 5.8: Cerebellar neuronal responses to yaw direction. **A)** Example unit tuned to CCW yaw direction (positive): top, yaw trace in deg/s; bottom, mean instantaneous firing rate, bin width 100 ms. **B-C)** Two example units preferentially responding to yaw-turning in the CW (**B**) or CCW (**C**) ; each data point is formed by more than 900 bins (100ms width); error bars are s.e.m.; inset bars indicate the modulation index values derived from the two curves. **D)** Example unit decreasing its activity in both yaw directions. **E)** Example unit that increases its activity in either yaw direction. **F)** Distribution of the difference in modulation index between the CW and CCW yaw direction. **G)** Population plot of modulation index values of each unit for the CW and CCW direction (n=311).

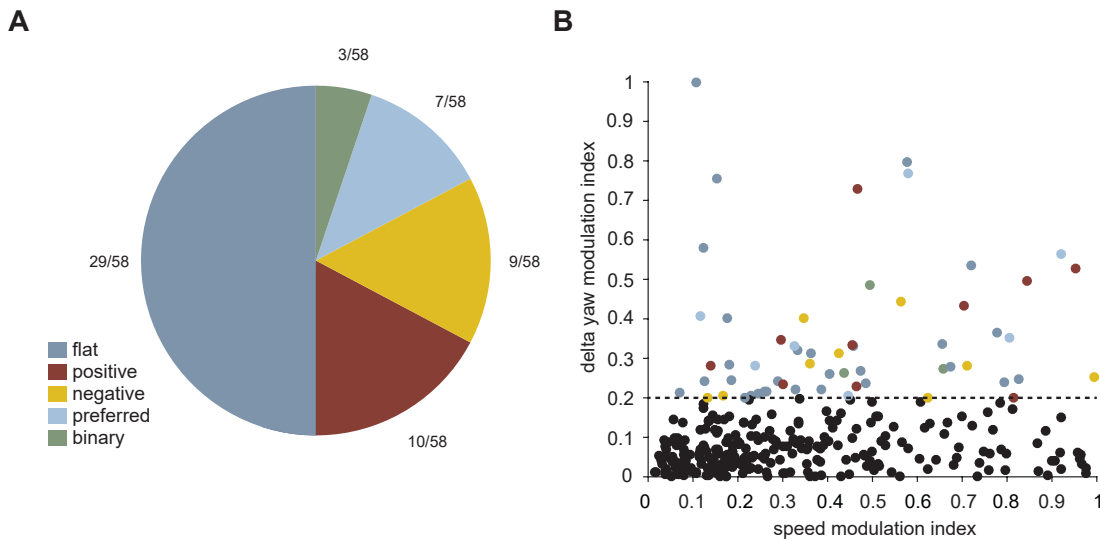


Figure 5.9: Yaw tuning responses with respect to speed tuning responses. **A)** Out of the 58 units with an absolute difference in modulation indexes between CW and CCW yaw tuning curve (delta yaw modulation index) larger than 0.2, more than half are not modulated by speed, while the rest are evenly spread amongst the other response categories. **B)** A large delta yaw modulation index is not related to the speed modulation index (n=311). The dashed line is the threshold that separates the 58 units represented in A.

5.5 Multiple kinematic parameters are represented in the activity of cerebellar units

I have described units tuned for speed of locomotion, yaw (including some tuned for direction of yaw motion), and phase of stepping cycle. It is important to determine whether these constitute separate classes of neurons, or if instead, each of the neurons displays tuning to a lesser or greater extent across each dimension.

For each cell, I therefore compared the modulation index for speed with the mean modulation index for CW and CCW yaw, and with the phase selectivity index. These are depicted in a tri-plot in figure 5.10A. It is apparent that speed, yaw and phase modulated units do not cluster in this space, but are instead distributed relatively uniformly. A similar picture arises when instead comparing the mutual information units convey about speed, yaw and step phase (see figure 5.10B). As speed and yaw are not independent - indeed, speed is comprised of both a pitch and a yaw component - I broke speed tuning up into these components and assessed them separately. Figure 5.10 C shows that units that conveyed substantial information about pitch also tended to convey substantial information about yaw (and that in fact, more of the speed

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information arose from yaw than from pitch signals). Even when units are separated by the putative cell class, I did not find bimodal distributions of information about speed, pitch, yaw nor step phase, see figure 5.10 D. I conclude that the cerebellar units examined were a relatively homogeneous population encoding all of these quantities to a greater or lesser extent, rather than a heterogeneous population comprising clusters of units encoding different kinematic variables.

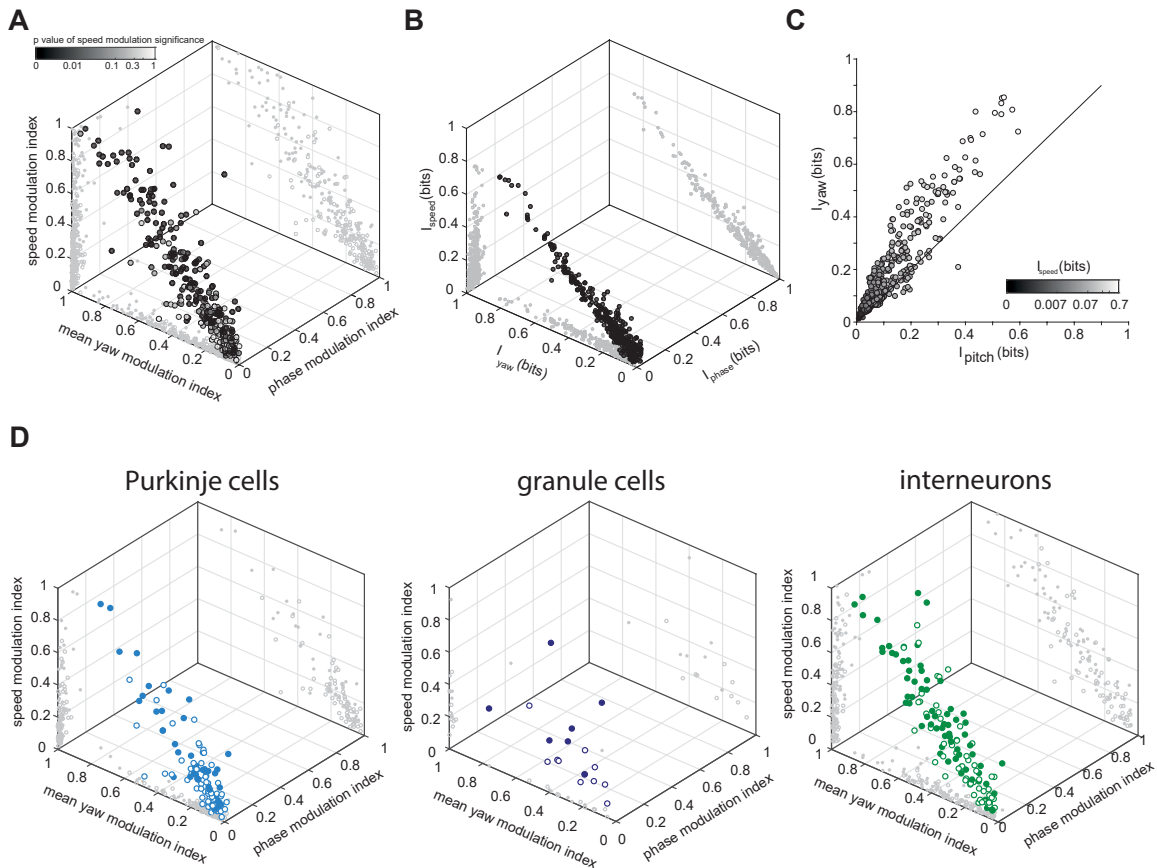


Figure 5.10: Modulation indexes and mutual information values of cerebellar units. **A)** Modulation indexes for speed, yaw and phase selectivity index. Colour coding indicates p value of test for significance of speed modulation. Mean yaw modulation index is the mean between the modulation indexes of the yaw tuning curves for clockwise and counter-clockwise directions. **B)** Mutual information values for speed, yaw and step cycle. **C)** Mutual information values of the two vectorial components of speed, pitch and yaw. Gray scale indicates the value of mutual information of firing rate and speed. **CD)** Modulation indexes as in A, separated by the putative cell type.

5.6 Cerebellar neurons better correlate with future values of locomotion

In order to understand whether the recorded units encode information about descending motor commands, I analysed the timing of the information conveyed by neuronal responses about locomotion speed, reasoning that for motor units, the firing rate should provide predictive information about locomotion speed, whereas for sensory units, the information should be largely retrospective. I computed the mutual information (Kraskov et al., 2004) between the firing rate and locomotion speed time courses for each neuron, for a range of imposed time lags. This approach has the advantage of computing the relationship between two variables (firing rate and kinematic parameter) without any assumption of linear correlation. Firing rates were shifted with respect to the speed signal by 10 ms from -500 to +500 ms (see figure 5.11A). While there were retrospective units (peak mutual information at negative time lag), the majority of speed modulated units showed a peak in the mutual information at a positive time lag of 108.1 ± 17.2 ms (mean $\pm$ s.e.m., $n=139$), suggesting that the neurons observed may encode descending motor signals rather than sensory feedback (Figure 5.11 B).

The values of maximal mutual information were significantly higher than those of non-modulated units ($p=2.3e^{-9}$, Mann-Whitney U-test): 0.24 ± 0.01 bits (mean $\pm$ s.e.m., $n=139$) for speed modulated units and 0.13 ± 0.01 bits (mean $\pm$ s.e.m., $n=172$) for unresponsive units. In fact, the lower information values - calculated at zero lag - for the latter class are consistent with the classification based on the tuning curve modulation approach (Figure 5.12).

There was no significant difference in the distributions of the mutual information at zero lag between units belonging to different putative cell classes - see figure 5.13 A. Also, the cell class did not seem to affect the time lag at which the maximal mutual information was recorded (see figure 5.13 B).

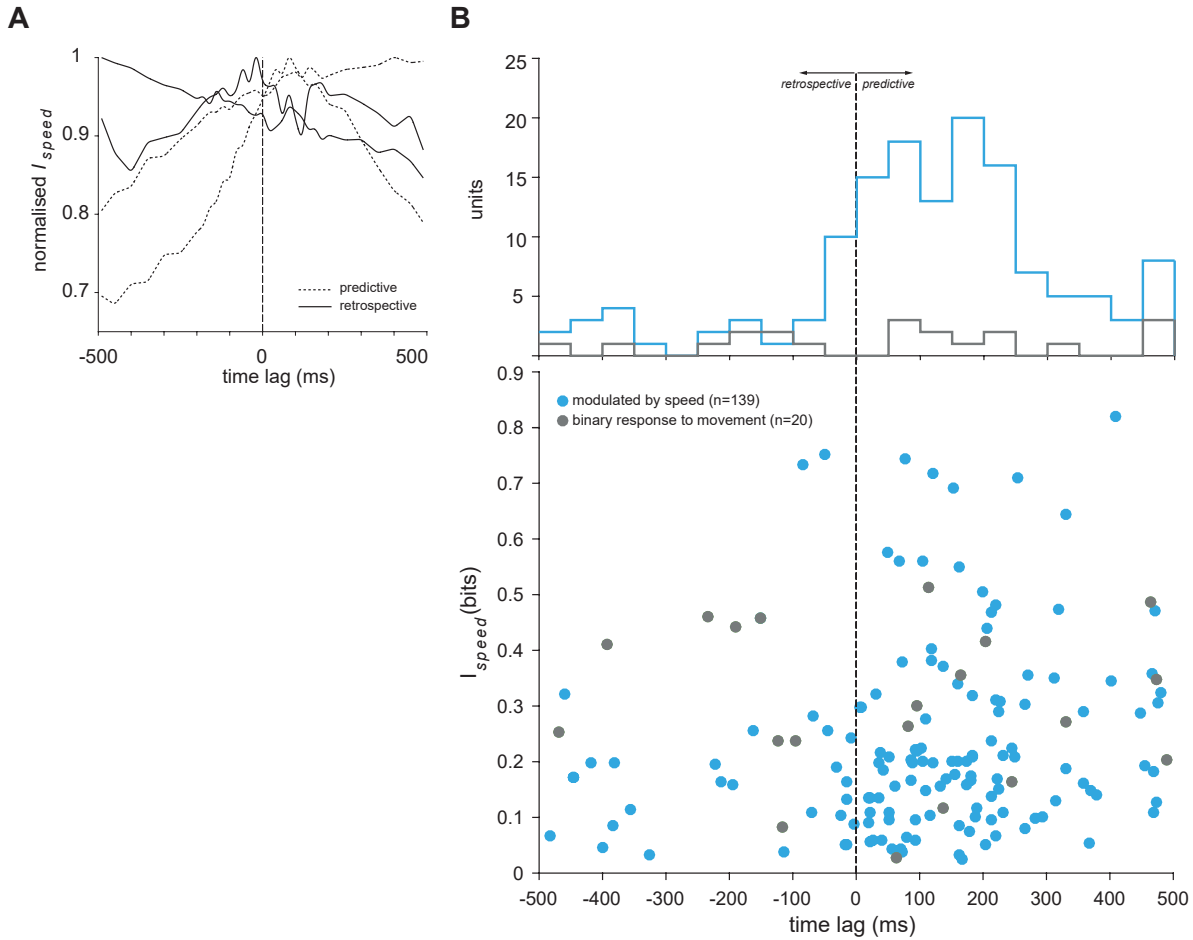


Figure 5.11: Mutual information of locomotion speed and firing rate. **(A)** Normalised mutual information of four example units for different time lags; predictive units are shown in dashed lines and retrospective units in solid lines; for each type, two examples are shown: one that either increases or decreases with time and one with a peak either before or after zero lag. **(B)** Top: Histogram of the distribution of the peaks at which maximal mutual information is found for units responsive to movement and modulated by speed (bin size = 50 ms). Bottom: maximal mutual information values of the units responsive to movement and modulated by speed with respect to the time lag.

5.7 Speed of locomotion can be linearly decoded from cerebellar neurons

It has been previously shown that neural activity of single Purkinje cells encode multiple kinematic parameters of multi-joint movements during arm reaching tasks in primates (Fortier et al., 1989; Fu et al., n.d.; Coltz et al., 1999; Roitman et al., 2005; Hewitt et al., 2011). Similarly, locomotion requires the coordination of multiple joints and muscles. Since all units were shown to

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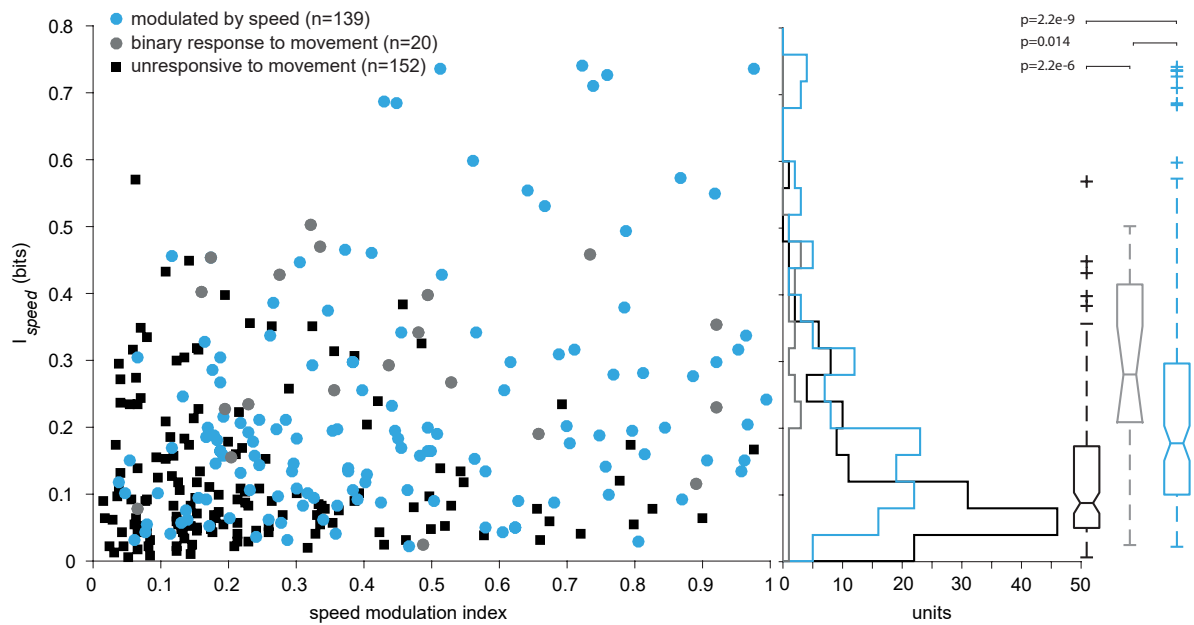


Figure 5.12: Mutual information versus speed modulation index. Mutual information of locomotion speed computed at zero lag as a function of the modulation index for speed modulated for all units. P values of Mann-Whitney U-test between the three distributions.

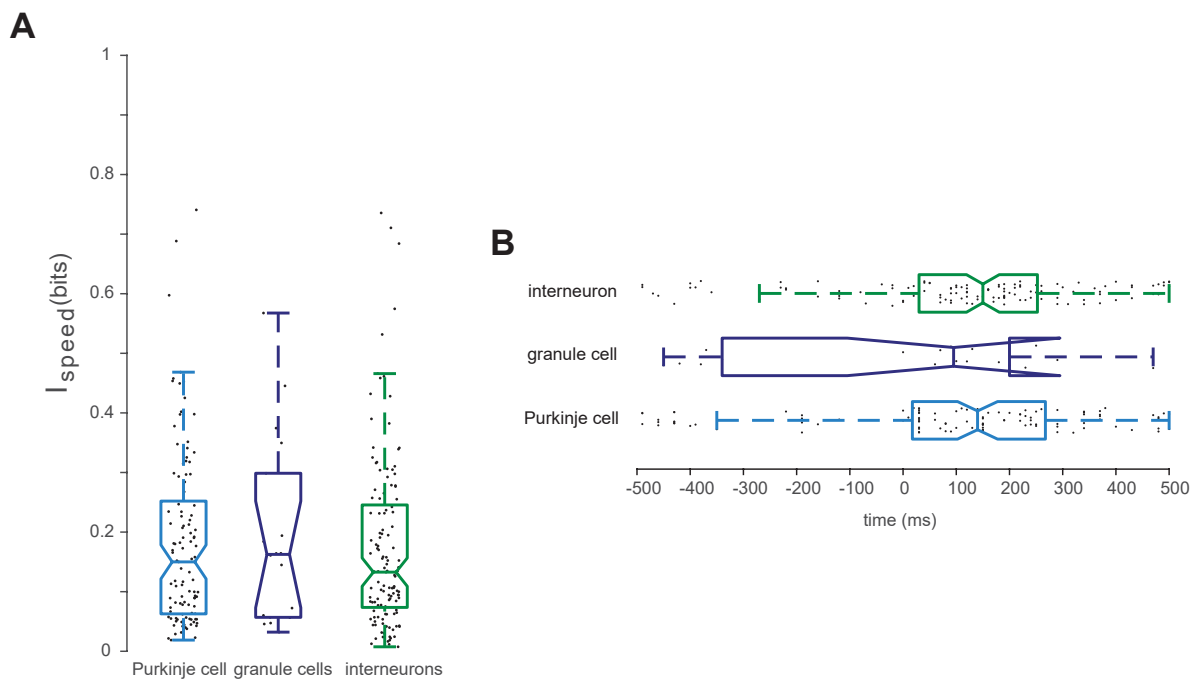


Figure 5.13: Mutual information of different cerebellar cell classes. **A)** Mutual information values at zero lag for Purkinje cells, granule cells, and interneurons. **B)** Time lag at which mutual information values is maximal for Purkinje cells, granule cells, and interneurons.

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encode multiple kinematics parameters to a greater or lesser extent, I set out to find out whether locomotion speed could be accurately reconstructed by the neural activity of single cerebellar neurons.

To this end, I implemented a simple linear decoder (Kropff et al., 2015) with the aim to reconstruct the locomotion speed time course from the firing rates of each unit (Figure 5.14 A). As a measure of the decoding accuracy, I used the Pearson correlation coefficient between the decoded and the original recorded trace. The decoder was trained on 70% of the data of each recording and tested on the remaining 30%.

First, I looked at whether the decoding accuracy was affected by the classification of units based on the tuning curve properties. As expected, on average, units responsive to movement provided a higher decoding performance compared to the units with a flat tuning curve (Figure 5.14 B). The distribution of correlation coefficients of responsive units was significantly higher than the one of flat units and there was no significant difference in the decoding performance for different putative cell classes.

The decoding accuracy was found to be affected by the width of the Gaussian filter used to smooth the firing rate and the speed traces. In fact, as the smoothing window for both the firing rate and the speed trace widened, the median accuracy of the decoder between the reconstructed and the original speed increased (Figure 5.14 C).

These results indicate that the cerebellar units are strongly modulated by the locomotion speed as a simple linear decoder based on a single unit firing rate can accurately reconstruct the locomotion speed. Although the median accuracy was shown to be higher for units responsive to movement, units identified as having a flat tuning curve could provide with a higher decoding performance than the units modulated by locomotion speed. This effect was still present when I checked whether the accuracy of the decoder was affected by non-linearities of units with a preferred speed or a binary response to speed - data not shown. Furthermore, the accuracy of the linear decoder increased as the smoothing window is widened suggesting that the firing rate modulation to locomotion speed might be due to a general behavioural state rather than to encoding of specific movement information such as swing or stance phase of the stepping cycle (Armstrong and Edgley, 1988; Arshavsky, Berkinblit, et al., 1972; Sauerbrei et al., 2015; Powell

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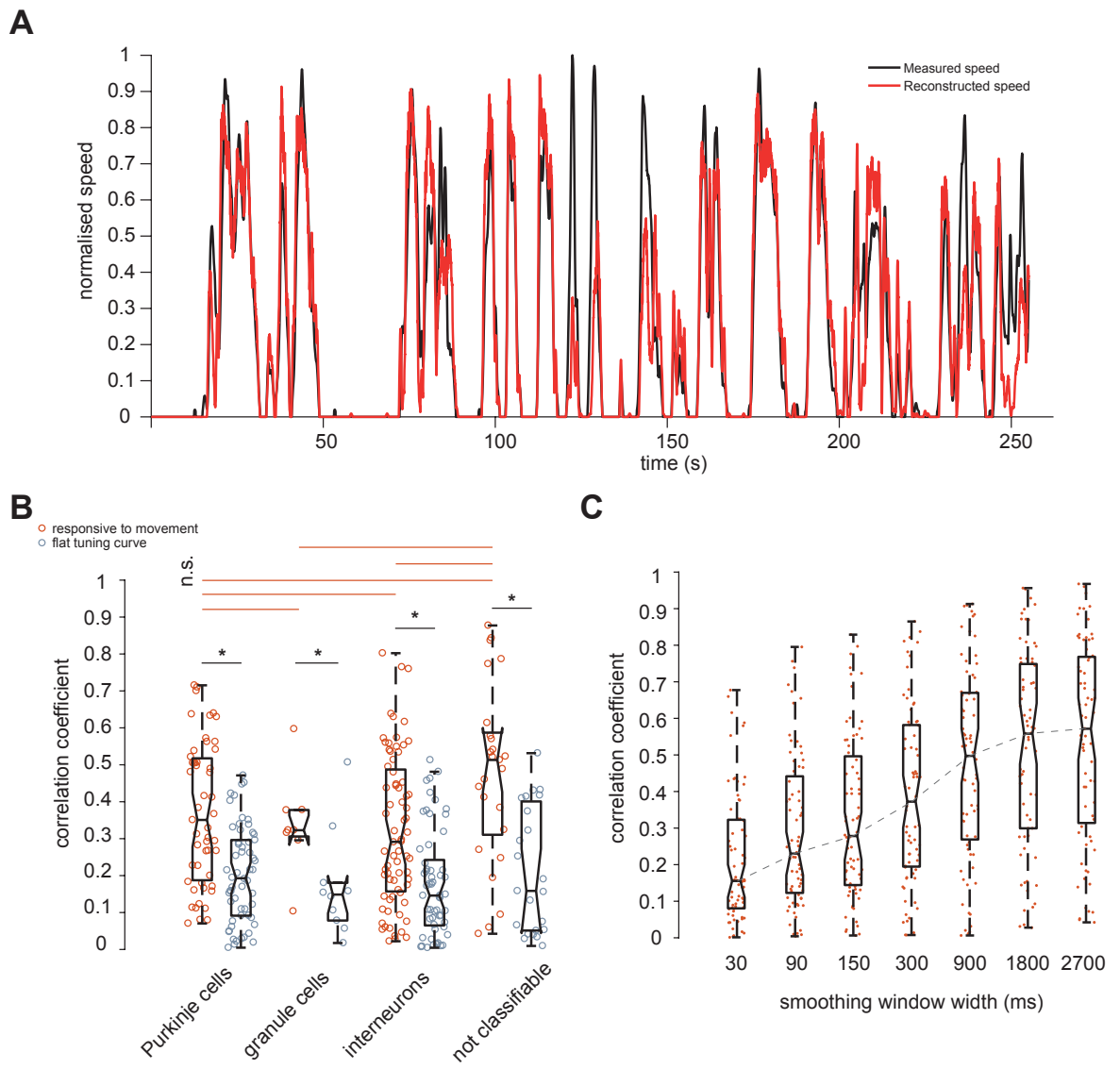


Figure 5.14: Decoding of single cerebellar neurons reconstruct speed. **A)** Time course of original and reconstructed locomotor speed signal. Firing rate and speed signals are sampled at 200Hz and smoothed with a Gaussian filter ($\sigma = 50$ ms, width = 150 ms). **B)** Reconstruction quality as a function of the putative cell class and modulation with locomotion speed. Box plots show first and third quartile with middle horizontal line indicating the median. Asterisks indicate significance; n.s. not significant. **C)** Median reconstruction quality as a function of the width of the smoothing window for units responsive to movement. Standard deviations are one third of the total widths indicated in the labels in the x axis.

et al., 2015)

5.8 Discussion

In this chapter, I looked at the neural activity of cerebellar neurons during locomotion behaviour. Combining a VR behavioural task with multi-unit electrophysiological recordings from the cerebellum offers the advantage of assessing the activity of multiple neighbouring neurons while maintaining a high degree of experimental control over behavioural parameters. These recordings from lobules V and VI of the vermis indicate that most cells recorded from this area are modulated by kinematic parameters of locomotion such as locomotion speed, yaw turning, and stepping cycle. These units did not belong to separate classes but were correlated with all these quantities.

Six per cent of the cells recorded showed significantly different firing rates during locomotion as opposed to rest (similar to the classical results of Armstrong and Edgley, 1984), a further 45% of neurons were specifically tuned for speed of locomotion. This included cells that increased their firing rate with increasing running speed (50 out of 311), cells that decreased their firing rate below the spontaneous (rest) level with increasing speed (51 out of 311), and cells showing a preferred locomotion speed (38 out of 311). These responses were not found to be due to visual feedback or an effect of the rhythmical modulation of the step cycle as reported in previous studies (Arshavsky, Berkinblit, et al., 1972; Armstrong and Edgley, 1984; Sauerbrei et al., 2015; Powell et al., 2015). Units belonging to different putative cerebellar cell classes were represented in all response profile groups. In addition, units recorded in close proximity, i.e. from electrodes within the same shank ($<140\ \mu\text{m}$), had heterogeneous response profiles. This was also true for pairs of putative Purkinje cells recorded from the same shank. The lack of functional and spatial clustering could be due to the fact that the recordings were not performed from cerebellar areas that had been selected according to a precise mapping protocol aimed at identifying, for example, units with cutaneous receptive fields on the mouse limbs.

By keeping the spherical treadmill free to rotate along any orientation, I aimed at creating a navigation paradigm congruent with a real-world scenario. Animals had to intentionally engage as many muscles relevant for locomotion as possible and were not constrained to run at fixed speeds. Because the animals were head-restrained, the vestibular inputs were also assumed to be negligible during yaw turning. The correlation with the direction of movement may be due to

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lateralised spinocerebellar inputs from muscles employed to steer clockwise or anti-clockwise. It was also found that cells with a preference for a turning direction (high difference in modulation index) were not highly modulated by speed, suggesting that speed and direction of locomotion might be relayed separately. A similar result was observed in macaque monkeys performing a visually guided tracking task (Roitman et al., 2005): Purkinje cells responded to position and direction of movement of the arm. An *ad hoc* behavioural task where the virtual reality environment requires the animal to make regular circular turns (almost mimicking the round arm-trajectory performed by monkeys) might enable to validate yaw-direction tuned cells.

Units tuned to speed of locomotion, yaw, and phase of stepping cycle did not constitute separate classes of neurons but, instead, they encoded all of these quantities to a greater or lesser extent. The mutual information of firing rate and kinematic parameters of locomotion revealed that the pool of neurons recorded came from a homogeneous population. Besides being in accordance with the analysis of speed, yaw and step cycle modulation - see figures 5.4, 5.7 B and 5.9, these findings can be justified by the fact that the recordings were not performed in areas with identified tactile receptive fields.

According to the information theoretic analysis carried out (figure 5.11), the majority of the units showed a greater predictive correlation with locomotion speed (~ 100 ms), suggesting they might be driven by internally generated signals. In fact, the cerebellum receives projections from the nucleus cuneiformis (Gioia and Bianchi, 1987) that, in turn, receives inputs from the mesencephalic locomotor region - MLR (Ryczko and Dubuc, 2013). Since the MLR is a region of the hind brain that is involved in initiating and modulating locomotion (Shik et al., 1966; Lee, Hoy, et al., 2014; Kiehn, 2016), the cerebellum might receive a copy of the motor signals sent to spinal locomotion centres (Orlovsky et al., 1999). I see this in agreement with computational theories, based on forward internal models, according to which the cerebellum uses an efference copy to compensate for slow sensory feedback (Wolpert et al., 1995a; Wolpert et al., 1995b; Pasalar et al., 2006). It is important to note, however, that locomotion speed is measured with velocity sensors on the treadmill so only the effect of limb movements onto the sphere is detected. For example, during movement onsets the movement to place the limb in position for the first step cannot be picked up by the velocity sensor. Although tracking of the spherical treadmill motion is

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an efficient approach for closed loops experiments, video tracking of mouse movements might help mitigating such issue (Powell et al., 2015; Sofroniew et al., 2014).

Remarkably, locomotion speed could be linearly reconstructed from the firing rate of single cerebellar neurons with high accuracy. The median accuracy of the decoder, defined as the Pearson's correlation coefficient between the actual and reconstructed speed, was measured to be higher for units modulated by locomotion. This also increased with the width of the filter used to smooth the firing rate and speed signals suggesting that the activity of the neurons recorded may be affected by slow changes of locomotion speed rather than quick locomotion-related movements. However, with the current dataset it is not possible to prove such hypothesis or if, instead, cerebellar units encode specific features of locomotion such as start/stop signals, muscle contractions, joint states, swing/stance phases of step cycles, etc.

Despite employing the latest classification algorithms to identify cell classes, I was unable to verify the cell identity and/or to recognise the cortical layer of recording. This imposes a limit to the information that can be gathered from these data about the role of single neurons within the cerebellar circuit. Furthermore, the data presented here come from units recorded from lobules V and VI, which are known to receive spinocerebellar inputs, however no receptive field mapping was performed beforehand. Such limitation, in fact, might explain the homogeneity of the response profiles across putative cell types and units in close proximity. Future experiments should therefore aim at identifying receptive fields in the cerebellar cortex, for instance, via topographical mapping with fluorescence imaging techniques based on either intrinsic or exogenous contrast agents (e.g. calcium indicators).

To the best of my knowledge, this is the first time MEA recordings are combined with a VR paradigm to investigate the neural bases of locomotion behaviour in the mouse cerebellum. This approach allowed to characterise the response profiles of cerebellar units with locomotion speed, yaw turning, and step cycle. Although the units did show a strong modulation with locomotion behaviour, it is not possible to determine if any cell type is specifically tuned to the kinematic parameters of locomotion analysed here because of the ill-defined sample of neurons. Moreover, it is likely that these units might encode specific features of locomotion and what was observed here was simply the modulation of such responses, similarly to how visual responses in the visual

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cortex are modulated by locomotion speed (Chiappe et al., 2010; Niell and Stryker, 2010; Lee, Hoy, et al., 2014; Saleem et al., 2013). For this reason, future experiments will benefit from improved methodology, in particular video tracking of behaviour, cerebellar input mapping and improved spike sorting techniques to help identifying cerebellar cell classes.

6

SUMMARY AND FUTURE DIRECTIONS

In this dissertation I have described the details of my research project aimed at understanding the role of cerebellum in sensorimotor control and advancing the field of experimental neuroscience. The main outcomes are the development of novel experimental paradigms, namely a virtual reality behavioural training protocol and a sensory perturbation system, and the characterisation of locomotion-related neural activity in the mouse cerebellum.

6.1 The MusoDrome

The use of VR systems in neuroscience allows the study of neural activity while subjects are engaged in behavioural tasks. This project capitalised on this capability to create a fast closed-loop VR apparatus for studying sensorimotor control in mice: the MusoDrome.

In the third chapter I presented the development and characterisation of the main features of the MusoDrome. The system uses an immersive visual stimulation based on a simple projection system whose specifications and configuration procedures were hereby outlined in detail.

For the first time, the VR update lags were fully characterised thanks to latency tests carried out using different tools such as a reference CRT monitor and a high-speed camera. The overall latency of the MusoDrome was measured to be approximately 46 ms, well within the lags of mouse visual feedback (Mauk and Buonomano, 2004). Although there are no latency data from

other rodent VR systems available to compare this value with, it is unlikely that such low latency is achieved by other systems that, for example, used two separate computers for the spherical treadmill tracking and the VR stimulus presentation (Dombeck, Harvey, et al., 2010; Harvey, Collman, et al., 2009; Takalo et al., 2012).

Crucial components for the mouse ergonomics, such as the spherical treadmill, were modelled with computer-aided design (CAD) software and manufactured using 3D printing. The main advantage of this technology is the parallel increase of throughput, reproducibility and quality. For instance, the air-supported spherical treadmill can be quickly and easily reproduced in other laboratories; because of the precise fabrication process the polystyrene sphere can rotate smoothly and relatively quietly (~49 dB) minimising acoustic noise during experiments and, consequently, distress for the animal.

The MusoDrome was integrated with a novel mechanical perturbation system to generate unexpected sensory stimulation onto the mouse whilst moving the virtual reality environment. This system makes use of high pressure air-jets to turn the spherical treadmill in either direction. The perturbation was designed to probe the locomotion pattern of the mice while they move in the VR environment. As with manipulandum devices commonly used in psychophysics (Wolpert and Ghahramani, 2000; Burdet et al., 2001; Pasalar et al., 2006; Selen et al., 2009), animals could be subjected to controlled perturbations while assessing the neural bases of visuomotor responses. The juxtaposition with responses to only visual perturbations has the potential to discern the extent by which these responses are driven by proprioceptive sensors and whether these prompt compensatory stabilisation reflexes.

To summarise, I developed a fast closed-loop VR reality system for studying sensorimotor control in mice. The MusoDrome is integrated with a novel perturbation paradigm that allows new visuomotor experiments in mice. The construction and configuration protocols are here outlined with the purpose of advancing the field of experimental neuroscience by a) establishing experimental standards for such tools and b) providing helpful information to laboratories intending to use similar MusoDrome systems.

VR apparatuses offer a highly controllable experimental setup for studying sensorimotor and cognitive functions. However, head-fixation may be the main limitation of using such methods.

Although VR experiments in rats allow the use of head-mountable recording chambers (Szuts et al., 2011), mouse experimentation can benefit from transgenic technology and optogenetics techniques. Therefore, further ethological validation of head-fixed experiments in VR systems is required, specifically with comparisons on freely behaving animals (Minderer et al., 2016).

6.2 VR behaviour and responses to mechanical perturbation

In the fourth chapter I described the VR behavioural training protocol and the behavioural responses to the mechanical perturbation.

Based on the chosen measures of performance, 75% of the subjects learnt to complete the VR task. The training was formalised in four main phases aimed at specific behaviours. In particular, the learnt avoidance of the lateral walls could only be achieved by lateral air-puffers that automatically engaged as soon as the subjects' VR position was too close the side walls, providing a haptic-like feedback. Indeed, the fraction of time per session during which the air-puffers were active significantly decreased following the introduction of the air-puffers. Navigating using the VR walls resulted in a more ethologically relevant behaviour and the visual input effectively reflected the locomotion pattern of the mice. Importantly, this condition was necessary for the application of the perturbation paradigm.

Analysis of behavioural responses to the mechanical perturbation suggests that mice corrected their locomotion trajectory based on the evaluation of their heading angle and not their closeness to the walls. Indeed, the data show that subjects, on average, corrected their VR position by limiting their lateral shift in the VR environment while they were still relatively far from the virtual walls. One explanation for this is that subjects used optic flow information to estimate their direction of motion and minimise rotation. Such behaviour is consistent with optic flow information processing used by humans during locomotion (Gibson, 1958; Warren et al., 2001; Bruggeman et al., 2007) and in insects (Krapp and Hengstenberg, 1996; Theobald et al., 2010)

The VR training protocol presented in this chapter establishes the basis for developing novel training protocols for assessing visuomotor control in VR systems. In addition, the behavioral responses of the mice during perturbation conditions a) prove the feasibility of the new mechanical

perturbation system and b) yielded preliminary results which provide insight into how mice might use visual feedback to adapt their locomotion.

Notably, 25% of the subjects did not learn the VR task and a long training duration was required (up to 25 sessions) for some of those which completed the training. The VR training protocol is relatively new and has been adapted from three previous studies (Dombeck, Harvey, et al., 2010; Harvey, Collman, et al., 2009; Schmidt-Hieber and Häusser, 2013). From this perspective, it may still require further optimisation. The animals' water restriction, for example, could be modified or removed. Another possible reason for lack of participation is the head-fixation, which clearly leads to an unnatural posture and interferes with head and eye movements involved in locomotion and gaze stabilisation. Further experiments should address both of these issues.

6.3 Characterisation of neural responses to locomotion in the mouse cerebellum

In chapter 5, I looked at the activity of cerebellar neurons during a virtual reality behavioural task. The results show that the majority of the units recorded from lobules V and VI of the cerebellar vermis were strongly modulated by kinematics parameters of locomotion, namely speed, turning, and stepping cycle.

Analysis of the neural activity revealed three classes of responses. Positively modulated units (50 out of 311) increased their activity with locomotion speed, whereas negatively modulated units (51 out of 311) decreased their firing rate with speed. In addition, I also found units whose activity peaked at a preferred speed (38 out of 311). Only 20 out of 311 units showed an increase in firing rate from resting periods that was not modulated by speed. There have been recent reports that revealed how locomotion modulates the activity of neurons in the visual (Niell and Stryker, 2010; Saleem et al., 2013) and medial entorhinal cortex (Kropff et al., 2015) but this is the first characterisation of such response profiles in cerebellar neurons.

A subset of units (58 out of 311) were found to respond selectively to yaw rotations in either direction during locomotion. These results are consistent with previous reports, in which simple spike activity of Purkinje cells differed between clockwise and anti-clockwise direction during

circular hand movements of monkey (Roitman et al., 2005). It is possible that these tuning properties may be the basis for cerebellar coordination of agonist and antagonist muscles during multi-joint movements. Further experiments would be required to validate these observations.

It has long been known that Purkinje cell activity is rhythmically modulated by inputs from the spinal tracts carrying information about the central pattern generator (CPG) network (Arshavsky, Berkinblit, et al., 1972; Armstrong and Edgley, 1984; Orlovsky et al., 1999; Powell et al., 2015; Sauerbrei et al., 2015). To verify whether the speed modulation was due to these inputs, I quantified the stepping cycle modulation of all units. Only 57 units were found to be modulated by stepping cycle and only 32 of these were also modulated by speed. These results suggest that locomotion speed modulation may not be an epiphenomenon of rhythmic modulation of the stepping cycle, as previously suggested (Sauerbrei et al., 2015).

Units tuned to speed of locomotion, yaw, and phase of stepping cycle did not constitute separate classes of neurons but, instead, responded to all of these quantities to a greater or lesser extent. The mutual information of firing rate and kinematic parameters of locomotion revealed that the pool of neurons recorded came from a heterogeneous population suggesting that cerebellar units may encode interdependent information about speed, laterality and type of muscles being recruited during locomotion. However, a more robust cell classification and recordings from cerebellar areas within receptive fields on the limb skin will be required to validate such conclusions and to elucidate how exactly the cerebellar network encodes locomotion-related information.

It was striking how accurately the locomotion speed could be reconstructed from the firing rate of some cerebellar neurons with a simple linear decoder. It would be useful for future work to assess how and if different types of cerebellar units combine these information to provide a more accurate estimate of the real speed of the animal, similarly to how activity of multiple Purkinje cells activity encode the saccade duration in monkeys (Thier et al., 2000).

The presence of speed modulation in the cerebellum is not surprising but its source is yet to be defined. Future work will elucidate if this is due to sensory afferent signals or to centrally generated signals that modulate the neural responses to sensory feedback during locomotion. For instance, modern gait analysis tools (Mendes et al., 2015) will make it possible to associate neural

activity with specific types of gait and features of locomotion (Bellardita and Kiehn, 2015). This knowledge will increase our understanding of how the cerebellar circuitry processes information and allow us to probe computational theories of sensorimotor integration.

The use of MEA probes limited the recognition of the cell identity of the units recorded. Although the classification algorithm that I used ascribed ~74% of the units to a cerebellar cell class, there is no ground truth for these units. In fact, future experiments should focus on comparing response profiles with cell classes. For example, by combining optogenetic techniques to selectively silence cerebellar cells it will be possible to assess the neural bases of locomotion behaviour or cerebellar conditions (Machado et al., 2015).

In this study, recordings were made from neurons of the cerebellar vermis, lobules V and VI but locomotion-related signals have been recorded in other cerebellar areas (such as paravermal lobule V, Armstrong and Edgley, 1984; Arshavsky, Berkinblit, et al., 1972; Orlovsky et al., 1999; Sauerbrei et al., 2015). Because of the peculiar fractured somatotopy and modular organization of the circuitry (see e.g. Apps and Hawkes, 2009), it is unclear how kinematic information processing is divided across zones and how their outputs are integrated by deep cerebellar nuclei to generate motor control. Perhaps such motor estimations are also relayed to, and exploited by, other nervous centres, to integrate behavioural information relevant for motor control and spatial navigation. In future work I hope that this may be elucidated.

6.4 Concluding remarks

The cerebellum is a fascinating structure whose *modus operandi* is still poorly understood. Thanks to technological advances in experimental techniques it is now possible to observe activity of multiple cerebellar neurons in awake preparations. I developed and applied novel methods to assess visuomotor control in mice advancing the field of experimental neuroscience. In addition, this is the first study that combines virtual reality behaviour with multi-unit electrophysiological recordings from the cerebellum. This work provided insight into the cerebellar function in motor control by showing tuning properties of cerebellar neurons to locomotion kinematic parameters.

The cerebellum is now regarded as being involved not only exclusively in motor behaviour

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but also in higher cognitive functions. In fact, an increasing body of evidence supports its role in functions such as semantic processing (Moberget et al., 2014), social behaviour (Fatemi et al., 2012) and other mental processes (Barton and Venditti, 2014; Ito, 2012). For this reason, understanding principles of cerebellar processing can shed light on the aetiology of mental conditions such as autism and possibly inspire new treatments. This prospect makes the study of the cerebellum, even its established role in sensorimotor function, a more appealing field for the years to come.

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APPENDIX

A.1 Materials and Reagents

Equipment

- Desktop computer running Windows XP or Windows 7 and the following programs installed: LabVIEW 2012 with all modules installed, Microsoft Visual Studio Express 2012, GLUT API version 3.7. **CRITICAL** The source code provided has been tested on Windows XP and Windows 7 using LabVIEW 2012 and Visual Studio Express 2012 (with GLUT API 3.67).
- 2 laser mice Razer Emperor (must have different serial numbers).
- Data acquisition PCI card (NI PCIe-6321, National Instruments Corporation).
- BNC connectors block (NI BNC-2090A, National Instruments Corporation).
- Cable for connecting BNC connector block to data acquisition PCI card (NI SHC68-68-EPM, National Instruments Corporation).
- 3D Digital projector. We used PJD6553w ViewSonic Corporation, USA.
- 8 aluminium beams 3m-long (RS Components, stock no. 289-9060).
- 8 t-slot nuts bugs (RS Components, stock no. 289-9212).

- 70 Angle brackets (RS Components, stock no. 289-9240).
- 2 swivel joints (RS components, stock no. 411-942).
- 2 aluminium sheets 2 m x 2 m (Smiths metal centres Ltd, www.smithmetal.com).
- Ceiling mount for 3D digital projector (ordered from www.ebay.com).
- 2 m x 1 m conductive fabrics (WaveTame Technologies Ltd, <http://www.wavetame.co.uk/>).
- Truncated dome for visual stimulus presentation (Talbot Design Ltd, www.talbotdesign.co.uk).
- Spherical treadmill (STL files are provided to print it in house or by external companies).
- Polystyrene spheres of 20 cm diameter (Poliemme s.n.c. Italy, www.poliemme.it).
- Polystyrene glue (Amazon.com, Aleene,Âs foamtastic).
- Head-plate holder (STL files are provided to print it in house or by external companies).
- Half-dome acrylic mirror (Signalnet Systems Ltd., UK).
- Flat acrylic mirror (RS components, stock no. 748-1302).
- Labjack size 200 mm x 200 mm, max height 290 mm (R&L Enterprises Ltd, catalogue no. 2036).
- Stainless steel head-plate implant for the subjects. We provide the sketch of our design so that can be fabricated by a mechanical workshop..
- Breadboard (Maplin electronics, product code AD-100).
- 3 transistors (Mouser electronics, manufacturer model no. BS170).
- 3 resistors 560 Ω , and 1 of resistance 100 Ω .
- 3 diodes (Mouser electronics, manufacturer model no. 1N4148).
- Infrared emitter (Mouser electronics, manufacturer model no. IR26-21C/L110/TR8).
- Infrared sensor (Mouser electronics, manufacturer model no. OPL-550).

- Plastic box to contain the circuits.
- 15 way socket and plug with hood (Maplin electronics, product code JW77J, JW78K, KE95D).
- 9 way socket and plug with hood (Maplin electronics, product code RK61R, RK60Q, KE94C).
- 4-mm sockets (Maplin electronics, product code N34AP).
- Nine 4-mm cord cables (Maplin electronics, product code ZB25C).
- Peristaltic water pump (Campden Instruments, Model 80204-0.5).
- Vacuum pump (Yujin Electric, KPV20A).
- 3 solenoid valves normally closed (Shako Co. Ltd, model PU220AR-01).
- Air regulator (Bee Fittings Limited, model no. R, 2500 litres per minute, 1/4 BSP connection size).
- Flexible nylon (PA11) air tubing (Bee Fittings Limited, model no. BLA/PA, 8 mm outer diameter, 5.5 mm inner diameter, 50 m length).
- 2 male stud fittings (Bee Fittings Limited, model no. PMST, 8 mm outer diameter, 1/4 BSP thread size).
- 2 equal tube tee fittings (Bee Fittings Limited, model no. PMST, 8 mm outer diameter).
- Silicone tubing 0.5 mm bore (RS components, stock no. 667-8438).
- Silicone tubing 4.8 mm bore (RS components, stock no. 667-8448).
- 2 copper tubes 1.1 mm inner diameter (Wonderland (Toys) Limited, model Albion Alloys CT2M).
- Base for copper tubes (STL files are provided to print it in house or by external companies).
- Water spout for delivering the reward (STL files are provided to print it in house or by external companies).

- Direct current power supply unit (RS components, stock no. 665-4906).
- 2 needles ().

Reagents Setup

Equipment setup

Preparation of the aluminium beams is indicated in details in the supplementary information. Acrylic mirror cuts. Glue or holes with drill. Computer installation of software

A.2 Main Structure

Chassis

Aluminium bars can be cut in a mechanical workshop by trained professionals or ordered already cut at custom dimensions by third companies (e.g. Bosch Rexroth, order list for all precut components is included in Equipment). Please note that the figures provided here are specific for the components we used to build our setup. Adjust these dimensions such that the positioning of the projector and the mirrors ensure the illumination of the entire internal surface of the dome.

1. After attaching a ceiling mount to the projector, mount this to the horizontal beam of the lint-like structure on what it will be the back of the screen dome (Fig. 3.2a). Regulates the height of the horizontal bar such that the projector lens is positioned at approx. 33cm from the table surface.
2. Mount two aluminium bars of the length 15cm on the edges of the flat mirror.
3. Mount the swivel joints on the central vertical bars in the back of the main structure (Fig. 3.2a). Space the vertical bars so that the flat mirror can be mounted between the swivel joints by the attached aluminium bars. Tilt these to 45 degrees.
4. Attach the half-dome mirror from its flange to a u-shaped base made of aluminium bars of dimensions 50cm by 25cm.

5. Add to the screen base five aluminium bars (2 x 50cm, 2 x 25.5cm and 1 x 71cm). They serve to position the half dome mirror, the spherical treadmill and air-puffers inside the dome.
6. Place the half-dome mirror with its base inside the main structure positioning the vertical anterior edge at 44cm from the flat mirror.
7. Place the labjack on the two central aluminium bars. The spherical treadmill is to be positioned onto this element whose height can be adjusted to accommodate subjects comfortably.
8. Place the spherical dome on the base making sure its vertical axis of symmetry is aligned with the centre of the base. This is to be aligned also with the sagittal plane of symmetry of the spherical mirror and of the projector lens.

Visual Stimulus Configuration

The following steps should guide towards the creation of a transformation map once all the hardware components of the system are assembled, the projector beam fully illuminates the demispherical dome and the computer is provided with all the software indicated as indicated in Equipment section.

1. Settings of the map parameters. The image below shows the pop-up window that appears at the start of the program *Main_meshmapping.vi*. Here, the dimensions of the dome and the resolution of the meshing map are to be inserted before the tracking procedure can start. I found that a grid of 4 rows and at least 17 columns for a projection surface of 90° by 240° offers a good compromise between time needed for the nodes tracking and accuracy of the map. The resolution of the map can be increased later once it is corrected for eventual mistakes.
2. Tracking of the Nodes. Position the laser pointer stand in the centre of the lab-jack so that it is also aligned with the vertical axis of symmetry of the dome screen. Adjust the height of the lab-jack so that the laser pointer is aligned with an imaginary radius of the sphere.

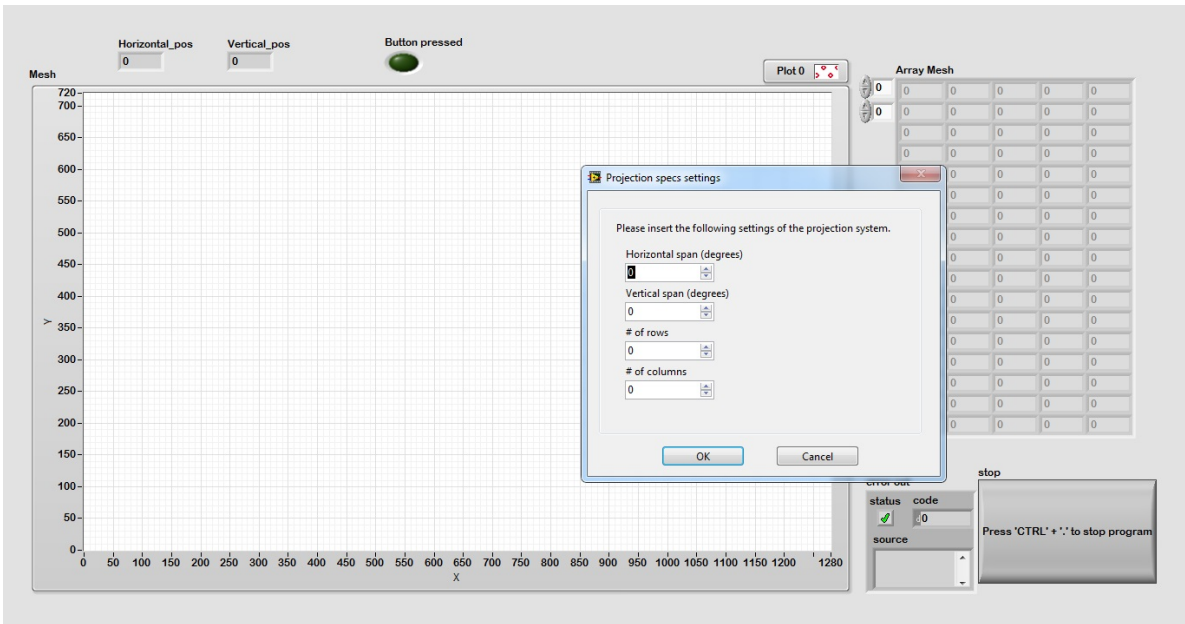


Figure A.1: Parameters configuration of LabVIEW program for generating projection map.

Mount the laser pointer such that is pointing towards the first node which has to be the bottom left one. Track the nodes by simply clicking with the left button of the mouse on the area of the screen where the laser beam is pointing to, see Fig.3.3a. The nodes are to be tracked from bottom to top row, left from right. After each click it is possible to confirm or cancel the record. Once it is confirmed, the pixels position of the mouse is saved in an array (right of the interface of the program) and visualised in a rectangular plot scaled to the projector resolution. Once all nodes are tracked, stop the program as indicated with *CTRL* + . . A text file with the mesh array is then saved.

3. Correct nodes position. Save numbers of nodes into C++ file *MainHeader.h* as indicated below. Change n_x and n_y to number of vertical and horizontal nodes respectively, see Fig. A.3. After having done this, re-compile the Visual Studio project *Mesh_tester*. This project is inside the folder *2_Read&CorrectMesh_Project\Mesh_tester*.

Open the program *MeshCorrector_main.vi* that is in the same folder. Load the *txt* file of the mesh generated in step 1 and run the program. The grid mesh appears on the white plot in normalised coordinates and, on the left, the actual array is displayed. The program displays a vertical or horizontal grating or a check-board onto the dome according to the

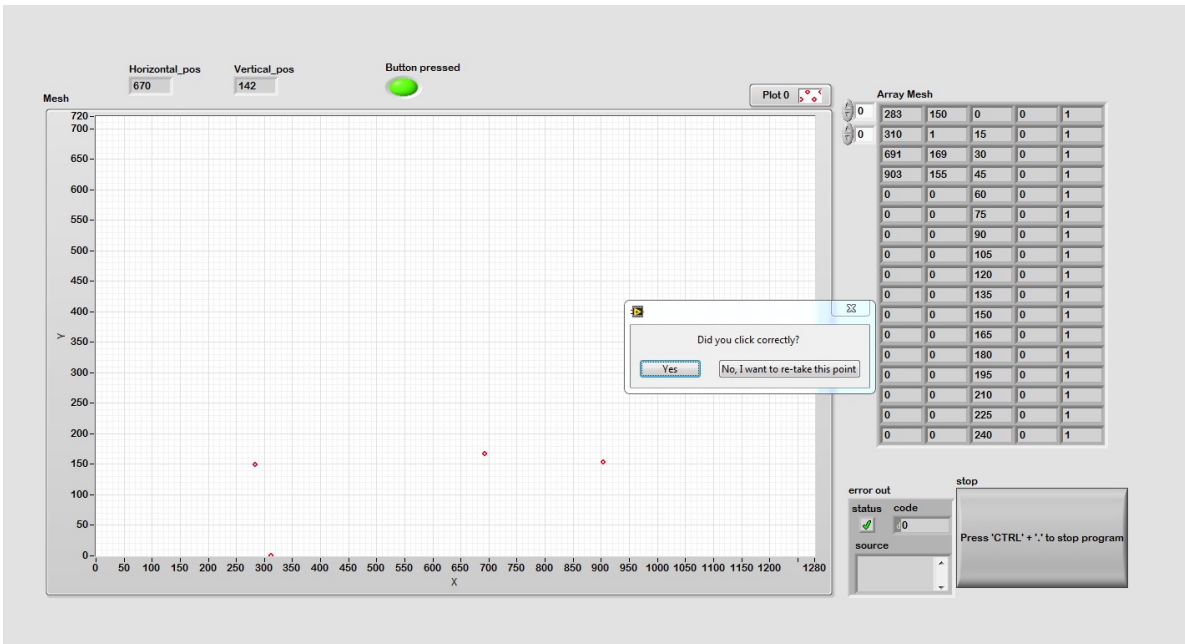


Figure A.2: Tracking of nodes of projection map with LabVIEW program.

```

////////////////////////////////////
////////////////////////////////////
////////////////////////////////////
////////// INSERT HERE NUMBER OF ROWS AND COLUMNS OF ORIGINAL MESH

# define nx 17
# define ny 4

extern int* Vert_Horiz;
extern int* cols;
extern int* rows;
extern int size;

```

Figure A.3: Parameters modification of c++ OpenGL code.

number selected on the box on the top. Should the gratings or check-board be distorted, click on nearest node to the distortion from the plot and move it around with arrow keys until grating is corrected. It is possible to modify the displacement distance of each key stroke by tuning in the apposite box, see Fig. A.4

4. Mesh resolution interpolation. Interpolate the mesh to increase the resolution and conformality of the map projection. A built-in function in the *MeshTester.vi* provides this option. Simply select the number of nodes along the vertical and horizontal directions that you want to use. The resolution of the mesh can also be modified with the *MeshTester.vi*.

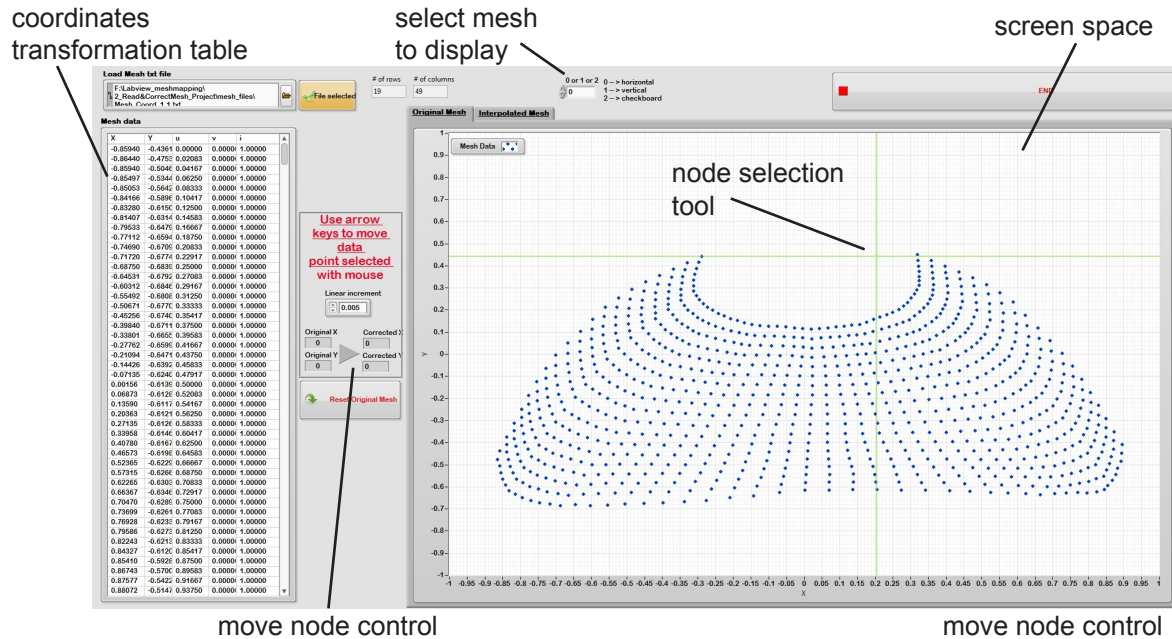


Figure A.4: LabVIEW program for correcting and interpolating projection map.

A.3 Spherical Treadmill

Hot-wire cutting device

The following steps should guide through the fabrication of the hot-wire cutting device.

1. Cut a wooden tablet of dimensions 300mm by 250mm , two smaller wooden boards of 50mm by 250mm to use to lift the first piece and two profiles long 200mm and two long 210mm (Fig. A.5a).
2. After attaching the two wooden boards on the bottom of the tablet attach the profiles on the upper side to form a square with side 200mm .
3. Drill two holes inside the square of appropriate dimensions to fit ceramic insulators being used. The first hole is in the geometric centre of the square and the second one at 9.5cm from the first one (Fig. A.5b).
4. Cut a segment of tungsten rod 30cm long. Bend the tungsten rod to form a quarter of a circumference arch with radius of approx. 9.5cm (Fig. A.5c).

5. Insert the extremes of the rod in the wooden board holes (Fig. A.5d). Use ceramic insulator tubes to avoid contact between the wood and the tungsten wire.
6. Solder each of these with a cable provided with 4-mm socket on the other end (Fig. A.5d).

Once the hot-wire cutter is built, a few tests should be made to check the electrical connections and the correct positioning of the ceramic insulators. Because the modelling of the tungsten wire is a manual process, its first shape may not be perfect. To compensate for this, some trials have to be made until a correct form of the wire is reached.

Another parameter to take into account is the intensity of the current. The temperature of the wire depends on this therefore it is important not to set it too high as this would melt the polystyrene too quickly. Because the temperature irradiates radially, the external surface of the sphere can be damaged.

Enlightening of the Polystyrene Spheres

Here is a description of the main step to enlighten a half-sphere. This procedure was tested with polystyrene sphere with specific weight of 33 kg/m^3 . As a general safety advice, this procedure should be done in conditions where personal and environmental safety precautions are applied to deal with hot material, toxic fumes and risks of fire.

1. Cut the slot from the edge of the half sphere that is needed to lock it with another half sphere. This is visible in Fig. A.5h.
2. Connect the terminals of the cutting device to the positive and negative terminals of a DC PSU capable of delivering at least 6-8A to test that the insulators are working correctly (Fig. A.5f). Remember to turn on the PSU only when you are ready to cut the sphere and turn it off as soon as you finish or for not longer than 2-3 minutes. Excessive heat can be hazardous; besides it damages the sphere.
3. Place the half sphere inside the 20-cm-by-20-cm slot of the hot wire cutting device (Fig. A.5e). Slowly lower the sphere until it touches the wood. Turn it around the ver-

tical axis at a constant speed to carve out the extra polystyrene material from the inside. This process should last about a minute.

4. Remove any extra material from the half sphere delicately to distribute the weight more evenly (Fig. A.5h).
5. Glue the half spheres together using the foam-specific glue and allow 24 hours to rest (Fig. A.5i).
6. Water-sand (sand paper nr.40) the sphere to smooth the central crease formed at the point of contact between the the halves and to improve the grip for the mice.

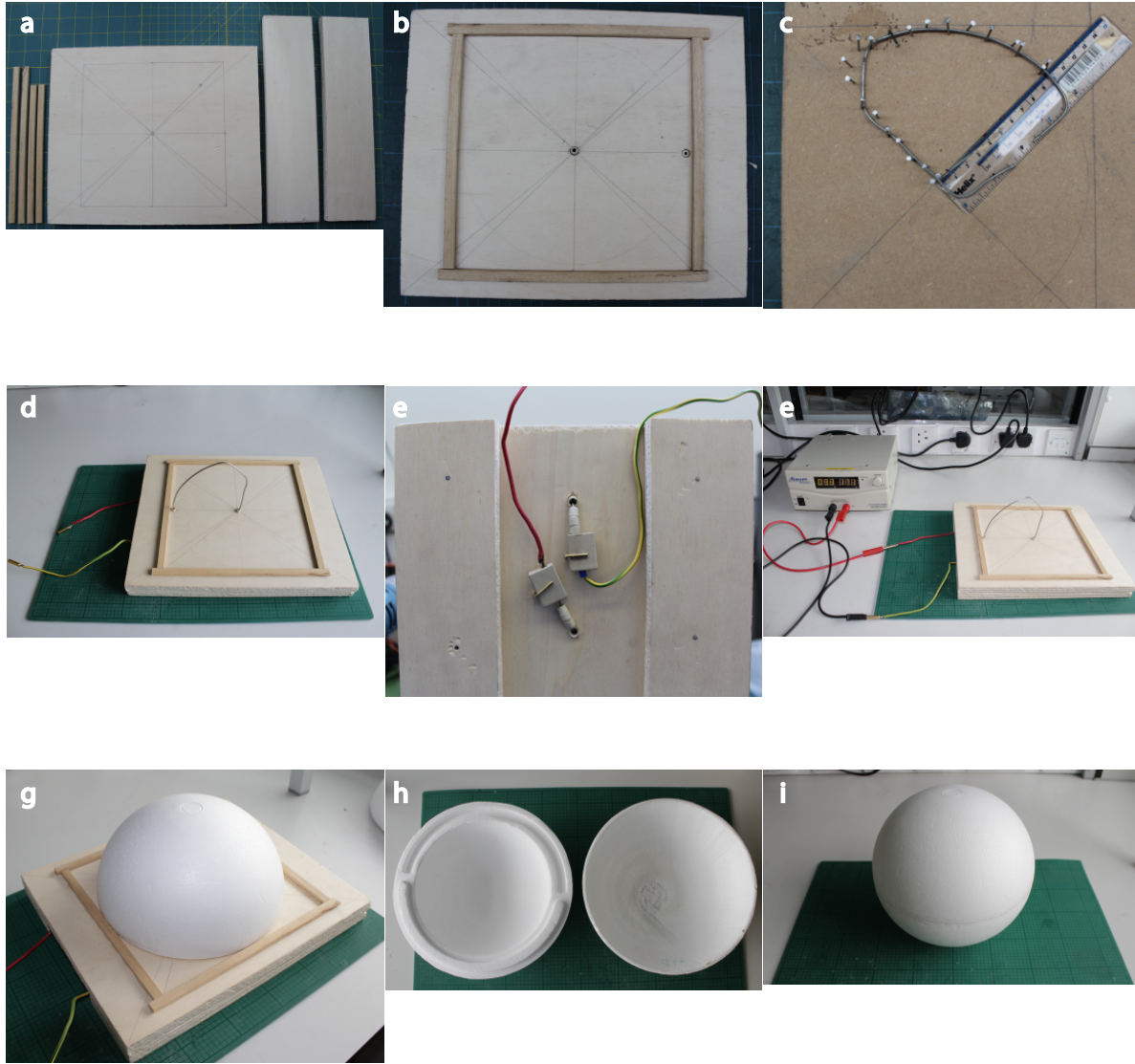


Figure A.5: Hot-wire cutter for lightening polystyrene spheres. (a) wooden tablets and profiles ready to be assembled. (b) Top view of the assembled hot-wire cutter. The two holes inside the square serve for positioning the tungsten wire. (c) Modelling of the tungsten wire into a quarter of circumference with radius 95mm . (d) The hot-wire cutting device complete with the tungsten wire and electrical connections. (e) Bottom view of the cutter. Ceramic terminal blocks are used to connect the tungsten wire to the electrical cables. (f) Testing the cutter with a direct current source. (g) A polystyrene hemisphere is placed on the cutter to be enlightened. The squared profile prevents horizontal displacement during the rotation of the hemisphere. (h) Two example hemispheres before and after the enlightening process. (i) The enlightened hemispheres are then glued together to form a sphere for the experiment.

Spherical treadmill base

The base of the spherical treadmill was 3D printed in three main parts. The STL files are stored here ([LINK TO ONLINE RESOURCES](#)), inside the folder *STLfiles*. In Fig. A.6, the main three parts are shown as 2D drawings and 3D views in their dimensions.

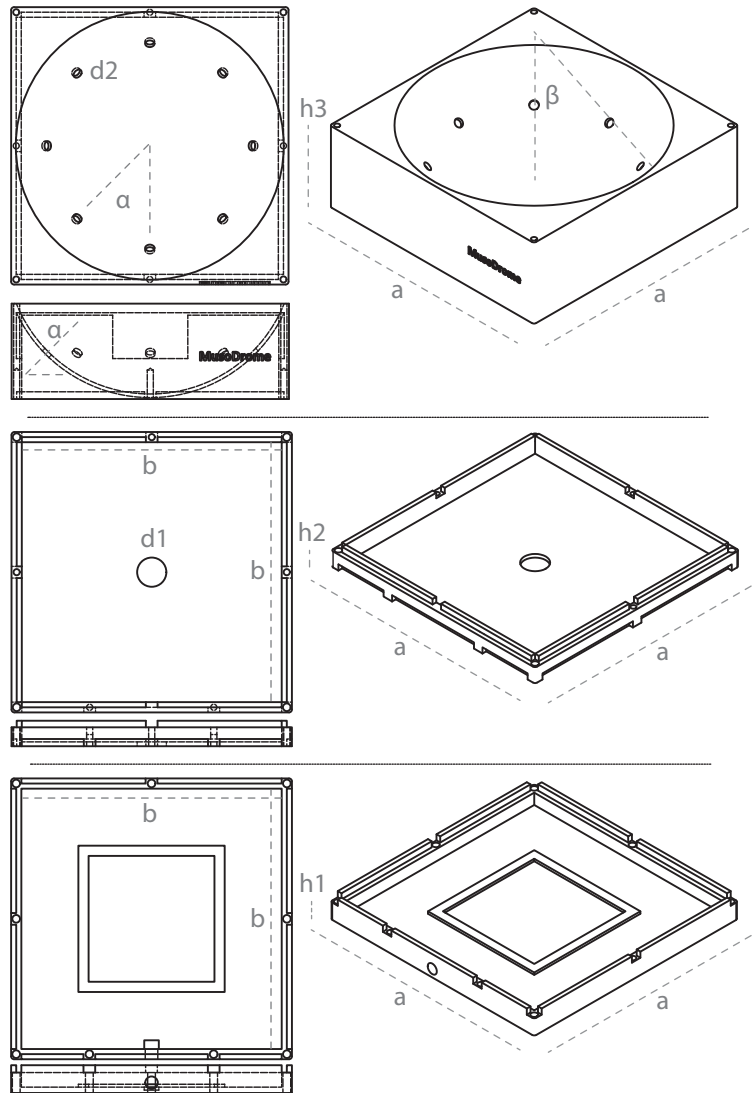


Figure A.6: Drawings of the spherical treadmill base. From bottom to top, orthogonal projections and perspective views of the main elements of the base. Dimensions are: $a = 192mm$; $b = 178mm$; $h1 = 14mm$; $h2 = 13.5mm$; $h3 = 66mm$; $d1 = 20mm$; $d2 = 6.5mm$; $\alpha = 45^\circ$; $\beta = 63^\circ$

Velocity sensors

The following procedure shows how the computer mice were disassembled and mounted on the spherical treadmill by means of a 3D printed enclosure. The PCBs' dimensions were measured with a caliper to precisely design their new enclosures.

1. Disassemble the computer mice from the original cases making sure the printed circuit boards are not damaged.
2. Place them in the rectangular 3D-printed cases (Fig. A.7).
3. Slide the velocity sensors inside their stands that mount on the corners of the treadmill (Fig. A.7).

Please note that the supports for the velocity sensors can only mount the PCBs of the computer mice we used (Razer Emperor). Given the modularity of the system, using other sensors is possible by creating *ad hoc* supports and apposite software control which might be different from the one we used.

Once the computer mice were connected to the host computer a few of their parameters were tuned to better suit the tracking of the sphere, namely:

- Polling rate : 1000 *Hz*.
- Sensitivity: 1000 *DPI* on both X and Y axes.
- Acceleration is set to 0 (zero) so that that gain of mouse movement is constant for any detected velocity.

After this, NI-VISA drivers were installed one mouse per time. The steps for doing this are explained in this tutorial: <http://www.ni.com/tutorial/4478/en/> . Please note that the USB port to which a mouse is connected must be always the same. The computer mice are now disconnected from the OS cursor and their signals can be read with the VISA commands in most programming languages. My code for reading their signal can be found here (LINK TO ONLINE RESOURCES), inside the folder *Software* under the name of *VISA_mice.vi*

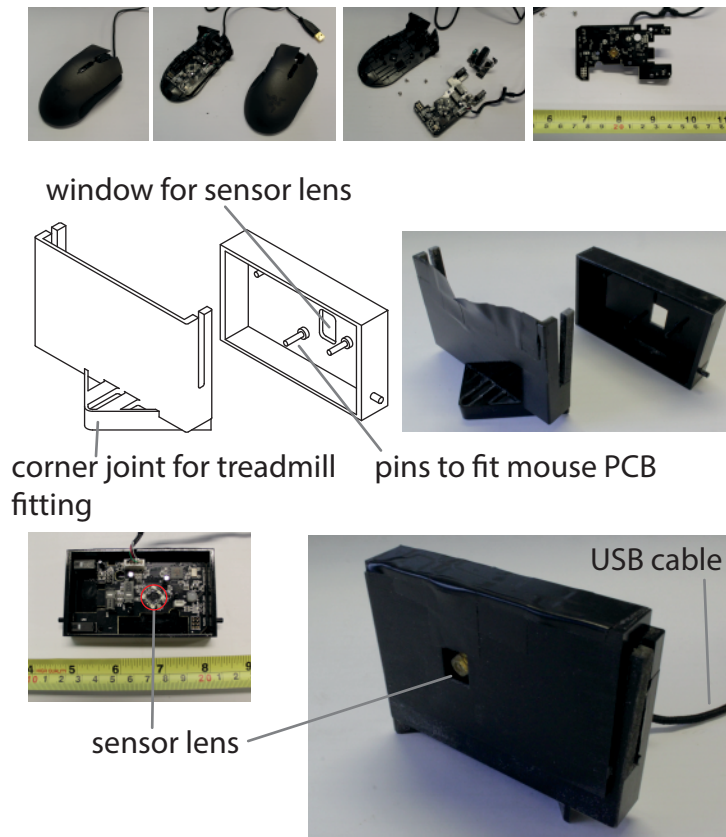


Figure A.7: Assemblage steps of the velocity sensors

Automated Reward System

1. Prepare a small tank for the water to be delivered to the subjects during the experiments. This can also be used as drain for the water removed from the water spout. Water in the reservoir should be changed every day to avoid stagnation and/or sedimentation that may cause obstructions of the tubing.
2. Mount the silicone tubing with bore 0.5mm into the peristaltic water pump and place one end inside the water reservoir. Connect the other end, using Leur adapters, to the upper (delivery) conduit of the 3D printed water spout. The diameter of the bore determines the water flow with which the pump activation time needs to be adjusted to ensure the desired quantity of water to the subjects. The pump used here (Campden Instruments model 80204-0.5) provides 0.025 ml per second with a 0.5 mm bore tube.

3. Use the silicone tubing with bore 4.8mm to connect the suction (lower) conduit of the water spout to the suction pump (use Leur adapters). Connect the outlet of the suction pump to a water reservoir. The bigger diameter of the silicone tube prevents eventual obstructions of the tubing itself and consequent failures of the suction pump.
4. Electrical connections: - Solder connector cables to the electrical components, namely the infrared emitter and the sensor, of the lick detection system on the water spout making sure the sensitive parts are correctly aligned through the holes near the licking spot, see Fig.3.7. The correct positioning of the IR sensor and emitter determines the accuracy of the lick event detection. - Connect the appropriate terminals of the water pump to a power unit and the control signal cable to the I/O data acquisition card. - Connect the suction pump to a switching circuit controlled via the I/O data acquisition card and power it as indicated in Fig. 4. - Test the pumps and the lick detection system with LabVIEW The fabrication of the air-tubing system should take approximately 1 to two days.
5. Connect a segment of air tubing to an air source capable of delivering more than 2 bars of pressure. Connect a three-way splitter to the other end (the schematic is shown Fig. 4).
6. Use two outputs of the splitter to deliver air to two solenoid valves. Use compatible (threading size and air tubing external diameter) fittings to plug the air tubes. The third output delivers air to the spherical treadmill (Fig. 4).The air tubing connected to the solenoid valves must be of the same length likewise the two air tubes that connect them to the air puffers. This is to ensure equal pressure drops on both sides.
7. Plug an air tubing to each of the solenoid valve outputs and connect them to the 3D printed bases of the air puffers.
8. Mount a copper tube on each of the 3D printed bases and direct it towards the subject. In this setup, they are kept at 100 mm from the subjects. Air-puffs are adverse stimuli that must be used in accordance with the animal care regulations.
9. Plug a pressure regulator in series with the third output of the three-way splitter to control the air pressure supporting the sphere inside the treadmill. The position of the regulator

should be as close as possible to the treadmill and easily reachable during the experiment so that the air pressure can be tuned for each subject at all times during the experiment. Close the regulator whenever the spherical treadmill is not in use.

10. Connect the solenoid valves to switching circuits controlled via the I/O data acquisition card and to power as indicated in Fig. 4.
11. Test the air-puffers system by using the Labview program provided in supplementary data.

A.4 MusoDrome control software

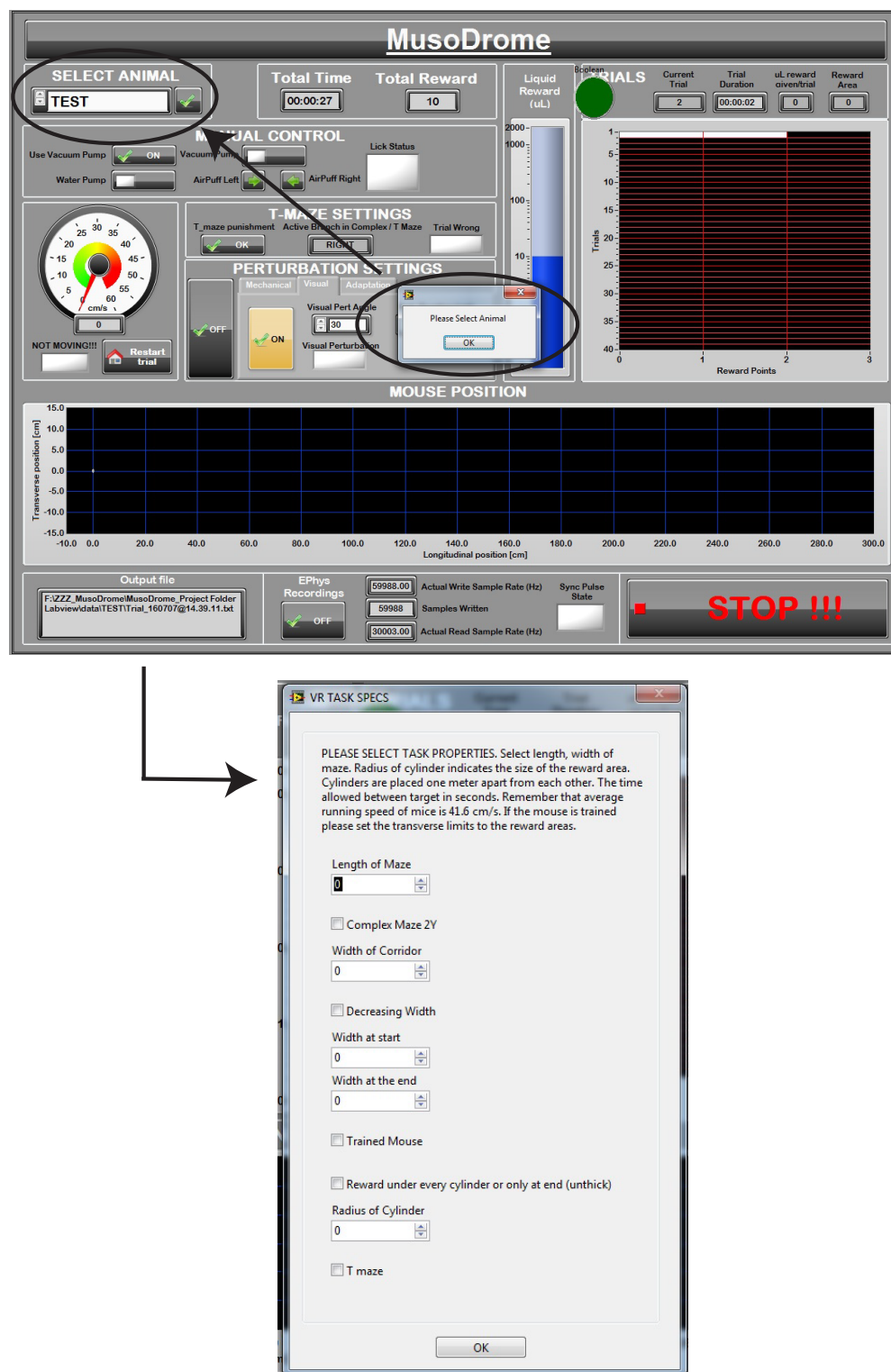


Figure A.8: Setting the parameters of a training session. Once the program is started, the user is prompted with a reminder message to select an animal from the list on the top left or create a new profile (not shown here). After the animal selection, the user can choose various parameters and the type of maze to present. Reward release can be chosen to be triggered by passing under every cylinder or only at the end.

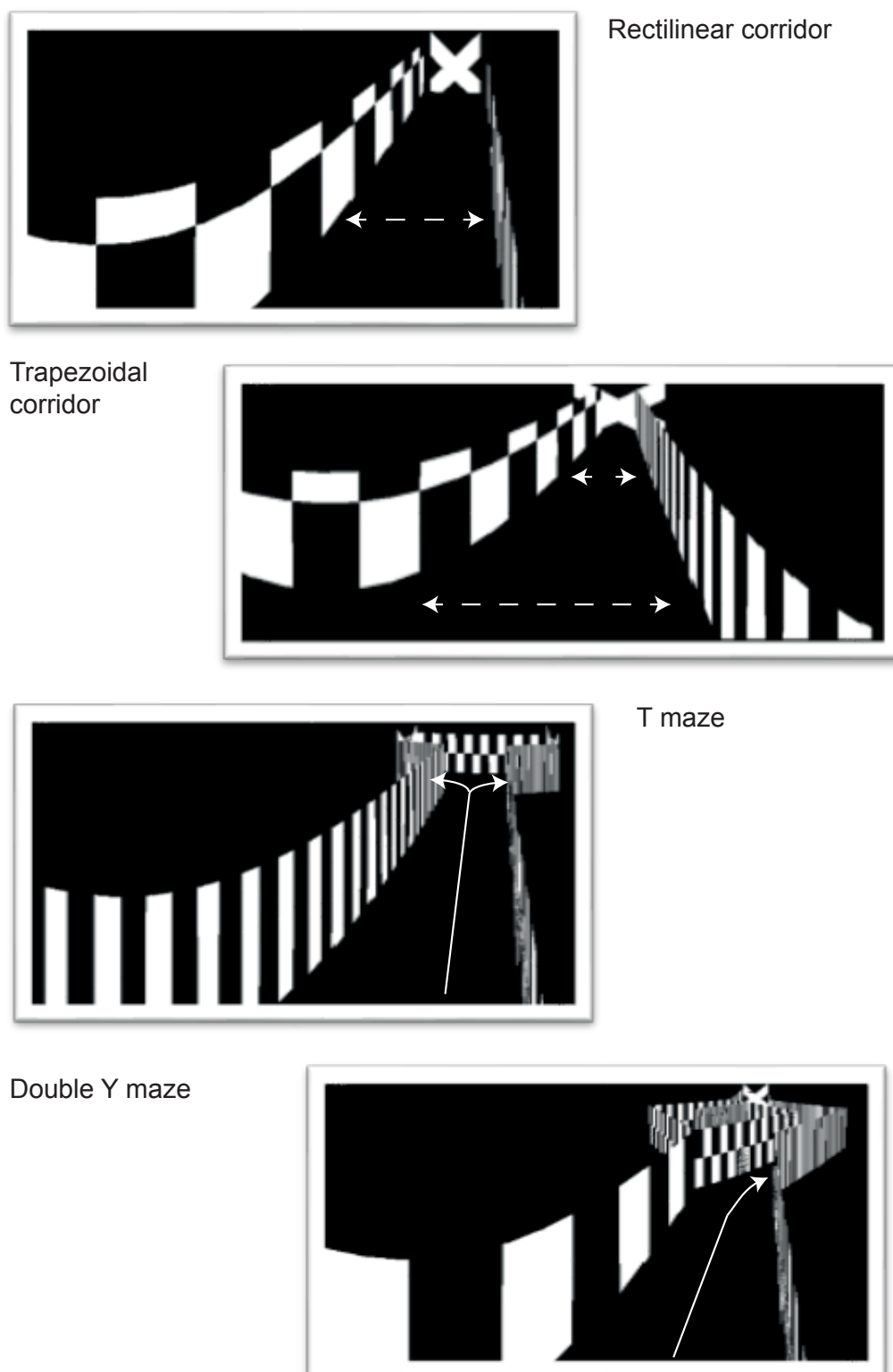


Figure A.9: MusoDrome mazes. The user can choose between 4 different mazes at the beginning of each session. A trapezoidal maze can be selected by ticking the option *decreasing width* in the *VR TASK SPECS* window. A T maze and a double-Y maze can be selected and adapted with specific paradigms (not defined here) for investigating different behaviours. Only one (randomly chosen) side of the double-Y corridor is open in any given trial.

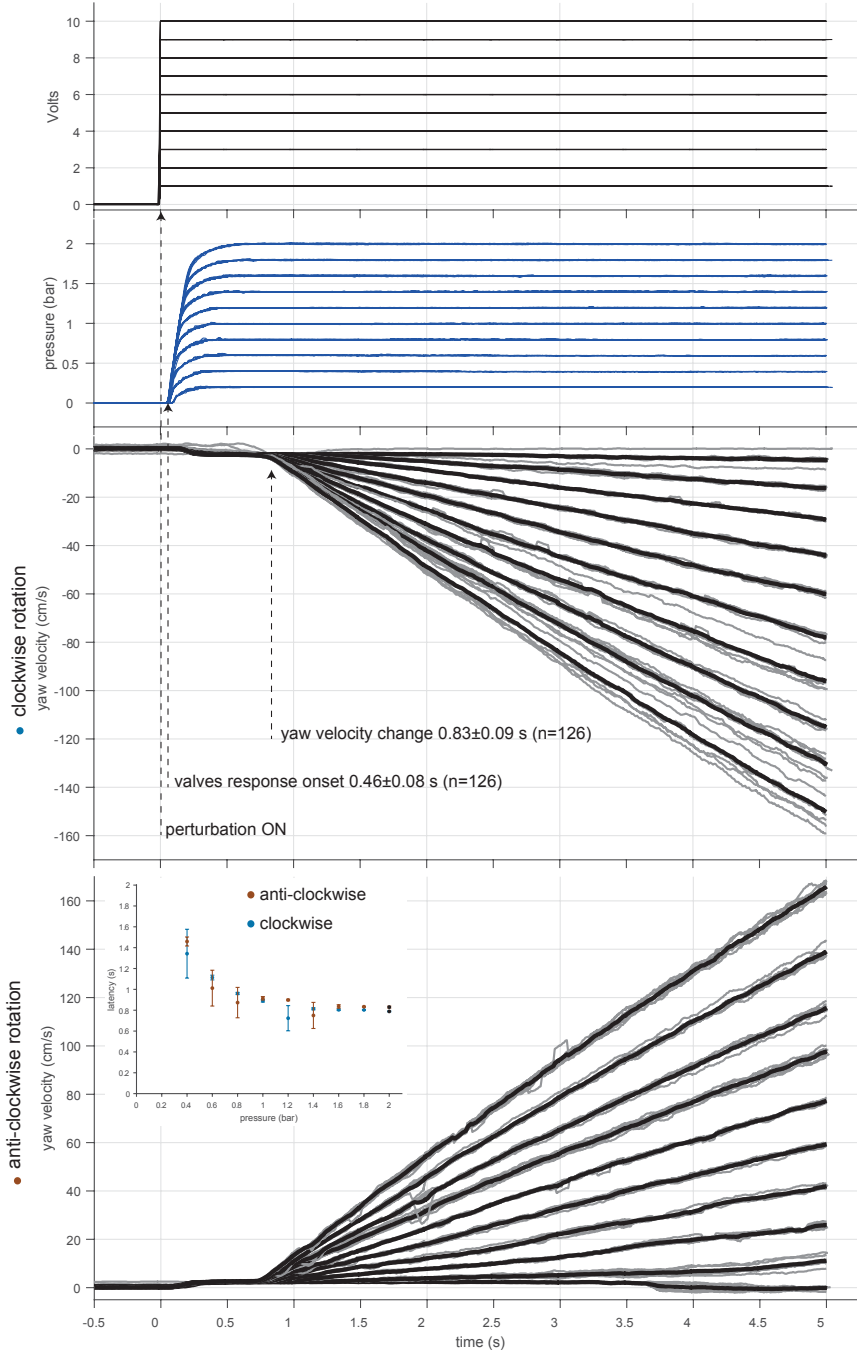


Figure A.11: Measure of latency of mechanical perturbation system. The analog signal controlling the pressure regulators is set at the desired value ($V = 1 : 1 : 10$) at time $t = 0$. For each value, seven tests were executed. The pressure builds up to the desired value following a dynamics consistent with a logistic function ($R^2 \geq 0.99$) for all values. The sphere starts rotating and increases the yaw velocity at a constant rate (a linear model describes every curve with $R^2 \geq 0.99$). Latency is evaluated as the time difference between control signal is set to 1, wheel movement onset and when pressure starts building ups. These are measured when their signals change by three times the standard deviations from the means preceding the wheel movement onset. The latency of the sphere movement onset is reported in the inset for all the pressure values. This is constant for values higher than 0.8bar or $4V$. Values for $1V$ were not reported as they did not cause sphere movements consistently. Values of latency are $\text{mean} \pm \text{s.e.m.}$

A.6 KlustaViewa parameters file

```
#####
# SpikeDetekt parameters #
#####

experiment_name = 'T012_EF_2neg'
raw_data_files = ['EXP_17062014_2.dat']
prb_file = '32chan4shankBuzsaki_geometry.prb'
nbits = 16
voltage_gain = 10.

sample_rate = 30000.
nchannels = 32

# Filtering
# -----
filter_low = 500. # Low pass frequency (Hz)
filter_high = 0.95 * .5 * sample_rate
filter_butter_order = 3 # Order of Butterworth filter.

filter_lfp_low = 0 # LFP filter low-pass frequency
filter_lfp_high = 500 # LFP filter high-pass frequency

# Chunks
# -----
chunk_size = int(1. * sample_rate) # 1 second
```

```

chunk_overlap = int(.015 * sample_rate) # 15 ms

# Spike detection
# -----

# Uniformly scattered chunks, for computing the threshold from the std of the
# signal across the whole recording.
nexcerpts = 50
excerpt_size = int(1. * sample_rate)
threshold_strong_std_factor = 4.
threshold_weak_std_factor = 2.
weight_power = 2.
detect_spikes = 'negative'
#precomputed_threshold = None


# Connected component
# -----

connected_component_join_size = int(.00005 * sample_rate)


# Spike extraction
# -----

extract_s_before = int(0.001 * sample_rate) # 1.0 ms
extract_s_after = int(0.001 * sample_rate) # 1.0 ms
waveforms_nsamples = extract_s_before + extract_s_after


# Features
# -----

nfeatures_per_channel = 3 # Number of features per channel.
pca_nwaveforms_max = 10000

```

```
#####
# KlustaKwik parameters #
#####

MaskStarts = 100

#MinClusters = 20

#MaxClusters = 110

MaxPossibleClusters = 500

FullStepEvery = 10

MaxIter = 10000

RandomSeed = 654

Debug = 0

SplitFirst = 20

SplitEvery = 100

PenaltyK = 0

PenaltyKLogN = 1

Subset = 1

PriorPoint = 1

SaveSorted = 0

SaveCovarianceMeans = 0

UseMaskedInitialConditions = 1

AssignToFirstClosestMask = 1

UseDistributional = 1

channel_groups = {
    # Shank 1
    0: {
# List of channels to keep for spike detection.
'channels': [0, 1, 2, 3, 4, 5, 6, 7],
```

```

# Adjacency graph. Dead channels will be automatically discarded
# by considering the corresponding subgraph.
'graph': [
    (0, 1), (0, 7),
    (7, 1), (7, 6),
    (1, 6), (1, 2),
    (6, 2), (6, 5),
    (2, 5), (2, 3),
    (5, 3), (5, 4),
    (3, 4),
],

# 2D positions of the channels, only for visualization purposes
# in KlustaViewa. The unit doesn't matter.
'geometry': {
7: (-287.75, 142),
    6: (-290.25, 102),
    5: (-292.75, 62),
    4: (-300, 22),
    3: (-303.5, 42),
    2: (-308.5, 82),
    1: (-311, 122),
    0: (-313.5, 162),
}
},

# Shank 2
1: {
# List of channels to keep for spike detection.
'channels': [8, 9, 10, 11, 12, 13, 14, 15],

```

```

# Adjacency graph. Dead channels will be automatically discarded
# by considering the corresponding subgraph.
'graph': [
    (8, 15), (8, 9),
        (15, 9), (15, 14),
        (9, 14), (9, 10),
        (14, 10), (14, 13),
        (10, 13), (10, 11),
        (13, 11), (13, 12),
        (11, 12),
],
# 2D positions of the channels, only for visualization purposes
# in KlustaViewa. The unit doesn't matter.
'geometry': {
    15: (-87.75, 142),
        14: (-90.25, 102),
        13: (-92.75, 62),
        12: (-100, 22),
        11: (-103.5, 42),
        10: (-108.5, 82),
        9: (-111, 122),
        8: (-113.5, 162),
}
},

# Shank 3
2: {
# List of channels to keep for spike detection.
'channels': [16, 17, 18, 19, 20, 21, 22, 23],

```



```

# Adjacency graph. Dead channels will be automatically discarded
# by considering the corresponding subgraph.
'graph': [
    (16, 23), (16, 17),
        (23, 17), (23, 22),
        (17, 22), (17, 18),
        (22, 18), (22, 21),
        (18, 21), (18, 19),
        (21, 19), (21, 20),
        (19, 20),
],
# 2D positions of the channels, only for visualization purposes
# in KlustaViewa. The unit doesn't matter.
'geometry': {
    23: (112.3, 142),
        22: (109.75, 102),
        21: (107.25, 62),
        20: (100, 22),
        19: (96.5, 42),
        18: (91.5, 82),
        17: (89, 122),
        16: (86.5, 162),
}
},

# Shank 4
3: {
# List of channels to keep for spike detection.
'channels': [24, 25, 26, 27, 28, 29, 30, 31],

```

```

# Adjacency graph. Dead channels will be automatically discarded
# by considering the corresponding subgraph.
'graph': [
    (24, 31), (24, 25),
        (31, 25), (31, 30),
        (25, 30), (25, 26),
        (30, 26), (30, 29),
        (26, 29), (26, 27),
        (29, 27), (29, 28),
        (27, 28),
],
# 2D positions of the channels, only for visualization purposes
# in KlustaViewa. The unit doesn't matter.
'geometry': {
    31: (312.25, 142),
        30: (309.75, 102),
        29: (307.25, 62),
        28: (300, 22),
        27: (296.5, 42),
        26: (291.5, 82),
        25: (289, 122),
        24: (286.5, 162),
}
}
}

```

A.7 Example of merging of five clusters

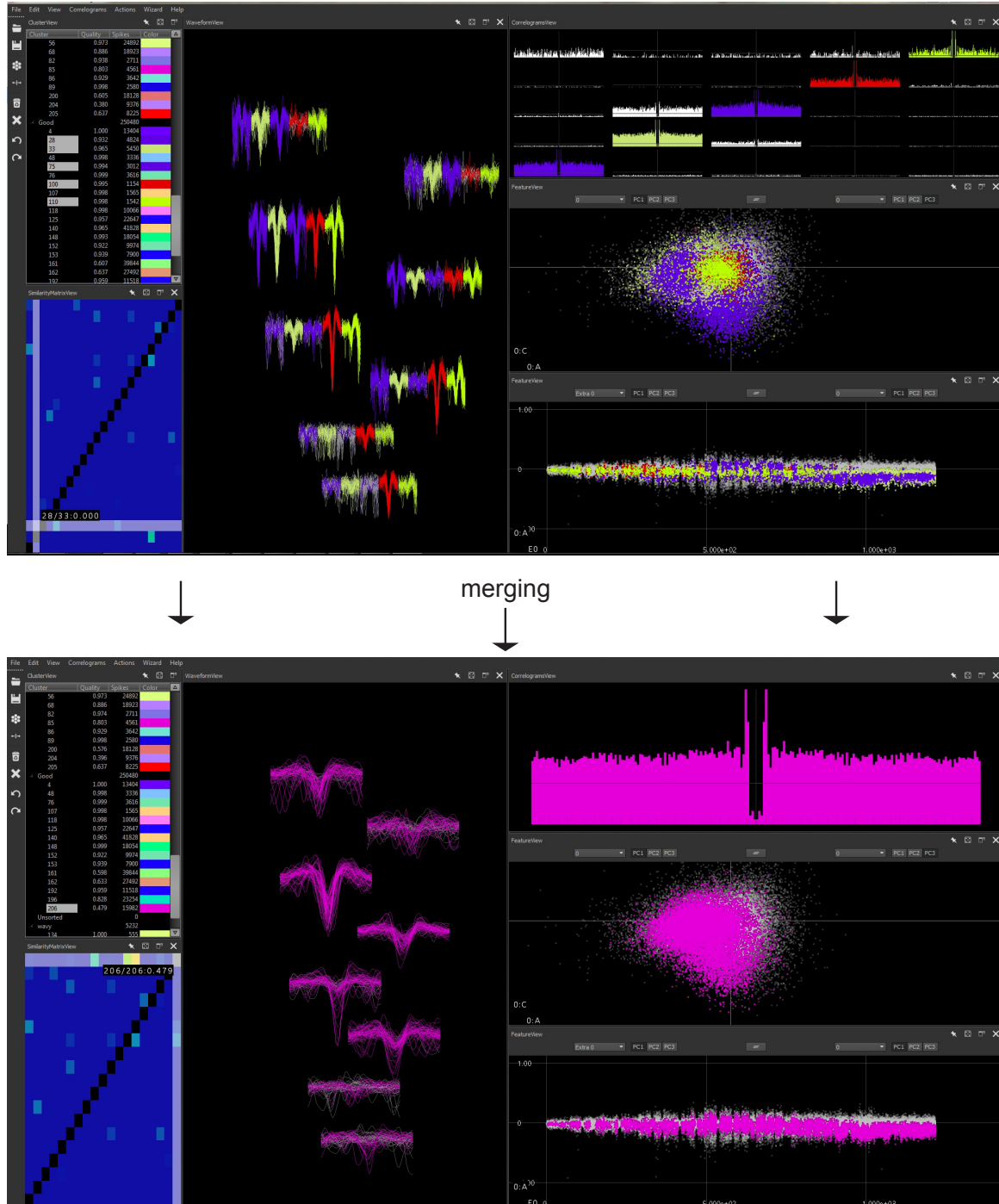


Figure A.12: Example of merging of five clusters. Five automatically identified clusters showed similar waveforms and clustering in feature space. Also, they appeared at different times during the recording as it can be observed from the top panel. After merging, the auto-correlogram still presented a deep central trough compared to its shoulders, the waveforms looked consistent, and the spike times were more homogeneously distributed throughout the recording.

A.8 Behavioural training

problem	reason	solution
subject does not participate	animal shows symptoms of fear (ears down) wrong positioning	habituate it to experimenter with more handling make sure front shoulders are high enough to allow steering movements, body slightly on the back of the sphere, regulate air pressure of <i>treadball</i> until the mouse can stay still without too much effort
	subject appears weak	check weight, give more water and/or suspend training if necessary
	mouse moves backward	remove the water spout; check head-fixation
mouse does not consume rewards	water spout not well positioned	adjust water spout position to make sure the subject reaches it easily
	water spout flooded does not consume it	empty it using vacuum pump avoid using the water spout and give water after session provided it does the task
poor performance	keeps one limb on water spout Mouse hit the walls too much	removes water spout increase air-puffers pressure; reduce or enlarge width of VR corridor
	visual stimulus too fast or slow	check gain of heading rotation and VR dimensions
	subject stops licking during session	move rewards to only the end; check water rewards consumed
	subject spins rather than walking	check position, <i>treadball</i> pressure, visual stimulus configuration
	does not participate	change daily order subjects for daily sessions
	starts completing trials late in the session	prolong session duration
sudden drop in performance	air pressure of <i>treadball</i> wrong	increase it or decrease it as needed
	air-puffers too strong find it hard to turn	reduce air pressure of air puffers make sure the sphere is clean and there are no feces between the base and the sphere of the <i>treadball</i>
	good start then stops moving during session	use longer VR corridor
	does not participate anymore	change daily order subjects for daily sessions; if experimenter changed, switch back to original
	technical problem	check all equipment is plugged to the mains and in correct working order

Table A.1: Troubleshooting for behavioural training. Series of possible solutions for problems encountered during this work. More problems might arise and multiple reasons might be at the source of single problems. Sometimes the training must be interrupted.

Cohort One

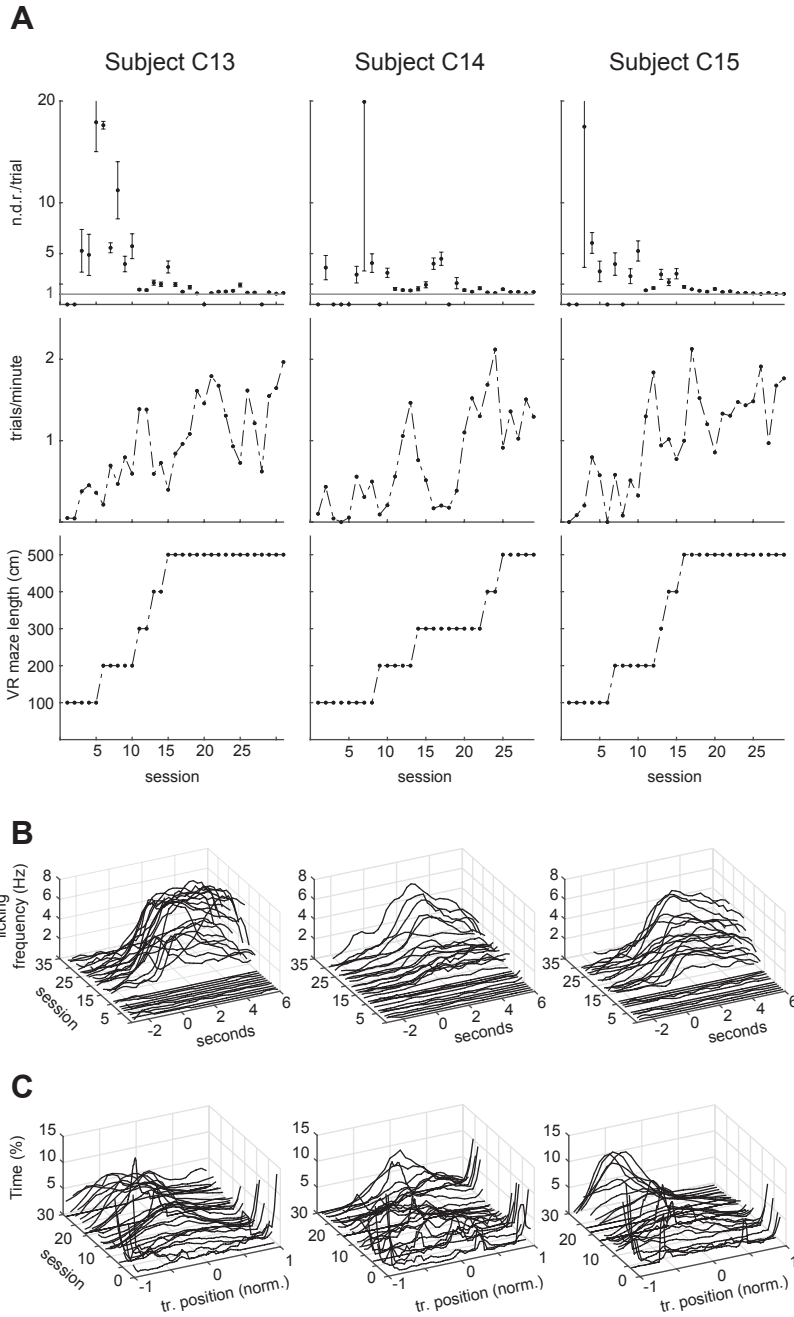


Figure A.13: Training progress of first cohort. **A)** Performance of mice measured during training sessions. Top: normalised distance ran per trial, error bars are s.e.m.; middle: trials completes per minute; bottom: VR corridor length for each session is an indication of training progress. **B)** Lick frequency for all sessions of the same subjects in A ordered in columns. When mice lick consistently in a session the curve shows a typical increase after the release of the water reward ($t=0$). **C)** Fraction of session time spent along the normalised transverse position of the VR corridor. If a mouse stays off the walls the curves should be high in the centre and next to zero at the sides.

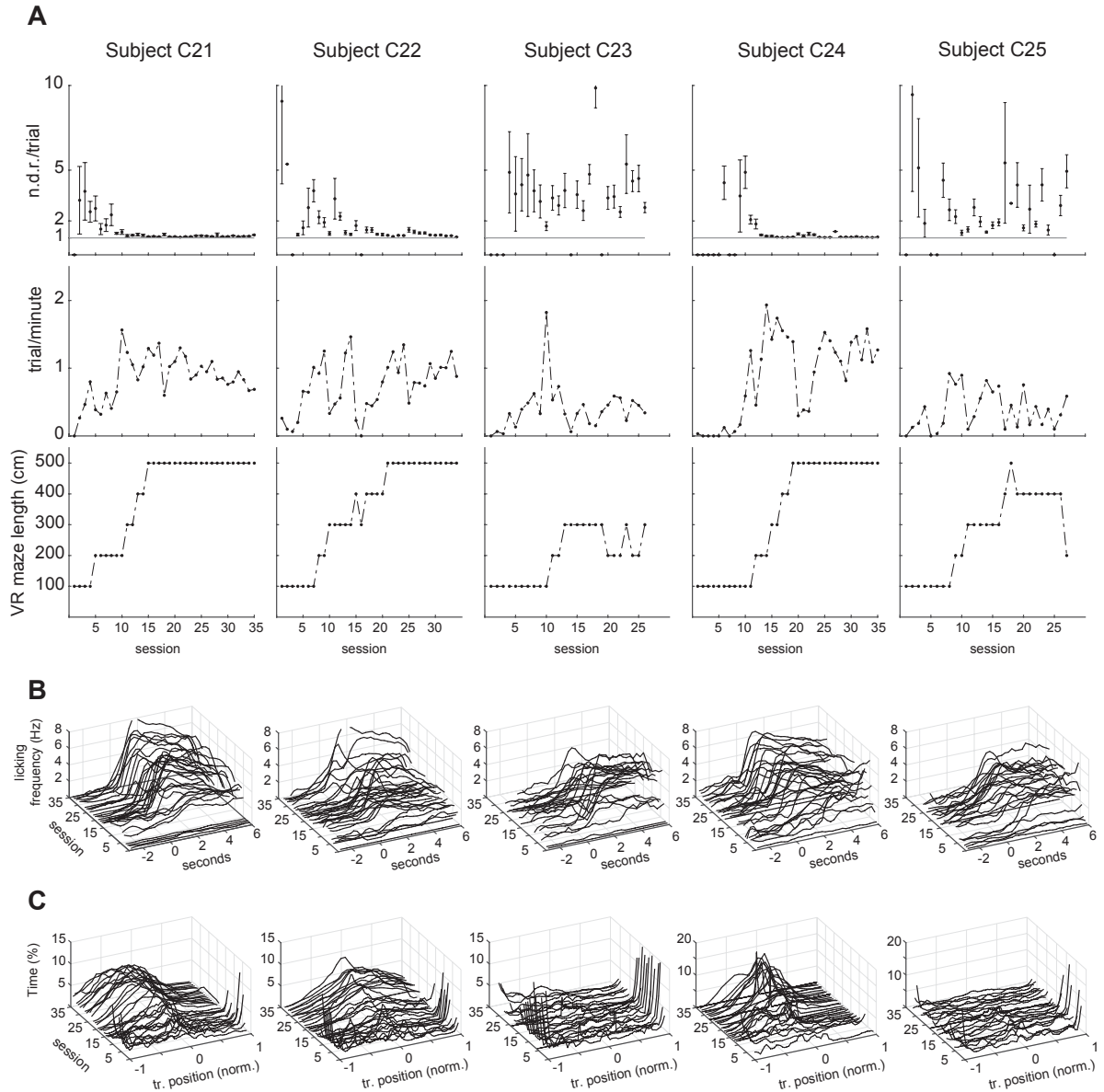
Cohort two

Figure A.14: Training progress of first cohort. **A)** Performance of mice measured during training sessions. Top: normalised distance ran per trial, error bars are s.e.m.; middle: trials completes per minute; bottom: VR corridor length for each session is an indication of training progress. **B)** Lick frequency for all sessions of the same subjects in A ordered in columns. When mice lick consistently in a session the curve shows a typical increase after the release of the water reward ($t=0$). **C)** Fraction of session time spent along the normalised transverse position of the VR corridor. If a mouse stays off the walls the curves should be high in the centre and next to zero at the sides.

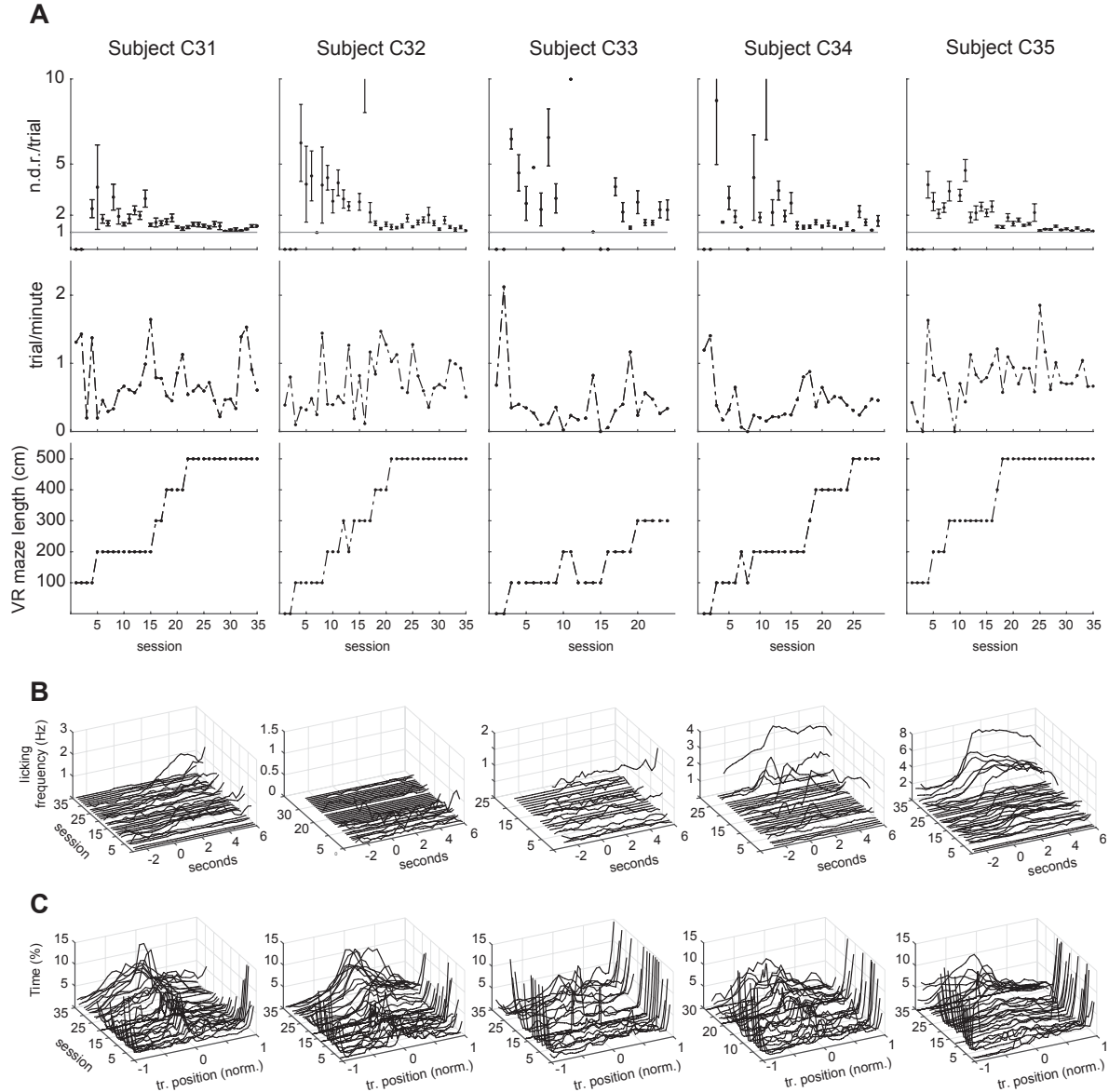
Cohort three

Figure A.15: Training progress of first cohort. **A)** Performance of mice measured during training sessions. Top: normalised distance ran per trial, error bars are s.e.m.; middle: trials completes per minute; bottom: VR corridor length for each session is an indication of training progress. **B)** Lick frequency for all sessions of the same subjects in A ordered in columns. When mice lick consistently in a session the curve shows a typical increase after the release of the water reward ($t=0$). **C)** Fraction of session time spent along the normalised transverse position of the VR corridor. If a mouse stays off the walls the curves should be high in the centre and next to zero at the sides.

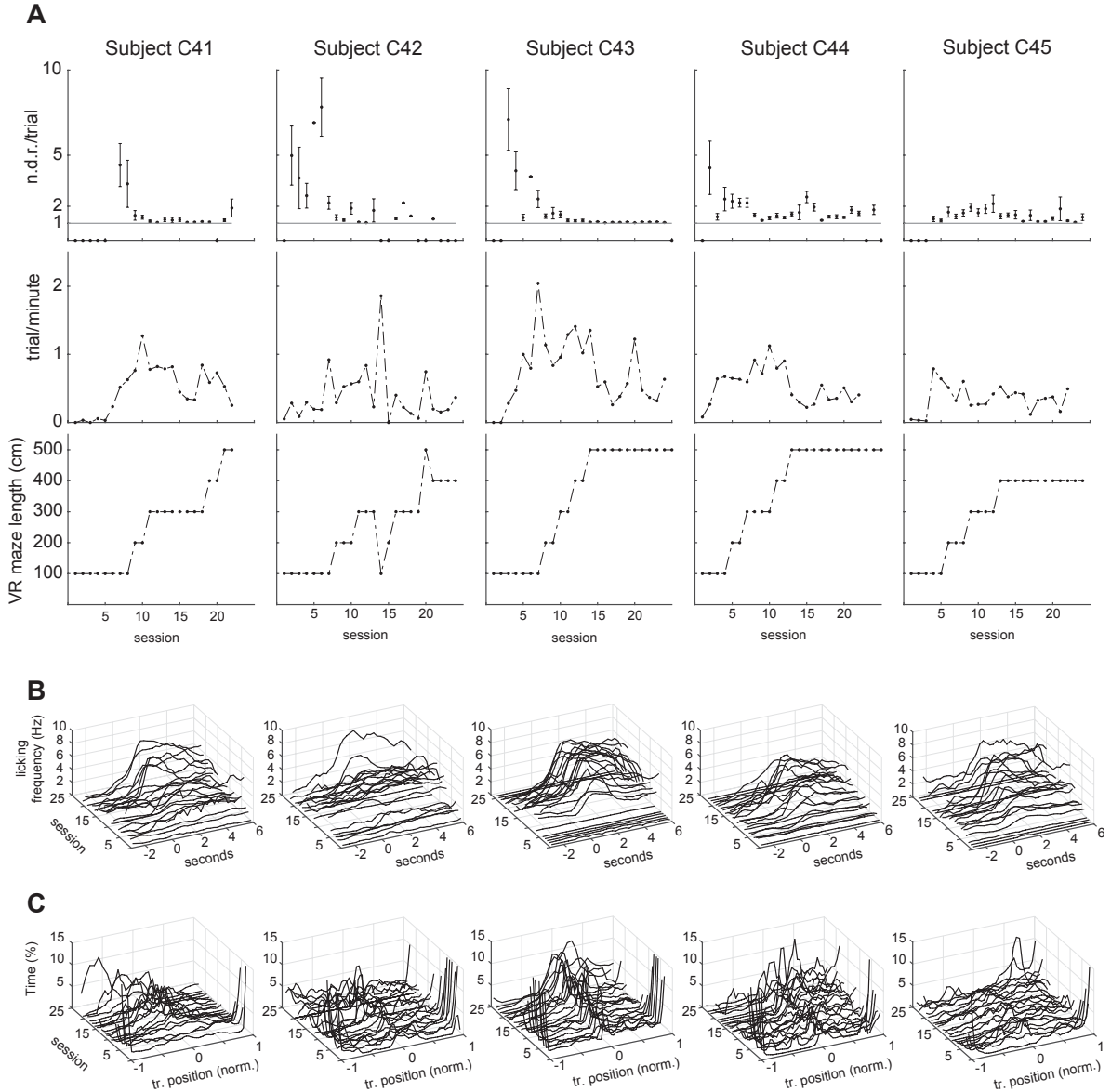
Cohort four

Figure A.16: Training progress of first cohort. **A)** Performance of mice measured during training sessions. Top: normalised distance ran per trial, error bars are s.e.m.; middle: trials completes per minute; bottom: VR corridor length for each session is an indication of training progress. **B)** Lick frequency for all sessions of the same subjects in A ordered in columns. When mice lick consistently in a session the curve shows a typical increase after the release of the water reward ($t=0$). **C)** Fraction of session time spent along the normalised transverse position of the VR corridor. If a mouse stays off the walls the curves should be high in the centre and next to zero at the sides.

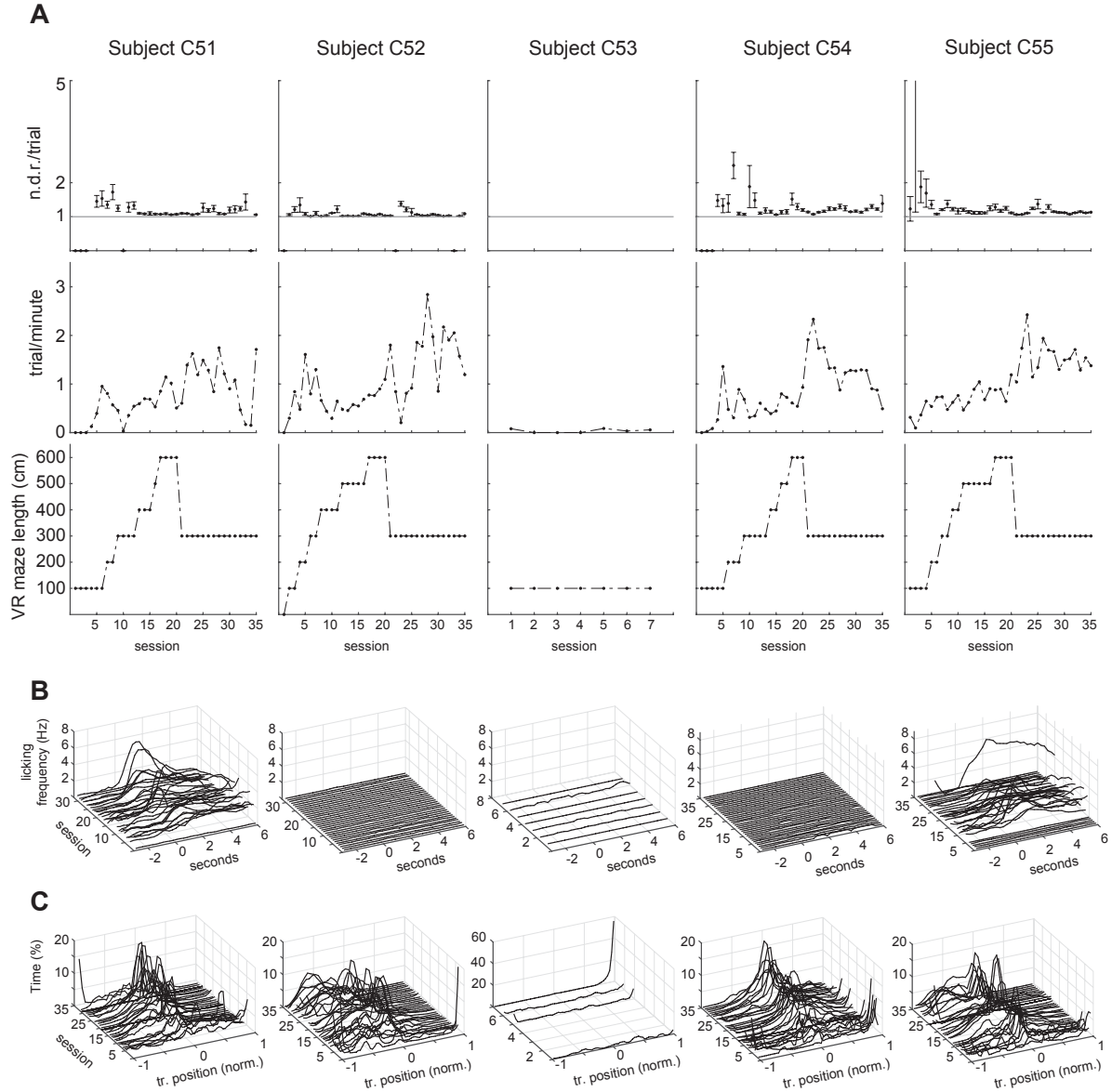
Cohort five

Figure A.17: Training progress of first cohort. A) Performance of mice measured during training sessions. Top: normalised distance ran per trial, error bars are s.e.m.; middle: trials completes per minute; bottom: VR corridor length for each session is an indication of training progress. **B)** Lick frequency for all sessions of the same subjects in A ordered in columns. When mice lick consistently in a session the curve shows a typical increase after the release of the water reward ($t=0$). **C)** Fraction of session time spent along the normalised transverse position of the VR corridor. If a mouse stays off the walls the curves should be high in the centre and next to zero at the sides.

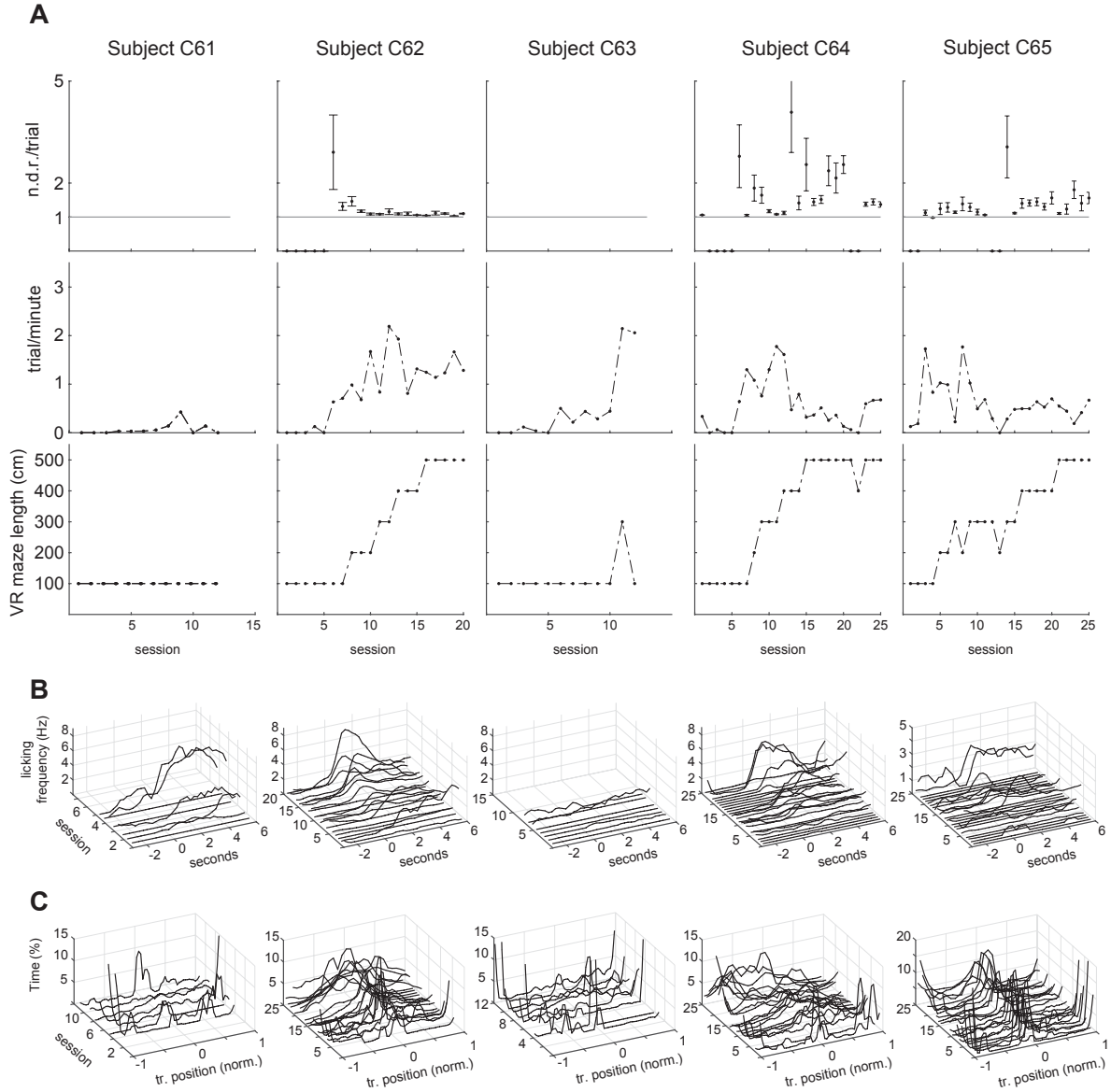
Cohort six

Figure A.18: Training progress of first cohort. **A)** Performance of mice measured during training sessions. Top: normalised distance ran per trial, error bars are s.e.m.; middle: trials completes per minute; bottom: VR corridor length for each session is an indication of training progress. **B)** Lick frequency for all sessions of the same subjects in A ordered in columns. When mice lick consistently in a session the curve shows a typical increase after the release of the water reward ($t=0$). **C)** Fraction of session time spent along the normalised transverse position of the VR corridor. If a mouse stays off the walls the curves should be high in the centre and next to zero at the sides.

Example of poor performance

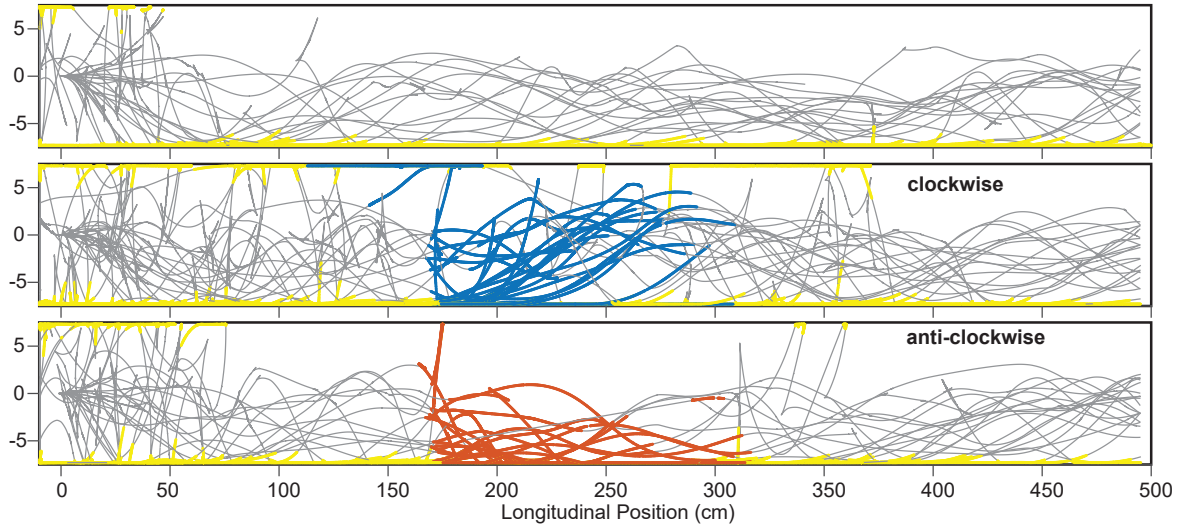


Figure A.19: Example session of poor performance. Trajectories clearly show the typical behaviour of this mouse (C31) running along the walls. Fraction of time during which air-puffers were active was 1% of the total duration of the session (seventh since perturbation paradigm was applied, session duration = 1346 seconds; 24 trials without perturbation, 23 trials with clockwise perturbation, 24 trials with anti-clockwise perturbation).

'E QUINDI USCIMMO A RIVEDERE LE STELLE'

(Inferno XXXIV, 139)