SUB-CORTICAL NEURAL CODING DURING
ACTIVE SENSATION IN THE MOUSE

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Abstract

Two fundamental questions in the investigation of any sensory system are what physical signals drive its primary sensory neurons and how such signals are encoded by the successive neural levels during natural behaviour. Due to the complexity of experiments with awake, actively sensing animals, most previous studies focused on anesthetized animals, where the motor component of sensation is abolished and therefore those questions are so far largely unanswered. The aim of this thesis is to exploit recent advance in electrophysiological, behavioural and computational techniques to address those questions in the sub-cortical whisker system of the mouse.

To determine the input to the whisker system, in Chapter 2 I recorded from primary whisker afferents (PWAs) of awake, head-fixed mice as they explored a pole with their whiskers, and simultaneously measured both whisker motion and forces with high-speed videography. To predict PWA firing, I used Generalised Linear Models. I found that PWA responses were poorly predicted by whisker angle, but well predicted by rotational force (moment) acting on the whiskers. This concept of “moment encoding” could account for the activity of PWAs under diverse conditions - whisking in air, active whisker-mediated touch and passive whisker deflection.

The discovery that PWAs encode moment raises the question of how mice employ moment to control their tactile behaviours. In Chapter 3 I therefore measured moment at the base of the whiskers of head-fixed mice, performing a novel behavioural task, which involved whisker-based object localisation. I then tested which features of moment during whisker-object touch could predict mouse choice. By using probabilistic classifiers, I discovered that mouse choices could be accurately predicted from moment magnitude and direction during touch, combined with a non-sensory variable - the mouse choice in the previous trial.

Finally, in Chapter 4 I asked how tactile coding generalized to whisker system sub-cortical brains regions during a natural active whisker-based behaviour. I therefore combined a naturalistic whisker-guided navigation task and extracellular recording with a novel generation of high density silicon probes (O3 Neuropixel probes) and studied how touch and locomotion were encoded by the whisker first (ventral posterior nucleus, VPM) and higher order thalamic relay (posterior complex, PO) and hypothalamic regions and (zona incerta, ZI). Using multiple linear regressions, I found that neurons in the relay nucleus VPM encoded not only touch, but also locomotion signals. Similarly, neurons in the higher-order regions PO and ZI were driven by both touch and locomotion. My study showed that in the awake animal, in the central part of the whisker system, peripheral signals were preserved, but were encoded concomitantly with motor variables, such as locomotion.

In summary, in this thesis I identified the mechanical variable representing the major sensory input to the whisker system. I showed that mice are able to employ it to guide behaviour and found that correlate of this signal was encoded by central neurons of the whisker system in VPM, PO and ZI, concomitantly with locomotion.
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1. General introduction
"La filosofia naturale è scritta in questo grandissimo libro che continuamente ci sta aperto innanzi agli occhi, io dico l'universo, ma non si può intendere se prima non s'impara a intender la lingua e conoscere i caratteri nei quali è scritto. Egli è scritto in lingua matematica, e i caratteri son triangoli, cerchi ed altre figure geometriche, senza i quali mezzi è impossibile a intenderne umanamente parola; senza questi è un aggirarsi vanamente per un oscuro labirinto."

“Philosophy is written in this grand book, the universe, which stands continually open to our gaze. But the book cannot be understood unless one first learns to comprehend the language and read the letters in which it is composed. It is written in the language of mathematics, and its characters are triangles, circles, and other geometric figures without which it is humanly impossible to understand a single word of it; without these, one wanders about in a dark labyrinth.” (Galilei, 1623).
1.1 GENERAL OVERVIEW

In the last decades many neuroscientists focused their attention on sensory cortex (Acsady, 2017) which, thanks to its higher experimental accessibility, has been extensively studied in awake behaving animals, leading to novel insights on its functions (O’Connor et al., 2010b, 2013; Saleem et al., 2013; Hires et al., 2015; Peron et al., 2015; Chorev et al., 2016; Yu et al., 2016a). However, although comprehensively studied in anesthetized animals, very little is known on the role played by sub-cortical components of sensory systems during behaviour. The general scope of this thesis is to investigate neural coding in the mouse sub-cortical whisker system (see paragraph 1.2, Diamond et al., 2008), by asking how peripheral sensory signals are represented by whisker sub-cortical neurons during natural behaviour.

The key step to understand how any sensory system operates is to determine what physical signals drive its sensory neurons during natural behaviour. The key condition to answer this question is to be able to precisely measure the physical input signals to the system. One complication of characterizing the inputs during behaviour is that sensation is an active process during which animals move their sensory organs – nose, eyes, antennae, fingers or whiskers (Gibson, 1962; Yarbus, 1967) in complex and unpredictable ways. Such active component of sensation has made so far hard to measure the inputs to the brain in awake animals and most of previous studies on sensory coding opted for anesthetized animals in which active component of sensation was suppressed by total anaesthesia. Therefore how sensory system operates during active sensation remained so far largely unanswered.

Thanks to recent improvement in experimental approaches and mathematical modelling, the whisker system of rodents is now the model of choice to study active sensation. First,
whisker-based sensation is active: rodents actively move their whiskers back and forth (in periodic cycles with a frequency of 1Hz to 20Hz, Carvell and Simons, 1990) and use them to navigate (Vincent, 1912; Mitchinson et al., 2007; Arkley et al., 2014; Sofroniew et al., 2014), detect objects (O’Connor et al., 2010a), estimate object distance, orientation and shape (Hutson and Masterton, 1986; Brecht et al., 1997; Krupa et al., 2001; Polley et al., 2005; Knutsen et al., 2006; Pammer et al., 2013; Sofroniew et al., 2014, 2015) and discriminate superficial textures (Guić-Robles et al., 1989; Carvell and Simons, 1990).

Second, whiskers have properties which make the measurement of the sensory input during active sensation experimentally tractable. Whiskers protrude from the skin and they are near-conical objects, typically 10 times longer than their base width (Williams and Kramer, 2010; Hires et al., 2016). This allows a whisker’s position and shape to be directly measured, accurately and non-invasively, in the awake, behaving animals, by high-speed imaging. There are then simple, but powerful, equations (detailed in the next sections) that express the relationship between the force applied to a conical rod and how much it bends (Birdwell et al. 2007, Pammer et al. 2013). By making appropriate measurements of whisker shape from imaging data, estimates of the mechanical forces acting on the base of the whisker shaft can thereby be derived.

In this thesis, I applied these methods and identified the primary sensory variable (“moment”) that PWAs encode during active sensation (Chapter 2). I then studied how moment is used to guide whisker-driven behaviour (Chapter 3) and, finally how tactile and motor variables were encoded by the central sub-cortical regions of the whisker system during a complex natural behaviour (whisker-guided navigation; for a more detailed description of thesis aims and contents see section 1.7).
The aim of this chapter is to introduce this new experimental paradigm by describing the basic approaches which have permitted the measurement of the mechanical whisker variables during active sensation (section 1.3) and the general anatomy and physiology of the whisker system (section 1.2). I will also review the main experimental approaches used in the past to study neural coding in the whisker system, using PWAs’ studies as representative example. Finally I will elucidate how the possibility of studying awake active behaviour measuring the sensory input to the whisker system, offers now the possibility of addressing previously unanswered questions of fundamental importance (section 1.5 and 1.6), which have been tackled in this thesis (section 1.7).

1.2 GENERAL ANATOMY AND PHYSIOLOGY OF THE WHISKER SYSTEM

The whisker system of rodents is an ensemble of interconnected neuronal structures, (Figure 1.1) which process the tactile information collected by whiskers or facial vibrissae (Diamond et al., 2008). Each whisker has one whisker follicle (Rice and Munger, 1986; Rice et al., 1993; Ebara et al., 2002) by which it is anchored in the snout skin (Figure 1.1, gray dots). Follicles are organized on the rodent's snout in a highly stereotyped manner (Diamond et al., 2008). They compose an array (called whisker pad) of 5 rows (A to E), and multiple arcs or columns (1 to 9). The 4 whiskers in the most posterior arc of the whisker pad are identified by the greek letters α, β, γ and δ (Dorfl, 1985).

Each whisker follicle is innervated by approximately 100-150 (Lee and Woolsey, 1975) pseudounipolar neurons (PWAs), which transduce stresses of the whisker follicle into action potentials. PWAs have been classified into different categories according to the micro-anatomical structure of their axon terminal (transverse lanceolate ending,
longitudinal lanceolate ending, club ending, superficial Merkel ending, deep Merkel ending, reticular ending, spiny ending, Ebara et al., 2002; Sakurai et al., 2013).

In the rodents’ brain, the sensory information transmitted by PWAs is processed and distributed by at least three parallel, tri-synaptic pathways: lemniscal pathway, extralemniscal pathway and paralemniscal pathway (Figure 1.1; Diamond et al., 2008; Moore et al., 2015).

Lemniscal (Figure 1.1, purple arrows) and extralemniscal pathways present a high degree of anatomical organization. In each of the relay/processing stations, located downstream of the TG, a somatotopic representation of the whisker pad has been observed. These representations consist in anatomically identifiable cluster of cells, called barrelettes in the pons, barreloids in the thalamus and barrels in layer 4 of somatosensory cortex S1 (lemniscal pathways only), whose neurons vigorously respond to mainly the deflection of a single whisker (Waite, 1973; Simons, 1978; Simons and Carvell, 1989; Minnery and Simons, 2003) in the anesthetized animal and to touch in the awake one (Moore et al., 2015; Yu et al., 2016a). Lemniscal barrelettes are located in the trigeminal nucleus principalis (PrV) and project to the dorsomedial part of barreloids in the controlateral ventral posteromedial (VPM) nucleus of the thalamus (Minnery and Simons, 2003; Diamond et al., 2008). Extralemniscal barrelettes occupy a different trigeminal nucleus (caudal part of the interpolaris trigeminal nucleus, SpVi) and project to the ventrolateral part of VPM barreloids (van der Loos, 1976). While lemniscal thalamic neurons send their axons predominantly to layer 4 barrels, (Minnery and Simons, 2003; Diamond et al., 2008), extralemniscal thalamic neurons project mainly to the septa between the barrels and to the secondary somatosensory cortex (S2; Diamond et al., 2008). Barrelettes and barreloids
contain approximately the same number of neurons as the number of PWAs, which innervate one follicle (250 units per whisker in the rat, Land and Simons, 1985). Conversely, the number of neurons increases dramatically in S1: the barrel itself includes 2000 neurons in the mouse, and the entire cortical column three times more (Welker and Van der Loos, 1986).

The paralemniscal pathway (Figure 1.1, green arrows) is only weakly somatotopically organized either in the pons (rostral part of SpVi) or in the thalamus or hypothalamus¹ (Posterior Complex, PO, and Zona Incerta, ZI). Differently from the lemniscal pathways, whose neurons project mainly to S1 and have single whisker receptive fields, PO and ZI neurons are characterized by a great variety of target structures and the neurons have mainly multi-whisker receptive fields (Diamond et al., 1992a, 1992b; Urbain and Deschênes, 2007; Jones, 2012). PO targets include motor cortex (M1), S1 and S2, auditory cortex, insular and ectorhinal cortex, dorso-lateral striatum and ZI (Deschênes et al., 1998; Ohno et al., 2012). ZI projects to many higher order thalamic nuclei and motor nuclei such as PO, the parafascicular nucleus, the anterior pretectal nucleus, ventral lateral nucleus, ventral medial nucleus, lateral posterior nucleus and lateral dorsal nucleus (Power et al., 1999; Barthó et al., 2002; Urbain and Deschênes, 2007). ZI is also characterized by long range projections to most cortical areas, brainstem, spinal cord, substantia nigra and contra-

¹ In this thesis I classified ZI as part of the hypothalamus, consistent with Allen Brain Atlas nomenclature system used in Chapter 4 and with Urbain and Deschênes (2007). However, it is worth to report that, according to other nomenclature systems, ZI has been classified as a part of the ventral thalamus (Jones, 2012).
lateral ZI (Mitrofanis and Mikuletic, 1999; Power et al., 1999; Jones, 2012; Watson et al., 2015).

Like lemniscal neurons, paralemniscal neurons respond to whisker stimulation in both anaesthetized and awake animals (Diamond et al., 1992a, 1992b; Urbain and Deschênes, 2007; Jones, 2012; Moore et al., 2015). However, a notable physiological difference between paralemniscal nuclei ZI and PO compared to the lemniscal nucleus VPM is dependency on cortical activity. While the sensitivity of VPM neurons to whisker deflection is largely independent of cortical manipulations, PO and ZI neuronal responses to whisker deflection are heavily dependent upon cortical state: inhibition of S1 and activation of M1 dramatically reduce responsiveness to whisker stimulation in PO and ZI respectively (Diamond et al., 1992a; Urbain and Deschênes, 2007).
Figure 1.1. The whisker system: lemniscal and paralemniscal pathways.

Schematic of the projections of lemniscal (purple arrows) and paralemniscal (green arrows) pathways from single whisker mechanoreceptors to cortex and various sub-cortical targets. Gray dots are whisker follicles. Extralemniscal pathway has been hidden here to reduce clutter, but see section 1.2 for an in depth anatomical description. For acronyms explanation see section 1.2. Adapted from Urbain et al. (2015).

1.3 APPROACHES TO QUANTIFY WHISKER SENSORY VARIABLES IN AWAKE ACTIVE SENSING RODENTS

1.3.1 A mechanical framework for whisker-based sensation

As a rodent move its whiskers in air or against object surfaces, the tissue in the follicle experiences stresses (Whiteley et al., 2015) that can trigger the activity of mechanoreceptors. In the absence of contact (‘whisking in air’), whisker mechanoreceptors are potentially susceptible to diverse forces, reflecting inertia of the whisker, contraction of facial muscles and viscoelasticity of the whisker pad tissue within which the follicle is anchored (see section 5.2 for in depth discussion). When a time-independent force is applied to a whisker, force onset triggers high-frequency vibration of the whisker (Neimark et al. 2003; Hartmann et al. 2003; Ritt et al. 2008; Wolfe et al. 2008; Boubenec et al. 2012), which rapidly decays to a static equilibrium, where the whisker bends against the object (Birdwell et al., 2007). In steady-state, the degree of bending depends on both the applied force and the whisker’s stiffness. During active whisking against an object, the relative importance of the dynamic and static effects depends on the material properties of the whisker, the whisker motion, the shape/texture/material of the object and where along the
whisker shaft the motion of the whisker is measured. Since mechanoreceptors sense stresses at the base of a whisker, it is motion here, rather than at the tip, that is most relevant to neural coding in PWAs. Whisking against a rough surface elicits dynamic ‘slip-stick’ effects that evoke neuronal responses (Arabzadeh et al., 2005; Wolfe et al., 2008; Jadhav et al., 2009), but whisking against a smooth surface such as a metal pole generally elicits only weak dynamic effects at the whisker base (Quist et al., 2014).

For whisking against smooth objects, a whisker’s shape can, aside from occasional slips, be approximated as a continuously changing steady-state, where the shape at any given time depends on the applied force at that time. This ‘quasi-static’ case is not universally applicable (e.g., rough textures) but, as detailed in the next section, it is the basis for a mechanically-rooted experimental paradigm (that I applied in Chapter 2 and 3) that has given substantial novel insight into somatosensation. Unless stated to the contrary, the approach applied in the thesis and discussed below assumes the quasi-static case.

1.3.2 Forces at the whisker base

Suppose a time-independent force is applied to a whisker. Such a force exerts a rotational effect on the whisker (‘moment’), which makes it bend around a pivot near its base. In steady state, the applied force and the moment are balanced by reaction force and reaction moment at the whisker base. In general, both the forces and moments are 3D, implying a total of 6 mechanical variables acting at the whisker base. However, 3D forces/moments are challenging to estimate (for progress, see Huet et al., 2015; Loft et al., 2016) and almost all studies to date, including the one reported in the thesis, have considered experimental conditions where whisker motion and whisker forces/moments are predominantly planar. Whisking motion occurs largely, but not entirely, in the horizontal plane defined by the two
eyes and the nose (Knutsen et al. 2008; Bermejo et al. 2002). Thus, when a rat/mouse whisks against a vertical surface, such as a pole, whisker-object contact force and whisker bending is largely in the horizontal plane: these effects can be measured by imaging in this plane. In this 2D case, whisker-object contact is characterised by 3 mechanical variables at the whisker-base: a 2-component applied force $\vec{F}$ directed at some angle in the horizontal plane and a moment $M_0$ (bending moment) directed about the vertical axis, normal to the horizontal plane (see Figure 1.2).

It is important to define $\vec{F}$ so that its relationship with mechanoreceptor activity is as direct as possible. Since mechanoreceptors are embedded in the whisker follicle, and since the follicle rotates rigidly during whisking (Bagdasarian et al., 2013), the natural coordinate system is a “whisker-centric” one (Pammer et al., 2013; Hartmann, 2015; Bush et al., 2016). The coordinate axes are ‘axial’ (pushing whisker into its follicle) and ‘lateral’ (pushing the whisker at 90 degrees to the follicle); the origin is at the base of the whisker (Figure 1.2). In whisker-centric coordinates, $\vec{F}$ is thus expressed in terms of an axial component $F_{ax}$ and a lateral one $F_{lat}$.

Whilst restricting analysis to the horizontal plane is experimentally advantageous, there are caveats. First, any vertical component of whisker motion (Knutsen et al. 2008) and any vertical component of bending (Huet et al. 2015) will be missed. Second, since whiskers are intrinsically curved and rotate about the axis of the follicle during whisking (Knutsen et al. 2008), imaging in the horizontal plane can register apparent changes in whisker shape that may be confused with bending. Third, any moment (torque) about the follicle axis will be missed (Huet et al., 2015). In 3D whisker-centric coordinates, the applied force has 1
axial component and 2 lateral components, the moment has 2 components of bending and 1 of torsion/roll about the long axis of the follicle (Huet et al. 2015).
Notation:
\( r_b \): quadratic Bezier curve fitted to the whisker base.
\( \kappa_b \): whisker curvature at the base.

Definition of whisker curvature at the base:
\[
\kappa = \frac{x''_b y'_b - x'_b y''_b}{(x''_b^2 + y''_b)^{3/2}}
\]
where \( x_b \) and \( y_b \) are horizontal/vertical coordinates of the image, \( s = [0, 1] \) parameterises \((x, y)\) location along the curve.

Notation:
\( M_0 \): bending moment at the whisker base.
\( \vec{F} \): applied force of object against whisker at the contact point.

Definition of applied force and moment:
\[
M_0 = F r_0 \sin(\phi)
\]
where \( r_0 \) and \( F \) are the modules of the “lever arm” vector \( \vec{r}_0 \) connecting the whisker base to the contact point and of the applied force vector \( \vec{F} \) respectively.

\[
F_{ax} = F \sin(\theta_{base} - \theta_{contact})
\]
\[
F_{lat} = F \cos(\theta_{base} - \theta_{contact})
\]
where \( \theta_{base} \) and \( \theta_{contact} \) are respectively the angle between the tangent to the whisker at its base and the horizontal, and the angle between the tangent to the whisker at the contact point and the horizontal.
**Figure 1.2. Mechanical framework for whisker-based sensation**

Whisker angle ($\theta$; orange), bending moment at the whisker base, ($\bar{M}_b$; cyan), applied force ($\bar{F}$; red) and its component axial force ($F_{ax}$; purple) and lateral force ($F_{lat}$; yellow) can be measured during active whisker object interaction, by filming the whisker at high-speed and processing each frame using whisker tracking algorithm. In this example, a quadratic Bezier curve ($r_s$; green) is fitted to the segment of the whisker near its base. Whisker angle is defined as the angle between the tangent to $r_s$ at the whisker base ($s=0$) and the horizontal. Whisker curvature ($\kappa_s$; blue) is computed from the Bezier quadratic fitting using the equation and is proportional to the reciprocal of the radius of the circle ($g$) which best fits the Bezier curve around point $s$ (blue dotted line). $F_{ax}$ and $F_{lat}$ are components of the applied force expressed in whisker centric coordinates. The trigonometric relation between the three variables can be computed measuring $\theta_{contact}$ and $\theta_{base}$ (adapted from Campagner et al., 2016).

### 1.3.3 Estimation of mechanical whisker variables

One of the drivers of recent progress has been the development of techniques for measuring these whisker mechanical variables experimentally in the awake, behaving animal. In this section, I outline the theoretical foundation.

Consider a whisker that is being bent due to contact with an object such as a pole. Whiskers are intrinsically curved: contact changes the whisker’s curvature with respect to its intrinsic (undeformed) value. As noted above, in steady-state, there is a simple relationship between the bending moment ($M_p$) about a point $p$ (located along the whisker shaft between the whisker base and the contact point) and the curvature $\kappa_p$ of the whisker at that point (Birdwell et al 2007). The fundamental equation is:

$$M_p(t) = \Delta k_p(t)E_p I_p$$  \hspace{1cm} (1.1)

Here $\Delta k_p(t)$ is the change in the whisker’s curvature compared to its intrinsic shape at time $t$ and $E_p I_p$ is a constant of proportionality (‘bending stiffness’; from this point on, to reduce
clutter, the \( t \) dependence is omitted). Equation 1.1 states simply that, the stiffer a whisker, the greater the moment implied by a given change in curvature. The constant consists of a factor \( E_p \) (‘Young’s modulus’) that depends only on the material composition of the whisker and a factor \( I_p \) (‘second moment of inertia’) that depends only on the geometry of the whisker. \( E_p \) has typical values of 2-5GPa (Hartmann, 2015), but may vary somewhat across whiskers and with \( p \) (Quist et al. 2011). \( I_p \) depends only on the cross-sectional area of the whisker at the point \( p \) and, for an object with circular cross-section, \( I_p = \frac{\pi}{4} a_p^4 \), where \( a_p \) is the radius of the whisker at point \( p \). Because \( I_p \) depends on the fourth power of the radius, the variation in stiffness along the whisker shaft is dramatic: for a typical whisker whose tip diameter is 10% of its base diameter, bending stiffness at the base is 10000 times greater at the base than at the tip.

Equation 1.1 is of fundamental, practical importance, since it indicates that mechanical variables associated with whisker touch can be estimated in an experimentally feasible manner by imaging a whisker’s shape. The simplest approach is to estimate the curvature change at the whisker base \( \Delta k_0 \) (Figure 1.2). Provided that, during the course of a video, \( \Delta k_0 \) is measured at the same point \( (p = 0) \) along the whisker shaft (so that \( I_0 \) is constant) this measure is proportional to \( M_0 \) (Equation 1.1). A second approach is possible if measurements of \( a_0 \) and \( E_0 \) are also available: in this case, \( I_0 \) can be calculated and thereby \( M_0 \) itself. However, since whiskers can grow over the time-course of a typical behavioural experiment (Ibrahim and Wright, 1975), and since the bending stiffness is sensitive to radius (a 10% change in \( a_0 \) changes \( I_0 \) by 46%), estimates of \( M_0 \) are more delicate. With both approaches, care must be taken to ensure that the location at which the curvature is measured is constant and as close as possible to the whisker base. Both these approaches
have been applied in Chapter 2, while in Chapter 3 we applied a variant of the former one. An alternative, more complex, approach is to model a whisker as a series of links connected by torsional springs and fit its parameters to imaging data (Quist et al., 2014). This approach has not been applied in this thesis; therefore it will not be discussed any further.

To complete the description of the forces acting on the whisker base, it is necessary to estimate the applied force \( \vec{F} \) (Pammer et al., 2013). The principle here is that a force applied to some point on a whisker exerts a moment \( M_0 \), which is the product of the force magnitude and distance between the whisker base and the line of action of the force. Thus, if this distance can be measured, the force magnitude can be obtained by working backwards from an estimate of \( M_0 \). An equivalent, and experimentally useful, form of this expression for \( M_0 \) is:

\[
M_0 = Fr_0 \sin(\phi) \quad (1.2)
\]

Here \( F \) is the magnitude of \( \vec{F} \), \( r_0 \) is the length of the lever arm vector \( \vec{r}_0 \) that connects the base of the whisker to the contact point and \( \phi \) is the angle between \( \vec{F} \) and \( \vec{r}_0 \) (see Figure 1.2). The equation expresses the fact that, to open a door, it is more effective to push in a direction normal to it (|\( \phi \)|=90°, |\( \sin(\phi) \)|=1) than at an angle (|\( \phi \)|<90°, |\( \sin(\phi) \)|<1) and more effective to situate the handle near the edge (large \( r_0 \)) than near the hinge (small \( r_0 \)). To obtain values for \( F_{ax} \) and \( F_{lat} \), it is necessary to know the direction of \( \vec{F} \). If friction can be approximated as zero, this direction is normal to the whisker at the contact point (Figure 1.2) and:

\[
F_{ax} = F\sin(\theta_{base} - \theta_{contact}) \quad (1.3)
\]

\[
F_{lat} = F\cos(\theta_{base} - \theta_{contact}) \quad (1.4)
\]
Here $\theta_{\text{base}}$ and $\theta_{\text{contact}}$ are angles that define the geometry of the contact (Figure 1.2). These quantities can be estimated from images of the whisker. Procedures to take account of friction have been proposed and, for whisking against smooth, metal poles, have little effect on the frictionless force estimates (Pammer et al., 2013; Hires et al., 2016; Huet and Hartmann, 2016).

1.3.4 Whisker imaging and tracking

Qualitative “cinematographic” analysis of whisking was first done by Welker (1964). However, to put the above theory into practice, it is necessary to obtain quantitative kinematic/mechanical information from individual video frames. In the first study to quantify whisker motion, the angle of whiskers to the head (‘whisker angle’) was measured by putting a transparency over the video monitor and tracing whiskers on to it by hand (Carvell and Simons, 1990). Later studies developed a variety of semi-automated techniques for measuring whisker kinematics (i.e., whisker angle) based on beam-break sensors (Bermejo et al., 1998, 2002; Arabzadeh et al., 2005; Wolfe et al., 2008; Jadhav et al., 2009; Khatri and Bermejo, 2009), computer-assisted, manual tracking (Grant et al., 2009), tracking the location of spots of dye (Knutsen et al., 2008; Venkatraman et al., 2009; Nashaat et al., 2017; Rigosa et al., 2017) and segmentation of whiskers from video (Voigts et al. 2008; Ritt et al. 2008; Perkon et al. 2011).

To estimate the key mechanical variables defined above, it is necessary to track not just whisker kinematics, but also whisker curvature. This has required machine vision techniques that extract the shape of one or more whiskers from imaging data (Knutsen et
al., 2005; O’Connor et al., 2010a; Clack et al., 2012; Pammer et al., 2013; Bale et al., 2015; Campagner et al., 2016). Application of machine vision has also made it feasible to measure whisker motion from high-speed video (500-1000 frame/s) on large data sets.

With these methods, the shape of a whisker is captured by fitting a parametric curve \( r_s = [x_s, y_s] \) to the image of a whisker (where \( s \) indicates location along the whisker; Figure 1.2). In principle, it is then straightforward to calculate the required curvature \( \kappa_s \) defined in Figure 1.2.

As noted above, it is forces at the base of the whisker that are most closely related to mechanotransduction. Thus, recent studies have focussed on estimation of the curvature near the base. The basal part of a whisker is most readily imaged (radius is comparatively large, moves most slowly and least out of the focal plane) and is well-approximated by a quadratic function (Quist and Hartmann, 2012; Pammer et al., 2013) which (in 2D) has only 6 free parameters. The basal part can either be described by refitting a quadratic function to this portion of the complete, tracked whisker (Clack et al., 2012; Pammer et al., 2013) or by restricting whisker tracking to the basal section of the whisker (Bale et al., 2015; Campagner et al., 2016). Whisker-object contact parameters, which are required in order to estimate \( F_{ax} \) and \( F_{lat} \), can be measured by tracking the segment of whisker near the contact point (Figure 1.2). In my thesis I applied the approaches described above to quantify whisker position and mechanical variables acting at the base of the whiskers in behaving mice.
1.4 PREVIOUS APPROACHES TO STUDY NEURAL CODING IN THE WHISKER SYSTEM OF RODENTS

Numerous studies, employing diverse experimental paradigms, have sought to investigate how physical signals associated with touch drive the responses of neurons in the whisker system. The bulk of these studies have focussed on the relationship between kinematic variables (whisker position, velocity, etc.) and neuronal activity. Next, I summarize, as an example of the previously applied techniques, the main experimental approaches used to study neural coding in PWAs of TG (which is the neural structure I investigate in Chapter 2). As explain more exhaustively in the General Discussion and Conclusion (section 5.3), similar approaches have also been applied to study several other regions of the whisker system listed in section 1.2. Only very recently approaches similar to the one described in section 1.3 have been successfully applied to study whisker sensory-motor cortex and thalamus (see Chapter 4 and section 5.3;(O’Connor et al., 2010b; Huber et al., 2012; Petreanu et al., 2012; Xu et al., 2012; Peron et al., 2015; Yu et al., 2016a).

1.4.1 Physiology of PWAs in anesthetized and awake rodents

1.4.1.1 Passive stimulation experiments

In the ‘passive stimulation’ paradigm (Zucker and Welker, 1969; Gibson and Welker, 1983), whisker movement is suppressed by global anaesthesia and sensory stimulation is applied by deflecting the whiskers, typically by means of mechanical actuators. Passive stimulation is useful to investigate questions for which a high degree of experimental control of the sensory input is essential. PWAs have been reported to be tuned to the direction (in the plane normal to the whisker shaft), the amplitude, the velocity or
acceleration of the deflection, but most are tuned to a weighted sum of amplitude and velocity, with different neurons having different weightings (Gibson and Welker, 1983; Lichtenstein et al., 1990; Shoykhet et al., 2000; Kwedyir-Afful et al., 2008; Bale and Petersen, 2009; Bale et al., 2013). There is also evidence that some PWAs respond to deflection in the axial direction (Stütgen et al., 2008).

Passive stimulation studies have also shown that neurons in the whisker system are capable of firing spikes timed with sub-millisecond precision (Petersen, et al. 2001; Panzeri et al. 2001; Arabzadeh et al.2005; Montemurro et al. 2007; Bale and Petersen 2009). A recent study showed, by high sampling rate electrophysiological recording (500 kHz), that the physical limits of spike timing precision are at the microsecond scale: spike timing jitter in PWAs in response to rapid whisker deflection was on average 17.4 μs with the most precise neurons exhibiting jitter of 5.1 μs (Bale et al. 2015).

1.4.1.2 Artificial whisking experiments

In the ‘artificial whisking’ paradigm (Zucker and Welker, 1969; Szwed et al. 2003; Arabzadeh et al. 2005; Lottem and Azouz 2011; Szwed et al. 2006; Wallach et al. 2016), whisker movement is produced by dissecting out the buccal motor branch of the facial nerve and electrically stimulating it, in an anaesthetised animal. Sensory stimulation is produced by positioning objects in the path of the whiskers. Artificial whisking (Zucker and Welker, 1969; Szwed et al., 2003) is useful since whisker-object contact forces are produced in a fashion that mimics the active character of awake, whisking behaviour, but can be controlled and repeated by the experimenter. The paradigm differs from natural whisking in that both intrinsic and extrinsic muscles are excited in phase (Szwed et al.,
2006) and that a fast unnatural component (83Hz) is added to whisker motion (Szwed et al., 2003, 2006).

Artificial whisking studies led to the first descriptions of how active whisker-object contact and whisking phase might affect PWA activity. Three neuronal subtypes have been reported: whisking neurons, touch neurons and whisking-touch neurons. Whisking neurons fire at specific phases of the whisking cycle (Szwed et al. 2003; Szwed et al. 2006; Wallach et al. 2016) and their response is invariant to weak whisker-object contact. Touch neurons fire only during contact and are subdivided into pressure neurons (fire for the entire duration of contact), contact neurons (fire only at onset of contact), detach neurons (fire only at offset of contact) and contact-detach neurons (fire at both onset and offset of contact). Whisking-touch neurons fire both during whisking (in a phase-locked fashion) and in response to contact (Szwed et al. 2003; Szwed et al. 2006). For most touch-sensitive PWAs, firing rate depends on radial pole location, decreasing with distance of contact from the face (Szwed et al., 2006).

1.4.1.3 Awake, behaving experiments

Lack of somatotopical organization (see section 1.2 and Leiser and Moxon, 2006), together with low cell density (about 8000 neurons/mm³, Sakurai et al., 2013, 60 times less than the density of granule cells in the mouse hippocampus, Richards et al., 2013), adds to the complexity of experimenting with awake animals, making electrophysiological recordings from TG of behaving animals particularly challenging. Two pioneering studies first succeeded in recording PWA activity from awake, behaving rats (Leiser and Moxon 2007; Khatri et al. 2009), but were limited by the whisker tracking tools available at the time. According to those studies, most PWAs fire at a higher rate during whisking than when the
whiskers are still, and some PWAs, particularly those with high firing rate during whisking, show phase-locked responses (Leiser and Moxon, 2007) and are also modulated by both whisking amplitude (Khatri et al. 2009) and whisking frequency (Leiser and Moxon, 2007). In addition, PWAs fire at a higher rate during whisker-object contact than during whisking in air (Leiser and Moxon, 2007).

To summarise, passive stimulation studies reported that the signals encoded by PWAs were kinematic variables (i.e. whisker angular position and its derivatives). Artificial whisking and awake studies observed tuning to kinematic variables during whisking in air: however, which sensory variables drive PWAs firing during touch was not understood.

As discussed above, ultimately, it has to be mechanical forces/moments acting on the whisker base that drive mechanotransduction. Until recently, however the relationship between whisker kinematics and mechanical variables (forces, moments, and proxies to them) during active touch was unknown. Hence it was unclear what mechanical variables, were actually encoded by PWAs, and therefore by the entire whisker system. In Chapter 2, I therefore recorded PWA activity while simultaneously estimating kinematics and mechanical variables, using the approach detailed in the above sections. This has resulted in the identification of the major driver of PWAs: the moment.

**1.5 MECHANICAL BASIS OF ACTIVE WHISKER-GUIDED BEHAVIOURS**

A further advantage of the experimental paradigm outlined in section 1.3 is that it allowed me to ask how the mechanical variables acting at the whisker base are used by mice to instruct their behaviours. As explained in section 1.3, only recently has it become possible to estimate mechanical variables during active sensing. Therefore, only very few studies
have so far tried to address such question (O’Connor et al., 2010a; Pammer et al., 2013), and the sensory signals used by rodents to guide their decisions are so far largely unknown. In particular, the fact that we identified moment as the sensory input to the system in Chapter 2 raises the question of how bending moment is used by mice to guide their behaviour.

A huge variety of behavioural tasks are available to study different aspects of whisker-based sensation (Maravall and Diamond, 2014). They include object detection (Mehta et al., 2007; O’Connor et al., 2010a; Guo et al., 2014a, 2014b), radial object localisation (Pammer et al., 2013), shape recognition (Brecht et al., 1997), texture discrimination (Guić-Robles et al., 1989; Carvell and Simons, 1990; Chen et al., 2013), anterior-posterior distance measurement (Knutsen et al., 2006), identification of object orientation (Polley et al., 2005), whisker-guided navigation (Sofroniew et al., 2014; Yu et al., 2016b), gap aperture measurement (Hutson and Masterton, 1986; Ghazanfar et al., 2001) and passive detection or discrimination of temporal sequences of passive whisker deflection (Sachidhanandam et al., 2013; Fassihi et al., 2014; Yang et al., 2015; Bale et al., 2017).

However, as discussed in section 3.2, none of them is suited to understand how moment is used by mice during behaviour. In Chapter 3, we therefore developed a novel whisker-based behavioural task and applied the paradigm described in section 1.3, to study how moment is exploited by mice to localize an object.

1.6 NEURAL CODING OF TOUCH AND MOTOR VARIABLES IN VPM, PO AND ZI DURING ACTIVE BEHAVIOUR

As explained at the outset of this introduction, the general aim of this thesis was to study how whisker sub-cortical neurons encode sensory signals in the awake animal, where the
motor component of sensation is preserved. In Chapter 2, I address this question at the first neural level of the system, the TG, but as explained in section 1.2, the sensory information collected by PWAs is transmitted to multiple neural structures via mono- or multi-synaptic pathways. Among whisker sub-cortical structures, the first order and higher order relay nuclei of the thalamus are of fundamental importance (Sherman and Guillery, 2006; Jones, 2012) since they are key building blocks of any ascending sensory pathway of mammals (Sherman and Guillery, 2006). However, how they operate during awake behaviour has only recently started to be addressed (Niell and Stryker, 2010; Erisken et al., 2014; Moore et al., 2015; Urbain et al., 2015; Roth et al., 2016; Yu et al., 2016a; Gutnisky et al., 2017) and still poorly understood. In Chapter 4, I therefore focused on the first order whisker relay nucleus VPM, on the high order whisker relay nucleus PO, and on its GABAergic modulator ZI (Calford, 1983; Calford and Aitkin, 1983; Barthó et al., 2002; Sherman and Guillery, 2006; Diamond et al., 2008; Lee, 2015; Watson et al., 2015). PO and ZI are characterized by high heterogeneity of input and output connectivity, which include sensory, motor and locomotion regions (see sections 1.2, 4.4.2 and 4.4.3 for detailed description; Mogenson et al., 1985; Diamond et al., 1992a; Lin et al., 1997; Power et al., 1999; Barthó et al., 2002; Trageser, 2004, 2006; Lavallee, 2005; Sherman and Guillery, 2006; Urbain and Deschênes, 2007; Hooks et al., 2013; Petersen, 2014; Urbain et al., 2015; Watson et al., 2015; Moore et al., 2015; Roth et al., 2016; Economo et al., 2016). The fact that PO and ZI had only rarely studied in awake rodents (Moore et al., 2015; Urbain et al., 2015) and never in locomoting animals, potentially drastically limits our understanding of their function. Recent developments in experimental techniques now make it possible to record neuronal activity from behaving mice running on spherical treadmill, while they navigate a tactile virtual corridor guided by their whiskers (Sofroniew et al., 2014, 2015).
By combining high-speed sensors and high-speed videography of the whiskers, it is then possible to monitor the mouse locomotion, measure whisker angle and estimate proxy for moment at the whisker base during the interaction of whiskers with the corridor walls (see Chapter 4 and Sofroniew et al., 2014). By recording neuronal activity during the task in Chapter 4, I studied VPM, PO and ZI encoded touch and locomotion during a natural behaviour.

1.7 RESULT CHAPTERS’ OUTLINE AND THESIS AIMS

In this thesis, I took advantage of the novel possibility offered by the whisker system of the mouse of measuring the sensory input in awake behaving animals, to study for the first time how sensory variables were encoded at different neural levels of the sub-cortical whisker system (TG, VPM, PO and ZI) in awake behaving animals and how such variables drive mouse behaviour.

In order to understand which kinematic and mechanical variables are encoded by PWAs and therefore to determine the nature of the sensory input to the whisker system in the awake animal, I recorded from PWAs of awake, head-fixed mice as they explored a pole with their whiskers (Chapter 2). Simultaneously I measured both whisker motion and forces with high-speed videography. Using Generalised Linear Models, I found that PWAs responses were poorly predicted by whisker angle, but well predicted by moment: both during touch and free-air whisker motion. Chapter 2 provides therefore the first electrophysiological evidence of which is the sensory input to the whisker system during active sensation, rooting it into the mechanical framework exposed above.
Having discovered that moment was the major driver of PWAs during touch, I then asked how this variable might be used by mice to solve a whisker-based behavioural task (Chapter 3). I therefore developed a novel behavioural task (the three-choice object localisation task), where mice had to localize the rostro-caudal location of an object with a single whisker. Using high-speed videography, I estimated bending moment during whisker-object touch and I found that mouse decision could be predicted from the direction and the magnitude of bending moment during touch plus a non-sensory component: the memory of the choice in the previous trial. I therefore found a direct link between mechanoreceptor encoding mechanisms and behaviour.

In Chapter 4 I studied whisker-guided locomotion in mice, while they used their whisker to navigate a corridor in a tactile virtual reality environment (Sofroniew et al., 2014). During this task I recorded neuronal activity with high density Neuropixel probes, from the sub-cortical whisker regions VPM, PO and ZI (Figure 1.1), while monitoring the mouse distance from the wall (a correlate of bending moment under this experimental condition, Sofroniew et al., 2014) and mouse locomotion velocity. Surprisingly, I found that the activity in the whisker regions VPM, PO and ZI were modulated not only, as expected, by tactile signals, but also by locomotion. I therefore concluded that, consistent with what expected from the findings of Chapter 2 and 3, tactile signals were encoded by the central part of the whisker system, however also non-whisker related signals (such as locomotion velocity), were encoded. Overall, in my thesis I identified the major sensory input to the whisker system in the awake animal, the moment; I showed its behavioural relevance and reported the encoding of tactile signal in the whisker primary thalamus and higher order thalamic and hypothalamic regions, concomitantly with locomotion.
1.8 JOURNAL FORMAT THESIS

This thesis is presented in the journal format. The rationale for submission in this format was the availability, at the start of the final year, of one already published paper and of two manuscripts in preparation. Having already written the two unpublished result chapters (Chapter 3 and 4) in a publishable format, will accelerate the process of their submission to scientific journals. While each manuscript stands alone as a piece of research, together they form a cohesive story on how tactile mechanical information is encoded by sub-cortical whisker pathway and drives mouse behaviour during active sensation.

The choice of the journal format is justified by the fact that I have played the primary role in all the aspects of the realization of the three results chapters. I am first author of the already published chapter (Chapter 2) and I will be co-first author of Chapter 3 and first author of Chapter 4. In particular, in:

Chapter 2 - I have designed the study together with Rasmus S. Petersen, performed the experiments, analyzed the data, and developed the experimental methods. Rasmus S. Petersen helped me with the implementation of the whisker tracking and the Generalized Linear Model algorithms, while Mathew H. Evans with the development of the mechanical variable estimation algorithm.

Chapter 3 - I have designed the study together with Rasmus S. Petersen and Karel Svoboda, performed the experiments, analyzed the data and developed the behavioural methods. Mathew H. Evans helped with the implementation of the whisker tracking algorithm, the touch detection algorithm and with the data curation process. During behavioural training I was supported by two lab technicians at the University of Manchester (Katarina Chlebikova...
and David Pettifer), and a lab technician at HHMI Janelia Research Campus (Luciana Walendy). Rasmus S. Petersen helped with the development of the software to control the behavioural setup.

Chapter 4 - I have designed the study together with Rasmus S. Petersen and Karel Svoboda, performed the experiments, analyzed the data, and developed the experimental methods. The idea for this project originated from a collaboration between Diego A. Gutnisky and Karel Svoboda, from HHMI Janelia Research Campus, Rasmus S. Petersen and me. The experimental setup was the same as the one used in Sofroniew et al. (2015) and the experiments were conducted at Karel Svoboda’s lab. During the project I have received technical support from the Applied Physics and Instrumentation Group and the Anatomy and Histology Team of HHMI Janelia Research Campus.

1.9 PUBLISHED CONTENT

This thesis contains chapters which have been published in scientific journals. Chapter 2 has been published as Campagner D, Evans MH, Bale MR, Erskine A, Petersen RS (2016). Prediction of primary somatosensory neuron activity during active tactile exploration. Elife 5:1–18. Part of the General Introduction (Chapter 1) and of the General Discussion and Conclusions (Chapter 5) has been published as What the whiskers tell the brain, Campagner D, Evans MH, Loft MSE and Petersen RS (2017) Neuroscience.
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2. Prediction of primary somatosensory neuron activity during active tactile exploration
2.1 ABSTRACT

Primary sensory neurons form the interface between world and brain. Their function is well-understood during passive stimulation but, under natural behaving conditions, sense organs are under active, motor control. In an attempt to predict primary neuron firing under natural conditions of sensorimotor integration, we recorded from primary mechanosensory neurons of awake, head-fixed mice as they explored a pole with their whiskers, and simultaneously measured both whisker motion and forces with high-speed videography. Using Generalised Linear Models, we found that primary neuron responses were poorly predicted by whisker angle, but well predicted by rotational forces acting on the whisker: both during touch and free-air whisker motion. These results are in apparent contrast to previous studies of passive stimulation, but could be reconciled by differences in the kinematics-force relationship between active and passive conditions. Thus, simple statistical models can predict rich neural activity elicited by natural, exploratory behaviour involving active movement of sense organs.
2.2 INTRODUCTION

A major challenge of sensory neuroscience is to understand the encoding properties of neurons to the point that their spiking activity can be predicted in the awake animal, during natural behaviour. However, accurate prediction is difficult without experimental control of stimulus parameters and, despite early studies of awake, behaving animals (Hubel, 1959), subsequent work has most often effected experimental control by employing anaesthesia and/or passive stimulation. However, the active character of sensation (Gibson, 1962; Yarbus, 1967), based on motor control of the sense organs, is lost in reduced preparations. Recent methodological advances permit a way forward: in the whisker system, it is now possible to record neuronal activity from an awake mouse, actively exploring the environment with its whiskers, whilst simultaneously measuring the fundamental sensory variables (whisker kinematics and mechanics) likely to influence neuronal activity (O’Connor et al., 2010b). Our aim here was to predict spikes fired by primary whisker neurons (PWNs)\(^1\) of awake mice engaged in natural, object exploration behaviour. The manner in which primary neurons encode sensory information fundamentally constrains all downstream neural processing (Lettvin et al., 1959). PWNs innervate mechanoreceptors located in the whisker follicles (Zucker and Welker, 1969; Rice et al., 1986). They are both functionally and morphologically diverse; including types responsive to whisker-object contact and/or whisker self-motion (Szwed et al., 2003; Ebara et al., 2002). PWNs project to the cerebral cortex, analogously to other modalities, via trisynaptic pathways through the brainstem and thalamus (Diamond et al., 2008).

\(^{1}\) Note that in this chapter we refer to ‘primary whisker afferents’ (PWAs), using the expression ‘primary whisker neurons’ (PWNs).
Here, we show that PWN responses are well-predicted by rotational force (‘moment’) acting on the whisker, while whisker angle is a poor predictor. Moment coding accounts for spiking during both whisker-object interaction and whisker motion in air. Moment coding can also account for findings in previous studies of passive stimulation in the anaesthetized animal; indicating that the same biomechanical framework can account for primary somatosensory neuron responses across diverse states. Our results provide a mechanical basis for linking receptor mechanisms to tactile behaviour.

2.3 RESULTS

2.3.1 Primary whisker neuron activity during object exploration is predicted by whisker bending moment

We recorded the activity of single PWNs from awake mice (Figure 2.1 A, E, Figure 2.5) as they actively explored a metal pole with their whiskers (N = 20 units). At the same time, we recorded whisker motion and whisker shape using high-speed videography (1000 frames/s; Figure 2.1 A, B and D). Before the start of each trial, the pole was moved to a randomly selected, rostro-caudal location. During this time, the whiskers were out of range of the pole. At the start of the trial, the pole was rapidly raised into the whisker field, leading to whisker-pole touch. As detailed below, PWNs were diverse, with some responding only to touch, others also to whisker motion. Since each PWN innervates a single whisker follicle, we tracked the ‘principal whisker’ of each recorded unit from frame to frame, and extracted both the angle and curvature of the principal whisker in each video frame (total 1,496,033 frames; Figure 2.1 B–E; Bale et al., 2015). Whiskers are intrinsically curved, and the bending moment on a whisker is proportional to how much this curvature changes due to object contact (Birdwell et al., 2007): we therefore used ‘curvature change’ as a proxy for
bending moment (O’Connor et al., 2010a). Whisker-pole contacts caused substantial whisker bending (curvature change), partially correlated with the whisker angle (Figures 2.1 E, 2.4 E) and, consistent with Szwed et al. (2003) and Leiser and Moxon (2007), robust spiking (Figures 2.1 E, 2.2 E).

Figure 2.1. Electrophysiological recording from single primary whisker units in awake, head-fixed mice and simultaneous measurement of whisker kinematics/mechanics.

**A.** Schematic of the preparation, showing a tungsten microelectrode array implanted into the trigeminal ganglion of a head-fixed mouse, whilst a metal pole is presented in one of a range of locations (arrows). Before the start of each trial, the pole was moved to a randomly selected, rostro-caudal location. During this time, the whiskers were out of range of the pole. At the start of the trial,
the pole was rapidly raised into the whisker field, leading to whisker-pole touch. Whisker movement and whisker-pole interactions were filmed with a high-speed camera.

**B, C.** Kinematic (whisker angle $\theta$) and mechanical (whisker curvature $\kappa$, moment $\vec{M}$, axial force $\vec{F}_{ax}$ and lateral force $\vec{F}_{lat}$) variables were measured for the principal whisker in each video frame.

When a whisker pushes against an object during protraction (as in panel D, red and cyan frames), curvature increases; when it pushes against an object during retraction (as in panels B and C), it decreases.

**D.** Individual video frames during free whisking (yellow and green) and whisker-pole touch (red and cyan) with tracker solutions for the target whisker (the principal whisker for the recorded unit, panel E) superimposed (coloured curve segments).

**E.** Time series of whisker angle, push angle and curvature change, together with simultaneously recorded spikes (black dots) and periods of whisker-pole contact (red bars). Coloured dots indicate times of correspondingly coloured frames in D.

To test between candidate encoding variables, our strategy was to determine how accurately it was possible to predict PWN activity from either the angular position or curvature change of each recorded unit’s principal whisker. To predict spikes from whisker state, we used Generalised Linear Models (GLMs; Figure 2.2 A). GLMs, driven by whisker angle, have previously been shown to provide a simple but accurate description of the response of PWNs to passive stimulation (Bale et al., 2013) and have mathematical properties ideal for robust parameter-fitting (Truccolo et al., 2005; Paninski et al., 2007).

For each recorded unit (median 69,672 frames and 550 spikes per unit), we computed the GLM parameters that best predicted the unit’s spike train given the whisker angle time series, using half the data as a training set for parameter-fitting (8 total fitted parameters - 5 for stimulus filter, 2 for history filter, 1 bias; Figure 2.8). We then assessed prediction
performance using the other half of the data as a testing set: we provided the GLM with the whisker angle time series as input and calculated the predicted spike train, evoked in response (section 2.5.9.3).

We then compared the recorded spike train to the GLM-predicted one (Figure 2.2 B–C) and quantified the similarity between the smoothed spike trains using the Pearson correlation coefficient (PCC). This is a stringent, single-trial measure of model prediction performance (Figure 2.7 B). We then repeated this entire procedure for the whisker curvature time series. Although angle GLMs predicted spike trains of a few units moderately well (2/20 units had PCC >0.5), they performed poorly for the majority (median PCC 0.06, IQR 0.019–0.3; Figure 2.2 B–D, orange). This was unlikely to be because of non-linear tuning to whisker angle, since quadratic GLMs fared only marginally better (median PCC 0.097, IQR 0.042–0.31; p=0.044, signed-rank test, Figure 2.2 A). In contrast, we found that, at the population level, the curvature GLMs were substantially more accurate than the angle GLMs (median PCC 0.52, IQR 0.22–0.66; p=0.0044, signed-rank test; Figures 2.2 B–D, blue) with prediction accuracy up to PCC 0.88. Curvature GLMs also predicted spikes during touch episodes significantly more accurately (median PCC 0.57, IQR 0.23–0.72) than did angle GLMs during non-touch episodes (median 0.06, IQR 0.02–0.35; p=0.005, signed-rank test).

At the level of individual units, 90% had above chance PCC and we termed these ‘curvature-sensitive’ (section 2.5.9.3). Of the curvature-sensitive units, 61% were sensitive to positive curvature change and 39% to negative curvature change (section 2.5.9.3).

A GLM performs a temporal filtering operation on its sensory stimulus input and the sensory feature(s) which it encodes is determined by this ‘stimulus filter’. The stimulus filters can, in principle, be complex, but we found that the ability of a GLM to predict
spikes from curvature change was fully explained by the simple case where the action of the stimulus filter is simply to multiply the sensory input by a gain factor (median 0.55, IQR 0.26–0.66; p=0.35 signed-rank test; Figure 2.7 C). This also indicated that, the result that curvature predicted PWN responses better than angle was robust to the number of fitted parameters. A GLM sensitive to instantaneous curvature has in fact only 4 parameters: 1 stimulus filter parameter, 2 history filter parameters and 1 bias.

The result was also robust to time-scale: prediction accuracy based on curvature was significantly greater than that based on angle for smoothing time-scales in the range 1–100 ms (signed-rank test, p<0.05, Bonferroni-corrected). Although the activity of most units was better predicted by whisker curvature change than by whisker angle, there was significant variability in prediction performance, and there were a few units for which the angle prediction performance was appreciable (Figure 2.2 D). However, we found that this could largely be attributed to redundancy. When a mouse whisks against an object, curvature change and angle fluctuate in concert (Birdwell et al., 2007; Bagdasarian et al., 2013; Pammer et al., 2013; Figures 2.1 E, 2.11 E–G). When we fitted GLMs using both curvature change and angle as input, these GLMs predicted the spike trains no more accurately (median PCC 0.53, IQR 0.40–0.62; p=0.067, signed-rank test; Figure 2.2 D) than GLMs based on curvature change alone. Moreover, on a unit-by-unit basis, for 65% of units, curvature change GLMs predicted spikes better than angle (signed-rank test, p<0.05, Bonferroni-corrected); only for 5% of units did angle predict spikes better than curvature change. GLMs based on curvature change also predicted spike trains more accurately than GLMs based on ‘push angle’ – the change in angle as the whisker pushes against an object (Figure 2.1 D; median PCC 0.25, IQR 0.04–0.45; p=0.006, signed-rank test). Moreover, prediction accuracy of GLMs fitted with both push angle and curvature change (median
PCC 0.52, IQR 0.2–0.69) inputs was no better than that of GLMs fitted with curvature alone (p=0.43, signed-rank test).

Figure 2.2. Primary whisker neurons encode whisker curvature, not whisker angle, during active sensation.

A. Schematic of the Generalized Linear Model (GLM).

B. For an example unit, whisker angle (top panel), whisker curvature change (middle panel) and simultaneously recorded spike train (bottom panel, black), together with predicted spike trains for the best-fitting angle GLM (bottom panel, orange) and curvature change GLM (bottom panel, blue). Spike trains discretized using 1-ms bins and smoothed with a 100 ms boxcar filter. Prediction performance (Pearson correlation coefficient, PCC) for this unit (curvature based GLM) was 0.59. Inset shows tuning curves for both GLMs, computed by convolving the relevant sensory time series.
(angle or curvature change) with the corresponding GLM stimulus filter to produce a time series of filter coefficients, and estimating the spiking probability as a function of filter coefficient (25 bins).

C. Analogous to panel B, for a second example unit. Prediction performance PCC for this unit was 0.74.

D. Prediction performance (PCC between predicted and recorded spike trains) compared for GLMs fitted with three different types of input: curvature change alone; angle alone; both curvature change and angle. Each blue/orange/green dot is the corresponding PCC for one unit: large black dots indicate median; error bars denote inter-quartile range (IQR). To test statistical significance of each unit's PCC, the GLM fitting procedure was repeated 10 times on spike trains subjected each time to a random time shift: magenta dots show these chance PCCs for the unit indicated by the magenta circle; the mean chance PCC was computed for each unit and the large grey dot shows the median across units. Black circles indicate units whose PCC was significantly different to chance (signed-rank test, Bonferroni-corrected, p<0.0025). To facilitate direct comparison between results for curvature change GLM and angle GLM, these are re-plotted in the inset.

E. Left: Firing rate during touch episodes compared to that during non-touch episodes for each unit, compared to corresponding predicted firing rates from each unit's curvature change GLM. Right: Medians across units: error bars denote IQR; * denotes differences significant at p<0.05 (signed-rank test).

In principle, neurons might also be sensitive to the axial force component (parallel to the whisker follicle) and/or lateral force component (orthogonal to axial) associated with whisker-object contact (Figure 2.1 C, Figure 2.6; Solomon and Hartmann, 2006; Pammer et al., 2013). We restricted our analysis to bending moment since, under our experimental conditions, axial/lateral force components were near-perfectly correlated with bending moment (Figure 2.9) and bending moment is likely to have a major influence on stresses in the follicle (Pammer et al., 2013).
To further test the curvature-encoding concept, we asked whether curvature GLMs could account for the response of PWNs to whisker-pole touch. To this end, we parsed the video data into episodes of ‘touch’ and ‘non-touch’. Units fired at a higher rate during touch than otherwise (Szwed et al., 2003; Leiser and Moxon, 2007). Without any further parameter adjustment, the curvature-based GLMs reproduced this effect (Figure 2.2 E): the correlation coefficient between recorded and GLM-predicted firing rate for touch episodes was 0.97. Collectively, the above results indicate that, during active touch, the best predictor of whisker primary afferent firing is not whisker angle but rather the bending moment.

2.3.2 Primary whisker neuronal activity during whisking is predicted by moment

During free whisking - in the absence of whisker-pole contact - whisker curvature, and therefore bending moment, changed little (Figure 2.1 E, Figure 2.4 F); consistent with previous studies (Knutsen et al., 2008; Quist et al., 2014). Yet, 50% of recorded units (‘whisking-sensitive units’) were significantly modulated by whisking amplitude (Figure 2.3 A). Consistent with Szwed et al. (2003), PWNs were diverse: 45% were curvature-sensitive (significant PCC for curvature based GLM) but not whisking-sensitive; 45% were both curvature- and whisking-sensitive; 5% were whisking-sensitive but not curvature-sensitive.

The presence of whisking sensitivity suggests that moment due to whisker bending is not the only force that influences PWN activity. A likely candidate is the moment associated with the rotational acceleration of a whisker: this moment is proportional to the whisker’s angular acceleration (Quist et al., 2014; section 2.9.1.2). Consistent with this possibility, we found that whisking sensitive units were tuned to angular acceleration (Figure 2.3 B) and that 50% of these were phase-modulated (Figure 2.3 C). Angular acceleration tuning was
diverse: some units fired to acceleration in a particular direction (rostral or caudal), whilst others responded to acceleration in both directions (Figure 2.3 B, Figure 2.10). Moreover, for whisking-sensitive units (but not whisking-insensitive ones), quadratic GLMs trained on data from non-touch episodes were able to predict spikes using whisker angle acceleration as input (Figure 2.3 D–E; whisking-sensitive units, median PCC 0.37, IQR 0.18–0.58; whisking-insensitive, median PCC -0.0071, IQR -0.035–0.041; p=0.0017 rank-sum test for whisking-sensitive vs whisking-insensitive units). For 70% of whisking-sensitive units, directional selectivity for acceleration was consistent with that for curvature. These findings indicate that, in the absence of whisker-object contact, responses of PWNs to whisking itself can be accounted for by sensitivity to the moment associated with angular whisker acceleration.
Figure 2.3. Primary whisker neurons encode whisker angular acceleration during free whisking.

A. Mean response of an example whisking-sensitive unit to whisking amplitude, computed during non-contact episodes (dark green, shaded area shows SEM) with regression line (black). Inset shows regression line slopes (median and IQR) for whisking sensitive (green) and whisking insensitive (grey) units. * indicates statistically significant rank-sum test (p=0.05).

B. Mean response of two example units as a function of angular acceleration. The dark brown unit is the same as that shown in A.

C. Mean response of two example units as a function of whisking phase. The dark pink unit is the same as that reported in A; the light pink unit is the same as that shown as light brown in B.
D. Excerpt of free whisking (orange) along with activity of an example, whisking-sensitive unit (black) and activity predicted by a GLM driven by angular acceleration (brown). The unit is the same as that shown in A.

E. GLM prediction accuracy (PCC) for all whisking sensitive (brown) and whisking insensitive units (grey). Bars and vertical lines denote median and IQR respectively.

### 2.3.3 Relation between kinematics and mechanics is different in active vs passive touch and has implications for neural encoding

We found, during active object exploration, that curvature change, but not whisker angle, predicts PWN firing. In apparent contrast, studies using passive whisker stimulation have reported that PWNs encode whisker angle and its temporal derivatives (Zucker and Welker, 1969; Gibson and Welker, 1983; Lichtenstein et al., 1990; Jones et al., 2004; Arabzadeh et al., 2005; Bale and Petersen, 2009; Lottem and Azouz, 2011; Bale et al., 2013). We wondered whether the discrepancy might be due to differences in whisker mechanics between passive and active stimulation conditions. To test this, we analysed the relationship between angle and curvature change during active touch and compared it to that during passive whisker stimulation. During active pole exploration, angle and curvature change were, overall, only loosely related (median correlation coefficient 0.20, IQR 0.079–0.39, Figures 2.4 D–E). Important contributory factors were that the angle-curvature relationship was both different for touch compared to non-touch (Figure 2.4 F) and dependent on object location (Figure 2.4 G). In contrast, during passive stimulation, whisker angle was near perfectly correlated with curvature change (for C2, correlation coefficients 0.96 and 0.94 respectively; similar results for C5; Figures 2.4 C–D, Figure 2.4 E, inset and Figure 2.11); consistent with properties of cantilevered beams (Birdwell et al., 2007).
Figure 2.4. Whisker angle and whisker curvature change are highly correlated during passive whisker deflection, but decoupled during active touch.

A. Whisker angle (top) and whisker curvature change (bottom) time series, due to passive, trapezoidal stimulation of C2 whisker in an anaesthetized mouse, estimated as mean over 10 repetitions. Note that error bars (showing SEM) are present but very small.

B. Corresponding data for low-pass filtered white noise (hereafter abbreviated to ‘white noise’) stimulation of the same whisker.

C. Cross-correlation between curvature change and angle during white noise stimulation, for C2 whisker.

D. Cross-correlation between angle and curvature change at zero lag, for both passive stimulation under anaesthesia and awake, active sensing (median of absolute cross-correlation for each unit; error bar denotes IQR).

E. Joint distribution of whisker angle and whisker curvature change in awake, behaving mice (1 ms sampling). Different colours denote data corresponding to different recorded units. Inset: Analogous plot for passive, white noise whisker deflection in an anaesthetised mouse. Different colours indicate data from different whiskers.

F. Joint distribution of angle and curvature change for an example recording from an awake behaving mouse, with samples registered during touch and non-touch distinguished by colour (1 ms sampling).

G. Touch data of F classified according to pole position (dot colour).

Simulations confirmed that, due to the tight relationship between the variables, a unit tuned purely to curvature change can appear tightly tuned to angle (Figure 2.12). The implication is that apparent sensitivity to whisker angle under passive stimulation conditions can be accounted for by moment-tuning.
2.4 DISCUSSION AND CONCLUSIONS

2.4.1 Prediction of spikes fired by sensory neurons under natural conditions

In the endeavour to understand how neurons encode and process sensory information, there is a basic tension between the desire for tight experimental control and the desire to study animals under natural, unconstrained conditions. Theories of sensory encoding suggest that neural circuits have evolved to operate efficiently under natural conditions (Simoncelli and Olshausen, 2001; Reinagel, 2001). Previous studies have succeeded in predicting/decoding spikes evoked by passive presentation of natural sensory stimuli to anaesthetized / immobilised animals (Lewen et al., 2001; Arabzadeh et al., 2005; Pillow et al., 2008; Mante et al., 2008; Lottem and Azouz, 2011; Bale et al., 2013), but it has been difficult to extend this approach to encompass natural, active movement of the sense organs. Here, we have addressed this general issue, taking advantage of experimental possibilities recently created in the whisker system (O’Connor et al., 2010a), and the ability of computational methods, such as GLMs, to uncover stimulus-response relationships even from data with complex statistical structure (Paninski et al., 2007; Fairhall and Sompolinsky, 2014).

Our main finding was that responses of PWNs, recorded as an awake mouse actively explores an object with its whiskers, can be predicted from the forces acting on the whiskers. Given that, for each unit, we were attempting to predict the entire ~70 s time course of activity, the variability of the behaviour of untrained mice (O’Connor et al., 2010a), and the lack of trial-averaging as a noise reduction strategy, it is remarkable that we found model prediction correlation coefficients up to 0.88. A challenge of studying neural coding under unconstrained, awake conditions is that sensory variables tend to correlate. A valuable feature of the GLM training procedure is that it takes such correlations into
account. We found that, although whisker angle predicted spikes for a subset of units, this effect was very largely explained by a curvature-coding model, together with the correlation between angle and curvature.

2.4.2 Mechanical framework for tactile coding

Pushing a whisker against an object triggers spiking in many PWNs (Szwed et al., 2003; 2006; Leiser and Moxon, 2007). Biomechanical modelling by Hartmann and co-workers accounts for this by a framework where the whisker is idealised as an elastic beam, cantilever-mounted in the skin (Birdwell et al., 2007; Quist et al., 2014). When such a beam pushes against an object, the beam bends, causing reaction forces at its base. Our data are in striking agreement with the general suggestion that mechanoreceptor activity is closely related to such reaction forces. Our results show that curvature change associated with contact-induced whisker bending, and acceleration associated with whisker rotation, predict PWN spiking. Our results also provide a mechanical basis for previous findings: our finding of subtypes of curvature-only and curvature-acceleration PWNs is consistent with previous reports of ‘touch’ and ‘whisking-touch’ units (Szwed et al., 2003; 2006). Thus, a common framework accounts for diverse PWN properties. Our finding that whisker angle predicts PWN spikes poorly indicates that whisker angle can change without modulating mechanotransduction in the follicle. This is consistent with evidence that, during artificial whisking, the follicle-shaft complex moves as a rigid unit (Bagdasarian et al., 2013). In apparent contrast, previous studies using passive stimulation in anaesthetised animals have consistently reported a tight relationship between whisker kinematics and PWN response. In the cantilever whisker model, passively induced changes in whisker angle correlate highly with whisker bending. We confirmed that this applies to real whiskers in vivo and
demonstrate that moment-sensitive units can thereby appear angle-tuned. In this way, moment-encoding can account for primary neuron responses not only during active touch but also under passive stimulation. More generally, our results highlight the importance of studying neurons under natural, active sensing conditions.

In this study, we considered PWN encoding under conditions of pole contact, since this is well-suited to reaction force estimation (O’Connor et al., 2010a; Pammer et al., 2013) and involves object-stimulus interactions on a ~100 ms time-scale that is conducive to single-trial analysis. Since whisker bending is ubiquitous in whisking behaviour, it is likely that our finding of curvature sensitivity is a general one. However, prediction performance varied across units, suggesting that other force components may also be encoded. Other experimental conditions – for example, textured surfaces – may involve multiple force components (Quist and Hartmann, 2012; Pammer et al., 2013; Bagdasarian et al., 2013) and/or encoding of information by spike timing on a finer time-scale (Panzeri et al., 2001; Petersen et al., 2001; Arabzadeh et al., 2005; Bale et al., 2015). It is axiomatic that mechanoreceptors are sensors of internal forces acting in the tissue within which they are embedded (Abraira and Ginty, 2013) and therefore valuable to be able to measure mechanical forces in the awake, behaving animal. In general, including the important case of primate hand-use, the complex biomechanics of skin makes force-estimation difficult (Phillips and Johnson, 1981). In contrast, for whiskers, the quasi-static relationship is relatively simple: the bending moment on a whisker is proportional to its curvature. This has the important implication that reaction forces can be directly estimated from videography in vivo (Birdwell et al., 2007; O’Connor et al., 2010a; Pammer et al., 2013). Our results are the first direct demonstration that such reaction forces drive primary sensory neuron responses – likely involving Piezo2 ion channels (Woo et al., 2014; Poole et al.,
2015; Whiteley et al., 2015) – and provide insight into how sensitivity to touch and self-motion arises in the somatosensory pathway (Szwed et al., 2003; Yu et al., 2006; Curtis and Kleinfeld, 2009; Khatri et al., 2009; O’Connor et al., 2010b; Huber et al., 2012; Petreanu et al., 2012; Peron et al., 2015; Yu et al. 2016).

2.4.3 Moment-based computations in tactile behaviour

Extraction of bending moment is a useful first step for many tactile computations. Large transients in bending moment signal object-touch events, and the magnitude of bending is inversely proportional to the radial distance of contact along the whisker (Solomon and Hartmann, 2006). As illustrated by our results on the statistics of active touch, if integrated with cues for whisker self-motion, whisker bending can be a cue to the 3D location of an object (Szwed et al., 2003; 2006; Birdwell et al., 2007; Bagdasarian et al., 2013; Pammer et al., 2013). Bending moment can permit wall following (Sofroniew et al., 2014) and, if integrated across whiskers, can in principle be used both to infer object shape (Solomon and Hartmann, 2006) and to map the spatial structure of the environment (Fox et al., 2012; Pearson et al., 2013).

We have shown that the responses of primary whisker neurons can be predicted, during natural behaviour that includes active motor control of the sense organ, from forces acting on the whiskers. These results provide a bridge linking receptor mechanisms to behaviour.
2.5 MATERIALS AND METHODS

All experimental protocols described in this chapter were approved by both United Kingdom Home Office national authorities and institutional ethical review.

2.5.1 Surgical procedure

Mice (C57; N=10; 6 weeks at time of implant) were anesthetized with isoflurane (2% by volume in O2), mounted in a stereotaxic apparatus (Narishige, London, UK) and body temperature maintained at 37°C using a homeothermic heating system. The skull was exposed and a titanium head-bar (19.1 x 3.2 x 1.3 mm; O’Connor et al., 2010a) was first attached to the skull ~1 mm posterior to lambda (Vetbond, St. Paul, MN), and then fixed in place with dental acrylic (Lang Dental, Wheeling, IL). A craniotomy was made (+0.5 to -1.5 mm posterior to bregma, 0-3 mm lateral) and sealed with silicone elastomer. A gold pin (0.031” gold-plated pins - World Precision Instrument) was inserted in the skull through a second smaller craniotomy and posed in contact with the dura mater surface. This pin was connected to the recording system ground during electrophysiological recording. Buprenorphine (0.1 mg/kg) was injected subcutaneously for postoperative analgesia and the mouse left to recover for at least 5 days.

2.5.2 Behavioural apparatus

Mice were studied in a pole exploration apparatus adapted from O’Connor et al., 2010a, but were not trained on any task. A mouse was placed inside a perspex tube (inner diameter 32 mm), which its head emerged at one end, and immobilised by fixing the head-bar to a custom mount holder. The whiskers were free of the tube at all times. The stimulus object was a 1.59 mm diameter metal pole, located ~3.5 mm lateral to the mouse’s snout. To allow
control of its anterior/posterior location, the pole was mounted on a frictionless linear slide (NDN 2-50.40, Schneeberger, Roggwil, Germany) and coupled to a linear stepper motor (NA08B30, Zaber, Vancouver, Canada). To allow vertical movement of the pole into and out of range of the whiskers, the pole/actuator assembly was mounted on a pneumatic linear slide (SLS-10-30-P-A, Festo, Northampton, UK), powered by compressed air. The airflow was controlled by a relay (Weidmüller, Richmond, VA). In this way, the pole moved rapidly (~0.15 s) into and out of range of the whiskers. The apparatus was controlled from Matlab via a real-time processor (RX8, TDT, Alachua, FL).

2.5.3 Electrophysiology

We recorded the activity of PWNs from awake mice in the following way. To permit reliable whisker tracking (see below), before each recording session, A, B and E whisker rows were trimmed to the level of the fur, under brief isoflurane anaesthesia. The silicone seal was removed and a 3/4 shank tungsten microelectrode array (FHC, Bowdoin, ME, recording electrodes 8 MW at 1 kHz, reference 1 MW; tip spacing ~500 mm) was lowered through the brain (angle 4° to vertical in the coronal plane) using a micromanipulator (PatchStar, Scientifica, Uckfield, UK) under isoflurane anaesthesia. Extracellular potentials were pre-amplified, digitised (24.4 kHz), filtered (band pass 300–3000 Hz) and acquired continuously to hard disk (RZ5, TDT). The trigeminal ganglion was encountered 6–7 mm vertically below the pial surface and whisker-response units identified by manual deflection of the whiskers with a small probe. Once a well-isolated unit was found, the whisker that it innervated (the ‘principal whisker’, PW) was identified by manual stimulation. To define the PW, we deflected not only untrimmed whiskers, but also the stubs of the trimmed whiskers. Any unit whose PW was a trimmed whisker was ignored. At this point,
anaesthesia was discontinued. Once the mouse was awake, we recorded neuronal activity during repeated presentations of the pole (‘trials’). Before the start of each trial, the pole was in the down position, out of reach of the whiskers. The pole was first moved anterior-posteriorly to a position chosen randomly out of a set of 11 possible positions, spanning a range ± 6 mm with respect to the resting position of the base of the PW. A trial was initiated by activating the pneumatic slide relay, thus moving the pole up into the whisker field, where it remained for 3 s before being lowered. At the end of a recording session, the microelectrode array was withdrawn, the craniotomy sealed with silicone elastomer, and the mouse returned to its home cage.

2.5.4 High-speed videography

Using the method of O’Connor et al. (2010a) to image whisker movement/shape, whiskers ipsilateral to the recorded ganglion were illuminated from below using a high-power infrared LED array (940 nm; LED 940-66-60, Roithner, Vienna, Austria) via a diffuser and condensing lens. The whiskers were imaged through a telecentric lens (55-349, Edmunds Optics, Barrington, NJ) mounted on a high speed camera (LTR2, Mikrotron, Unterschleissheim, Germany; 1000 frames/s, 0.4 ms exposure time). The field of view of the whiskers was 350 x 350 pixels, with pixel width 0.057 mm.

2.5.5 Response to touch and non-touch events

Mouse whisking behaviour during the awake recording was segmented into ‘touch’, and ‘non-touch’ episodes. Touches between the PW of each unit and the pole were detected manually in each frame of the high-speed video. A frame was scored as touch if no background pixels were visible between the pole silhouette and the whisker. Any frame not
scored as a touch was scored as non-touch. Touch and non-touch firing rates for a given unit were computed by averaging activity over all corresponding episodes.

2.5.6 Whisker tracking

Since the trigeminal ganglion lacks topography, it is difficult to target units that innervate a specific whisker, and therefore desirable for a whisker tracker to be robust to the presence of multiple rows of whiskers. However, since neurons in the ganglion innervate individual whiskers, it is sufficient to track only one whisker (the PW) for each recorded neuron. To extract kinematic/mechanical whisker information, we therefore developed a whisker tracker (‘WhiskerMan’; Bale et al., 2015) whose design criteria, different to those of other trackers (Perkon et al., 2011; Clack et al., 2012), were to: (1) be robust to whisker cross-over events; (2) track a single, target whisker; (3) track the proximal segment of the whisker shaft. The shape of the target whisker segment was described by a quadratic Bezier curve \( r(t,s) \) (a good approximation away from the zone of whisker-object contact; Quist and Hartmann, 2012; Pammer et al., 2013): \( r(t,s) = [x(t,s), y(t,s)] \), where \( x, y \) are horizontal/vertical coordinates of the image, \( s = [0,..,1] \) parameterises \((x,y)\) location along the curve and \( t \) is time. We fitted such a Bezier curve to the target whisker in each image frame using a local, gradient-based search. The initial conditions for the search were determined by extrapolating the solution curves from the previous two frames, assuming locally constant, angular velocity. The combination of the low-parameter whisker description and the targeted, local search made the algorithm robust to whisker cross-over events. The ‘base’ of the target whisker was defined as the intersection between the extrapolated Bezier curve and the snout contour (estimated as described in Bale et al.,
The solution curve in each frame was visually checked and the curves manually adjusted to correct occasional errors.

2.5.7 Estimation of kinematic/force parameters

The whisker angle (θ) in each frame was measured as the angle between the tangent to the whisker curve at the base and the anterior-posterior axis (Figure 2.1C - top). Whisker curvature (κ) was measured at the base as $\frac{xy'yt - xyt'y'}{\sqrt{(x^2 + y^2)^3}}$, where $x'$, $y'$ and $x''$, $y''$ are the first and second derivatives of the functions $x(s)$ and $y(s)$ with respect to $s$ (Figure 2.1 C - top). Since reaction force at the whisker base reflects changes in whisker curvature, rather than the intrinsic (unforced) curvature (Birdwell et al., 2007), we computed ‘curvature change’ $\Delta \kappa = \kappa - \kappa_{int}$, where $\kappa_{int}$, the intrinsic curvature, was estimated as the average of $\kappa$ in the first 100 ms of the trial (before pole contact; O’Connor et al., 2010a; 2010b). During free whisking, whisker angle oscillated with the characteristic whisking rhythm, but curvature changed little. The small changes in whisker curvature during free whisking were consistent with torsional effects (Knutsen et al., 2008). We estimated the number of whisking cycles from the duration of touch/non-touch episodes and the whisking frequency: median 419 whisking cycles per unit during touch periods; 415 during non-touch periods.

Under conditions of whisking against a smooth surface, such as in the present study, the quasi-static framework of Birdwell et al. (2007) applies. $\Delta \kappa$, measured, at the base of a whisker, in the horizontal plane, is proportional to the component of bending moment in that plane. We used $\Delta \kappa$ as a proxy for bending moment. Bending moment ($M$), Axial ($\vec{F}_{ax}$) and lateral force ($\vec{F}_{lat}$) at the whisker base were calculated, during periods of whisker-pole
contact, using the method of Pammer et al. (2013), using published data on areal moment of inertia of mouse whiskers (Pammer et al., 2013), along with whisker-pole contact location (see Figure 2.6 for details). Pole location, in the horizontal plane, in each frame, was identified as the peak of a 2D convolution between the video image and a circular pole template. To localise whisker pole contact, the whisker tracker was used to fit the distal segment of the whisker close to the pole, seeded by extrapolation from the whisker tracking solution for the proximal whisker segment, described above. Whisker-pole contact location was defined as the point where this distal curve segment was closest to the detected pole centre. Pole and contact locations were verified by visual inspection.

As expressed by Newton’s second law of rotational motion, the moment – or torque – of a rigid body, rotating in a plane, is proportional to the body’s angular acceleration. During free whisking, a whisker behaves approximately as a rigid body and, for the whiskers considered in this study, their motion is predominantly in the horizontal plane (Bermejo et al., 2002; Knutsen et al., 2008). Thus, to assess whether such a moment is encoded by PWNs, we measured angular whisker acceleration during free whisking as a proxy. Acceleration was calculated from the whisker angle time series after smoothing with a Savitzky-Golay filter (polynomial order 5; frame size 31 ms).

Push angle – the change in angle as a whisker pushes against an object - was measured during touch epochs. For each touch episode, we determined the value of the angle in the frame before touch onset and subtracted this from the whisker angles during the touch.
2.5.8 Passive whisker deflection

To determine how whiskers move/bend in response to passive deflection under anaesthesia, a mouse was anesthetized (isoflurane 2%) and placed in the head-fixation apparatus. Individual whiskers (C2 and C5 trimmed to 5 mm) were mechanically deflected using a piezoelectric actuator as previously described (Bale et al., 2013; 2015). All other whiskers were trimmed to the level of the fur. Each whisker, in turn, was inserted into a snugly fitting plastic tube attached to the actuator, such that the whisker entered the tube 2 mm from the face. Two stimuli were generated via a real time processor (TDT, RX8): (1) a 10 Hz trapezoidal wave (duration 3 s, amplitude 8°); (2) Gaussian white noise (duration 3 s, smoothed by convolution with a decaying exponential: time constant 10 ms; amplitude SD 2.1°). During the stimulation, the whiskers were imaged as detailed above (1000 frames/s, 0.2 ms exposure time).

2.5.9 Electrophysiological data analysis

2.5.9.1 Spike sorting

Single units (N=20) were isolated from the extracellular recordings as previously described, by thresholding and clustering in the space of 3-5 principal components using a mixture model (Bale and Petersen, 2009). A putative unit was only accepted if (1) its inter-spike interval histogram exhibited a clear absolute refractory period and (2) its waveform shape was consistent between the anaesthetised and awake phases of the recording.

2.5.9.2 Responses to whisking without touch

To test whether a unit responded to whisking itself, we extracted non-touch episodes as detailed above and computed time series of whisking amplitude and phase by band-pass
filtering the whisker angle time series (6–30 Hz) and computing the Hilbert transform (Kleinfeld and Deschenes, 2011).

Amplitudes were discretised (30 equi-populated bins) and the spiking data used to compute amplitude tuning functions. Phases for bins where the amplitude exceeded a given threshold were discretised (8 equi-populated bins) and used to construct phase tuning functions. To determine whether a unit was significantly amplitude-tuned, we fitted a regression line to its amplitude tuning curve and tested whether the slope was statistically significantly different to 0 (p=0.0025, Bonferroni-corrected). To determine whether a unit was significantly phase-tuned, we computed the maximum value of its phase tuning curve and compared this to the distribution of maxima of chance tuning functions. Chance tuning functions were obtained by randomly shifting the recorded spike sequences by 3000–8000 ms and recomputing tuning functions (500 times). A unit was considered phase tuned if its tuning function maximum (computed using amplitude threshold of 2°) exceeded the 95th percentile of the shuffled distribution.

Acceleration tuning curves were quantified, for each unit, as follows. First, an acceleration tuning curve was estimated (as above). Units typically responded to both positive and negative accelerations, but with unequal weighting between them. To capture this, we fitted the following regression model to the tuning curve:

\[ r_i = \mu_0 + \mu_1 |a_i| + \mu_2 \Delta_i |a_i| \]

Here, for each bin i of the tuning curve, \( r_i \) was the firing rate and \( a_i \) was the acceleration; \( \mu_0, \mu_1 \) and \( \mu_2 \) were regression coefficients; the term \( \Delta_i \) (\( \Delta_i=1 \) if \( a_i<0 \), \( \Delta_i=0 \) otherwise) allowed for asymmetric responses to negative and positive acceleration. Based on its best-
fitting regression coefficients (p=0.05), units were classified as: having ‘preference for negative acceleration’, if $\mu_2$ was significantly $>0$; having ‘preference for positive acceleration’, if $\mu_2$ was significantly $<0$; as having ‘no preferred direction’ if both $\mu_1$ was significantly $>0$, and $\mu_2$ was not significantly different from 0; and as ‘not acceleration sensitive’ if neither $\mu_1$ nor $\mu_2$ were significantly different from 0.

2.5.9.3 Generalised Linear Model (GLM)

To investigate how well PWNs encode a given sensory variable (e.g., whisker angle, curvature), we fitted single unit activity to a GLM (Nelder and Wedderburn, 1972; Truccolo et al., 2005; Paninski et al., 2007), using methods similar to Bale et al., 2013. For each unit, a ‘stimulus’ time series ($x$) (whisker angle or whisker curvature change) and a simultaneously recorded spike time series ($n$) were discretized into 1 ms bins: $x_t$ and $n_t$ denote respectively the stimulus value and spike count (0 or 1) in bin $t$. GLMs express how the expected spike count of a unit depends both on the recent stimulus history and on the unit’s recent spiking history. The standard functional form of the model we used was:

$$y_t = f(\vec{k}^T \vec{x}_t + \vec{h}^T \vec{n}_t + b)$$

Here $n_t$, the output in bin $t$, was a Bernoulli (spike or no-spike) random variable. The probability of a spike in bin $t$, $y_t$, depended on three terms: (1) the dot product between the stimulus history vector $\vec{x}_t = (x_{t-Lk+1}, ..., x_t)$ and a ‘stimulus filter’ $\vec{k}$ (length $L_k = 5$); (2) the dot product between the spike history vector $\vec{n} = (n_{t-Lh+1}, ..., n_t)$ and a ‘spike history filter’ $\vec{h}$ (length $L_h = 2$); (3) a constant bias $b$, which sets the spontaneous firing rate. $f(\cdot)$ was the logistic function $f(z) = (1 + e^{-z})^{-1}$. The preferred direction of the GLM is determined by the sign of the stimulus filter. Positive (negative) $k$ coefficients tend to make
positive (negative) stimuli trigger spikes. Since we found that GLM performance was just as good with \( L_k = 1 \) as \( L_k = 5 \) (Figure 2.7 C), we used results from the \( L_k = 1 \) case to define selectivity to curvature change direction: positive \( k \) implies selectivity for positive curvature change; negative \( k \) selectivity for negative curvature change. When a whisker pushed against an object during protraction, curvature increased; when it pushed against an object during retraction, it decreased. To consider whether units might encode multiple sensory variables (e.g., both whisker angle and whisker curvature change), we used a GLM with multiple stimulus history terms, one for each sensory variable:

\[
y_t = f(k^T \vec{x}_{t;1} + k^T \vec{x}_{t;2} + \vec{h}^T \vec{n}_s + b)
\]

Here the indices 1, 2 label the sensory variables.

Training and testing of the GLM were done using a cross-validation procedure. For each unit, half of the trials were assigned randomly to a training set and half to a testing set. The training set was used to fit the parameters \((\vec{k}, \vec{h} \text{ and } b)\), while the testing set was used to quantify the similarity between the spike train of the recorded unit and that predicted by the GLM. GLM fitting was achieved by finding the parameter values \((\vec{k}, \vec{h} \text{ and } b)\), which minimized a cost function consisting of the sum of the negative log-likelihood and a regularizing term \(-\alpha \|\vec{k}\|\). For all units, model prediction performance on the test set was robust to variation of \(\alpha\) over several orders of magnitude; \(\alpha\) was therefore set to a standard value of 0.01. To quantify the performance of the model, the sensory time series of the testing set was used as input to the best-fitting GLM to generate a ‘predicted’ spike train in response. Both real and predicted spike trains were then smoothed by convolution with a 100 ms box-car filter and the similarity between them quantified by the Pearson correlation.
coefficient (PCC). For each unit, the entire training/testing procedure was repeated for 10 random choices of training/testing set and the final prediction accuracy defined as the median of the 10 resulting PCC values. Data from these 10 samples were also used to test whether an individual unit exhibited statistically significant prediction performance for different sensory features. To test whether the results were robust to the smoothing time-scale, the above procedure was repeated for a range of box-car smoothing filters (1, 5, 10, 20, 50, 70 ms). To test whether a given ‘actual’ PCC was statistically significant, we tested the null hypothesis that it could be explained by random firing at the same time-averaged rate as that of the recorded unit. To this end, the recorded spike sequences were randomly shifted by 3000–8000 ms and the training/testing procedure above applied to this surrogate data. This was repeated 10 times and the resulting chance PCCs compared to the actual PCC using a signed-rank test, $p=0.0025$ (Bonferroni-corrected). This analysis was used to classify units as being ‘curvature-sensitive’.

2.5.9.4 Quadratic GLM

To test whether the units might exhibit nonlinear dependence on the stimulus parameters, we adapted the GLM defined above (Equation 1) to include quadratic stimulus variables (Rajan et al., 2013). This was important to assess whisker angular acceleration during free whisking, since a subset of units exhibited U-shaped acceleration tuning functions (Figure 2.10 and 2.3 B). Given a stimulus time series $x_t$, the quadratic stimulus history vector was $[x_{t-L+1}^2, \ldots, x_t, x_{t-L+1}^2, \ldots, x_t^2]$. Fitting methods were otherwise identical to those detailed above.
2.5.9.5 Effect of angle-curvature correlations on apparent neuronal stimulus encoding in the passive stimulation protocol.

If, in a given recording, sensory variable X correlates with sensory variable Y, a neuron responsive purely to X will tend to appear tuned to Y. To investigate whether such an effect might produce apparent sensitivity to whisker angle in the passive stimulation paradigm, we simulated the response of curvature-tuned neurons to the whisker curvature change time series measured during passive white noise stimulation. To minimise free parameters, constrained GLMs (4 free parameters) were used, sensitive either to instantaneous curvature ($\gamma = [\gamma]$) or to its first order derivative ($\gamma = [\gamma[-1 1]]$) where $\gamma$ was a signed, gain parameter. Parameters ($\gamma$, $b$ and $\gamma$), were adjusted to produce two spike trains (one for training, the other for testing) with a realistic white noise induced firing rate (~50 spikes/s; Bale et al., 2013). We then attempted to predict the simulated, curvature-evoked (training) spike train by fitting GLMs (length 5 stimulus filter, 8 free parameters) using as input either angle or curvature change. Cross-validated model accuracy was computed as the PCC between the predicted spike train and the testing spike train (both smoothed by convolution with a 5 ms box-car).

2.5.9.6 Effect of single-trial approach on GLM prediction performance

The objective of encoding models, such as GLMs, is to obtain an accurate description of the mapping between a stimulus and the neuronal spike trains it evokes. Since the random component of a neuron’s response is inherently unpredictable, the best any model can do is to predict the probability of the spike train. To enable this, encoding models have generally (with few exceptions; Park et al., 2014) been applied to a ‘repeated-trials’ paradigm, where a stimulus sequence (e.g., frozen white noise) is repeated on multiple ‘trials’ (Arabzadeh et
Model accuracy can then be quantified, largely free of contamination from random response variability, by comparing (using PCC or otherwise) the trial-averaged response of the model to the trial-averaged response of the neuron. In contrast, in the present study of awake, actively whisking mice, the precise stimulus (time series of whisker angle/curvature) was inevitably different on every pole presentation: there were no precisely repeated trials to average over. Our standard model performance metric (PCC) was computed by comparing the response on a single long, concatenated ‘trial’ with the corresponding GLM predicted response. Such a PCC is downwards biased by random response variability.

To gauge the approximate magnitude of this downward bias, we used a simulation approach. By simulating the response of model neurons, we could deliver identical, repeated trials and thereby compare model prediction performance by a metric based on trial-averaging with that based on the single-trial approach. To this end, for each recorded unit, we used the best-fitting curvature change GLM to generate 100 trials of spike trains evoked by the curvature time series measured for that unit. Data from the first of these trials was used to fit the parameters of a minimal ‘refitted GLM’ (stimulus filter length 1, spike history filter length 2; bias; total 4 free parameters), and the single-trial performance quantified, using the approach of the main text (Figure 2.7 B, left). Next, we used the refitted GLM to generate 100 repeated trials of spike trains evoked by the curvature time series. Repeated-trials performance was then quantified as the PCC between PSTHs obtained by trial-averaging (Figure 2.7 B, right).
2.6 SUPPLEMENTARY FIGURES:

Figure 2.5. Electrophysiological recording from primary whisker neurons of awake, head-fixed mice.

Extracellular potential recorded from the same single unit during both anaesthetized and awake epochs. Spikes belonging to the cluster of the target unit are shown by black triangles. Inset shows overlay of all waveforms belonging to this cluster.
Axial ($F_{ax}$) and lateral ($F_{lat}$) force components at the whisker base were calculated, in each video frame where there were whisker-pole contacts, as follows (Pammer et al., 2013). First, the point of whisker-pole contact was located (see section 2.5.7). The direction of the force was then calculated as the normal to the whisker tangent at the contact point (Pammer et al., 2013). Moment at the base $M$ was calculated from the whisker curvature at the base (see section 2.5.7) and then the magnitude $F$ of $\vec{F}$ was derived from the definition of moment:

$$F = \frac{M}{r \sin(\varphi)}$$

where $r$ is the magnitude of the lever arm vector $\vec{r}$ from whisker base to contact point, and $\varphi$ is the angle between $\vec{r}$ and $\vec{F}$. The components $F_{ax}$ and $F_{lat}$ were then found by projecting $\vec{F}$ onto the tangent and normal to the whisker at its base, respectively:

$$F_{ax} = F \sin(\theta_{base} - \theta_{contact})$$

$$F_{lat} = F \cos(\theta_{base} - \theta_{contact})$$
Here \( \theta_{\text{base}} \) is the angle between the tangent to the whisker at its base and the horizontal;

\( \theta_{\text{contact}} \) is the angle between \( \vec{F} \) and the tangent to the whisker at the contact point.
**Figure 2.7.** Effect on GLM performance of quadratic input terms, simulated repeated trials and minimal stimulus filters.

**A.** Angle GLM prediction performance is robust to addition of quadratic stimulus-dependence. Prediction accuracy (PCC) for standard angle GLM (same data as Figure 2.4 D of main text) in comparison to quadratic GLM (see section 2.5.9.4). Black dots denote medians, error bars IQR.

**B.** Single-trial GLM prediction accuracy is limited by neuronal response variability. Prediction accuracy (PCC) for simulated neurons. Each simulated neuron is the best-fitting GLM, based on instantaneous curvature change, for its corresponding recorded unit (see section 2.5.9.6). Prediction accuracy is quantified both using the single-trial approach of the main text and using a repeated-trial method only possible by virtue of using a simulation. Black dots denote medians, error bars IQR.

**C.** Prediction accuracy of curvature-based GLMs is accounted for by tuning to instantaneous curvature change. Recorded spike train (upper left) and curvature-predicted spike trains (lower left) both for a ‘curvature history’ GLM with a length 5 stimulus filter, as used in Figure 2.2, and for an ‘instantaneous curvature’ GLM with a length 1 stimulus filter. Data for unit 2 of Figure 2.2 C. Prediction accuracy of the curvature history GLM compared to that of the instantaneous curvature GLM for every recorded unit (right).

**D.** Tuning curves for curvature change (blue) and angle (orange) of unit 1 and unit 2 in Figure 2.2.
Figure 2.8. Example filters for curvature-based GLMs.

Stimulus filter, history filter and bias term of curvature-based GLMs for two units (A, B), fitted as described in section 2.5.9.3. Both units had negative history filters (in the 2 ms preceding a spike), consistent with refractoriness. The stimulus filter of unit B was negative (in the 5 ms preceding a spike), indicating sensitivity to negative curvature change. The stimulus filter of unit A was biphasic, but with positive integral, indicating sensitivity both to positive curvature change and to positive curvature change derivative. Under our stimulus conditions, dominated by slow (~100 ms) time-scale whisker-pole interactions, the former effect was dominant; derivative-sensitivity had relatively little impact on spike prediction.
Figure 2.9. Moment is near-perfectly correlated with axial/lateral contact force components during pole exploration.

**A.** Two example time series for simultaneously measured whisker angle, bending moment, lateral force and axial force (see section 2.5.7). Red bars indicate episodes of whisker-pole contact.

**B.** Joint distribution of bending moment and lateral force (left), compared to that of bending moment and axial force (right), for the same recording shown in A. Moment was highly linearly correlated with lateral force (median absolute correlation coefficient across units 0.995, IQR 0.99–1.00, median $R^2$ of linear fit 0.99, IQR 0.97–1.00), and highly quadratically correlated with axial force.
(median $R^2$ of quadratic fit 0.94, IQR 0.85–0.98). This indicates that, during our conditions of pole exploration, axial force and lateral force are both redundant with moment.
Figure 2.10. Whisking-sensitive units exhibit heterogeneous selectivity to angular acceleration.

For each whisker-sensitive unit, an acceleration tuning curve was estimated (Figure 2.3 B). Tuning to positive (negative) acceleration was quantified by the slope of a regression line fitted to the positive (negative) half of the acceleration tuning curve. In general, units responded to both positive and negative accelerations, but to different degrees. Statistical tests, based on regression coefficients, detailed in section 2.5.9.2, were used to differentiate the different types of unit.
Figure 2.11. Measurement of whisker bending during passive whisker deflection.

**A.** Four video frames taken during trapezoidal, passive whisker stimulation with whisker tracker solutions overlaid (coloured lines).

**B.** Curvature change (left) and corresponding tracker solutions (right) during a 45 ms episode. Coloured dots mark the times of the example frames in panel A and shading from blue to aqua indicates curvature change. This whisker had negative intrinsic curvature. As the actuator applied force to the whisker, the whisker straightened up and the curvature increased.
Figure 2.12. Correlations between angle and curvature change during passive whisker stimulation can make curvature-tuned units appear angle-tuned.

The data of Figure 2.4 show a strong correlation between whisker angle and whisker curvature during passive stimulation of the whisker. To test whether this correlation might make curvature-tuned units appear angle-tuned, we used a simulation approach. This allowed us to generate responses from idealised neurons whose true tuning was known, by construction, to be only to curvature. We simulated responses of such neurons to the curvature change time series obtained from passive white noise stimulation (A1-2). We then trained a GLM to predict these curvature-
evoked spikes using only whisker angle as input (A3-A4). Despite being fed the ‘wrong’ input, this GLM was able to predict the spikes accurately (for C2 whisker, angle PCC was 0.90, curvature change PCC 0.94; results similar for C5; C). This result was robust to different choices of feature tuning (B-C). (A1) Whisker curvature change caused by the white noise stimulus applied to C2 whisker of an anaesthetized mouse (same data as main text Figure 2.4 B, repeated for clarity). (A2) Spike train evoked by a simulated curvature-tuned neuron in response to the stimulus in A1 (a GLM with the position filter shown in left panel of A5). (A3) Whisker angle time series corresponding to panel A1. (A4) Target response (black) compared to predicted response from best-fitting GLMs using either angle (orange) or curvature change (blue) as input. (A5) Left. Stimulus filter used to generate the spike train of panel A2. Middle-Right. Best-fitting stimulus filters (normalised to unit length) for GLMs trained on the spikes of panel A2 and the angle time series of panel A3 or the curvature change time series of panel A1 respectively. (B1-5) Results analogous to A1-5 for a simulated neuron tuned to curvature velocity. (C) Quantification of the GLM predictions shown in panels A4-B4.
2.7 REFERENCES


3. Mechanosensory basis of tactile object localisation behaviour
3.1 ABSTRACT:

Sensation is an active process, where animals purposively move their sensory organs to collect sensory information from the environment. The whisker system of rodents is a well established model to study active sensation, but precisely which sensory information drives whisker-guided behaviours is still largely unknown. The recent discovery that primary whisker afferents encode a mechanical signal (bending moment) raises the question of how rodents employ bending moment to control their behaviours. We therefore measured (1000 Hz) bending moment at the base of the whiskers of head-fixed mice, performing a novel behavioural task, during which they use one whisker to localise a pole presented in one of three anterior-posterior locations. We then tested which features of bending moment could predict mouse behaviour at single trial level, using a classifier-based approach. We discovered that mouse correct choices could be accurately predicted from bending moment magnitude and direction, while mouse errors could be predicted from the mouse choice in the previous trial, revealing a prominent history-dependent behaviour. Our study shows that bending moment can account for mice choices during object localization and provides a functional explanation for the encoding of bending moment magnitude and direction in the whisker ascending pathways.
3.2 INTRODUCTION

A major goal in the investigation of any sensory system is to understand what physical signals drive its primary sensory neurons during natural behaviour. In order to achieve this, it is necessary to accurately measure the sensory input to the brain during behaviour. However, sensation is an active process, where animals move their sense organs (Gibson, 1962; Yarbus, 1967), and until recently, measuring the sensory information collected by a moving sensory organ with high temporal precision was experimentally intractable. Thanks to technical improvements, the major physical signal that drives primary whisker afferents, PWAs (Dorfl, 1985; Rice et al., 1993; Ebara et al., 2002) during active sensing has been identified: it is a rotational force associated with whisker-object contact (bending moment, Campagner et al., 2016; Severson et al., 2017). This raises the question of how bending moment is used by mice to guide their behaviour.

Many behavioural tasks (Hutson and Masterton, 1986; Guić-Robles et al., 1989; Carvell and Simons, 1990; Brecht et al., 1997; Ghazanfar et al., 2001; Polley et al., 2005; Knutsen et al., 2006; Mehta et al., 2007; O’Connor et al., 2010a; Pammer et al., 2013; Sachidhanandam et al., 2013; Chen et al., 2013; Sofroniew et al., 2014; Fassihi et al., 2014; Guo et al., 2014a, 2014b; Yang et al., 2015; Yu et al., 2016b; Bale et al., 2017) are available to study different aspects of whisker-based sensation (Maravall and Diamond, 2014). However, none of them can be employed to address the question posed above: many of those tasks involve freely moving, multi-whiskered rats, but bending moment estimation from whiskers requires head- or body- restrained rodents (preferably mice) with one or few whiskers (Clack et al., 2012; Pammer et al., 2013; Bush et al., 2016; Campagner et al., 2016; Severson et al., 2017). Also, in multi-whisker tasks, interpretation of results is
complicated by the fact that the animal’s behaviour might depend on any of several whiskers. Finally, some single whisker or single row object detection tasks in head-fixed mice have been developed (O’Connor et al., 2010a; Guo et al., 2014a) and they suggested that a large transient in bending moment could be used as a cue to detect object. However, novel tasks are required to gain a deeper understanding of which precise bending moment features mice have access to during behaviour.

In this study, we therefore developed a novel, three-choice object localisation task in head-fixed mice with a single whisker, and used high-speed imaging and computer vision to measure touch and correlates of bending moment during performance of the task. We then asked if bending moment was used by the mice to solve the task. Using classifiers, we were able to accurately predict mouse correct choices based on bending moment magnitude and direction during touch. In contrast, only a small fraction of error trials could be predicted from bending moment, with the majority of them better predicted from the choice of the mouse in the previous trial. Overall, our findings demonstrate how bending moment – the major driver of PWAs – is employed by mice to solve a behavioural task, identifying a direct link between mechanoreceptor mechanisms and behaviour.

3.3 RESULTS

3.3.1 The three-choice object localisation task

In order to investigate how bending moment drives mouse behaviour during an active tactile discrimination task, we trained mice (N=5) to perform a novel, three-choice object localisation task (Figure 3.1 B). Head-fixed mice were trained to use one whisker to localise a metal pole in a dark, sound-proof enclosure (under infra-red illumination). On
any given trial, the pole was presented in one of three locations (anterior, middle or posterior) along the anterior-posterior axis of the mouse (Figure 3.1 A, B). Mice were trained to associate each pole location with a unique response: to lick the left lick port (‘left lick’), to lick the right lick port (‘right lick’) or to refrain from licking (‘no lick’). There were, therefore, 9 possible trial-choice outcomes, 3 correct and 6 incorrect (Figure 3.1 A-C). In the rest of the paper, we assign to each choice the name of the pole location for which that choice, if taken, would be correct. For example, we refer to ‘posterior choice’ as that particular choice that would lead to a correct outcome, when the pole is presented in the posterior location (i.e. right lick in Figure 3.1 B).

The use of a task with three pole locations aimed to challenge mice to adopt more sophisticated strategies than presence/absence of touch (O’Connor et al., 2010a). Therefore, we expected mice to employ strategies based on fine grain decoding of sensory input features.

Mice were first trained with all whiskers (Figure 3.2 A and 3.7 A). Whiskers were progressively trimmed until, in the final phase of training (‘full task’), mice performed the task with only one whisker (Figure 3.2 A and 3.7 A, dark purple dots). This allowed us to control and measure the sensory input available to the animal to perform the task. Mice learned the full task in 36 ± 12 days of training (mean ± SD across mice) and performed on average 179 ± 63 trials per day (Figure 3.2 A and 3.7 B). We quantified a mouse’s ‘task performance’ as the proportion of trials on which its choice was correct. Mice reached stable performance of 0.74 ± 0.08 (mean ± SD, Figure 3.2 B), which was significantly above chance (Figure 3.2 B; chance confidence interval: 0.34 ± 0.003, see section 3.5.6.1).
Performance was high at all three pole locations (posterior 0.77 ± 0.07; middle 0.75 ± 0.06; anterior 0.70 ± 0.06). There was, on average, no significant effect of pole location on performance, (Figure 3.4 A; one-way ANOVA: F=1.3, p=0.31), and each pole location could be identified significantly above chance at single mouse level (Figure 3.4 A).

To verify that mice relied on their whiskers to perform the task, we trimmed the whiskers of fully trained mice and retested them on the task. As expected, performance dropped significantly (t-test; p = 0.0013), from 0.72 ± 0.04 pre-trim (5 trimming tests, performance averaged over 5 sessions prior to trim, Figure 3.2 C-D purple dots) to 0.36 ± 0.02 post-trim (average over 2-3 sessions post-trim, Figure 3.2 C-D black dots). Moreover, the post-trim performance was statistically indistinguishable from chance (95% confidence interval of chance was 0.28-0.38; Figure 3.2 C-D). Collectively, these results indicate that mice can learn a challenging, 3-choice object localisation task, using mechanosensory input from a single whisker.
Figure 3.1. The three-choice object localisation task

A. Left: Schematic of the experimental preparation, showing a head-fixed mouse, whilst the metal pole was presented in one of the three locations (circles), and the two lick ports. Both lick ports and pole location are colour coded consistently with panel B. Whisker movements and whisker-pole interactions were filmed with a high-speed camera (1000 frame/s). Right: Schematic of one correct go trial to illustrate the trial structure. Coloured bars: duration of different periods which occurred during the trial (for the meaning of different terms see section 4.5.3.1). Sensory variables were recorded by filming the whisker and extracting whisker angle (orange) and whisker curvature (blue) time series, and by detecting the period of whisker pole interaction (touches, purple and green). Mouse choice was monitored by measuring the first lick time (cyan).

B. Schematic of the three-choice object localisation tasks (see section 3.5.3 for detailed description).

C. Summary plot of mouse behaviour during an example experimental session. Whisker angle (left) and whisker curvature (right) for each whisker tracked trial (see section 3.5.5 and Figures 3.8 and 3.9) of the session are shown. In the top two panels, trials are sorted according to the order they were presented during the sessions. In the bottom two panels, trials were sorted according to pole location, and then for a given pole location sorted by mouse choice. Pole location and choices are colour coded consistently with panel A. Correct trials are indicated by a green line in the right column. First lick (red and blue dots), first touch purple dots and pole onset times (yellow dotted line), have been overlaid to the whisker angle and curvature time series of the respective trial.
Figure 3.2. The three-choice object localisation task can be learnt by mice and is whisker-dependent.

A. **Top:** Single mouse example showing the task performance for all the days in which the mouse was trained in the task. Analogous data for other mice are reported in Figure 3.7. The mouse in the example was initially trained with all its whiskers intact (pink dots). The number of whiskers was gradually decreased by trimming them to C row (light purple dots) and then to one whisker (dark purple). Finally, all the whiskers were trimmed after the mouse has been trained in the full task (black dots). Coloured lines indicate the protocol the mouse was trained to in the respective day: lick (Cyan), go – no go (red), lick left- lick right (green), lick left - lick right – no lick (gold) protocol (see section 4.5.3.2 for detail description of each protocol). When cyan and red lines overlap, it
indicates that the mouse switched from lick protocol to go – no go protocol during the same
behavioural session. **Bottom**: Total number of trials per day.

**B.** Asymptotic performance for each mouse during AB trials of the full task with single whisker (see
section 4.5.6.1 for session selection criteria and section 3.5.3.2 for definition of AB). Gray dots and
error bars represent chance interval for the respective session (purple dot). Note that all purple dots
are above the respective chance interval. Black dots: mean, black error bars: SD.

**C.** Task performance during AB trials in each of the 5 sessions before whisker trimming, during
which mice were performing the full task with a single whisker (dark purple dots) and task
performance during AB trials in each of the 2 (or 3) sessions after whisker trimming (black dots). **D.**
Grand mean of each group of no whisker sessions (black) and the respective group of five pre-
trimming (dark purple) sessions.* indicates p = 0.0013 in one tailed paired t-test. Dotted line:
average chance range for single whisker (light purple) and no whisker groups (gray). Error bars:
SD.

3.3.2 Whisking strategy during the three-choice object localisation task

The fact that mice performed the task using a single whisker drastically limits the possible
sensory input available to the mice to localise the pole, and makes it possible to measure
that input on a trial by trial basis. To investigate how mice were solving the task, we used
high-speed imaging (1000 frames/s) to estimate both whisker movement and bending
moment during whisker-pole touch (Figure 3.1 A and C). Due to the very high volume of
imaging data, for each fully trained mouse, we selected 7.4 ± 2.7 sessions for detailed
analysis; comprising 761 ± 175 trials per mouse, where the animal was performing the ‘full
task’ with a single whisker (see Figure 3.8 and 3.9 for details). Performance in these
sessions (0.74 ± 0.05) was consistent with the analysis above.
We tracked the location and shape of the whisker in every frame of the selected sessions (total >10⁷ video frames; Figure 3.1 A, C, Figure 3.7; section 3.5.5). To quantify whisker movement (‘kinematics’), we extracted the angle of the whisker at its base (see section 3.5.5). To estimate bending moment on the whisker due to object touch, we extracted the curvature of the whisker near its base (see section 3.5.5).

Consistent with previous work on two-choice pole detection tasks (O’Connor et al., 2010a), mice adopted a stereotyped whisking strategy. At the start of a trial, prior to pole movement, mice whisked little (Figure 3.1 A, C and 3.3 A, B). Pole movement elicited robust whisking in all mice (Figure 3.3 B, significant increase in whisking amplitude in 200ms interval before and after pole onset, t-test p = 4·10⁻⁴).
**Figure 3.3. Mice implement stereotyped and reliable whisking motor strategy.**

**A.** Whisking amplitude times series in all whisker tracked trials for an example mouse, aligned to pole onset time. Vertical broken line indicates pole onset time.

**B.** Mean (thick line) + SD (thin line) whisking amplitude across all whisker tracked trials of each mouse, aligned to pole onset time. Vertical broken line indicates pole onset time.

### 3.3.3 Touch detection is not sufficient to solve the task

Mice learn to solve two-choice pole detection tasks (O’Connor et al., 2010a) by positioning their whiskers near one of the pole locations. In this way, whisker-object touch occurs much more frequently at one location and the presence/absence of touch becomes a reliable cue to pole location. As stated above, our task was designed to study how bending moment during touch drives behaviour. It was therefore essential to verify that the presence/absence of touch was not a sufficient cue to explain mouse task performance. To this end we applied a touch detection algorithm (see section 3.5.5) to the imaging data to register whether or not a mouse touched the pole with its whisker on each trial (the outcome was manually checked on all trials). We found that touches occurred at all pole locations: almost always at both posterior (0.87 ± 0.10) and middle (0.93 ± 0.05) locations; less often (0.46 ± 0.15) at the anterior location (one-way ANOVA: F = 27.2, p=3.5∙10⁻⁵). Since mice were able to accurately discriminate middle from posterior locations (Figure 3.4 A), the presence/absence of touch is unlikely to provide a sufficient cue. However, since touches were less frequent at the anterior location compared to the middle/posterior ones, it is possible that the presence/absence of touch could account for some degree of the animals’ performance (Figure 3.4 B). To test this, we computed the ability of an ideal statistical
observer of the presence/absence of touch to predict pole location, by training a probabilistic classifier on touch data measured from the mice (see sections 3.5.6.3 and 3.5.6.4). We measured classifier performance as the proportion of trials for which it predicted the pole location correctly. Since the classifier is an ideal observer, its performance sets an upper bound to the level of performance a mouse might achieve given the presence/absence of touch as a cue (see section 3.5.6.4 for details). We measured classifier-mouse choice consistency as the fraction of trials in which mouse and classifier made the same choice.

As expected, we found that classifier performance (0.53 ± 0.06) was significantly lower than that of the mice (0.74 ± 0.05; t-test p = 1.8⋅10^{-4}) and that classifier-mouse choice consistency was, mediocre, albeit above chance (red dots in Figure 3.4 C, right column; see section 3.5.6.4), (0.53 ± 0.07; Figure 3.4 C). This confirms that mice cannot solve the three-choice object localisation task simply using the presence/absence of touch, indicating that the task challenges the mice to implement more sophisticated tactile strategies.
Figure 3.4. Presence or absence of touch poorly predicts mouse choice

A. Conditional probability of correct choice given the pole location for each mouse (coloured lines). Broken coloured lines indicate the respective chance interval (5000 shufflings, 95% confidence interval) for each mouse.

B. Mean probability of touch given the pole location. Gray lines represent individual mice’s values. Black dots: mean. Error bars: SD.
C. Mouse performances (left), touch classifier performance (middle) and touch classifier-mouse choice consistency (right). Red dots indicate that the classifier performance or classifier-mouse choice consistency was significantly higher than chance. Black dots: mean. Error bars: SD. *: paired t-test $p = 1.8 \times 10^{-4}$.

3.3.4 Bending moment direction and magnitude predict mouse behaviour

What additional touch signals might be guiding mouse behaviour? Electrophysiological studies have shown that PWAs communicate much more information than just the presence/absence of touch: PWAs are sensitive to both the direction and magnitude of the bending moment associated with whisker-object contact (Szwed et al., 2003; Bush et al., 2016; Campagner et al., 2016; Severson et al., 2017). We therefore hypothesised that the three-choice object localisation task might bring out the mice’s ability to guide their decision-making using the direction and/or magnitude of bending moment that is latent in simpler tasks (O’Connor et al., 2010a).

To test the potential influence of bending moment direction, we classified each trial according to whether the first whisker-pole touch occurred during protraction or retraction. Retraction and protraction touches would cause variations in bending moment with opposite directions (Pammer et al., 2013; Peron et al., 2015). In this way, the ‘touch type’ on each trial was scored as either ‘no touch’ (Figure 3.5 A, gold), ‘protraction touch’ (Figure 3.5 A, green) or ‘retraction touch’ (figure 3.5 A, purple). We only analysed the first touch since, as we verified for a representative subset of the data, the first touch was a good proxy for subsequent touches (see section 3.5.5). The vast majority of trials (84%) had at most 3 touches, 94% of second touches were identical to first touches and 98% of third
touches were identical to second touches. There was therefore negligible difference in the information content of the first versus subsequent touches.

We found that, at every pole location, there was a significant difference in the incidence of different touch types (Figure 3.5 A; one-way ANOVAs p <10^{-5}). The posterior pole location tended to elicit retraction touches; the middle location protraction touches and the anterior location no touch or protraction touches (Figure 3.1 B, 3.5 A). This suggests that mouse whisking strategy, after the initial whisk (Figure 3.3A), was to adjust whisking set point (Huber et al., 2012), so that posterior contacts were typically made during retraction; middle contacts during protraction and, if the anterior location was reached at all, it was also during protraction. These data indicate that the direction of touch is a potential cue to pole location. To test whether direction might be a useful cue, we used the classifier approach to quantify how well pole location can be predicted from touch type. We found that the touch type classifier not only performed significantly better than the presence/absence touch classifier (0.67 ±0.05 vs 0.53 ± 0.06, t-test, p = 0.0011), but also that its classifier-mouse choice consistency was significantly higher (0.63 ± 0.09 vs 0.53 ± 0.07, p = 0.0066). However, the touch type classifier performed significantly worse than the mice (0.74 ±0.05, t-test, p = 5.5·10^{-4}), indicating that mice had access to additional sensory information beyond touch type.
To account for this performance gap, we investigated the possibility that mice might be able to use bending moment magnitude, in addition to direction, as a cue. As a simple and robust, scalar measure of bending moment during the first touch, we computed $\Delta \kappa_{95}$ (section 3.5.6.3). In our coordinate system, protraction touch caused the whisker to bend
with positive $\Delta \kappa$ and retraction touch to bend with negative $\Delta \kappa$. Thus, touch at the posterior pole location was associated with negative $\Delta \kappa_{95}$, whereas touch at the middle pole location was associated with positive $\Delta \kappa_{95}$. Anterior pole location was also associated with positive $\Delta \kappa_{95}$. However, it was substantially weaker than that for middle pole location. Therefore, $\Delta \kappa_{95}$ is a potential cue to pole location (Figure 3.5 C - D).

To quantify the value of $\Delta \kappa_{95}$ as a cue to the task, we again used the classifier approach. We constructed a classifier with input of both $\Delta \kappa_{95}$ and touch type (see sections 3.5.6.3 and 3.5.6.4), and trained it to predict pole location as above. This classifier performed as well as the mice (0.72 ± 0.03 and 0.74 ± 0.05 respectively; t-test $p = 0.21$) and significantly better than the touch type classifier (t-test, $p = 0.038$). Classifier-mouse choice consistency was 0.66 ± 0.05. For the three mice with the highest touch type classifier-mouse choice consistency, the effect of adding $\Delta \kappa_{95}$ as an input to the touch type only classifier, was negligible. In contrast, for the two mice with the lowest touch type classifier-mouse choice consistency, adding $\Delta \kappa_{95}$ improved classifier-mouse choice consistency (Figure 3.5 F). Our findings indicate that bending moment strength and direction account for mouse choices during the task; however sensory strategies were heterogeneous among mice, with different mice assigning different weights to magnitude and direction of bending moment.

To get further insight into mice decision making strategy, we investigated error trials (Figure 3.1 C). One possibility is that errors were sensory driven – e.g., due to an unusual touch on a particular trial. Alternatively, errors might be internally driven – e.g., due to fluctuations in mouse attention. To test between these possibilities, we asked whether the touch type – $\Delta \kappa_{95}$ classifier could predict mouse choice on error trials. To this end, we split the trials into those on which a mouse chose correctly (‘correct trials’) and those on which
it did not (‘error trials’). Consistently with the data reported above, the classifier was highly accurate on correct trials (0.77 ± 0.03). However we found that the classifier performed poorly on error trials: on average, classifier-mouse choice consistency on error trials was significantly lower than that on correct trials (0.35 ± 0.1, paired t-test p = 3.7·10⁻⁴) and for only one mouse the classifier-mouse choice consistency on error trials was above chance (Figure 3.5 E right, red dot). The result that choices on error trials could not, in general, be predicted from sensory variables suggests that there is an important internal contribution to errors.

3.3.5 Choice history predicts behaviour on error trials

To investigate the nature of the errors, we examined the time sequence of choices (Figure 3.6 A). We found that mice showed a striking tendency to ‘perseverate’ (Figure 3.6 A-C) - that is, a tendency to make the same choice on consecutive trials. Across all trials, the average probability of perseveration across mice was 0.43 ± 0.03, (Figure 3.6 C, light blue dot) and the probability of perseveration of each individual mouse was significantly higher than chance (Figure 3.6 C, 95% chance confidence interval for each mouse indicated by black error bars, section 4.5.6.5).

When we separately analysed perseveration during correct and error trials, we noticed a striking asymmetry: the probability of perseveration on error trials, but not on correct trials, was above chance for all individual mice (all single mice values in Figure 3.6 C are outside 95% confidence interval, but not for correct trials). The average probability of perseveration during error trials (0.63 ± 0.02) was significantly greater than the probability of perseveration during correct trials (0.36 ± 0.02, paired t-test p=1.43·10⁻⁵). Thus, a strikingly high number of choices on error trials originated from perseveration (Figure 3.6
C). Since it was significantly more likely that a mouse perseverated if the choice in the previous trial led to a correct outcome (Figure 3.10 A), the most common perseverating behaviour observed was that, when a mouse got a certain trial correct by making a certain choice, it had a tendency to repeat that choice. This suggests that the difficulty of predicting mouse choice on error trials from sensory signals is largely due to a substantial internal (that is, not sensory-driven) component to the mouse’s decision-making.

We then asked whether the knowledge of the choice in the previous trial would improve the capacity of the classifier to predict error trials. To this end, we considered a new predictor variable called choice history (section 3.5.6.3) and trained classifiers based on choice history to predict mouse choice. Consistent with the observed perseverating behaviour, the choice history classifier performed well on error trials (0.58 ± 0.08), predicting mouse choice above chance levels for all mice during error trials, but performed significantly worst on correct trials (0.41±0.03, Figure 3.6 D, t-test p = 8.8·10⁻³). In contrast, a classifier with access to sensory information (either Δκ₉₅ and touch type or Δκ₉₅, touch type and choice history classifier) could predict choices on correct trials accurately (0.77 ± 0.02, 0.74 ± 0.04), but failed to predict choices on error trials (0.35 ± 0.10 and 0.41 ± 0.08; t-test p = 2.7·10⁻⁴ and p =8.7·10⁻⁵ respectively; Figure 3.6 D).

These results indicate that two competing mechanisms governed mouse behaviour during the task: an internally-driven (history-dependent) and a sensory-drive (bending moment-dependent) one. If, in a given trial, the history-driven mechanism succeeded, it was more likely that the outcome was an error, on the contrary, if the sensory-driven mechanism succeeded, the outcome was more likely correct.
Figure 3.6. Choice history predicts error trials
**A.** Sequence of 31 consecutive trials performed by an example mouse. Red, blue and gray rectangles indicate trials in which the pole location was anterior, middle or posterior (the top row), or in which the mouse made a posterior, middle or anterior choice (bottom row). Triangles indicate error trials, dark red triangle indicate error trials in which the choices in the previous and current trial were identical (i.e. the mouse perseverated).

**B.** Probability of perseveration for the example mouse in A under different conditions: considering all trials (light blue), correct trials only (orange) or error trials only (dark red).

**C.** Same plot as in B showing population data from all mice. Dots: mean, error bar: SD (not visible since smaller than the dots' diameter). * indicates paired t-test, p ≤ 0.0167 (Bonferroni correction, n = 3). Black bars indicate chance interval (10000 shuffling, 95% confidence interval).

**D.** Performance of classifiers predicting mouse choice in all trials (left), correct trials only (middle) and error trials only (right). Blue, green and yellow dots indicate mean values for $\Delta \kappa_{95}$ - touch type classifier, $\Delta \kappa_{95}$ - touch type - choice history classifier and choice history classifier respectively. Small dots are single mice values. Red indicates that the classifier performance value for the mouse was above chance. Error bar are SD. * indicates p ≤ 0.05, t-test.

### 3.4 DISCUSSION AND CONCLUSIONS

The recent discovery that PWAs of mice are driven by bending moment (Bush et al., 2016; Campagner et al., 2016, 2017; Severson et al., 2017) raises the question of how these animals use this mechanical signal to guide their behaviour. Here we tested how mice, trained in a novel three-choice object localisation task, find the position of an object along their anterior-posterior axis. We discovered that bending moment direction and magnitude could explain the vast majority of mouse correct choices, but only a small fraction of errors. Conversely, error trials could be explained by a history-dependent behaviour, during which mice repeated in the current trial the same choice as the previous trial.
3.4.1 Decision making during active sensation

Animals base their decisions on the sensory information they extract from the environment. Hence, when studying decision making, a fundamental step is to establish the sensory input which precedes each choice. However, in most decision making paradigms, sensory cues are typically externally controlled and there is no direct measurement of the sensory input. Decision making under active sensing conditions is much harder to study, since the sensory input to a moving sensory organ is under the control of the animal and, only indirectly, of the experimenter. A striking case is the one of the visual system, where saccadic and fixational eye movements can dramatically affect the visual input to the retina of awake animals (Rucci and Victor, 2015). In absence of careful measurement of eye position, the approximation that what reaches the retina photoreceptors at a given time closely matches presented stimulus, may be conducive to erroneous estimate of the visual information available to the animal brain and therefore to misleading conclusion on what drives choice (Rucci and Victor, 2015). Similarly to what happen with eyes, animals move their whiskers in a fairly unpredictable and complex manner while they are exploring objects, and the way in which whiskers are moved drastically affects the sensory signals (Carvell and Simons, 1990; Mitchinson et al., 2007; O’Connor et al., 2010a; Arkley et al., 2014; Sofroniew et al., 2014). However, a great advantage offered by the whisker system is that, thanks to recent technological progress, the problem of measuring sensory signals during behaviour has been substantially overcome: it is now possible to combine high speed imaging of the whiskers with computer vision and mathematical modelling to estimate the mechanical forces/moments which constitute the sensory input during active touch (O’Connor et al., 2010a; Clack et al., 2012; Pammer et al., 2013; Bush et al., 2016; Campagner et al., 2016;
Severson et al., 2017). Therefore the whisker system now constitutes a powerful model to study decision making under active conditions.

3.4.2 Mechanical basis of anterior-posterior object localisation

Recent biomechanical modelling studies (Yang and Hartmann, 2016; Huet et al., 2017) have shown that, in principle, information of bending moment direction and magnitude at the base of a tapered whisker during touch is sufficient to uniquely localise the anterior-posterior location of an object. A previous study in awake rats (Bagdasarian et al., 2013) suggested that the change in the global bending of the whisker (i.e. curvature computed over a substantial part of the whisker shaft) due to touch with a metal pole inversely correlates with the position of the pole along the anterior-posterior axis. However, whether or not rodents actually exploit bending moment magnitude and direction during object localisation was unknown. By estimating mechanical variables in vivo during the task, we showed that bending moment magnitude and direction can account for how mice localise object along anterior-posterior axis. Moreover, they complement previous work which have shown that whisker-based radial object localisation was performed by mice combining bending moment and axial force information (Pammer et al., 2013).

3.4.3 Neural basis of anterior-posterior object localisation

Neurons responsive to bending moment magnitude and direction are widely distributed in the whisker system (Campagner et al., 2017). Neurons sensitive to bending moment magnitude have been reported in the PWAs (Campagner et al., 2016; Severson et al., 2017), whisker thalamus (Yu et al., 2016a; Gutnisky et al., 2017), barrel cortex (O’Connor et al., 2010b; Hires et al., 2015; Peron et al., 2015; Yu et al., 2016a) and primary motor cortex.
(Huber et al., 2012; Petreanu et al., 2012; Xu et al., 2012) of awake mice. Studies in anesthetized rodents have reported sensitivity to the direction of whisker deflection, and therefore to bending moment direction (Campagner et al., 2016), in PWAs, whisker brainstem, whisker thalamus and barrel cortex (Zucker and Welker, 1969; Waite, 1973; Simons, 1978; Gibson and Welker, 1983; Lichtenstein et al., 1(Kwon et al., 2017)990; Minnery and Simons, 2003; Petersen et al., 2008; Bale and Petersen, 2009; Bale et al., 2013; Martini et al., 2017). Furthermore, neurons sensitive to bending moment direction have been directly observed in the barrel cortex of awake mice (Peron et al., 2015; Kwon et al., 2017). However, the functional importance of bending moment magnitude and direction encoding in the whisker system was unclear and it had not been tested if mice could exploit such variables during behaviour. Our study links electrophysiological evidence to behaviour by showing that the direction and the magnitude of bending moment can account for mouse choices during object localisation.

3.4.4 Neural mechanisms and behavioural relevance of history-dependent choices

All mice in our study showed a strong perseverating behaviour, in which the mouse carried over the memory of the previous choice into the next trial where it repeated the same behaviour, ignoring the available sensory information. The effect of previous trial choice on behaviour has been rarely studied in rodents and, as far as our knowledge, it has been previously reported only in mice performing a visual detection task (Busse et al., 2011). For the first time, we showed that an internally generated signal - the memory of the choice in the previous trial - could affect mouse behaviour during an active discriminative task.

The significance of perseveration during a behavioural task is still elusive in rodents. However, serial dependence effects are well-established in human perceptual decision
making. They may reflect the fact that, although maladaptive in laboratory tasks, where the sequence of trials is random, they may be adaptive in the natural environment where the recent past is typically a good predictor of the future (Snyder et al., 2015). Indeed, fMRI studies of visual tasks, have shown that stimuli from previous trials can bias activity in visual cortex (Kiyonaga et al., 2017), but the neural mechanism that shifts the choice balance toward listening to the memory of previous trial instead of to the sensory input of the current trial is unknown. Recent studies in the frontal orienting fields (FOF) and the posterior parietal cortex (PPC, Marcos and Harvey, 2016; Scott et al., 2017) of rats have provided the first evidence for the existence of neurons able to store the memory of the choice and of the behavioural outcome of the previous trial for many seconds. Our task therefore provides a useful tool to study the neural basis of choice history dependence in a controlled and highly repeatable manner. Neural recording from somatosensory and higher order cortices (e.g., mouse homologous of FOF and PPC) and selective inactivation of these areas during the three-choice object localisation may shed new light on how perseveration arises during a behaviour and what mechanisms may regulate the switching from a sensory-driven to internally-driven choice.

More in general, the three-choice object localisation task presents several features that make it ideal for simultaneously studying multiple dimensions of neural codes, previously unexplored. The task has a longer training time than the object detection task (O’Connor et al., 2010a), indicating a higher degree of complexity. It requires fine grain decoding of the bending moment direction and magnitude, in order to correctly identify pole location, which therefore permits the study of how mechanical forces are encoded in the brain and how they affect behaviour. In addition, the task cannot be solved by simply sampling one of the three pole locations, but requires active whisking to contact the pole in at least two of
the three locations. This makes it suitable for studying motor control of goal-directed whisker movements. Finally, internally driven, memory-based behaviours, like perseveration, spontaneously emerge during training. This allows the task to be used for studying superior cognitive functions in higher order cortical areas such as FOF and PPC, in a well-controlled manner.

3.4.5 Summary and conclusions:

We have developed a novel whisker based three-choice object localisation task which revealed that bending moment magnitude and direction can account for how mice localise objects. Our study provides a behavioural explanation of why bending moment is encoded in the whisker system, from peripheral mechanoreceptors to cortex.

3.5 MATERIALS AND METHODS

All experimental protocols described in this section were approved by both United Kingdom Home Office national authorities and institutional ethical review.

3.5.1 Surgical procedure and water restriction

Mice (C57; N=5; 6 weeks at time of implant) were implanted with a titanium head-bar as detailed in (Campagner et al., 2016). After the surgery, mice were left to recover for at least 5 days before starting water restriction (1.5 ml water/day). Mouse training began 7 to 10 days after the start of water restriction.

3.5.2 Behavioural apparatus

Mice were trained in a dark, sound-proof enclosure adapted from (O’Connor et al., 2010a). Briefly, a head-fixed mouse was placed inside a perspex tube, from which its head emerged
at one end. The stimulus object was a 1.59 mm diameter metal pole which could be moved along the anterior-posterior axis of the mouse by a linear stepper motor (NA08B30, Zaber, Vancouver, Canada). To allow vertical movement of the pole into and out of range of the whiskers, the pole was mounted on a pneumatic linear slide (SLS-10-30-P-A, Festo, Northampton, UK), powered by compressed air. The apparatus was controlled from Matlab. Mouse response was monitored by two lick ports located anterior to its mouth.

Licks were detected as described in O’Connor et al., 2010a (Figure 3.1 A and B). Each lick port consisted of a metal tube connected to a water reservoir via a computer-controlled solenoid valve (LHDA1233215H, Lee Company). Lick port position was monitored using an infrared camera (N08CX- Sentient) and adjusted using a micromanipulator.

### 3.5.3 Behavioural task

Head-fixed mice were trained to locate a metal pole using their whiskers and to report its position by licking (Figure 3.1 B). On each trial, the pole was presented in one of three anterior-posterior locations (posterior, middle and anterior). On trials when the pole was middle or posterior (‘go left location’ or ‘go right location’), the correct response was for the mouse to lick at one of the two lick ports. In 3 cases (mouse id: 32, 33 and 34), mice were rewarded for licking at the right lick port when the pole was posterior, and for licking at the left lick port when the pole was at the middle location. In 2 other cases (36 and 38) the contingency was reversed. Incorrect responses (licking the wrong side or not licking at all) were punished by timeout (Figure 3.1 B, hourglass symbol). On trials where the pole was anterior (‘no go location’), the correct response was to refrain from licking. Incorrect responses on no go trials (licking) were punished by timeout and punishment tone (frequency 1 kHz; Figure 3.1 B, speaker symbol).
3.5.3.1 Trial structure

Each trial consisted of three successive periods, which occurred for all pole location/mouse choice combinations (i.e. pole out of reach period, grace period and response period) and two which occurred only for specific pole location/mouse choice combination (i.e. drink period and time out period). See Figure 3.1 A for a visual representation of different trial periods.

Pole out of reach period: Each trial started with the pole out of whisker reach. Licks during this period were ignored.

Grace period: a short time period (typically 0.5 s for the full task), which followed the pole onset time, during which any licks were ignored. As in related previous studies, the sound caused by opening of the pneumatic valve which drove pole upwards tended to trigger reflexive licks, unrelated to mouse choice (O’Connor et al., 2010a; Guo et al., 2014a). The pole came within whisker reach after ~0.15 s from the start of the grace period.

Response period: the time period during which the mouse licking was detected and could control reward. It started at the end of the grace period and lasted for up to 2 s in the full task. During the response period the pole was in reach.

Drink period: A drink period was initiated if the mouse made the correct choice in go trials and its onset was triggered by the first lick to occur within the response period. We used different durations over the course of the experiments (typically 0.5- 2 s). The drink period allowed the mouse to consume the water reward before starting the next trial. The end of the drink period triggered withdrawal of the pole and terminated the trial.
Time-out period: To punish mice for incorrect responses, a time out period was used. This consisted of prolonging the trial duration by a fixed amount of time (typically 2-10 s, O’Connor et al., 2010a; Guo et al., 2014a)

3.5.3.2 Training protocols

The mouse learning process was subdivided into several successive steps (protocols) of increasing complexity, following (O’Connor et al., 2010a; Guo et al., 2014a). Transition from one protocol of the training to the next was performed only if the mouse showed stable high performance (typically ~70%) on at least two consecutive days (see Figure 3.2 A and 3.7 A

The typical sequence of training protocols was as follows:

1) Lick

Here, mice were trained to associate whisker-pole contact with availability of water reward from the lick port. Whenever the pole moved up into one of the two go locations, a drop of water was delivered. After a few trials, mice started to lick in response to the pole movement, triggering water delivery via the lick sensor.

2) Go – no go

Here, the pole was presented in one of two alternative locations: the posterior go location and the anterior no go location. Only one lick port was within reach. The mouse was rewarded for licking when the pole was presented in the go location. The mouse was punished (by timeout) for both false alarms (licking on no go trials), or for misses (not licking on go trials). When the mouse reached high stable performance typically (~70%
correct performance) with its full whisker array, all whiskers, except for C row, were trimmed to fur level. Such whisker configuration was maintained by frequent re-trimming in the successive days/weeks. If trimming caused a drop in mouse performance, training in AB go – no go continued with a single row until mouse performance returned to its pre-trimming level.

3) Lick left-lick right

Here, the pole was presented in one of two alternative go locations: the posterior go location or the middle go location. Both lick ports were within reach. The mouse was rewarded for licking a designated lick port when the pole was presented in the posterior location, and the other lick port when the pole was presented in the middle location. The mouse was punished by timeout for either licking the non-designated lick port or not licking.

4) Lick left-lick right-no lick (‘full task’)

The task included three pole location: posterior, middle and anterior, and 3 behavioural outcomes: lick left, lick right and do not lick. Once performance reached (typically 70%) correct, all whiskers except one (C1 or C2) were trimmed to the level of the fur. This is the final configuration of the task and we refer to it as ‘full task’.

On each protocol which followed the lick protocol, the mouse was first trained using the ‘On policy’ and subsequently switched to the ‘AB policy’:

On policy: Trials were presented in blocks of the same pole location. The pole location was switched only once the mouse performed a criterion number of consecutive trials (typically 3-8) correctly.
**AB policy:** The type of each trial was determined randomly, subject to the constraint that runs of the same pole location were typically limited to a maximum of three. We either used same probability for each trial type (most sessions) or same probability for go and no go trials. During early training, probabilities could be adjusted in order to correct mouse bias.

During a typical training session in the full task, a few trials at the beginning of the session were presented using the On policy, to remind the mouse of the reward-pole location contingency, before switching to the AB policy (Figure 3.8).

3.5.4 High-speed whisker imaging

Whiskers were imaged using same method described in (Campagner et al., 2016). Briefly, whiskers ipsilateral to the pole were illuminated from below using an infrared (940 nm) LED array, in order avoid visual cues to pole location. Whiskers were imaged in the horizontal plane using a high speed camera (1000 frames/s, 0.4 ms exposure time).

3.5.5 Whisker tracking and touch detection

The large number of trials and sessions imaged necessitated a fast whisker tracking approach requiring minimal user intervention. In this study we only tracked those sessions in which the mice performed the task at criterion with a single whisker (ca $10^7$ frames, see Figure 3.8 and 3.9). To extract whisker position/shape from the high-speed imaging, we first applied the ‘Whisk’ whisker tracker (Clack et al., 2012). The tracker output was then checked by an automated quality-control program to identify misclassified or poorly tracked video frames, based on expected whisker length and location within the image.
Whisker bending (curvature) and whisker position (whisker angle) were computed at the intersection of the whisker and a face-fur-mask by fitting a quadratic curve to the masked portion of tracked whisker following Pammer et al. (2013). Whisker angle was defined as the angle tangent to the whisker with respect to the anterior-posterior axis of the mouse (0° in the posterior direction, 180° in the anterior one).

Given the high number of video frames, in order to detect the onset and offset times of whisker-pole contact with millisecond accuracy, we developed a semi-automatic, human-in-the-loop touch detection GUI. Pole location in each video frame was determined by convolution with a circular pole template. The minimum distance between pole centre and tracked whisker was calculated in each frame and putative touches identified as when this distance was lower than a user-defined threshold. The user then used the GUI to confirm putative touches and to curate their timing to frame-rate precision. On each trial, the first touch was classified as protraction or retraction based on the phase of the Hilbert transform of whisker angle time series (Kleinfeld and Deschênes, 2011) and manual curation. During touch curation, whisker tracking output was also visually inspected. For one session, (150 trials) we detected and classified as protraction or retraction all the touches.

If a frame failed quality-control, that frame was classified as ‘dropped’. If dropped frames occurred during the first touch, that trial was either re-tracked or discarded. Curvature/angle of occasional, isolated dropped frames was corrected by interpolation.
3.5.6 Behavioural and imaging data analysis

3.5.6.1 Quantification of learning time and asymptotic performance of mice

In this study mouse performance (‘task performance’) was quantified as the proportion of trials in which mouse choice was correct during a session. In order to assess performance, we considered AB trials of single whisker sessions in which the mouse was performing the full task and compared the actual performance to that expected if the mouse responded randomly. To this end, we shuffled the pole location sequence with respect to the mouse choice sequence and computed the proportion of correct trials. By repeating this procedure 10000 times, we estimated the mean and 95% confidence interval on performance attributable to chance. We considered a mouse to have learnt the task when performance exceeded the 95% chance confidence interval on 3 consecutive sessions. We defined asymptotic performance as the performance averaged over 8 consecutive, above-chance sessions as close as possible to the end of training (32, 36 and 38) or just before second whisker trimming (33 and 34; Figure 3.2 A and 3.7 A).

3.5.6.2 Analysis of whisker movement

In order to quantify whisker movement during the task we computed time series of whisking amplitude (Kleinfeld and Deschênes, 2011) from whisker angle as in Campagner et al., 2016.

3.5.6.3 Classifiers: input and output variables

In order to investigate the extent to which one or more sensory variables (e.g., bending moment magnitude or direction) might account for a mouse’s decision-making, we used a classifier approach. We term the candidate sensory variables ‘predictor variables’ or
‘predictor’. To this end, we trained classifiers to predict pole location or mouse choice based on one or more predictor variables obtained from the whisker tracking and touch scoring procedures detailed above (section 3.5.5).

The predictor variables were:

- **Touch**: a binary variable scoring presence or absence of whisker-pole touch on a given trial.

- **Touch type**: a 3-valued variable scoring whether touch was absent or, if present, whether first touch in a trial occurred during retraction or protraction (see section 3.5.5).

- **$\Delta \kappa_{95}$**: a continuous variable related to bending moment during first touch. During whisker-object touch, a whisker bends and the change ($\Delta \kappa$) in curvature ($\kappa$) at a given point along the shaft, compared to that of the undeformed whisker, is proportional to the bending moment (Birdwell et al., 2007; Campagner et al., 2017). Within a touch episode, $\Delta \kappa$ of each frame was computed by subtracting from $\kappa$ the median curvature in the 6 ms before touch onset. For each touch episode, we calculated the 5th and 95th percentiles of the distribution of $\Delta \kappa$. $\Delta \kappa_{95}$ was set equal to whichever of these two percentiles had greater absolute value. If no touch occurred during the trial, $\Delta \kappa_{95}$ was, by definition, 0.

- **Choice history**: A 6-valued variable indicating both the mouse’s choice in a given trial and whether or not it was correct (i.e., posterior, middle or anterior correct; posterior, middle or anterior incorrect).
3.5.6.4 Classifiers: training and testing procedure

To attempt to classify pole location from predictor variables, we used a Maximum a Posteriori (MAP) approach (implemented in Matlab using the function fitnbc). For each mouse, the training/testing data consisted of a vector $Y$ specifying the pole location ($y$) on each of $T$ trials and a predictor matrix $X$. $y$ was a 3-valued quantity (corresponding to anterior, middle or posterior location classes, indexed by $k$). $X$ specified the value of each of $P$ predictor variables ($x_1, x_2, ..., x_P$) on each trial.

As detailed below, we used the training data to estimate, for each trial, $\hat{P}(y = k|x_1, ..., x_P)$ – the posterior probability that pole location was class $k$, given the predictors:

$$\hat{P}(y = k|x_1, ..., x_P) = \frac{\pi(y = k) \prod_{j=1}^{P} P(x_j|y = k)}{\sum_{k=1}^{K} \pi(y = k) \prod_{j=1}^{P} P(x_j|y = k)}$$

Here $\pi(y=k)$ is the prior probability of pole location $y$ (determined from relative frequencies within the training set) and $P(x_j|y=k)$ is the probability of predictor value $x_j$ conditional on pole location. The predictors were assumed to be conditionally independent given pole location. For each trial, the pole location predicted by the classifier was set to that with the maximal posterior probability over $k$.

The distributions of categorical predictors were described by multinomials; those of continuous predictors were approximated as Gaussians. Gaussian approximation was validated by ensuring that classifier accuracy did not increase if continuous distributions were described non-parametrically by Gaussian kernels.

To avoid overfitting, we evaluated the classifier 10 times using a different training and testing set each time. The training set was constituted by a random selection of 90% of the
trials and the testing set by the remaining 10%. Classifier chance level, and confidence interval on it, was computed using a shuffling method, as described above for the mouse data (50 iterations).

We used four different metrics to quantify classification performance. They were:

*Classifier performance*: proportion of trials for which the classifier correctly predicted pole location.

*Classifier mouse-choice consistency*: proportion of trials for which the mouse and the classifier made the same choice.

*Classifier mouse-choice consistency during error (or correct) trials*: proportion of error (or correct) trials for which the mouse and the classifier made the same choice.

We also trained classifiers to predict mouse choice instead of pole location. We used the same procedure described above, replacing pole location data with mouse choice data as $Y$.

Performance was quantified using the following metrics:

*Classifier performance*: proportion of trials in which the classifier correctly predicted mouse choice.

*Classifier performance during error trials (or correct trials)*: proportion of error (or correct) trials in which the classifier correctly predicted mouse choice.

### 3.5.6.5 Quantification of perseveration

Probability of perseveration was computed as the proportion of whisker tracked trials in which the choices in the current and in the previous trial were identical.

Probability of perseveration during error trials was computed as proportion of incorrect whisker tracked trials (all triangles in Figure 3.6 A) in which the choices in the previous
and current trial were identical (dark red triangles in Figure 3.6 A). Probability of perseveration during correct trials was computed applying the same method described for error trials to correct trials. Chance levels for probability of perseveration were computed by random shuffling as described above.
3.6 SUPPLEMENTARY FIGURES:

A

Mouse id: 32

Task performance

Mouse id: 34

Number of trials

Mouse id: 36

Task performance

Mouse id: 38

Number of trials

Days of training

Days of training

All whisksers
Single whisker
C - row
No whiskers

B

Number of trials per day

Mouse id

147
Figure 3.7. The three-choice object localization task. Data from mice not shown in Figure 3.2 A and total number of trials for each mouse.

A. Learning curves for mice not shown in figure 3.2 A. Note that in two mice (36 and 38), whiskers were trimmed to C row at the conclusion of lick protocol (cyan bar). In one mouse (38) go-no go protocol (red bar) was skipped. After the mouse learnt the lick protocol, we immediately introduced the lick left-lick right protocol (green bar). The no go location was introduced with ‘the full task’ (gold bar).

B. Total number of trials per day performed by each mouse (black dots: mean, error bars: SD).
Figure 3.8. Summary data of the whisker tracked sessions.

A. Task performance in the sessions where we tracked the whisker, considering only whisker tracked trials (top), all the AB trials (middle) and all the trials in the session (bottom). Dotted lines
represent the bounds of the chance level for each mouse and session (10000 shufflings, 95% confidence interval).

**B.** Number of trials for each of the performances computed in A.
Figure 3.9. Pole location vs mouse choice confusion matrices for whisker tracked trials

Confusion matrices summarizing the mouse choice given each of the pole locations for every mouse, using whisker tracked trials. White numbers are the number of times in which the mouse made the choice in the row given the pole location in the column. Gray levels indicate the conditional probability of making a certain choice given the pole location. The total number of trials in which the mouse made each choice is reported on the left side of each row. The total number of trials in which the pole was presented in a given location is reported under each column.
Figure 3.10. Effect of previous trial outcome on perseveration during error trials and performances of choice history classifiers when predicting pole location.

A. Probability of perseveration during error trials considering separately if the previous trial was a correct or an error trial. *: paired t-test $p = 3.4 \cdot 10^{-4}$. Black error bars indicate chance intervals of each mouse (10000 shufflings, 95% confidence interval).

B. Classifier performance, classifier-mouse choice consistency, classifier-mouse choice consistency during correct trials and classifier-mouse choice consistency during error trials for classifiers predicting pole location. Blue, green and yellow dots indicate average values for $\Delta k_{95}$ - touch type classifier, $\Delta k_{95}$ - touch type - choice history classifier and choice history classifier respectively. By definition, choice history classifier can poorly predict pole location; therefore we used a classifier predicting mouse choice in the analysis reported in figure 3.6D. Small dots are single mice values. Red indicates that the classifier performance value for that mouse was above chance. Error bar are SD. * $p \leq 0.05$, paired t-test.
3.7 REFERENCES


Dorfl J (1985) The innervation of the mystacial region of the white mouse: A topographical


4. Mixed encoding of touch and locomotion by first and higher order thalamic nuclei and zona incerta
4.1 ABSTRACT

Thalamic first and higher order relay nuclei are key building blocks of any mammalian sensory pathway. However, while they have been widely studied in the anesthetized animal, their functions in the awake behaving animal are still largely unknown. To study how sensory and locomotion signals were encoded by first order and the higher order thalamic relays, and the GABAergic modulator of higher order relays ZI (zona incerta), we combined a whisker-guided navigation task in tactile virtual reality with high density 384-site extracellular recording techniques (O3 Neuropixel high density silicon probes), to record from thalamic whisker system nuclei VPM (ventral posteromedial nucleus), PO (posterior complex) and ZI. Probe design allowed recording from 17 additional regions located in hippocampus, thalamus, hypothalamus and midbrain. Surprisingly, we found that neurons in the first order relay VPM encoded not only sensory signals (touch), but also motor signals (locomotion). Similarly, the higher-order nucleus PO and ZI encoded both signals. Finally, we observed that locomotion signals were exceptionally widely encoded in the brain, across all the recorded brain regions. Our results indicate that, during whisker guided navigation, contrary to expectation, the function of first and higher order thalamic nuclei cannot be neatly separated into sensory and locomotion. Moreover, encoding of both tactile sensory and locomotion signal is widespread.
4.2 INTRODUCTION

Most sensory systems include not only a first order relay nuclei of the thalamus (somatosensory VPM, visual lateral geniculate nucleus and auditory ventral division of the auditory medial geniculate nucleus), but also higher-order thalamic nuclei (somatosensory PO, visual pulvinar and auditory dorsal and medial division of the medial geniculate nucleus) and their multimodal GABAergic modulator ZI (Calford, 1983; Calford and Aitkin, 1983; Barthó et al., 2002; Sherman and Guillery, 2006; Diamond et al., 2008; Lee, 2015; Watson et al., 2015). First order, higher order relay nuclei and ZI have been well-studied under anesthetized condition (Waite, 1973; Ito, 1988; Simons and Carvell, 1989; Armstrong-James and Callahan, 1991; Diamond et al., 1992a, 1992b; Pinto et al., 2000; Sherman and Guillery, 2002, 2006; Trageser, 2004, 2006; Lavallee, 2005; Urbain and Deschênes, 2007a; Montemurro et al., 2007; Petersen et al., 2008; Bale and Petersen, 2009; Saalmann and Kastner, 2011). These studies found that first order relays have an essential role for conveying sensory input from periphery to cortex (Sherman and Guillery, 2006; Jones, 2012). Conversely, the role of the higher-order thalamic relays and ZI is less understood. In fact, under anaesthesia, such regions are characterized by less prominent sensory-evoked responses, which are strongly dependent on cortex (Diamond et al., 1992a; Sherman and Guillery, 2006; Urbain and Deschênes, 2007a; Jones, 2012). Higher-order thalamic relays and ZI are also characterised by high heterogeneity of inputs and outputs, which include not only sensory, but also motor and locomotion regions (Mogenson et al., 1985; Diamond et al., 1992b; Lin et al., 1997; Power et al., 1999; Grieve et al., 2000; Barthó et al., 2002; Trageser, 2004, 2006; Lavallee, 2005; Sherman and Guillery, 2006; Urbain and Deschênes, 2007a; Wurtz et al., 2011; Hooks et al., 2013; Petersen, 2014; Urbain et al., 2015; Watson et al., 2015; Moore et al., 2015; Roth et al., 2016; Economo et
al., 2016). However, motor functions such as locomotion cannot be studied in anesthetized preparation and therefore have been so far poorly investigated (Roth et al., 2016).

In the whisker system, the first order relay VPM receives whisker-tactile input via a projection from the brainstem trigeminal nucleus principalis (Diamond et al., 2008) and its neurons respond vigorously both to deflection of one or multiple whiskers (Waite, 1973; Ito, 1988; Simons and Carvell, 1989; Armstrong-James and Callahan, 1991; Pinto et al., 2000; Sherman and Guillery, 2006; Yu et al., 2006; Montemurro et al., 2007; Petersen et al., 2008; Bale and Petersen, 2009) and to whisker-object touch (Moore et al., 2015; Yu et al., 2016). The higher order relay PO and ZI receive ascending whisker-tactile input via a projection from the spinal trigeminal nucleus interpolaris of the brainstem (Diamond et al., 2008) and, less prominently, respond to whisker deflection (Diamond et al., 1992a, 1992b; Ahissar et al., 2000; Urbain and Deschênes, 2007a) and touch (Yu et al., 2006; Moore et al., 2015). In the anesthetized animal, ZI acts as a strong modulator of PO neuronal responses to whisker deflection. ZI tonically inhibits PO neurons via GABAergic long range projections (Barthó et al., 2002; Trageser, 2004; Lavallee, 2005), but this inhibition can be removed by the activation of a recurrent inhibitory circuit within ZI (Urbain and Deschênes, 2007a), controlled by primary motor cortex (Urbain and Deschênes, 2007a) and other brain regions including the superior colliculi (Watson et al., 2015).

Here we took advantage of the rodent whisker system to study neural coding in thalamic primary and higher order relay nuclei and ZI during a whisker-guided navigation task, where mice used their whiskers to follow a winding corridor in a tactile virtual reality environment (Sofroniew et al., 2014). We chose this task because, to be solved, it requires mice to combine locomotion, whisker motion and to collect tactile information using their
whiskers (Sofroniew et al., 2014). By measuring salient whisker-sensory and locomotion variables during the task, and correlating them with neuronal activity, we aimed to get new insight into both sensory and motor functions of VPM, PO and ZI.

To simultaneously record neuronal activity from VPM, POM and ZI during the task, we used new, high density 384-recording site silicon probes (O3 Neuropixel probes, Jun et al., 2017; Mora Lopez et al., 2017). Thanks to the probe design, we were able to sample widespread brain activity: a total of 17 other thalamic and peri-thalamic regions, including hippocampus, hypothalamus and midbrain.

Surprisingly, we found that the neuronal activity in the relay nucleus VPM was modulated by not only whisker tactile signals, but also by locomotion signals. Similarly, the higher-order regions PO and ZI were also modulated by both tactile and locomotion signals. Indeed, locomotion signals were exceptionally widely distributed in the brain, including non-whisker thalamic nuclei, hippocampus, midbrain and hypothalamus. Whisker tactile signals were also widely distributed, but less prominently.

### 4.3 RESULTS

#### 4.3.1 Large-scale multi-regional silicon probe recordings during whisker-guided locomotion

To simultaneously study how sensory whisker-related signals and locomotion signals are encoded in the whisker thalamus and adjacent regions, we recorded neuronal activity (N = 6 recording sessions) from trained mice (N = 3), while they performed a whisker guided navigation task (Figure 4.1 A, 4.1 B and 4.1 C). Mice were head-fixed on a spherical treadmill in a tactile virtual reality environment (Figure 4.1 A; Sofroniew et al., 2014,
2015), constituted by a winding corridor, whose walls were located on both side of the mouse head (see section 4.5.2). The walls were moved in real-time based on the movements of the spherical treadmill: when mice ran towards a wall, a motor moved the wall closer to the animal; when mice ran away from a wall, a motor moved the wall away. During the task, mice learnt to use their whiskers to correctly estimate their distance from the corridor walls (brow line in Figure 4.1 A; Sofroniew et al., 2014) and to transform that information into the correct value of lateral velocity (yellow vector in figure 4.1A right panel), to maintain their position in the centre of the corridor, and of forward velocity (orange vector in figure 4.1A right panel), to reach in time the end of the corridor and get a water reward (Figure 4.1 E6 and see section 4.5.2).

Mice were trained (closed loop experiment) to navigate 5 different types of corridors (5° and 10° turn left, 5° and 10° turn right or straight corridor, Figure 4.1 C) and an ‘open field’ (walls not present) using their whiskers (Figure 4.1 B, for details see section 4.5.2 and Sofroniew et al., 2014, 2015). Mice performed on average 136 ± 35 (mean ± SD, Figure 4.1 E5) trials per session, travelling a total distance of 109.2 ± 34.7 m (Figure 4.1 E6). Mice obtained a reward in 74% ± 12% of the trials (Figure 4.1 E6).

We took advantage of the controlled experimental conditions provided by head fixation in a virtual reality environment to precisely monitor mouse behaviour. As a measure of the mouse’s motor output we extracted the lateral and forward components of mouse locomotion velocity. As a measure of the sensory input we extracted the distance between the right wall and the mouse right whisker pad (Figure 4.1 A, right panel).

To decrease the correlation between the time series of the recorded behavioural variables, a key condition for the multiple linear regression analysis employed in this study (see
sections 4.3.2 and 4.5.5.3), we performed an open loop experiment (Sofroniew et al., 2015) after the closed loop experiment. During the open loop experiment the walls were moved independently from the spherical treadmill motion (Figure 4.1 D; see section 4.5.2). During open loop experiments, mice were presented with on average $62 \pm 4$ open loop trials per experimental session. During open loop experiments, mice exhibited locomotion (total travelled distance $26.8 \pm 8.6$ m). During both closed loop and open loop experiments mice (Figure 4.1 C3, D3, E1, E2 and E4) experienced a wide range of forward velocities, lateral velocities and wall distances (Figure 4.1 E1-E3), alternating periods of running to periods of quiescence (Figure 4.1 B2, C2, D2, E1-2). High variance of behavioural variables was a fundamental prerequisite to be able to tease apart encoding of different variables using the multiple linear regression analysis (see sections 4.3.2 and 4.5.5.3).

In order to maximize the number of neurons recorded from VPM, PO and ZI in a single coronal penetration (Figure 4.2 B, 4.6 lower panel and 4.7), we recorded neuronal activity (Figure 4.2 B) during both closed loop and open loop experiments using O3 Neuropixel probes (Jun et al., 2017; Mora Lopez et al., 2017). These probes had the virtue of permitting recording from a linear track of 3.84 mm of neural tissue (~80% of the dorso-ventral thickness of the mouse brain), by densely sampling it with 2 recording sites every 20 μm. This allowed us to construct a high-resolution map of neuronal activity across multiple brain regions (Figure 4.2 A, 4.2 D, 4.7, Table 4.1). We recorded in total 659 ($110 \pm 28$ per recording session) well-isolated single units, (Figure 4.2 B-C) from VPM, PO and ZI (Figure 4.2 A, D and 4.6) and 17 additional brain regions (located in hippocampus, thalamus, midbrain and hypothalamus, Table 4.1, Figure 4.2 A, D and 4.6).
Figure 4.1. Behavioural variables during whisker-guided navigation

A. Schematic of the experimental apparatus (left panel). We recorded neuronal activity from head fixed mice while they navigated a tactile virtual reality corridor using their whiskers, which were filmed by a high-speed camera (500Hz). Mouse behaviour was monitored by recording (sampling rate 500Hz) mouse forward velocity (orange), lateral velocity (yellow) and wall distance (brown; right panel).

B1. During closed loop experiments six different types of trial were presented. In closed loop-wall absent, mouse navigate an ‘open field’ with no wall present. B2. Forward speed and lateral speed time series during an example in a closed loop wall absent trial. Cyan line indicates the time in which the water reward was provided. B3. Mouse position during each of the closed loop–wall absent trials in the example session. 0 indicates the position of the mouse at trial start. Dotted line indicates the trial in B2.

C1. In closed loop - wall present mice navigate a 30 mm wide corridor using their whisker. Wall position changed according to spherical treadmill motion. C2. Same as B2 for a closed loop wall present trial. In this case also wall distance time series is reported (brown) C3. Same as B3 for closed loop wall present trials. Colours indicate trial type.

D1. In open loop condition walls were moved independently from the spherical treadmill motion. During each trial, the wall was moved close to mouse snout, kept at a fixed distance for 2.4 s and then moved away. 4 different distances were tested for each open loop experiment (shades of pink). D2. Same as B2 for an example open loop trial. D3. Same as B3, for open loop trials. Colours indicate trial type. E. Mean ± standard deviation of forward velocity (1), lateral velocity (2) and wall distance (3) for each mouse (coloured dots and bars) and across recording sessions (black circles and bars). Mean and standard deviation across recording sessions for total travel distance (4), total number of trials (5) and fraction of rewarded trials (6).
Figure 4.2. Large-scale multi-regional extracellular recordings from the sub-cortical mouse brain

A. Schematic representation of the O3 Neuropixel probe approximate trajectory (black line) from most superficial recording site to probe’s tip and of the macro-region encountered by the probe during an example experimental session.

B. Example raw voltage traces recorded by adjacent probe sites (Band pass filter 300Hz – 3000Hz) in ZI. Action potentials fired by the same single unit reported in C top left, have been indicated with black triangles.

C. Top left: 1000 randomly sampled waveforms (gray), from the single unit shown in B, sampled from the probe site with the largest mean waveform peak to through amplitude. Black line indicates the mean of the plotted waveforms. Top right: Auto-correlogram of spike timing of the same single unit shown in C top left and B. Bottom left: Distribution of the mean waveform amplitudes (computed as in C top left) of all 659 recorded units. Bottom right: Median of the auto-correlograms for all 659 single units (shaded area, inter-quartile range).
D. Raster plot of 138 single units simultaneously recorded from an awake mouse in the same recording session of A and B. Colours indicate the brain macro-region in which the units were located, following the colour code in A.

4.3.2. First and higher order thalamic relays and ZI encode a combination of locomotion velocities and wall distance

We asked how wall distance and locomotion velocity were encoded by VPM, PO and ZI neurons. When a whisker touches an object it bends and this causes an increase in the activity of the mechanoreceptors located at the whisker base (Bush et al., 2016; Campagner et al., 2016; Severson et al., 2017). Wall distance inversely correlates with whisker bending (Sofroniew et al., 2014) and therefore when the wall is closer to the whisker pad, it is likely that peripheral mechanoreceptors will fire at high rates, signalling the presence of whisker-wall touches. We would therefore expect to see wall distance modulation in touch-sensitive neurons of the whisker system (Moore et al., 2015; Yu et al., 2016; Gutnisky et al., 2017).

Figure 4.3A shows the activity of two example neurons recorded from the whisker system region ZI, during closed loop trials with the walls present (left), absent (middle) or during open loop trials (right).

As expected from a neuron located in the whisker system region ZI, activity of example neuron 11 and wall distance changed in concert (Pearson’s correlation coefficient between wall distance and firing rate: $PCC = 0.62; p<10^{-6}$): when the right wall was close to the mouse whisker pad (low wall distance), neuron firing rate was almost zero; when the wall was far from the snout (high wall distance) the neuron exhibited a higher firing rate (Figure 4.3A, left panel). Surprisingly, the same neuron was also responsive to locomotion (forward velocity and firing rate $PCC = 0.14, p<10^{-6}$: when the neuron was not inhibited by
low wall distance, its firing rate was modulated by locomotion velocity (see closed loop -walls absent period in Figure 4.3A, left panel). This neuron showed therefore combined sensitivity to wall distance and locomotion velocity. In contrast, the activity of example neuron 1 correlated with locomotion velocity, but not with wall distance (forward velocity and firing rate $PCC= 0.82$, $p < 10^{-6}$; wall distance and firing rate $PCC = -0.03$, $p = 0.12$).

These two example neurons suggest the presence of an unexpected locomotion signal in the whisker system region ZI, which is encoded together with tactile signals or alone.

To systematically evaluate the effect of each behavioural variable on neuronal activity across all the recorded neurons we needed an analytical method able to account for the correlation between behavioural variables (mean pairwise PCC magnitudes between all behavioural variables reported in figure 4.3 C across all sessions, $0.17 \pm 0.03$, mean±sd). Controlling for correlation between behavioural variables is of fundamental importance to avoid that a neuron might appear artefactually modulated by a given experimental variable, just because that variable is correlated with the real driver of the neuron (Campagner et al., 2016 and section 4.4.5). We therefore fitted multiple linear regression models to the data (Wooldridge, 2012; Martini et al., 2017). This method allowed in fact to measure the effect of each behavioural variable on neuronal activity, after that the effect of all others behavioural variables on the considered one have been netted out (Wooldridge, 2012). In other words, multiple linear regression provides a way of measuring how much neuronal activity changed when one behavioural variable changed, keeping all the others constant. Firing rate of the neuron was the dependent variable of the linear regression while the rectified positive and negative component of forward, lateral velocities, and wall distance were the predictors (see section 4.5.5.3). The magnitude of a regression coefficient indicated how strong the effect of the respective behavioural variable on firing rate was.
The sign of the regression coefficients indicated whether the effect of the behavioural variable was to increase or decrease the firing rate. A neuron was considered tuned to a given sensory variable if the p-value associated with the respective regression coefficient was significant at $p = 0.05$ level, after correcting for multiple comparisons (Figure 4.3 B, see Martini et al., 2017 and section 4.5.5.3 for details).

Multiple linear regression results were consistent with the correlations reported above: neuron 11 firing rate was strongly correlated with wall distance ($PCC = 0.62$) and the regression coefficient associated to wall distance was high (12.12). Neuron 11 activity was also correlated with forward locomotion velocity, but less strongly than with wall distance ($PCC = 0.12$). Consistently regression coefficient for positive forward velocity was low (1.2), but significant (Figure 4.3 B, left panel, bottom arrow). Similarly neuron 1 locomotion velocity $PCC$ was 0.82 and respective regression coefficient was 12.3 (Figure 4.3 B, left panel, top arrow). Our analytical method was therefore able to capture neuronal sensitivity in a meaningful and interpretable way, even when multiple sensory variables were encoded as in neuron 11. At the population level, 60% (of 117) ZI neurons, were tuned to wall distance (Figure 4.3 B-C). Unexpectedly, 85% were tuned to forward locomotion velocity (see Figure 4.3 B-C) and 85% were tuned to lateral locomotion velocity. 60% of ZI neurons were tuned to both locomotion velocity and wall distance (Figure 4.3 C).

A similar pattern was observed also in VPM and PO. 32% (of 106) VPM neurons and 51% (of 208 PO neurons) were tuned to wall distance. However, 79% and 84% were tuned to forward velocity (see Figure 4.3 B-C) and 76% and 81% were tuned to lateral velocity. 32% and 51% were tuned to locomotion velocity and wall distance (Figure 4.3 B and C).
Surprisingly for whisker system regions, no neurons tuned to only wall distance, and not to locomotion, were observed (Figure 4.3 C). We therefore identified two categories of neurons: neurons sensitive to both locomotion and wall distance (32%, 51% and 61% of VPM, PO and ZI neurons respectively; for an example see neuron 11 of Figure 4.3A) and neurons purely sensitive to locomotion (58%, 44% and 37% of VPM, PO and ZI neurons respectively; for an example see neuron 1 of Figure 4.3A).

The effect of a behavioural variable on firing rate could be either an increase or a decrease. We therefore asked whether the effect of running and wall distance was to inhibit or activate each neuron. We used the coefficient value of the positive component of forward velocity as a proxy for running. The percentage of neurons inhibited or activated by running and wall distance was consistent across ZI, VPM and PO: that activated by running was 52%, 57% and 53% respectively; inhibited by running, 24%, 12% and 25%; activated by wall distance, 30%, 21% and 25%; inhibited by wall distance, 30%, 11% and 26% (Figure 4.3 C).

In all the brain regions (hippocampus, hypothalamus, midbrain and non-whisker thalamus; Table 4.1) we recorded from, the majority of neurons encoded lateral or forward velocity (mean ± standard deviation of percentage of neurons which significantly encoded lateral velocity, 78% ± 20% and forward velocity, 88% ± 12% across brain regions), while on average about half neurons encoded wall distance (54 ± 20%, Figure 4.3 C, 4.3 D). On average, 52% ± 14% of neurons were activated by running (see neurons 1 and 11 as examples, Figure 4.3 A and 4.3B), while 27% ± 19% were inhibited. 18% ± 16% were activated by wall distance (see neuron 11 as examples, Figure 4.3A and 4.3B), while 35% ± 20% were inhibited (Figure 4.3 E). On average, half of the neurons in each brain region
showed mixed encoding of both velocity and wall distance (Figure 4.3 D). In summary, we observed an exceptionally widespread encoding of locomotion signal across all the recorded brain regions.

4.3.3. Whisking amplitude encoding is widely distributed in the brain and cannot account for velocity encoding

Previous sections showed a surprising result: sub-cortical whisker system contained many neurons sensitive either to locomotion velocity alone (e.g., Figure 4.3 A) or in combination with wall distance (e.g. Figure 4.3 B). However, when mice start running, they also move their whiskers (Arkley et al., 2014; Sofroniew et al., 2014) and encoding of whisking had been reported in multiple whisker system regions (Leiser and Moxon, 2007; Khatri and Bermejo, 2009; Huber et al., 2012; Petreanu et al., 2012; Moore et al., 2015; Peron et al., 2015b; Urbain et al., 2015; Campagner et al., 2016; Yu et al., 2016). We therefore wondered whether the locomotion velocity tuning observed in VPM, PO and ZI might be an artefact of the correlation between locomotion velocity and whisking. To address this, during two recording sessions, we extracted from whisker video data the time series of whisker angular position (see section 4.5.5.2) during wall absent trials (Figure 4.1 A, left panel and 4.4 A) and we found that PCC between positive component of forward velocity and whisking amplitude was 0.59 and 0.7 in the two whisker tracked sessions We then fitted a multiple, linear regression model as before, with whisking amplitude and locomotion velocity as predictors (see sections 4.5.51 and 4.5.5.3 for details).

If the sensitivity to velocity were a by-product of correlation between whisking amplitude and locomotion velocity, only the coefficients for whisking amplitude should be significant.
However, we found that in PO, ZI and VPM neurons, sensitivity to locomotion velocity persisted even after controlling for whisking amplitude: PO, ZI and VPM exhibited pure locomotion velocity (light brown bar, Figure 4.4 B) neurons, and neurons which encoded both whisking amplitude and locomotion velocity (magenta bar, Figure 4.4 B). As expected for whisker system regions (Moore et al., 2015; Urbain et al., 2015; Yu et al., 2016), pure whisking amplitude neurons were also observed (Figure 4.4 B, green bar). However, as we observed for wall distance, neural encoding of whisking amplitude was not limited to whisker system regions only, but was shared across many brain regions not classically considered part of the whisker system (Figure 4.4 C and D).
Figure 4.3. Encoding of locomotion and wall distance is widespread in the sub-cortical brain

A. Mouse forward velocity (orange), lateral velocity (yellow), wall distance (brown) and simultaneously recorded neuronal activity (black) of two example neurons. For each neuron a concatenated sequence of closed loop – wall present (left), closed loop wall absent (middle) and open loop trials (right) are shown. Note that during the experiment the presentation of closed loop trials with and without walls was temporally intermingled. Titles indicate the brain region in which the neurons were located and the adjusted $R^2$ of the linear regression model fitted with the regression variables indicated in B. Numbers in the titles indicates the ranking of the neurons in B, left panel.

B. Model adjusted $R^2$ and regression coefficients of each sensory variable of the linear regression model based on the two rectified forward velocities (forward velocity; pos., forward velocity; neg.), the two rectified lateral velocities (lateral velocity; pos., lateral velocity; neg.) and wall distance, for all neurons recorded in ZI (left), VPM(middle) and PO (right). For other brain regions adjusted $R^2$'s, see Figure 4.6, top panel. Neurons are sorted according to the adjusted $R^2$, from largest to smallest. Gray rectangles indicated that the respective regression coefficient was not significant at 0.05, after applying Benjamini–Hochberg–Yekutieli procedure for controlling the false discovery rate (section 4.5.5.3). For visual purpose, colour scale of regression coefficient has been truncated at ±16.

C. Fraction of neurons in each recorded brain region (y-axis), which had: no significant coefficients (non sensitive), at least one significant coefficient for lateral velocity (lateral velocity sensitive), at least one significant coefficient for forward velocity (forward velocity sensitive), significant coefficient for wall distance (wall distance sensitive), at least one significant coefficient for velocity but not for wall distance (locomotion only sensitive), only significant coefficient for wall distance (wall distance only sensitive), both locomotion and wall distance sensitive (mixed sensitivity), or which were activated (or inhibited) by running or by wall distance. Coloured rectangles indicate the brain macro-region to which each the brain region was assigned.

D. Mean and standard deviation across brain regions for all the quantities computed in C, left panel. Dots are brain regions.
E. Mean and standard deviation across brain regions for all the quantities computed in C, right panel. Dots are brain regions.
Figure 4.4. Encoding of locomotion velocity cannot be explained by whisking amplitude.

A. Top: Mouse forward velocity (orange), lateral velocity (yellow) and whisking amplitude (green) and simultaneously recorded neuronal activity (black) of an example ZI neuron. Bottom: Regression coefficients of each sensory variable of the linear regression model. Model adjusted $R^2$ is reported in the title of the top panel. Gray rectangles indicated that the regression coefficient was not significant (as in Figure 4.3 B).

B. Fraction of neurons in VPM, ZI and PO, which had no significant coefficients (non sensitive, black), a significant coefficient for whisking amplitude, but not for any of the velocity variables (whisking amplitude only sensitive, green), at least one significant coefficient for lateral or forward velocity, but not for whisking amplitude (velocity only sensitive, light brown), significant coefficient for whisking amplitude and for at least one of the velocity variables (amplitude and velocity sensitive, magenta).

C. Same measures of panel B, for all the neurons recorded during whisker tracked sessions. Coloured rectangles indicate brain macro-regions.

D. Mean and standard deviation of the quantities computed in C across brain regions. Dots indicate individual brain regions.
4.3.4. Strong neural encoding of velocity and wall distance was sparse

Neurons which encoded locomotion velocity or wall distance were well represented in all the brain regions we recorded from (on average only 5% of the neurons per regions were not sensitive to any variable, Figure 4.3D). This observation was surprising since it might suggest that a large proportion of the mouse brain might be devoted to the implementation of a single, although complex, behaviour like whisker-guided locomotion (dense code, Harris and Mrsic-Flogel, 2013). An alternative possibility would be that, although many neurons were recruited during the task, the strength of the coding in most cells was weak (sparse code, Harris and Mrsic-Flogel, 2013), leaving them available to encode also other variables. The linear regression approach used in previous paragraphs provided a direct way of testing between the dense code and the sparse code scenarios, by examining the value of the regression coefficients. We observed that the distribution of lateral, forward and wall distance significant coefficient magnitudes were highly skewed (Figure 4.5A). This indicates that only a minority of neurons were strongly sensitive to the behavioural variables.

The above result also implied that many of the significant coefficients that we observed were small therefore potentially more susceptible to estimation errors and able to explain only a small proportion of the firing rate variance. We then asked whether our observation of widespread sensitivity of locomotion and wall distance was robust if we focused only on those ‘best-fitted neurons’ (BFNs) whose responses were most accurately explained by the regression model based on locomotion velocities and wall distance (top 25th percentile adjusted $R^2$, section 4.5.5.4). We discovered that, in VPM, PO and ZI, all (93 of 93) BFNs
were significantly tuned to locomotion and a large proportion of them (68 of 93) to wall distance (Figure 4.5 B), confirming the findings reported above.

We performed the same analysis for other brain regions by grouping them into macro-regions: hippocampus, other thalamic, other hypothalamic and midbrain (Table 4.1). Our analysis confirmed that, among the BFNs, all the neurons (72 of 72) were tuned to locomotion and a large fraction of them (53 of 72) to wall distance (Figure 4.5 B, top panel). We conclude that the widespread encoding of locomotion and wall distance reported in Figure 4.3 C was present even when we redefined the dataset including only those neurons whose activity was well-explained by wall distance and locomotion.
Figure 4.5. Robustness checks on widespread locomotion and wall distance encoding

A. Distribution of significant regression coefficient magnitudes for forward velocity, lateral velocity and wall distance (note that for lateral and forward velocities only the largest between the positive and negative rectified terms for each neuron was included). Dotted line indicated the median of each distribution.
**B. Top:** Fraction of BFNs (see section 4.5.5.4) sensitive to at least one of rectified velocity component variables (locomotion sensitive) or to wall distance (wall distance sensitive), in each of the brain regions reported in the bottom panel. **Bottom:** Number of BFNs in VPM, PO, ZI and in the macro-regions hippocampus, midbrain, hypothalamus (after excluding ZI) and thalamus (after excluding VPM and PO).

### 4.4 DISCUSSION AND CONCLUSIONS

Thalamic first order and higher order relay nuclei and ZI have been widely studied in anesthetized animals (Waite, 1973; Ito, 1988; Simons and Carvell, 1989; Armstrong-James and Callahan, 1991; Diamond et al., 1992a, 1992b; Pinto et al., 2000; Sherman and Guillery, 2002, 2006; Trageser, 2004, 2006; Lavallee, 2005; Urbain and Deschênes, 2007a; Montemurro et al., 2007; Petersen et al., 2008; Bale and Petersen, 2009; Saalmann and Kastner, 2011) and, more recently, in awake non-locomoting animals (Poulet et al., 2012; Moore et al., 2015; Urbain et al., 2015; Yu et al., 2016) but very little is known about how those regions encode locomotion and sensory information during navigation (Niell and Stryker, 2010; Erisken et al., 2014; Roth et al., 2016). By taking advantage of the novel opportunity offered by Neuropixel probes (Jun et al., 2017; Mora Lopez et al., 2017), we recorded from first order and higher order whisker relays and ZI, plus from 17 other perithalamic sub-cortical regions during whisker-guided navigation. Consistently with previous studies, VPM, PO and ZI responded to whisker related signals during the task (Moore et al., 2015; Urbain et al., 2015). However our recordings revealed novel and unexpected encoding of locomotion, never observed before in the whisker thalamus and ZI. Furthermore, widespread locomotion encoding was not a feature specific to the whisker system: high fractions of neurons sensitive to locomotion were observed in all recorded
brain regions. Finally we observed that whisker related variables were not encoded only in the whisker system, but in most of the other brain regions.

4.4.1 VPM, PO and ZI encode locomotion

Previous recordings from somatosensory thalamus of awake mice identified sensitivity to a whisker motor signal (Poulet et al., 2012; Moore et al., 2015; Urbain et al., 2015; Yu et al., 2016), which persisted after removing the somatosensory input from the whisker pad by infraorbital nerve transection (Poulet et al., 2012). We found that a second type of motor signal (locomotion) was encoded by the vast majority of the neurons in VPM, PO and ZI regions. Our results provide therefore additional evidence for motor encoding in somatosensory thalamus.

The presence of a locomotion signal was particularly surprising in VPM. VPM in fact does not receive, with the exception of a limited portion of dorsal VPM very close to VPM/PO border (Urbain and Deschênes, 2007b), direct projections from motor regions. However, consistent with our findings, locomotion encoding have been reported also in first order visual thalamus (Erisken et al., 2014; Roth et al., 2016).

ZI and PO receive input from motor regions and their activity is modulated by them (Mogenson et al., 1985; Mitrofanis and Mikuletic, 1999; Trageser, 2006; Urbain and Deschênes, 2007a; Urbain et al., 2015; Economo et al., 2016). Consistent with that, we observed encoding of a motor signal (locomotion). One recent study (Roth et al., 2016), reported locomotion encoding also in higher order visual thalamus, while lesion study suggested that ZI might be involved in locomotion (Mogenson et al., 1985).
What could be the function of locomotion encoding in the whisker system? It is still unclear. However interesting insight may come from a comparison with locomotion encoding in the visual system. In vision, locomotion encoding enhances the amplitude of visual responses (Niell and Stryker, 2010), promotes spatial integration over wider regions of the visual field (Ayaz et al., 2013) and allows the computation of mismatch between the optic flow and animal speed (Keller et al., 2012; Roth et al., 2016). New studies will be necessary to elucidate whether similar mechanisms are also implemented by the whisker system.

In general, the presence of locomotion encoding in first order and higher order thalamus indicates that thalamic function is more complex than just relaying sensory information (Acsady, 2017). Increasing evidence is now available that consistently shows that first and higher order sensory thalamus can be modulated by non-sensory signal, besides locomotion, such as attention (O’Connor et al., 2002), motor commands or arousal state (Poulet et al., 2012; Urbain et al., 2015).

4.4.2 Neural basis of widely distributed locomotion velocity encoding

We found that locomotion encoding was not limited to the whisker system, but was also observed in all recorded brain regions including thalamus, midbrain, hippocampus and hypothalamus. These results are consistent with – and extend – previous studies in suggesting that locomotion encoding is extremely widespread. Locomotion encoding has been previously reported in primary somatosensory cortex (Chorev et al., 2016), primary visual cortex (Niell and Stryker, 2010; Ayaz et al., 2013; Bennett et al., 2013; Polack et al., 2013; Saleem et al., 2013; Erisken et al., 2014), primary motor cortex (Schiemann et al., 2015), visual thalamus (Erisken et al., 2014; Roth et al., 2016), hippocampus (McNaughton
et al., 1983; Czurkó et al., 1999; Lee et al., 2014; Kropff et al., 2015; Whalley, 2015), entorhinal cortex (Kropff et al., 2015) and substantia nigra (Howe and Dombeck, 2016).

The source of such signals has not been fully elucidated. In general, two possible coexisting sources of locomotion signal have been proposed: a sensory source and an internally generated source (von Holst, 1954). One type of locomotion signal of sensory source, which may be observed in PO and ZI, but not in VPM, may be the one of non-whisker somatosensory neurons. PO and ZI both contain somatosensory neurons whose receptive fields are located on the paws (Diamond et al., 1992a; Nicolelis et al., 1992), and whose activity could therefore potentially correlate with locomotion. Another locomotion signal of sensory origin may instead be whisker-related. Such signal has been reported in primary somatosensory cortex, where locomotion velocity signals could originate from tactile activity triggered by the contact of E row / trident whiskers with the floor (Chorev et al., 2016), or in primary visual cortex and visual thalamus as an effect of the optic flow (Saleem et al., 2013; Erisken et al., 2014). Might then the locomotion signal we observed in whisker thalamus be of sensory origin? If a neuron were responsive to (E row or trident) whisker-object touch, it may have been modulated by both wall touches and by treadmill touches, if they occurred (Chorev et al., 2016). Treadmill touches would likely have been more frequent and intense when velocity was high, implying that such a neuron might potentially both encode wall distance and animal locomotion velocity. Consistent with this possibility, neurons sensitive to whisker deflection as well as to relative speed between animal and ground have been reported in the trident whisker cortex of anesthetized rats (Chorev et al., 2016). On the other hand, due to the mouse head position in the experimental apparatus and to the anatomical organization of the whisker array, it is unlikely that more than a small minority of whiskers touched the treadmill surface during
the task. Moreover, such a sensory-based explanation does not account for the presence of pure locomotion sensitive neurons. Therefore, the locomotion signal observed in VPM may also be driven by non-sensory sources. The existence of locomotion signals of non-sensory origin has been suggested also in the primary whisker trident cortex, since it is twice as likely to find a speed sensitive neuron in a freely moving rat than in an anesthetized one, whose whiskers are passively deflected by a drifting ground (Chorev et al., 2016). Also, neurons responsive to locomotion velocity have been observed in the absence of sensory information (complete darkness) both in primary visual cortex and thalamus (Saleem et al., 2013; Erisken et al., 2014). This suggests the existence of additional sources of locomotion velocity information than the sensory one.

The second possible mechanism is an internally generated locomotion velocity signal. Motor structures such as motor cortex (Aldes, 1988; Schiemann et al., 2015; Urbain et al., 2015; Economo et al., 2016) or the mesencephalic locomotor region (MLR; Shik et al., 1966; Grillner et al., 2008; Lee et al., 2014; Roseberry et al., 2016) project directly to some of the recorded regions (including PO, ZI, VM, VAL; see Table 4.1) and might transmit a corollary discharge of motor commands to them (Shik ML et al., 1966; Aldes, 1988; Trageser, 2006; Urbain and Deschénes, 2007a; Grillner et al., 2008; Lee et al., 2014; Schiemann et al., 2015; Urbain et al., 2015; Economo et al., 2016; Roseberry et al., 2016). Furthermore, there is evidence of projection from motor cortex and the mesencephalic locomotor region to highly connected structures like the cholinergic basal forebrain (Lee et al., 2014) , ZI (Lin et al., 1997; Mitrofanis and Mikuletic, 1999; Barthó et al., 2002; Trageser, 2006; Urbain and Deschénes, 2007a) and PO (Urbain et al., 2015). In this way, motor signals might also be indirectly distributed to cortex, thalamus, hippocampus and hypothalamus, complementing the widespread direct innervation provided by MLR (Curro
Dossi et al., 1991; Steriade et al., 1991; Lu et al., 1993; Munk et al., 1996; Erişir et al., 1997; Power et al., 1999; Barthó et al., 2002; Jones et al., 2004; Lavallee, 2005; Trageser, 2006; Urbain and Deschénes, 2007a; Pignatelli et al., 2012; Hooks et al., 2013; Roseberry et al., 2016). Consistent with this possibility, a recent study showed that basal forebrain cholinergic axon terminals can modulate activity in primary somatosensory cortex and are activated by motor action (Eggermann et al., 2014).

To summarize, locomotion sensitivity may be distributed to multiple brain regions by highly interconnected structures (such as ZI or basal forebrain) under the control of motor centres like primary motor cortex and the mesencephalic locomotion region or directly by the latter.

4.4.3 Neural basis of widely distributed whisking amplitude and wall distance encoding

In this study, we measured two whisker related variables: wall distance (Sofroniew et al., 2014, 2015) and whisking amplitude (Kleinfeld and Deschénes, 2011). Sensitivity to touch and whisking in awake rodents has been reported before in whisker thalamus (Moore et al., 2015; Urbain et al., 2015; Yu et al., 2016) and ZI (Moore et al., 2015). We observed tuning to whisker-related variables in regions in which it was not expected (e.g., hippocampus, high order visual thalamus, motor thalamus, midbrain and non-ZI hypothalamic nuclei).

Both electrophysiological and anatomical evidence support the hypothesis that long range projections are a mechanism through which neural signals, including somatosensory, motor and visual ones, can be widely distributed in the brain (Huber et al., 2012; Petreanu et al., 2012; Vélez-Fort et al., 2014; Li et al., 2015). Therefore, similarly to what we suggest for locomotion encoding, sensitivity to whisker-related variables may be distributed by widely projecting whisker regions such as ZI (Mitrofanis and Mikuletic, 1999; Power et al., 1999;
4.4.4 Novel and more naturalistic tasks can help to reveal brain function

Activity of neurons in hippocampus, thalamus, midbrain and hypothalamus were never recorded before during the behavioural task studied here. In particular, VPM, PO and ZI were previously studied only in anesthetized or awake, non-locomoting animals. As stated above, we have observed, for the first time, widespread locomotion and tactile encoding. The novelty of our results may be due to the fact that we have studied these brain regions under conditions that are natural, in the sense that the mouse was free to locomote. It has recently been pointed out that the functional properties of even extensively studied brain regions, such as thalamus (Acsady, 2017), may vary according to which behavioural task the animal performs and that the complexity and the ethological plausibility of the behaviour may be critical to unveiling dimensions of neural coding that might be hidden during unnatural behavioural tasks (Olshausen and Field, 2004; Sofroniew et al., 2014; Krakauer et al., 2017). Our study is consistent with this idea since it shows that neural recordings during a task which includes an intrinsic feature of natural behaviour (locomotion), revealed novel coding properties.

4.4.5 Methodological implications of studying awake animals

Studying neural coding during natural behaviour poses new experimental and data analysis challenges. First of all, measuring neuronal activity and behavioural variables in a behaving animal is difficult. However, thanks to technical improvement and novel experimental methods involving head fixation (O’Connor et al., 2010; Sofroniew et al., 2014; Scott et al.,
2017), it is now possible to study awake behaviour under controlled conditions, by studying
an animal during behavioural tasks with high speed videography and/or measuring salient
experimental variables using sensors (O’Connor et al., 2010; Peron et al., 2015b; Sofroniew
et al., 2015; Campagner et al., 2016; Yu et al., 2016; Severson et al., 2017). However, in
contrast to typical anesthetized preparations, experimental variables measured during
awake behaviour, such as, for example, whisking amplitude and locomotion velocity (see
section 4.3.3) can exhibit awkward statistical properties (correlations over both variables
and time). Such correlations make it more difficult to establish the effect of each measured
variable on neuronal activity. It therefore becomes fundamental to use data analysis
methods that guard against artefactual outcomes: namely, a neuron might appear modulated
by a given experimental variable simply because that variable is correlated with the real
driver of the neuron (Campagner et al., 2016).

One way of dealing with such correlation issues is multiple linear regression (Wooldridge,
2012; Martini et al., 2017). However, although this method is in general robust to
correlation among experimental variables (Wooldridge, 2012), it can break down when
such correlation is close to 1 (multicollinearity, Wooldridge, 2012; Fox and Guestrin,
2015). In this case, the estimation error of the regression coefficients increases to the point
that true effects can be missed. In our study, this would translate into an increased
likelihood of neurons appearing untuned to a given variable. The problem is exacerbated
when the number of data samples used to fit the regression model is low. Multicollinearity
has important consequences for the interpretation of the apparent discrepancy between the
fraction of non-sensitive neurons observed in figure 4.3 and 4.4. The linear regression
model fitted in Figure 4.4 used only a subset of the trials (only closed loop - walls absent)
and therefore was more susceptible to multicollinearity. In addition, the whisking amplitude
variable used in the regression is known to be highly correlated with locomotion speed (see section 4.3.3, Arkley et al., 2014; Sofroniew et al., 2014). We observed an increase of non-sensitive neurons in the analysis of Figure 4.4 compare to the one of Figure 4.3. The latter was more robust to multicollinearity due to a larger amount of data and lower correlation between predictors. It is therefore possible that the fraction of amplitude and velocity sensitive neurons reported in Figure 4.3 is underestimated. Future analysis involving more advanced computational approaches such as ridge regression or Bayesian regression could be used to complement the standard linear regression approach, making the analysis more robust to correlation among predictors (Fox and Guestrin, 2015).

4.4.6 Importance of simultaneous recordings in system neuroscience

When comparing two or more brain regions non-simultaneously recorded, apparent differences in neuronal tuning properties can occur as an artefact of differences in experimental conditions. This is a particular issue in studies of awake, behaving animals where correlations between measured behavioural variables might differ substantially across recording sessions. Our study has the virtue of simultaneously recording from at least 8 brain regions distributed in at least 3 macro-regions during each experimental session. Therefore the effect of experimental condition variability was mitigated, permitting rigorous comparison of coding properties across different brain regions. Future recording techniques which will permit simultaneous recording of even a larger number of sub-cortical neurons and brain regions in behaving animals will produce even more robust results.

4.4.7 Large scale recording reveals novel properties of sub-cortical neural coding

Reductionist descriptions (Olshausen and Field, 2004) of brain physiology often assigned
particular functions to specific anatomical brain structures: for example, that primary somatosensory and visual cortices are mainly composed of neurons modulated purely by tactile or visual stimuli respectively.

Novel experimental techniques, like large scale calcium imaging in awake behaving rodents (Peron et al., 2015a), that permit simultaneous recordings of hundreds to thousands of neurons and many behavioural variables, reveal a more complex picture, which challenges the reductionist view. For example, at least half of the neurons of the primary visual cortex do not respond to visual stimulation (Olshausen and Field, 2004; Bonin et al., 2011) and some of the neurons are modulated only by non-visual features (e.g., locomotion velocity, see paragraph 4.4.2). In primary whisker motor cortex, neurons responsive to whisker motion have been observed together with neurons responsive to touch, a sensory variable, and licking (Huber et al., 2012; Petreanu et al., 2012).

By using a large-scale electrophysiological recording techniques (Neuropixel probes; Jun et al., 2017; Mora Lopez et al., 2017), we reported here an analogous situation in the subcortical part of the brain. For example, we observed that regions which are classically associated with the whisker system (VPM, PO and ZI) and therefore expected to contain mainly neurons which respond to sensory variables like whisker touch and/or to whisker motion, contain also neurons purely sensitive to locomotion. in addition, neural coding of whisker related variables (whisking amplitude and wall distance) was not only limited to the whisker system (Diamond et al., 2008), but was observed in most regions measured (Figure 4.3 and 4.4). Neural coding in the sub-cortical brain is therefore heterogeneous within each brain region: both locomotion only sensitive neurons and locomotion and wall distance sensitive neurons were observed in each brain region. However, when we compare
across multiple brain regions, we also observed that the same sensory variables were
encoded by many neurons in many brain regions. Therefore neural coding was, at the same
time, homogeneous across multiple brain regions.

4.4.8 Conclusions

In conclusion, during whisker-guided navigation, there appears to be no neat separation into
pure sensory and pure locomotion regions, but both first order thalamic relay and higher-
order regions are modulated by a combination of tactile sensory and locomotion signals.

4.5 MATERIALS AND METHODS

All procedures were in accordance with protocols approved by the Howard Hughes
Medical Institute Janelia Research Campus Institutional Animal Care and Use Committee.

4.5.1 Surgical procedure and water restriction

Six VGAT – Chr2-EYFP mice (isoflurane, 1–2%) were kept on a thermal blanket (Harvard
Apparatus) to maintain body temperature. We removed the scalp and periosteum over the
dorsal surface of the skull and marked the main sutures, bregma and lambda landmarks
(Paxinos, G, Franklin, 2004) and the coordinate of the edges of the craniotomy. We then
applied a thin layer of cyanoacrylate adhesive (Krazy Glue, Elmer’s Products Inc.) to the
exposed skull. Two metal ground and reference pins connected to micro-wires (platinum–
iridium; A-M Systems #776000) were fixed to the skull using dental acrylic (Lang Dental,
Wheeling, IL), so that the micro-wires could penetrate in the brain through two tiny
 craniotomies. A custom made titanium head-post was fixed to the skull, just posterior to
lambda, using cyanoacrylate adhesive and dental acrylic. The exposed skull was covered
with Kwik-Cast (World Precision Instruments). Buprenorphine HCl (0.1 mg/kg,
intraperitoneal injection; Bedford Laboratories) was used for postoperative analgesia. Ketoprofen (5 mg/kg, subcutaneous injection; Fort Dodge Animal Health) was used at the time of surgery and postoperatively to reduce inflammation. After the surgery, mice were allowed to recover for at least 3 days with free access to water before water restriction (1 ml/day). Mice were water deprived for 3 to 7 days before starting the training.

4.5.2 Whisker-guided navigation task

Head-fixed mice were trained to navigate a tactile virtual reality environment (corridor of 3 cm width) using their whiskers as previously described in Sofroniew et al., 2014, 2015. The behavioural setup consisted of an air-supported spherical treadmill and two motorized walls (microscope slides, Figure 4.1 A), located on both sides of the mouse head. For each recording session we performed two different kinds of experiments. First, a closed loop experiment (Figure 4.1 B and C) in which the wall motion was coupled to the spherical treadmill motion and therefore when mice ran towards a wall, a motor moved the wall closer to the animal; when mice ran away from a wall, a motor moved the wall away. After, an open loop experiment was performed (Figure 4.1 D), in which the walls were moved to a fixed position independently of spherical treadmill motion (Sofroniew et al., 2014, 2015).

During the closed loop experiment, six different types of trial were presented interleaved: straight corridor, 5° and 10° right or left turning corridor or an ‘open field’ (walls were absent). Mice received a water reward, via a computer controlled lick port, if they ran for 90 cm in the forward direction. For those trials in which the walls were present, mice had to be located in the central 1 cm of the corridor, when they reached the 90th cm, in order to receive the water reward. The trial was terminated if mice ran for 100 cm or after 10 second
from the start of the trial. Unless stated to the contrary, the period after water delivery was excluded from subsequent analysis.

Open loop trials lasted 6 s and started with the right wall at 3 cm from the mouse right whisker pad, and consisted of ~1.5 s of the wall moving in towards a fixed distance from the mouse (plateau), where it remained for ~2.4 s and ~1.5 s of the wall moving away from the animal. Before and after the wall moved, wall distance was fixed at 3 cm for 0.3 s. Four different wall distances were used at plateaus: 7 mm, 12 mm, 17 mm and 22 mm. In one mouse, wall distance was instead 5mm, 10mm, 15mm and 20 mm to compensate for head bar medio-lateral position asymmetry. In two mice, whiskers were trimmed to C row before the last recording session.

Mouse behaviour was monitored by recording at 500Hz the following variables: mouse forward and lateral locomotion velocity, lateral distance between the right wall and the mouse right whisker pad (Figure 4.1 A, right panel) and filming the whiskers top-down view by using an infrared sensitive high-speed camera (Mikrotron, MC1362) coupled to a telecentric lens (Edmund Optics, no. 58-257). Mouse velocities was estimated from ball rotation using two cameras containing chips that measure optic flow (Avago Technologies, ADNS-6090; see Sofroniew et al., 2014 for details).

4.5.3 Electrophysiological recording

Mice were trained for about 10-16 days before the first recording session. Mice were anesthetized (isoflurane, 1–2%) and a craniotomy was drilled following the marks made on the skull during the head-bar implant. Craniotomy was covered with 1% agarose gel made
in cortex buffer and Kwik-Cast (World Precision Instruments). Mice were left to recover for at least 2 hours before starting the recording session.

We used novel generation of high density silicon probes (O3 Neuropixel probe, Jun et al., 2017; Mora Lopez et al., 2017) to acutely record from a 3.84 mm track of neural tissue with 384 recording sites (2 recording sites every 20μm). The raw voltage was sampled at 30000 Hz and the signal was band pass filtered (300-5000 Hz). Before starting the recording, the probe was lowered vertically up to a sub-pial depth of ~5 mm. After the recording, the probe was removed, the craniotomy was sealed with agarose gel and Kwik-Cast and the mouse was returned into its cage. For each mouse 1 to 3 penetrations (1 penetration a day) were performed.

4.5.4 Histology

Following the final recording session, mice were deeply anesthetized and then perfused with 0.1 M sodium phosphate buffer followed by 4% paraformaldehyde (in 0.1 M phosphate buffer, pH 7.4). The brain was immersed in fixative for at least 24 h before sectioning. The brain was sectioned in 50 μm - tick coronal slices. In half of the slices cytochrome oxidase staining was performed to reveal anatomical details. Half of the slices were instead left unstained to maintained EYFP - associated fluorescence, which highlighted GABAergic neural structures. Before penetration the probe was painted with DiI in order to reconstruct penetration trajectory. Recording site location was assigned according to Allen Mouse Brain Reference Atlas (© 2015 Allen Institute for Brain Science. Allen Brain Atlas API. Available from: brain-map.org/api/index.html), using a custom Matlab routine. White matter-reach regions like the medial lemniscus, the fields of Forel, the cerebral peduncle and other fibre tracts were excluded from the analysis.
4.5.5 Behavioural and electrophysiological data analysis

Six recording sessions from three separate mice were spike sorted. Only the data from those sessions have been included in the study.

4.5.5.1 Spike sorting

Spikes emitted by a given single unit were detected and clustered automatically by using the automatic spike sorting algorithm Kilosort (Pachitariu et al., 2016) and manually curated using Phy (https://github.com/kwikteam/phy). Recording site arrangement on the probe (Jun et al., 2017; Mora Lopez et al., 2017) permit ‘tetrode’ effect exploitation during spike sorting: the same unit typically appeared on 5-10 recording sites (also compare with Jun et al., 2017). Only units that exhibited a clear absolute refractory period and that fired at least 100 spikes during both closed and open loop protocols were included in subsequent analysis.

4.5.5.2 Whisker tracking

For 2 recording sessions, whisker angle was measured in each video frame of no wall trials using WhiskerMan (Bale et al., 2015). Whisking amplitude was computed by applying the Hilbert transform to the band pass filtered (6 Hz -30Hz) whisker angle time series (Kleinfeld and Deschénes, 2011; Campagner et al., 2016). Whisker angle was defined as in Bale et al. (2015) as the angle between the tangent to the whisker contour at the base and the anterior-posterior axis.
4.5.5.3 Multiple linear regression

In order to quantify the effect of each measured behavioural variable on the firing rate of the neuron, while simultaneously controlling for correlation between variables, we fit linear multiple regression models to the data (Wooldridge, 2012; Martini et al., 2017). In order to match the stride frequency (Sofroniew et al., 2014), we concatenated all closed loop and open loop trials in a given the session and discretized them in 250 ms bins. This generates the time series of the firing rate ($FR$). Similarly, we concatenated behavioural variables time series and subsampled them at 4Hz. We then fit the following linear regression model to the data:

$$FR = \beta_0 + \beta_1 v_{FW;\text{pos}} + \beta_2 v_{FW;\text{neg}} + \beta_3 v_{Lt;\text{pos}} + \beta_4 v_{Lt;\text{neg}} + \beta_5 w + \varepsilon \quad (1)$$

where $v_{FW}$, $v_{Lt}$ and $w$ were the subsampled time series of forward velocity, lateral velocity and wall distance respectively. $Pos$ (or $Neg$) indicated that the variable was rectified by setting to zero values which were less than or greater than zero respectively. This allowed us to capture potential non-monotonic relation between velocity components and firing rate. In order to make regression coefficients for different variables comparable, each variable was standardized by dividing it by its standard deviation. Regression coefficients were then normalized so that a value of 1 corresponded to a 1 Hz variation of the firing rate of the neuron, given a 1 standard deviation variation of the predictor. For wall absent trials, wall distance was set to 40 mm.

Analysis of whisker-tracked trials was performed using a similar approach. Since the analysis included only trials in which the wall was absent, we did not include wall distance variable in the model. Instead, we replaced it with whisking amplitude.
We used as criterion to establish if a neuron encoded or not a given sensory variable, the significance of the p-value of the regression coefficient, corrected for false positives due to multiple comparisons using the procedure of Martini et al., 2017 (Benjamini–Hochberg–Yekutieli procedure, implemented using fdr_bh Matlab function written by David Groppe; Benjamini and Hochberg, 1995; Benjamini and Yekutieli, 2001). The number of comparisons was considered equal to the product of the total number of neurons and the total number of predictors of the multiple regression. The magnitude of the coefficient was used to quantify the strength of the modulation.

4.5.5.4 Best-fitted neuron analysis

Best-fitted neurons (BFNs) were identified by computing the 75th percentile of the adjusted $R^2$ distributions (equal to 0.1), obtained from fitting the locomotion velocities and wall distance based multiple regression model (equation 1) to each neuron. A neuron was classified as BFN if its adjusted $R^2$ was greater than or equal to the 75th percentile.

4.6 SUPPLEMENTARY MATERIAL

<table>
<thead>
<tr>
<th>Brain region acronym</th>
<th>Brain region name</th>
<th>Brain macro-region name</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1</td>
<td>Field CA1</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>CA3</td>
<td>Field CA3</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>DG-mo</td>
<td>Dentate gyrus, molecular layer</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td>Location</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td>----------</td>
</tr>
<tr>
<td><strong>DG-po</strong></td>
<td>Dentate gyrus, polymorph layer</td>
<td>Hippocampus</td>
</tr>
<tr>
<td><strong>DG-sg</strong></td>
<td>Dentate gyrus, granule cell layer</td>
<td>Hippocampus</td>
</tr>
<tr>
<td><strong>LD</strong></td>
<td>Lateral dorsal nucleus of thalamus</td>
<td>Thalamus</td>
</tr>
<tr>
<td><strong>LP</strong></td>
<td>Lateral posterior nucleus of thalamus</td>
<td>Thalamus</td>
</tr>
<tr>
<td><strong>PO</strong></td>
<td>Posterior complex of the thalamus</td>
<td>Thalamus</td>
</tr>
<tr>
<td><strong>VPM</strong></td>
<td>Ventral posteromedial nucleus of the thalamus</td>
<td>Thalamus</td>
</tr>
<tr>
<td><strong>VPLpc</strong></td>
<td>Ventral posterolateral nucleus of the thalamus, parvicellular part</td>
<td>Thalamus</td>
</tr>
<tr>
<td><strong>VAL</strong></td>
<td>Ventral anterior-lateral complex of the thalamus</td>
<td>Thalamus</td>
</tr>
<tr>
<td><strong>VM</strong></td>
<td>Ventral medial nucleus of the thalamus</td>
<td>Thalamus</td>
</tr>
<tr>
<td><strong>SPFp</strong></td>
<td>Subparafascicular nucleus,</td>
<td>Thalamus</td>
</tr>
</tbody>
</table>


Table 4.1

List of brain region recorded in the study. Acronym (first column), brain region name (second column) and macro brain region in which it is located according to Allen Brain Atlas nomenclature (© 2015 Allen Institute for Brain Science. Allen Brain Atlas API. Available from: brain-map.org/api/index.html). Background colours indicate macro-brain region (hippocampus-pink; thalamus-blue; midbrain-purple; hypothalamus-green). In the current study we classified ZI as part of the hypothalamus, consistently with Allen Brain Atlas nomenclature and with (Urbain and Deschênes, 2007a). However, it is worth to report that, according to other nomenclature system, ZI has been classified as a part of the ventral thalamus (Jones, 2012).

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Brain Region Name</th>
<th>Macro Brain Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNC</td>
<td>Substantia nigra, compact part</td>
<td>Midbrain</td>
</tr>
<tr>
<td>SNR</td>
<td>Substantia nigra, reticular part</td>
<td>Midbrain</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
<td>Midbrain</td>
</tr>
<tr>
<td>MRN</td>
<td>Midbrain reticular nucleus</td>
<td>Midbrain</td>
</tr>
<tr>
<td>LHA</td>
<td>Lateral hypothalamic area</td>
<td>Hypothalamus</td>
</tr>
<tr>
<td>ZI</td>
<td>Zona incerta</td>
<td>Hypothalamus</td>
</tr>
<tr>
<td>STN</td>
<td>Subthalamic nucleus</td>
<td>Hypothalamus</td>
</tr>
</tbody>
</table>
Figure 4.6. Summary of linear regression model fitting results and total number of neurons per brain region.

**Top:** Median and inter-quartile range across the neurons of each brain region of the adjusted $R^2$ of the multiple regression model fitted using the following predictor: positive and negative components of forward and lateral velocity and wall distance as predictors. Gray dots indicate single neurons.

**Bottom:** Total number of neurons recorded in each brain region. Colours indicated the brain macro – region (Hippocampus, thalamus, midbrain and hypothalamus).
Figure 4.7. Large scale extracellular recording from VPM, PO and ZI

A. Same as in Figure 4.3 A, but indicating which neurons were located in VPM, PO and ZI.

B. Same as in Figure 4.3 B with a zoomed in panel indicating the respecting location of VPM, PO and ZI.
4.7 REFERENCES


Calford MB (1983) The parcellation of the medial geniculate body of the cat defined by the


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Moore JD, Mercer Lindsay N, Deschenes M, Kleinfeld D (2015) Vibrissa Self-Motion and Touch Are Reliably Encoded along the Same Somatosensory Pathway from Brainstem


5. General Discussion and Conclusions
5.1 GENERAL OVERVIEW

This thesis established a new experimental paradigm applicable to study neural coding of any active sensory system. It includes three main steps: 1) identification of the sensory input to the system during awake behaviour; 2) assessment of the behavioural relevance of the identified sensory input; 3) studying how the sensory input is encoded by central brain structures during natural behaviour. Such procedure has been applied to the whisker system of the mouse.

In Chapter 2 I have elucidated the input to the whisker system during active sensation by identifying the major mechanical drivers of PWAs (primary whisker afferents): the moment at the whisker base. As discussed below (section 5.2), our findings solved a decades-long debate on what constitute the sensory input to the whisker system and unified under a single mechanical framework half century of electrophysiological studies on neural coding in the whisker system of anesthetized and awake rodents. Determining the input to the sensory system is of fundamental importance to understand the functions of the system itself, since the nature of the input constrains the computations of central brain structures (section 5.3).

Having identified the sensory input to the system raises the question how mice exploit it to drive tactile-based behaviours. In Chapter 3, I therefore designed a novel behavioural task (the three-choice object localisation task) and I showed that moment magnitude and direction could account for how the mice solved a whisker-based task.
Having established what drives the PWAs, I asked how downstream neurons respond during complex natural tactile behaviour (whisker-guide navigation, Sofroniew et al., 2014), focusing, in particular, on poorly understood higher-order thalamic nucleus PO (posterior complex), its GABAergic modulator ZI (zona incerta) and the first order thalamic nucleus VPM (ventral posteromedial nucleus). I discovered that in the central part of the whisker system both whisker-mediated touch and non-whisker related variable (like locomotion velocity) were encoded.

In the sections below, I will discuss the main points outlined above and I will illustrate the new challenges that scientists have to face when studying sensory coding in awake behaving rodents. Finally I will summarize the main criticisms to my work and elucidate how they can be tackled and solved in future studies.

5.2 MOMENT ENCODING HYPOTHESIS ACCOUNTS FOR PREVIOUS FINDINGS OF PASSIVE STIMULATION, ARTIFICIAL WHISKING AND AWAKE EXPERIMENTS

My work presented in Chapter 2 and published as Campagner et al. (2016), later corroborated by two other studies (Bush et al., 2016; Severson et al., 2017), has identified moment as the primary driver of PWAs activity. I will discuss below how this discovery has fundamentally changed our view on neural coding in the whisker system and reconciled, under a single mechanical framework, observations on kinematic sensitivity and touch responsiveness reported in previous studies in anesthetised and awake rodents (section 1.4.1). Consistent with the notation of the General Introduction (Chapter 1), I will refer here to bending moment at the whisker base as $M_0$. 
5.2.1 Awake, behaving experiments

As reported in section 1.4.1.3 and in Chapter 2, PWAs activity has been studied in awake rodents while their whiskers interacted with objects or while whisking in air.

5.2.1.1 Whisker-object contact

The major finding from awake animals (Chapter 2) is that PWA firing rate can be predicted, using statistical models, from time series of either $M_0$ or its proxy $\Delta \kappa_0$ (see section 2.3.1, Figure 2.2 and Bush et al., 2016; Severson et al., 2017). There is consistent evidence, from the temporal profile of GLM parameters (Figure 2.8 A) and from prediction accuracy (Severson et al., 2017) for coding of both $M_0$ and its temporal derivative $\dot{M}_0$. The relative importance of $M_0$ and $\dot{M}_0$ for predicting activity varies from neuron to neuron, and is likely to depend on the extent to which rapid bending changes occur during the conditions of a particular recording.

An apparent discrepancy between the findings reported in Chapter 2 and one subsequent study (Bush et al., 2016) concerns how well PWA activity can be predicted from kinematics. In Chapter 2 I reported that mechanical variables predict PWA activity better than kinematic ones, whereas Bush et al (2016) reported no difference in awake rodents. One potential source of discrepancy is that the studies quantified kinematics differently. However, discrepancy remains even when comparing results where kinematics were measured in a similar way (as $\theta_{push}$ - the change in angle of whisker to head during an episode of whisker-object touch, see Figure 2.1 E). Instead, an important factor is likely to be the degree to which mechanical and kinematic variables were correlated in the conditions of the two studies. Bush et al. (their awake recordings) used experimental
conditions where $\theta_{push}$ was tightly coupled to $M_0$. In contrast I decoupled the variables by varying anterior-posterior object location and was therefore able to tease the roles of the variables apart, finding greater predictive power for mechanical variables compared to kinematic ones. Consistent with this account, when Bush et al. (2016) decoupled the variables under anaesthetised conditions, they also found greater predictive power for mechanical variables.

5.2.1.2 Whisking in air

Most PWAs fire at a higher rate during whisking than when the whiskers are still, and some of them, particularly those with high firing rate during whisking, show phase-locked responses (Figure 2.3, Leiser and Moxon, 2007; Severson et al., 2017). What might be the mechanical basis of this activity? Whiskers are controlled by intrinsic and extrinsic muscles (Berg and Kleinfeld, 2003). To make a whisker move, the muscle force must overcome resistance from both the mass of the whisker (inertial force) and the springiness/viscosity of mystacial pad tissue (viscoelastic force, Mitchinson et al., 2004; Lottem and Azouz, 2011; Severson et al., 2017). In principle, any of these forces, alone or in combination, could drive follicular mechanoreceptors. I consider first the inertial force. Due to taper, the 70% or so of a whisker closest to the base is relatively stiff and moves as a rigid object during whisking (Quist et al., 2014). Hence its angular acceleration is expected to be proportional to moment (Quist et al., 2014). The hallmark of a moment-sensitive neuron is therefore that its firing rate should increase with angular whisker acceleration. Consistent with this, the firing rate of some PWAs can be predicted, during free whisking, from angular whisker acceleration (see section and 2.3.2, Figure 2.3 and Severson et al., 2017). Since acceleration varies during the whisking cycle (the precise phase relationship depending on the exact
waveform of the cycle), moment-coding can potentially explain phase-selectivity of PWAs: indeed, for some PWAs, phase-tuning can be predicted from acceleration (Severson et al., 2017). Since it takes more acceleration to move a whisker with greater amplitude and greater frequency, moment-coding can also explain correlation between PWA firing rate and both whisking amplitude (Khatri et al. 2009) and whisking frequency (Leiser and Moxon, 2007). Phase tuning can be predicted with increased accuracy using a multivariate model that includes both acceleration and other kinematic variables as inputs (Severson et al., 2017): this may reflect influence of viscoelastic forces on the mechanoreceptors.

An elegant test of whether PWAs are sensitive to inertial moment is based on the fact that, if the mass of a whisker is changed, the firing rate of an inertia-sensitive neuron, at a given angular acceleration, should change correspondingly (Severson et al., 2017). This prediction was confirmed by progressive trimming of the whiskers in a subset of PWAs. Because of the low mass of whiskers (of order 10-100 micrograms) and, due to taper, the concentration of this mass near the base, moment at the whisker base – as inferred by modelling – is likely to be orders of magnitude smaller than those generated by whisker-object contact (Quist et al., 2014). This suggests that at least some whisker mechanoreceptors are exquisitely sensitive.

However, the response of PWAs to whisking in air cannot be entirely explained by sensitivity to inertial moment. Some PWAs do not change firing rate after whisker trimming (Severson et al., 2017) and the activity of a minority of whisking sensitive PWAs cannot be well-predicted by angular acceleration (see section and 2.3.2 and Figure 2.3). What might be driving the activity of the non-inertia coding PWAs? Severson et al. (2017) found that PWAs, whose firing rates were unaffected by whisker trimming, were phase-
locked to the EMG of either intrinsic or extrinsic muscles. Collectively, these results indicate that activity of PWAs during whisking in air can largely be explained by sensitivity to either inertial moment or muscle contraction force.

In summary, the electrophysiological recordings from awake animals reported in Chapter 2 and subsequent studies (Bush et al., 2016; Severson et al., 2017) indicate that the major driver of PWAs is moment.

5.2.2 Passive stimulation experiments

As explain in section 1.4.1.1, passive stimulation experiments have provided much data concerning the relationship between whisker kinematics and PWA activity, showing that PWAs encodes a combination of amplitude and velocity of deflection (Zucker and Welker, 1969; Gibson and Welker, 1983; Lichtenstein et al., 1990; Shoykhet et al., 2000; Jones et al., 2004; Leiser and Moxon, 2006; Kwegyir-Afful et al., 2008; Bale and Petersen, 2009; Storchi et al., 2012; Bale et al., 2013; Maravall et al., 2013). It has, however, been unclear what mechanical variables drive PWA responses under these conditions. In a typical experiment, the whisker is trimmed to a length of a few mm and the whisker stump is deflected laterally by an actuator. According to the mechanical framework discussed in the General Introduction (section 1.3), the actuator applies a force on the whisker shaft, which exerts a moment that makes the whisker bend. However, in a typical passive stimulation experiment, the whisker appears to rotate as a rigid body; no bending is apparent (at least by eye). One possibility is that the theory makes some assumption that fails to generalise in vivo. However, another possibility is suggested by considering the mechanics of the experiment. Trimming results in a short whisker stump. Since whiskers are tapered, the stump is the thickest, and stiffest, part of a whisker. Equation 1.1 therefore implies that
there may be an appreciable bending moment, but that the associated change in curvature of the stump is slight due to its stiffness. To test between these possibilities, in Chapter 2 (see section 2.3.3) I used high speed imaging to measure how the shape of a whisker stump changes during passive stimulation. I measured small but reliable changes in whisker curvature during stimulation and, by varying the magnitude of the deflection, showed that bending correlates linearly with deflection angle (correlation coefficient ~0.95, Figure 2.4). This confirms that passive deflection does bend a whisker in vivo, as predicted by the mechanical theory. The significance of these findings is the implication that, under typical passive stimulation conditions, there is a simple relationship between kinematic and mechanical whisker variables: whisker deflection angle is proportional to $M_0$. This relationship allows kinematic tuning results from passive stimulation studies to be reinterpreted within the whisker mechanical framework. In fact, if deflection angle is proportional to $M_0$, which also implies that deflection velocity is proportional to $\dot{M}_0$, then PWAs are tuned to the direction of bending within the lateral plane and are sensitive to a weighted sum of $M_0$ and $\dot{M}_0$, as observed during active touch (section 5.2.1). Similarly, a recent study reported that PWA responses to passive deflection of whiskers are well-predicted by the time series of $M_0$ (Bush et al., 2016). In summary, the framework of whisker mechanics can account for many results from passive stimulation by the hypothesis that PWAs encode moment and its temporal derivative.

5.2.3 Artificial whisking experiments

As explained in section 1.4.1.2, artificial whisking experiments reported the presence of PWAs responsive to touch only (with different properties), both whisking and touch and whisking only. How might these artificial whisking data be explained by the whisker
mechanics and moment encoding? Since modelling suggests whisker forces to be substantially weaker during free whisking compared to contact (Quist et al., 2014), some of the diversity in neuron types can be accounted for by the empirical observation that PWAs differ in their response threshold (Zucker and Welker, 1969). Neurons with low deflection thresholds would be expected to respond to both whisking and touch; neurons with higher threshold only to touch. Responses to whisker-object contact can be accounted for by the hypothesis that PWAs encode $M_0$ in the following way (Birdwell et al., 2007). A neuron sensitive to $M_0$ should respond to touch, since touch causes bending. Diversity in touch responses can be accounted for by variation in the extent to which neurons weight $M_0$ compared to $\dot{M}_0$. Neurons with high weighting towards $M_0$ would be expected to respond throughout contact (pressure type). Neurons with high weighting to $\dot{M}_0$ would be expected to respond primarily to contact onset or offset, depending on the directionality of their sensitivity (contact, detach or contact-detach types). Due to the marked variation in stiffness along the shaft of a tapered whisker, the $M_0$ produced by a given contact is much weaker for contact on a distal part of the whisker compared to a proximal part. Thus neurons sensitive to $M_0$ would be expected to fire at a higher rate to proximal compared to distal contacts, as observed by (Szwed et al., 2006). In summary, how PWAs respond to whisker-object contact during artificial whisking can be accounted for in a straightforward way by moment coding. The origin of pure whisking neurons is on the contrary less clear. In fact, although studies of artificial whisking have reported a substantial number of ‘whisking neurons’ (Szwed et al., 2003), studies of awake mice have found neurons sensitive to whisking, but not touch, to be rare (see section 2.3.2 and Severson et al., 2017). The reasons for this discrepancy are unclear, but note that, in artificial whisking studies, a ‘whisking neuron’ is defined as a neuron that is insensitive to weak contact (Knutsen and
Ahissar, 2009; Wallach et al., 2017). In the awake animal, where both weak and strong contacts occur (see for an example Figure 2.4), such a neuron might be classified as sensitive to both whisking and touch. A tentative mechanical explanation for whisking neurons is that they might be responsive not only to muscular contraction (Severson et al., 2017), but also to moment with high threshold (thereby responding only to strong contacts). Finally, to explain the prominent phase modulation in whisking and whisking and touch neurons (Szwed et al., 2003; Wallach et al., 2016) the same argument made for the awake case is valid (sections 5.2.2).

**5.3 TRANSFORMATIONS OF MECHANICAL CODING ACROSS THE ASCENDING SOMATOSENSORY PATHWAY**

Part of the significance of the progress in understanding what PWAs encode, is that it provides a baseline for understanding how downstream circuits transform the ascending drive, and use it as the basis for behaviourally-oriented computations. In this section, therefore, I compare how mechanical signals are encoded in the periphery to how they are encoded in the central part of the whisker system. I will both discuss the existing literature and our findings reported in Chapter 4 concerning moment sensitivity in PO, VPM and ZI during whisker-guided navigation.

In one respect, central encoding is similar to the peripheral one. Studies of both barrel cortex and motor cortex have shown widespread encoding of whisker base curvature – and therefore of $M_0$ (O’Connor et al., 2010b, 2013; Huber et al., 2012; Petreanu et al., 2012; Chen et al., 2013; Yu et al., 2016). Although no study has yet directly tested for $\dot{M}_0$ tuning in cortex, there is indirect evidence. First, layer 4 neurons exhibit a robust, transient response to touch (Hires et al., 2015): since touch involves sudden onset of whisker
bending, and hence high, transient $\dot{M}_0$, this response is likely to correlate better with $\dot{M}_0$ than $M_0$. Second, passive stimulation studies have consistently shown, in anaesthetised animals, that cortical neurons encode the velocity of deflection angle (Pinto et al., 2000; Arabzadeh et al., 2003; Maravall et al., 2007; Estebanez et al., 2012). As discussed above, since deflection angle correlates tightly with whisker bending under passive stimulation conditions (Figure 2.4 and 2.10), this is consistent with cortical encoding of $\dot{M}_0$.

A very similar reasoning can be applied to sub-cortical whisker structure (VPM, PO and ZI), were both responses to touch in the awake animal (Moore et al., 2015; Yu et al., 2016) and response to passive whisker deflection in the anesthetized animal has been observed (Waite, 1973; Simons and Carvell, 1989; Diamond et al., 1992; Urbain and Deschênes, 2007; Petersen et al., 2008; Watson et al., 2015). In chapter 4, I observed that, during a whisker guided wall-following task VPM, PO and ZI neurons encoded the distance between the wall and the mouse snout. Since wall distance inversely correlates with whisker curvature (Sofroniew et al., 2014), and therefore with $M_0$ (Pammer et al., 2013), my results provide further evidence supporting moment encoding in the sub-cortical whisker system.

In another respect, encoding in central and peripheral structures differs. Studies of actively sensing animals demonstrate that whisking elicits robust ‘self-motion’ activity in PWAs, even in the absence of touch (see Chapter 2 and Szwed et al., 2003, 2006; Leiser and Moxon, 2007; Khatri and Bermejo, 2009; Wallach et al., 2016; Severson et al., 2017). This implies that central circuits are faced with an important signal detection problem: how to distinguish spikes generated by contact with external objects from spikes generated by self-motion of the sense organ. Whisking-evoked activity remains prominent in both brainstem
and thalamus (Moore et al., 2015; Urbain et al., 2015; Yu et al., 2016 and also Figure 4.4 in thalamic neurons). However, in barrel cortex, there is a marked transformation, by which the layer 4 response to touch is robust, but that to self-motion is substantially attenuated (Hires et al., 2015). During whisking against a pole, 70% of spikes occur within 40 ms of touch onset; Hires et al., 2015). Yu et al. (2016) have identified circuitry inside layer 4 that accomplishes this transformation. The basic principle, first proposed by Simons and colleagues to account for responses to whisker deflection in anaesthetised rats (Pinto et al., 2003), is that, whereas self-motion elicits relatively asynchronous firing across thalamic neurons, touch elicits a precisely timed volley of synchronous thalamic activity. The thalamic touch response is likely to reflect the strong sensitivity of thalamic neurons to $M_\theta$ (Petersen et al., 2008; Bale et al., 2015). This synchronous volley can activate layer 4 excitatory neurons before the gate is shut by feedforward inhibition (Gutnisky et al., 2017).

Evidence is beginning to emerge for distinct functions of whisker cortical and sub-cortical system beyond transmission of peripheral touch information. I observed a strong non-whisker related signal encoded by the sub-cortical whisker system neurons, which was locomotion velocity (Chapter 4). This was encoded concomitantly with touch signal or alone. The origin of such signal is discussed in sections 4.4.1 and 4.4.2. Locomotion signals are not the only examples of non-whisker related variables encoded in the whisker system. Other examples are reported below. First, in the extragranular layers of the barrel cortex, the touch response is much less distinct than that in layer 4 or in thalamus, suggesting that the activity of layer 5 neurons is strongly influenced by non-sensory inputs (Hires et al., 2015). One important component of this is likely to be of motor origin (Xu et al., 2012). Second, and perhaps related, it has been observed that barrel cortex neurons of mice,
trained to detect single whisker deflections, exhibit non-sensory driven spiking, which correlates with animal choice (Yang et al., 2015). Finally, studies that selectively record from barrel cortex neurons that project to primary motor cortex or secondary somatosensory cortex have reported intriguing evidence for differential representation of touch signals across the two pathways that is task-dependent (Chen et al., 2013).

5.4 BEHAVIOURAL RELEVANCE OF MOMENT

My findings of Chapters 2 and 4, together with the vast body of literature discussed in section 5.2 and 5.3, provided evidence for the presence of neurons in the entire whisker ascending pathways, which are sensitive to moment (both to its magnitude and direction), suggesting that it might be an important mechanical variable underlying touch-based behaviour. Previous behavioural and robotics studies had indicated that extraction of moment would be in fact a useful first step for many tactile computations: large transients in moment could signal object-touch events (O’Connor et al., 2010a), and the magnitude of bending is inversely proportional to the radial distance of contact along the whisker (Solomon and Hartmann, 2011). As explained in chapter 4, bending moment could potentially permit wall following (Sofroniew et al., 2014, 2015) and if integrated across whiskers, could in principle be used both to infer object shape (Solomon and Hartmann, 2006) and to map the spatial structure of the environment (Fox et al., 2012; Pearson et al., 2013).

However, direct investigation of how rodents might use moment to drive behaviour have been lacking, due to the previous difficulty of imaging whiskers and estimating mechanical variables during active touch (see section 1.3). Only recently evidence is starting to emerge. The first examples have been provided by O’Connor et al. (2010a), which suggested that
large transients in moment magnitude at the whisker base could be used by mice as a cue to detect object and by Pammer et al., (2013), where authors concluded that radial object localisation could be performed by mice based on axial force (see Figure 1.2) and bending moment magnitude during whisker-object touch. However, very little is known on the mechanical basis of object localisation along the anterior-posterior axis during behaviour and on the behavioural role played by bending moment direction. One previous study (Bagdasarian et al., 2013) suggested that in the awake rat the change in the global curvature of the whisker (i.e. curvature of a substantial part of the whisker shaft) due to touch with a metal pole inversely correlates with the position of the pole along the anterior-posterior axis. Mathematical modelling suggests that bending moment magnitude and direction should, taken together, provide sufficient cue to uniquely localise object along the anterior-posterior axis (Yang and Hartmann, 2016; Huet et al., 2017). In Chapter 3, I tested this hypothesis and I found that bending moment magnitude and direction could account for mouse behaviour during anterior-posterior object localisation. This discovery links therefore the theoretical prediction with empirical electrophysiological observations of encoding of moment magnitude and direction in the whisker system (see section 5.3), by showing how moment can provide signals to guide mouse behaviour.

5.5 CORRELATION AMONG SENSORY VARIABLES POSES NEW CHALLENGES WHEN STUDYING AWAKE ANIMALS

In any sensory system, a major challenge is to understand the principles of sensory coding in the awake, behaving animal (Hubel, 1959; O’Connor et al., 2010b, 2013; Saleem et al., 2013; Peron et al., 2015; Yu et al., 2016). In this thesis, I therefore extensively used experimental preparations which involved awake animals. When studying active sensory
system in the awake animals, in contrast to the passive stimulation paradigm in anesthetized animals, sensory variables cannot be closely controlled, but they can be measured and statistical models can be used to attempt to infer the relation between one or more sensory variables and neuronal activity (see Chapter 2 for an example). However, an important and widely applicable caveat is that sensory variables measured from awake animals typically have statistical properties that are awkward to the experimenter (correlations over both variables and time). For example, as shown in Figure 2.4, whisker angle correlates with whisker bending during whisker-based object exploration or, as in Figure 4.4, whisking amplitude correlates with locomotion during running. Thus, in a traditional tuning curve analysis, or univariate regression, a neuron that is tuned purely to bending might appear also to be tuned to angle, simply as an artefact of such correlation (Figure 2.4 and 2.12). Multiple regression (used in Chapter 4) and extensions to it, such as GLMs (used in Chapter 2), can handle correlation, provided that it is not too strong. However, beyond a certain correlation strength (‘multicollinearity’), even with these methods, it becomes impossible to tease the influence of different sensory variables apart, and results can be misleading, particularly if the data sample is small (Wooldridge, 2012, and see Figure 2.12 or 4.4 for examples and section 4.4.5 for in depth discussion). It is therefore important, when an experiment which involves the use of awake animals is designed, to take into account strategies to decouple, as much as possible, the sensory/behavioural variables of interest. In Chapter 2, decoupling of whisker angle and curvature has been achieved by varying the spatial location of the object that the animal whisks against. In Chapter 4, I took advantage of the virtual reality environment to decouple the wall distance and mouse locomotion velocities. This was achieved by recording the same neurons during two experimental paradigms, one in which wall motion were coupled to mouse locomotion
velocities (‘closed loop’) and one in which walls were moved independently from mouse locomotion velocities (‘open loop’).

5.6 FUTURE WORKS

Each chapter of my thesis opens new directions of investigation.

In Chapter 2 PWA encoding was studied in mice exploring a smooth metal pole. This sensory stimulus, despite the advantage of making force estimation problem tractable (see section 1.3.1), has the limitation that it samples a fairly limited space of possible types of whisker-objects interactions. In particular, fast events, as slip-sticks (see section 1.3.1), were rare under my experimental conditions. However, there are situations, such as whiskering against textured surfaces, which are characterized by such fast transient movements, and slips-stick events are known to evoke PWA activity (Arabzadeh et al., 2005; Wolfe et al., 2008; Jadhav et al., 2009). A similar caveat applies to most studies on whisker-based somatosensation in the awake animal, including both those of PWAs (Bush et al., 2016; Severson et al., 2017) and those of central regions (O’Connor et al., 2010b; Huber et al., 2012; Petreanu et al., 2012; Xu et al., 2012; Peron et al., 2015; Yu et al., 2016). No study to date has tested the moment encoding hypothesis using stimulus objects with more complex texture/geometry. Future studies should therefore attempt to record from PWAs of mice whisking against textured surfaces, while simultaneously estimating forces at the whisker base. The aim would be to test whether moment encoding hypothesis generalize also to more complex and transient whisker-object interactions or, if instead, under such conditions, other mechanical variables (poorly described by the quasi-static framework) play a prominent role. Such studies would require further development of the existing mechanical modelling framework in order to include frictional effects (see section
1.3.3). There is in fact evidence that including/excluding friction doesn’t affect the results of the mechanical modelling framework if the stimulus is a smooth pole (see section 1.3.3). Friction is therefore ignored by the mechanical framework applied in this thesis. In contrast, friction and more in general adhesion effects are likely to have a prominent mechanical role when whiskers interact with rough surfaces.

Concerning the work reported in Chapter 3, one main limitation is that, due to the complexity of the task and to the water restriction regime, significantly milder than that applied in analogous studies (O’Connor et al., 2010a; Guo et al., 2014b), training duration was long and only five mice learnt the ‘full task’ (Figure 3.2 and 3.7). Low number partially reduced the statistical power of some of the analyses performed in Chapter 3 and limited the feasibility of performing low success-rate experiments such as barrelloid- or barrel-specific electrophysiological recording on trained animals (see below). In the future, it is important to reduce the amount of water per day available to mice. This would increase the animals’ motivation, speed up training and increase the number of trials per day, likely increasing the proportion of mice which will learn the ‘full task’. Interesting target regions for electrophysiological recordings would be the somatosensory thalamus and the barrel cortex, with the aim of studying how moment-sensitive neurons respond during the task, and how moment information is combined at population level to represent pole locations and instruct mouse choice. I would expect to be able to predict, from a population of cortical and/or thalamic moment-sensitive neurons, the pole location and, therefore, most mouse choices during correct trials (see Figure 3.5). What would happen during error trials is intriguing and harder to predict. During error trials, mouse behaviour is poorly predictable from bending moment and best predicted by non-sensory variables such as the choice in the previous trial (see Figure 3.6). In Chapter 3, I hypothesis the existence a
neural mechanism that shifts the choice balance between memory of previous trials and sensory input of the current trial. However, the neural substrate of such a switching mechanism is unknown. I speculate that a top-down signal originating from regions homologous to rat FOF and PPC (see section 3.4.4), may change the coding properties thalamic and cortical whisker neurons leading to a change in behaviour during error trials. Alternatively, FOF or PPC may operate on behavioural output regions such as ALM or VM (Guo et al., 2014a, 2017; Li et al., 2015). A key experiment would be to simultaneously record from whisker system regions or from ALM and VM and simultaneously inactivate FOF or PPC, and test how inactivation affects both recorded neuronal activity and mouse behaviour.

An important limitation of Chapter 4 is that whisker guided navigation in virtual reality may differ from that in a freely moving mouse. Further work is required to test whether the observed widespread sensitivity to locomotion and touch generalizes to freely moving mice, or if it is an artefact of head-fixed tactile virtual reality. An experiment to answer this question would have to solve challenging technical issues such as high speed whisker imaging and tracking (see section 1.3.4) in freely moving mice, as well as fast and accurate measurement of mice locomotion velocity and of the distance of their whisker pad from objects. In addition, differently from what was done in Chapter 4, such an experiment would require chronic electrophysiological recordings. Chronic recording with Neuropixel probes were not feasible when I performed the experiments reported in Chapter 4, due to the fact that the probes had limited availability. However, this could be achievable in the near future.
A second interesting new line of investigation originating from the results of Chapter 4, would be to identify the sources of the widespread locomotion encoding observed (Figure 4.3). In section 4.4.1 and 4.4.3 I identified candidate sources for such signal, including sensory, motor or cholinergic systems. In order to study the contribution of whisker-related sensory input, a new key experiment would be to repeat the recordings of Chapter 4 in mice whose infraorbital nerve (Dorfl, 1985; Poulet et al., 2012; Yu et al., 2016) has been transected. Simultaneous electrophysiological recording and selective optogenetic inactivation of putative sources, while monitoring the effect of inactivation on neuronal activity and mouse behaviour, would help to elucidate, for each recording brain region, the internal sources that contribute to its sensitivity to locomotion.
5.7 REFERENCES


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