Classe di Scienze

Corso di perfezionamento in
Neuroscienze

XXXI ciclo

Early, sustained and broadly-tuned discharge of fast-spiking interneurons in the premotor cortex during action planning

Settore Scientifico Disciplinare **BIO/09**

Candidato
dr. Nadia Giordano

Relatore/i
prof. Matteo Caleo

Supervisore interno
prof. Antonino Cattaneo

Anno accademico 2020/2021

Abstract **3**

1. Introduction **5**

1.1 Voluntary Movement and Preparatory Activity 5

1.2 The Premotor Cortex 7

1.2.1 The Rodent Premotor Cortex 9

1.3 The Rostral Forelimb Area 10

1.3.1 Behavioural Paradigms for Assessing Forelimb Motor Function 12

1.4 The Anterior-Lateral Motor Cortex 13

1.4.1 The Licking Behaviour in Rodents 17

1.4.2 ALM Pathways 19

1.5 The role of Inhibition in the Motor Control 21

1.6 Electrophysiological Approach: Single-Units Recordings 25

1.6.1 Spike sorting 26

1.7 Action Understanding: Mirror Neurons System 27

1.7.1 Searching for Mirror Neurons in Rodents 27

1.7.2 Social Facilitation 29

2. Aim of the project **32**

3. Materials and Methods **34**

3.1 Animals 34

3.2 Surgery Procedure and Animal Preparation 34

3.3 Behavioural Training and Data Analysis 35

3.4 Single-Unit Recording 36

3.5 Single Unit Spike Sorting 36

3.6 Data Analysis 37

3.7 Information Content 39

3.8 Social Facilitation Test 40

3.9 Statistics and data 42

4. Results **43**

4.1 Activity of PNs and FSNs in the ALM during licking 43

4.2 FSNs display less tuned responses during single vs. multiple licks 48

4.3 FSNs show earlier and more sustained activation than PNs during licking 49

4.4 Information content of Firing Rate 53

4.5 Layer-specific responses of PNs and FSNs 56

4.6 Direct comparison of the neuronal responses of PNs and FSNs during two types of movement 57

4.7 FSNs exhibit lower selectivity than PNs for licking behaviour and forelimb retraction 58

4.8 Onset and duration of discharge of PNs and FSNs during licking and forelimb pulling 60

4.9 Modulation of neuronal discharges of PNs and FSNs by nearby licking and pulling events62

4.10 Social Facilitation during Licking Behaviour 63

5. Discussion **68**

5.1 Distinct roles of PNs and FSNs during action planning 68

5.2 Social facilitation and identification of mirror neurons in mice: ongoing experiments .. 73

6. Bibliography **76**

7. Appendix **99**

Abstract

Preparatory neural activity in premotor areas is critical for planning and execution of voluntary movements. Previous studies in monkeys and mice have revealed how the discharges of pyramidal, excitatory neurons (PNs) encode a motor plan for an upcoming movement (Afshar et al., 2011; Chen et al., 2017; Li et al., 2015). However, the contribution of GABAergic interneurons, specifically fast-spiking interneurons (FSNs), to voluntary movements remains poorly understood.

Putative premotor areas involved in action planning have been demonstrated in rodents. In particular, in mice, a premotor area controlling voluntary licking has been identified in the anterior-lateral motor cortex (ALM) (Komiyama et al., 2010). Also, ALM partially overlaps with the rostral forelimb area (RFA), the previously defined premotor region involved in the control of paw movement in rats and mice (Rouiller et al., 1993; Tennant et al., 2011).

To understand the excitatory-inhibitory microcircuit involved in action planning, here I compare directly the response properties of PNs and FSNs during licking behaviour and forelimb retraction in the mouse. Recordings are carried out with both acute electrodes and chronic microelectrode arrays from both the two premotor areas, i.e. the ALM – responsible for licking –, and RFA – involved in paw movement.

Specifically, in a first set of experiments, I used head-restrained mice that spontaneously lick a reward delivered at random intervals from a drinking spout. Mice voluntary performed either single isolated or a burst of consecutive licks, which I categorized, *a posteriori*, in single (= 1 lick) and multiple licks (≥ 3 licks). During the task, I extracellularly recorded single units' activity from ALM, using acute *in vivo* electrophysiology. I identified putative PNs and FSNs, based on well-established features of their waveforms, and investigated their functional properties during the movement.

Unexpectedly, I report that optogenetically-verified FSNs showed an earlier and more sustained activation than PNs. In particular, most of the neurons' activity anticipated the licking onset, consistently with an involvement of the ALM in movement planning. The majority of the neurons (~90%) increased their firing frequency in correspondence with the movement, but suppressive modulations were also observed in a subset of units. For both PNs and FSNs, I found significantly greater discharge during multiple than single licks and the peak discharge was significantly delayed for both subclasses during multiple licking events. However, FSNs modulated their activity about 100ms earlier than PNs. Furthermore, almost all FSNs showed

a peak in their response before the beginning of the sequence of licks. Analysis of mean information content confirms that FSNs predict licking onset not only significantly better, but even earlier, than PNs.

Chronic electrode arrays covering both the ALM and RFA were next used to simultaneously probe neural responses during (i) licking and (ii) forelimb pulling in a robotic device (Spalletti et al., 2017). I report that most of the FSNs respond with a stereotyped increase in their firing rates during both licking and pulling. In stark contrast, PNs show a variety of behaviours, dependent on movement type. At least for a minority of them, licking behaviour and forelimb retraction are represented as two different motor acts, reaching significant levels in the PNs. Accordingly, computational analysis shows that PNs carry more independent information than FSNs.

Altogether, these data indicate that a global rise of GABAergic inhibition mediated by FSNs firing contributes to early action planning.

Next, encouraged by the deeper understanding of the cortical microcircuits underlying movement planning in mice, I exploited this knowledge to explore more complex mechanisms, as action understanding. The neural circuits that integrate performed and observed actions have been found in the premotor cortex of monkeys and named as 'mirror neurons system' (di Pellegrino et al., 1992). Recently, the presence of mirror neurons have been demonstrated in rodents in the anterior cingulate cortex (Carrillo et al., 2019), but whether they could contribute to action understanding in the premotor cortex is still unclear.

At behavioural level, the observation of actions can actually lead, in some cases, to the repetition of those same actions. This phenomenon has been named social facilitation, and the underlying motor program has been attributed to the mirror system (Ferrari et al., 2005).

Here, I set up a behavioural task similar to the one exploited in monkeys to explore social facilitation in mice. I took advantage of licking behaviour to set up the social facilitation experiment. Therefore, head-restrained mice were allowed to lick water from a feeding needle. I found that mice can actually facilitated to lick more when another individual was engaged in the same action, supporting the hypothesis of a social facilitation in mouse.

Altogether these results indicate that the observers' behaviour was actually influenced by the demonstrators' one, laying the groundwork for the study of mirror neurons in mice at cellular level.

1. Introduction

1.1 Voluntary Movement and Preparatory Activity

Voluntary movement is ensured by one of the most elaborate circuitries in our body. It involves the coordinated activity of many different neurons, located both in the cerebral cortex and in the spinal cord. However, the heart of the voluntary movement is essentially in the motor cortex.

The cortical structures most involved in motor control include a primary motor area, M1, a nonprimary motor cortex, the supplementary area, and, lateral to that and rostral to M1, the premotor cortex.

Voluntary movements involve at least two stages, i.e. planning and execution. Specifically, the motor planning of the action involves the decision of what is the action or series of action to perform to fulfil an intention, linking past events to future movements. On the other hand, execution orchestrates actual movement, with central information-processing stages that culminate in the activation of muscles (Flanders et al., 1992; Ghez et al., 1997; Gordon et al., 1994; Requin et al., 1988). Neural correlates of motor planning, referred here as “preparatory activity”, anticipate movement execution and have selectivity for specific movements (such as saccade location, or movement direction of the hand, wrist, or tongue).

Studies of nearly every cortical area, involved in arm movement, have attempted to identify the neural pathways specific to planning or execution. It is widely assumed that early planning stages involve cognitive processes, such as identification of stimulus properties and saliency, and response selection, that are initiated by sensory signals but are not causally related to muscle activation and so are theoretically dissociable from overt motor output (Lecas et al., 1986; Rosenbaum et al., 1983). This provides the rationale for instructed-delay tasks in which a preparatory signal provides information about a desired movement whose execution must be delayed until a subsequent response signal. This allows the subject to plan the signalled attributes of the ensuing movement and consequently reduce the amount of information processing needed after the go signal. These studies show that movement planning and execution are not completely segregated at the level of single neurons or neuronal population in a given cortical area. Some neurons discharge only during the planning phase of the task, whereas others are active mainly during the execution and others show activity changes during both stages (**Figure I1**).

In nonhuman primates, preparatory activity has been detected in the primary motor cortex (Alexander and Crutcher, 1990; Riehle and Requin, 1989; Tanji and Evarts, 1976), the premotor or supplemental motor cortex (Alexander and Crutcher, 1990; Churchland, 2006; Riehle and Requin, 1989), but also in the frontal eye field (FEF) (Bruce and Goldberg, 1985; Hanes and Schall, 1996), the parietal cortex (Gnadt and Andersen, 1988; Maimon and Assad, 2006), the striatum (Alexander and Crutcher, 1990; Ding and Gold, 2010), the superior colliculus (Goldberg and Wurtz, 1972), the motor-related thalamus (Tanaka, 2007), and the cerebellum (Ohmae et al., 2017). Within each of these brain regions, neurons show activity patterns with diverse dynamics. The major difference between cortical areas is whether the predominant neural activity is correlated with planning and execution. The premotor areas typically contain more neurons that are strongly activated during the planning stage (Crammond and Kalaska, 2000).

A subset of these preparatory neurons ramp in anticipation of movement onset (Bruce and Goldberg, 1985; Funahashi et al., 1989; Riehle and Requin, 1989). Indeed, as a neural correlate of motor planning, preparatory activity has to meet three criteria (Riehle and Requin, 1993):

1. Changes in neural spike rate must precede movement initiation;
2. Neural activity must be selective for specific movements, such as saccade location, or movement direction of the hand, wrist or tongue;
3. Details of the neural activity predict aspects of the subsequent movement execution, such as reaction time.

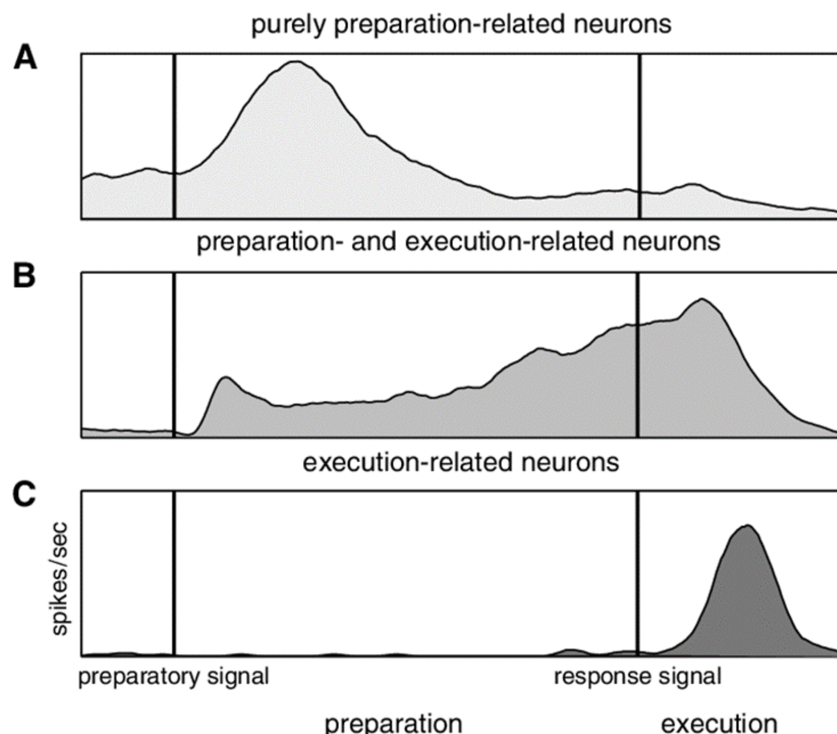

Figure I1. Neural process related to movement preparation and movement execution dissociate in time using a instructed-delay task. A sensory preparatory signal instructs the subject where to perform a movement (preparatory signal, vertical line at left) and when to move (response signal, vertical line at right). The knowledge provided by the first signal permits the subject to plan the upcoming movement. Changes in activity, which occur after the preparatory signal, but before the second signal are presumed to be neuronal correlates of the planning stage. Histograms show the response of three premotor cortex neurons. The activity in **A** appears to be strictly related to the planning phase of the task, as there is no execution-related activity after the response signal in the instructed-delay task. The **B** cell shows a different degree of activity related to both planning and execution; whereas in **C** the activity is limited to the execution of the movement.

1.2 The Premotor Cortex

The premotor cortex has been identified to be the principal core for movement planning, preparation and the sensory guidance of a movement, acting as a conductor to orchestrate the network activity of the rest of the motor modules on a moment-by-moment basis (Churchland, 2006; Godschalk et al., 1985; Guo et al., 2014b; Murakami et al., 2014; Weinrich and Wise, 1982). In contrast, the primary motor cortex is closer to movement execution (Wise and Mauritz, 1985). While primary motor cortex neurons discharge mainly during execution, premotor cortex contains more neurons that are strongly activated during the planning stage.

In 1905, Campbell was the first one to introduce the concept of a premotor cortex, while the first use of the term was proposed by Hines (1929) and adopted later by Fulton and his collaborators (Bucy and Fulton, 1933; Fulton, 1937, 1935, 1932; Jacobsen, 1935; Kennard et al., 1934). However, their "premotor area" contained the supplementary motor cortex, as it was later defined by physiological methods (Penfield, 1951; Woolsey et al., 1952).

The premotor region is located in the frontal cortex, commonly designed as motor field and lacks a clearly defined internal granular layer, layer IV, and thus is referred to as agranular frontal cortex.

Neural activity consistent with motor planning was first recorded in non-human primates (Alexander and Crutcher, 1990; Crutcher and Alexander, 1990). Monkeys were instructed to pull or push a lever, but only after a delayed go cue. A subset of neurons in the primary motor cortex increased their activity seconds before the go cue. This activity was selective for the movement. On error trials (i.e., a wrong movement in response to an instruction), the activity still reflected the future movement rather than the instruction, a key signature of preparatory activity. The upper motor neurons in the premotor cortex influence motor behaviour both through extensive reciprocal connections with the primary motor cortex, and directly via axons that project through the corticobulbar and corticospinal pathways to influence local circuit and lower motor neurons of the brainstem and spinal cord. Indeed, over 30% of the axons in the corticospinal tract arise from neurons in the premotor cortex. In general, a variety of

experiments indicate that the premotor cortex uses information from other cortical regions to select movements appropriate to the context of the action.

Substantial interest in the premotor cortex has focused on motor programming, one aspect of motor set. Roland et colleagues (Roland et al., 1980) have speculated on the basis of their regional cerebral blood flow studies in man that the "premotor areas are activated when a new motor program is established or a previously learned motor program is modulated", i.e. during nonrepetitive voluntary movements, or when a program is "changed on the basis of sensory information, as will be the case during exploratory manipulation of objects" (Rizzolatti et al., 1983, 1981). Based on their studies of macaque monkeys, it has also been suggested a role for premotor cortex in programming ("praxic") functions for complex movements, with subregions of the premotor cortex involved in the organization of specific motor acts, such as bringing food to the mouth by hand.

Functions of the premotor cortex are usually considered in terms of the lateral and medial components of this region. As many as 65% of the neurons in the lateral premotor cortex have responses that are linked in time to the occurrence of movements; as in the primary motor area, many of these cells fire most strongly in association with movements made in a specific direction (Cisek et al., 2003). However, these neurons are especially important in conditional motor tasks. Thus, in contrast to the neurons in the primary motor area, when a monkey is trained to reach in different directions in response to a visual cue, the appropriately tuned lateral premotor neurons begin to fire at the appearance of the cue, well before the monkey receives a signal to actually make the movement (Chen et al., 1995). As the animal learns to associate a new visual cue with the movement, appropriately tuned neurons begin to increase their rate of discharge in the interval between the cue and the onset of the signal to perform the movement. Rather than directly commanding the initiation of a movement, these neurons appear to encode the monkey's intention to perform a particular movement; thus, they seem to be particularly involved in the selection of movements based on external events (Chen et al., 1995; Weinrich and Wise, 1982).

Further evidence that the lateral premotor area is concerned with movement selection comes from studies of the effects of cortical damage on motor behaviour. Lesions in this region severely impair the ability of monkeys to perform visually cued conditional tasks, even though they can still respond to the visual stimulus and can perform the same movement in a different setting. Similarly, patients with frontal lobe damage have difficulty learning to select a particular movement to be performed in response to a visual cue, even though they understand the instructions and can perform the movements. Individuals with lesions in the premotor cortex may also have difficulty performing movements in response to verbal commands (Rizzolatti and Fabbri-Destro, 2009).

The medial premotor cortex, like the lateral area, mediates the selection of movements. However, this region appears to be specialized for initiating movements specified by internal rather than external cues (Chen et al., 1995). In contrast to lesions in the lateral premotor area, removal of the medial premotor area in a monkey reduces the number of self-initiated or “spontaneous” movements the animal makes, whereas the ability to execute movements in response to external cues remains largely intact (Thaler et al., 1995). Imaging studies suggest that this cortical region in humans functions in much the same way. For example, PET scans show that the medial region of the premotor cortex is activated when the subjects perform motor sequences from memory (i.e., without relying on an external instruction) (Sakai et al., 2002). In accord with this evidence, single unit recordings in monkeys indicate that many neurons in the medial premotor cortex begin to discharge one or two seconds before the onset of a self-initiated movement (Baker et al., 1999; Jerjian et al., 2020).

In summary, both the lateral and medial areas of the premotor cortex are intimately involved in selecting a specific movement or sequence of movements from the repertoire of possible movements. The function of the areas differs, however, in the relative contributions of external and internal cues to the selection process.

1.2.1 The Rodent Premotor Cortex

More recently, preparatory activity has been studied in rodents. Although rodent motor behaviours are less sophisticated compared to non-human primates, rodents allow a more comprehensive analysis of neural activity and a wider range of experimental manipulations. In particular, two putative premotor areas have been identified in mice and rats: the rostral forelimb area, mainly involved in the forelimb movement, and the anterior lateral motor cortex, involved in the movement of the mouth/tongue region (Guo et al., 2014b; Komiyama et al., 2010; Rouiller et al., 1993; Tennant et al., 2011).

Evidence for a second, putative premotor area in frontal cortex of rats first came from the report of Neafsey and Sievert (Neafsey and Sievert, 1982), which indicated that a forelimb movement could be evoked from a second cortical region in addition to the forelimb portion of M1. The second forelimb region was rostral to the M1 forelimb region, and clearly separate from it. Neurons in this area project to hind limb regions of the spinal cord, as well as spinal cord and brain stem regions devoted to the forelimb and vibrissae (Li et al., 1990). This second motor region in rats resembles the supplementary motor area (SMA) or premotor cortex (PM) of primates (Neafsey and Sievert, 1982; Rouiller et al., 1993). It has firstly been found within the medial agranular field (AGm) of rats. However, as M1 now appears to include part of AGm, a clear architectonic distinction between M1 and the second motor area has not been made. Other studies in rats indicate that microstimulation of the field results in less muscle activity

and a slower build-up of muscle activity, when measured electromyographically, than when M1 is stimulated (Liang et al., 1993). In addition, there is evidence that the somatotopy of the second motor area is less plastic as forelimb reach training does not result in the same extent of reorganization as is observed in M1 (Kleim et al., 1998).

1.3 The Rostral Forelimb Area

The newly identified area in rats was defined as the rostral forelimb area (RFA) to distinguish it from the caudal forelimb area (CFA). CFA has connections mainly with the ventrolateral thalamic nucleus, and RFA has connections mainly with the ventromedial nucleus. The RFA corresponds to the location where a population of corticospinal neurons is labelled by an injection of tracers into the cervical spinal cord. However, both forelimb motor fields are interconnected, and RFA has more dense interconnections with insular cortex (Rouiller et al., 1993).

Since its first identification in rats, RFA has been extensively investigated in rodent species, specifically, there are suggestive intracortical microstimulation (ICMS) evidence for this motor representation in mice (Li and Waters, 1991; Pronichev and Lenkov, 1998). In particular, smaller forelimb representation has been found at about 2.5 mm anterior to bregma (**Figure I2**). Cytoarchitectural analyses to characterize the mouse motor map reveal that the mouse motor cortex resembles, in its general organization, those found also in other species, such as rats (Donoghue and Wise, 1982), cats (Asanuma and Sakata, 1967), and monkeys (Nudo et al., 1996). Specifically, it has general similarities to the rat motor cortex, including its relative size, location to other movement representations, and, in particular, the existence of the 2 distinct forelimb areas, RFA and CFA (Tennant et al., 2011).

Mice RFA and CFA motor maps comprise a large representation of both digits and the elbow or the wrist movement, even if the first are more represented in CFA and the second in RFA; in RFA movement thresholds of digits were higher than those of wrist and elbow, despite the greater prevalence of digit-responsive sites (Tennant et al., 2011).

The majority of the mouse motor map was found in the agranular field, but it extended into adjacent cytoarchitecturally distinct regions. The medial AG is characterized by a compact layer II and a pale-staining layer III, while the lateral AG is characterized by more homogenous superficial layers and a broad layer V that contains particularly large pyramidal cells. The granular field is characterized by the presence of densely packed granule cells in layer IV (Bates and Killackey, 1984; Donoghue and Wise, 1982; Neafsey et al., 1986). An intermediate zone, named overlap cortex, is characterized, cytoarchitecturally, by the presence of both

densely packed granule cells in layer IV and large, widely spaced layer V pyramidal cells (Brecht et al., 2004).

Specifically, the most prevalent location of forelimb representations was in the lateral AG, however, approximately half of the RFA was in the medial one and approximately half of the CFA in the overlap cortex. Based on cytoarchitectural and ICMS studies of rats (Donoghue and Wise, 1982; Kleim et al., 1998), the RFA has approximately the same placement relative to cytoarchitecture in both rats and mice. Moreover, in rats and in mice – using Nissl-stained coronal sections –, the posterolateral portion of the CFA is known to overlap with the anteromedial portion of the forelimb somatosensory map (Donoghue and Wise, 1982; Hall and Lindholm, 1974). This area is cytoarchitecturally distinct from either M1 or S1 areas, as it contains both a distinct layer IV with densely packed granular cells and layer V characteristics of lateral AG (Donoghue and Wise, 1982; Tennant et al., 2011). This pattern was also described by Caviness (Caviness, 1975) as characteristic of the interface of frontal and parietal cortex in mice.

Beyond the cytoarchitecture, it has been found that the RFA displayed a slightly higher current threshold for evoking limb movements as compared to the CFA (Alia et al., 2016). Moreover, the two homotopic RFAs are connected through excitatory transcallosal transmission, which drives local GABAergic neurons with consequent GABA-B dependent inhibition of cortical neurons (Spalletti et al., 2017).

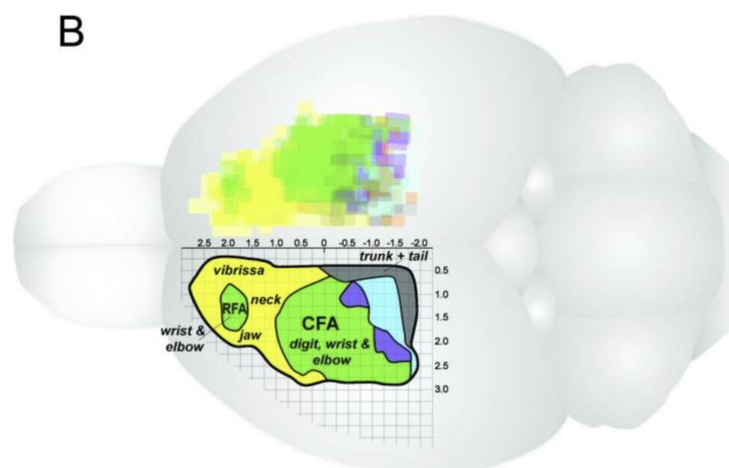

Figure I2. Motor maps of naive animals obtained by the intracortical microstimulation (ICMS) technique following a grid of stimulation sites. A schematic representation of the dorsal surface of the mouse cortex showing movement representation regions simplified by colour coding all forelimb-responsive sites as green and head movement representations as yellow. Transparent overlays of all maps are shown in the right hemisphere (top). The left hemisphere approximates their relative positions and sizes. Numbers are millimetre distances relative to bregma (point 0 on the horizontal axis). Adapted from Tennant et al. (2011).

1.3.1 Behavioural Paradigms for Assessing Forelimb Motor Function

The presence in mice of motor and premotor areas resembling those of primates has shifted the attention on well-controlled behavioural paradigms suited for head-fixed mice that, at the same time, have ethological and translational value.

Joystick or lever tasks emulating human behaviour have been employed in mice, yet most of them constrain the behavioural repertoire to pull or push movements. Specifically, in some experiments a lever press beyond the set threshold during an auditory cue was rewarded with water; the motor cortex is necessary for this task, as lesions before training prevented the emergence of movement and acute inactivation by pharmacology or optogenetics impaired task performance (Peters et al., 2014). A pull lever task has also been used in head-restrained mice; it required the maintenance of grasping, pulling, and holding, which are all forelimb movements, during a certain period of time to receive a water drop (Hira et al., 2013). Moreover, a two-choice forelimb joystick task, in which the motor action was separated from the sensory stimulation by a delay period, has also been implemented in mice. CFA and RFA of trained mice has been demonstrated to be involved during the stimulus delivery for correct choice and during the answer period for motor execution, since their transient silencing with optogenetic inactivation affected different task phases (Morandell and Huber, 2017).

Similarly to primates, rodents perform forelimb skilled movements for manipulating and reaching objects, which share many common traits with those of primates. Indeed, the close resemblance of the reach-to-grasp sequence supports the hypothesis that reaching behaviour is homologous in rodents and primates (Sacrey et al., 2009; Whishaw et al., 1992) and has led to the development of widely used food pellet reaching tasks for rats and mice. Moreover, rodent models of Parkinson's disease, Huntington's disease, and stroke display impairments in reaching performance similar to human patients. Skilled reaching for food pellets has therefore been proposed as a translational tool for investigating neurological diseases (Klein et al., 2012) and is widely used to study motor skill learning (Chen et al., 2014; Harms et al., 2008; Kleim et al., 2004; Xu et al., 2009). Reaching for pellets in mice currently has also been used under head-fixed conditions (Guo et al., 2015; Whishaw et al., 2017).

Forelimb movements in mice can be successfully monitored using a robotic platform (M-platform), designed to train mice during forelimb retraction tasks (Pasquini et al., 2018; Spalletti et al., 2014). The M-platform was exploited for its ability to extract kinetic and kinematic parameters of the retraction movement, in healthy and pathological conditions. Indeed, head-restrained mice can be trained to perform intensive and highly repeatable exercises by retracting their forelimb previously extended by a linear actuator. Forces exerted during the task, time required for task execution (t-target), number of submovements and

attempts can be quantified for each trial. Also, the M-platform allows to perform an isometric task, which provides additional parameters associated with the maximal force the animals is able to generate. This system is combined with advanced techniques, such as optogenetics and *in vivo* electrophysiology, to allow investigation at cellular level underlying the forelimb movements (Pasquini et al., 2018).

Moreover, a directional reaching paradigm (reaching for water droplets) has been proposed in mice (Galiñanes et al., 2018). This task combines the reach-to-grasp movement and the convenience of water-based training in mice, with the multi-directionality and cue-guided principles of the primate tasks. Reaching for water is quickly learned and easily implemented. Head-fixed mice use a chemosensory system to locate water droplets and reaching is strongly affected by motor cortex inactivation. Importantly, layer 2/3 neurons of the motor cortex display direction-selective responses, as shown by calcium imaging in behaving animals (Galiñanes et al., 2018).

1.4 The Anterior-Lateral Motor Cortex

In 2010 Karel Svoboda and his group identified two non-overlapping candidate tongue motor cortical areas controlling licking: the anterior-lateral motor cortex (ALM) and posterior-medial motor cortex (PMM) in mice. Inactivation of either area impaired licking, showing their requirement for voluntary licking (Komiyama T et al., 2010).

These authors described a new procedure for a licking behavioural training in mice. In each trial, mice were exposed to one of several sensory stimuli and had to choose one of two responses based on the sensory stimuli (**Figure I3**). Specifically, mice used their whiskers to detect and locate a vertical pole during a head-fixed condition, that facilitated precise measurements of the dynamics of whiskers and their interactions with objects. The measurement of the locations of object features is a critical aspect of object identification and navigation. The vertical pole was presented in one of two positions (anterior or posterior) during a sample epoch. During a subsequent delay epoch, mice planned the upcoming response. An auditory “go” cue signalled the beginning of the response epoch, and the behavioural choice was signalled by mice touching a water port with their tongue, by licking one of two ports (posterior→ lick right; anterior→ lick left). Mice were water restricted, and thus motivated by thirst. Mice performed many hundreds of behavioural trials per session for water rewards (Guo et al., 2014a).

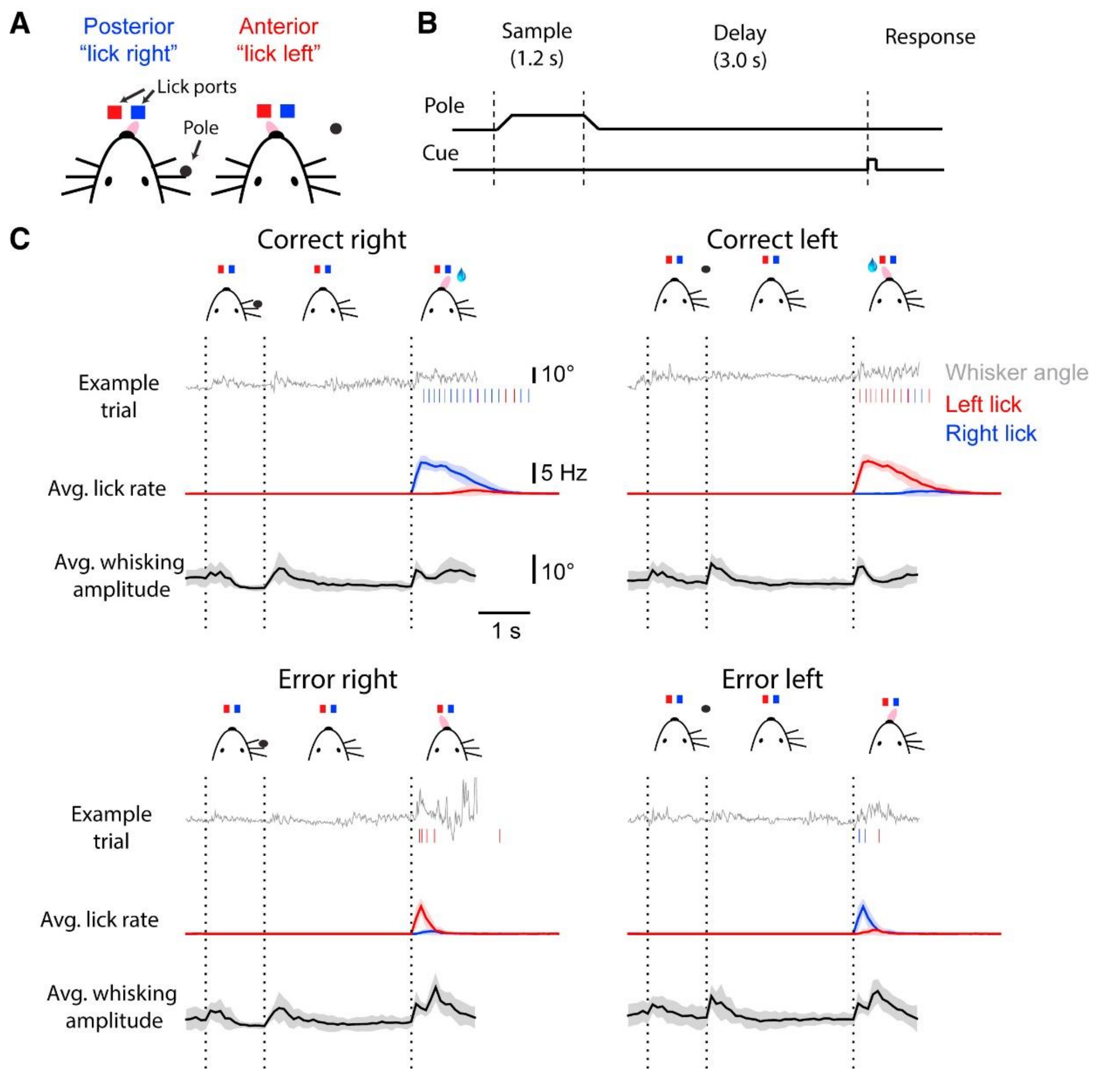

Figure I3. **A**. Mice were trained to lick the right (blue) lickport for the posterior pole location or the left (red) lickport for the anterior pole location. **B**. The pole was within reach of the whiskers during the sample epoch (1.2 s). Mice responded with licking after a delay (3 s) and an auditory cue. Licking the correct port was rewarded with water. Licking the wrong port was neither rewarded nor punished. **C**. Whisker and tongue movements tracked for the four trial types (correct right, CR; correct left, CL; error right, ER; error left, EL). Shaded area indicates standard deviation (SD). Adapted from Chen et al., 2017.

The two identified areas for licking behaviour both project to the brainstem reticular formation, where rhythmic licking is generated (Travers et al., 1997). Moreover, the projections of ALM pyramidal tract neurons overlap with regions of superior colliculus (Rossi et al., 2016) that have been implicated in the control of licking. ALM was first identified as the brain area with the lowest microstimulation threshold for activating rhythmic licking in rats (Travers et al., 1997) and mice (Komiyama et al., 2010), and may play roles in controlling cognitive aspects of tongue movements, similar to the roles of frontal eye fields in the control of eye movements (Bruce and Goldberg, 1985; Funahashi et al., 1989; Schall and Thompson, 1999). ALM also overlaps with RFA, the previously defined premotor region in rats and mice involved in the control of limb movement (Rouiller et al., 1993; Tennant et al., 2011).

ALM contains a large fraction of neurons with preparatory activity (**Figure I4**), predicting upcoming movements (Guo et al., 2014b), similar to primate premotor cortex (Alexander and Crutcher, 1990; Riehle and Requin, 1989; Shenoy et al., 2013; Tanji and Evarts, 1976). These functional data, as well as anatomical studies (Reep et al., 1987; Rouiller et al., 1993), suggest that ALM is homologous to the premotor cortex in non-human primates. Indeed, although ALM is studied in the context of licking, ALM and nearby motor cortical areas also have roles in planning other movements (Erlich et al., 2011; Murakami et al., 2014; Sul et al., 2011).

Simultaneous recordings of multiple neurons have provided a comprehensive description of the population dynamics underlying motor planning. The preparatory activity in ALM has been demonstrated to be able to predict the directional licking (Chen et al., 2017; Guo et al., 2014b); in particular, in head-restrained mice performing the previous described task, in which there is a tactile delayed-response licking (Guo et al., 2014b), using calcium imaging and electrophysiological recordings, there was a large proportion of neurons exhibited preparatory activity with precise directionality. The preparatory activity for licking direction appeared earliest in the ALM and remained localized to ALM until movement onset. A large proportion of ALM neurons showed preparatory activity, especially in layer V, and ALM neurons selective for either movement direction were intermingled in approximately equal proportions in both hemispheres (Erlich et al., 2011; Li et al., 2015).

These results were supported by optogenetic manipulations (Guo et al., 2014b; Li et al., 2015; Svoboda and Li, 2018). Indeed, in mice, ALM was first defined in an optogenetic inactivation screen (Guo et al., 2014b). It has been demonstrated that transient unilateral inactivation of ALM, but not surrounding cortical areas, during motor preparation impaired upcoming licking in the contralateral direction, causing an ipsiversive bias in future licking direction, without impairing licking in general (Guo et al., 2014b); and transient unilateral activation of ALM pyramidal tract neurons had persistent effects on ALM population activity and biases the direction of future licking in the contralateral direction, even in untrained mice (Li et al., 2015).

Moreover, inactivation of ALM biased the future movement only when applied right before movement onset, but was ineffective if it terminated more than several hundred milliseconds earlier (Li et al., 2016), similar to the microstimulation experiments in primates (Churchland and Shenoy, 2007) or to the effects of the spatial contralesional neglect observed after unilateral lesion of the primate premotor cortex (Kerkhoff, 2001; Rizzolatti et al., 1983).

Transient bilateral inactivation of ALM perturbed preparatory activity and randomized licking direction. After inactivation, activity recovered on average, but selectivity did not. Over time, populations of neurons trace out a trajectory in activity space, where each neuron corresponds to a dimension of activity space. These activity trajectories occupy a low-dimensional manifold (Gao and Ganguli, 2015). The detailed structure of the activity trajectories during motor

planning predicts parameters of the future movements (Li et al., 2016; Shenoy et al., 2013). The endpoints of trajectories could reflect optimal "initial conditions" for specific planned movements (Churchland, 2006).

Dynamics of populations of ALM neurons were explored in several types of delayed-response tasks, showing that ALM is required for motor planning independent of the sensory modality. Indeed, ALM neurons showed direction-selective ramping activity for more tasks, involving different sensory modality, auditory or tactile. Although there was heterogeneity in the dynamics across individual ALM neurons, many features of ALM activity were similar across the tasks, and similar to findings in nonhuman primates and rats, including direction selectivity, the properties of ramping activity, the dimensionality of the neural dynamics, and the spike-count correlations across neurons (Inagaki et al., 2018). High spike-count correlations among neurons sharing the same coding preference indicate that such neurons have strong functional connections or receive the same inputs.

The preparatory activity in ALM was not static but evolved with complex dynamics: subsets of neurons showed selective activity during the sample epoch whereas other neurons showed "bumps" of activity during different times of the delay epoch (**Figure I4a**). Despite these fluctuating responses at the level of the single neuron, selectivity for the upcoming movement remained stable at the level of the population (**Figure I4b**). On average, selectivity emerged in the sample epoch and ramped up throughout the delay epoch, reaching a maximum at the beginning of the response epoch (**Figure I4b**). This ramping activity in ALM during the delay epoch is similar to the ramping activity reported in frontal (Hanes and Schall, 1996; Murakami et al., 2014), parietal (Harvey et al., 2012; Maimon and Assad, 2006; Roitman and Shadlen, 2002), and motor cortex (Erlich et al., 2011; Thura and Cisek, 2014), anticipating voluntary movements in primates and rodents. The ALM preparatory activity could provide a substrate for the maintenance of the motor plan during the delay epoch. Information about the upcoming movement is likely coded at the level of the population (Harvey et al., 2012; Laurent, 2002; Murakami and Mainen, 2015; Shenoy et al., 2013).

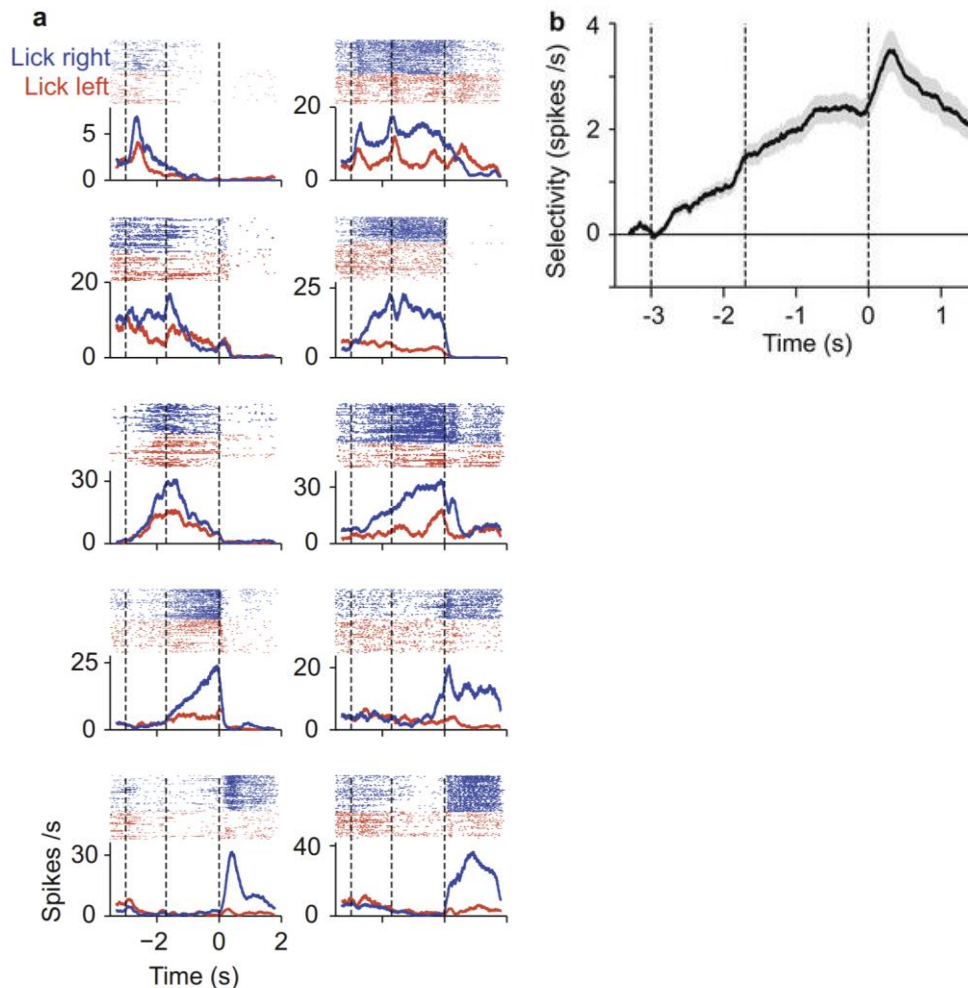

Figure I4. a. Head-fixed tactile decision behaviour and involved cortical regions. (a) Nine examples of ALM neurons. Top, spike raster. Bottom, PSTH. Correct “lick right” (blue) and “lick left” (red) trials only. Dashed lines, behavioural epochs. **b.** Average ALM population selectivity in spike rate (± s.e.m. across neurons, bootstrap). Selectivity is the difference in spike rate between the preferred and non-preferred movements. Time zero is the onset of the go cue. Averaging window, 200 ms. Vertical dotted lines represent the beginning of the sample (whiskers vertical pole localization), delay and response (go signal) epochs. Figure adapted from Guo et al., 2014b.

1.4.1 The Licking Behaviour in Rodents

For many animals the consummatory phase of ingestion is characterized by rhythmic oromotor activity. Functional morphologists have described these motor patterns as an intricate sequence of movements produced by the tongue, jaws and related structures. Variations in the motor sequence of consumption reflect both evolutionarily imposed adaptation of the musculo-skeletal system and the moment to moment adaptation to the changing nature of the ingesta (Thexton, 1992). Thus, the consumption of solid food by chewing requires continuous sensory monitoring to optimally position food between the teeth, resulting in more stages of processing compared to fluid consumption. Fluids do not require mechanical repositioning per se, but like solid food are transferred to the back of the tongue

for swallowing by the cyclic, coordinated action of many of the same muscles used to chew. Thus, the ingestion of fluids by licking appears simpler than mastication, but expresses many of the fundamental components of a common motor pattern used to consume all varieties of food (Thexton, 1992; Zeigler, 1991).

Common to orolingual movements, there are rhythmical alternations between lingual protrudor and retractor muscles, the close functional coupling between lingual and masticator muscles during individual lick cycles, and coordination between licking and other oral motor function, particularly swallowing. Voluntary water-bottle licking (**Figure I5**) is characterized by rhythmic alternations of lingual protrudor and retractor muscles at about 6–8 Hz.

Licking typically occurs in bursts at species-specific modal frequencies, posing the question of the neural origin of this modal frequency. Crucially, the mean number of licks per cluster (lick cluster size) is not random, but instead is lawfully related to the nature of the solution being consumed: lick cluster size shows a positive, monotonic relationship to the concentration of palatable fluids such as sucrose (e.g., Davis & Smith, 1992; Spector, Klumpp, & Kaplan, 1998), while lick cluster size decreases monotonically with increasing concentrations of unpalatable quinine solutions (Hsiao and Fan, 1993; Spector and St. John, 1998).

Although it is atypical for rats to emit a single lick in isolation, they can do so spontaneously but also under specific conditions. For example, rats trained to alternate licking between two water-bottle spouts emit nearly three licks per spout, despite withdrawal of the spout after just one lick cycle, i.e. there is a tendency for one to two extra licks to be directed at the vacant site (Mamedov and Bureš, 1990). If, after training in the alternation procedure, one of the spouts no longer contains water, only one lick is directed at the empty site, i.e. sensory feedback indicating the lack of water inhibits further licks.

There is some evidence that the 6–8 Hz modal licking frequency for rodents is controlled by a central timing mechanism that is somewhat impervious to disturbance. Indeed, repetitive tongue and jaw movements are controlled by a network of brainstem neurons forming a central pattern generator (Travers et al., 1997) located in lateral medullary reticular formation, receiving descending (orbital) cortical input (either directly or indirectly) and having the necessary oromotor connectivity and sensorimotor responsiveness to be potential neurons in a central pattern generator for licking/mastication. (Chen et al., 2001). This central pattern generator is itself modulated by olivocerebellar neuronal firing that is time locked to licking events (Bryant et al., 2010; Cao et al., 2012; Welsh et al., 1995). Moreover, differences in lick rate among different strains of mice have been exploited for mapping quantitative trait loci that are involved in controlling licking behaviour and for identifying the genes that modulate the rhythm generated by the central pattern generators located in the brainstem (Boughter et al., 2012, 2007).

Lick sensors are routinely employed to monitor the licking behaviour. How do they work? Piezoelectric or electrical contact In numerous experiments they are used for counting the number of licks that are emitted, for detecting responses in operant conditioning and producing scheduled consequences. They are valuable tools in the investigation of micro- and macro behavioural aspects of licking (Davis, 1996; Davis and Smith, 1992; Fowler and Mortell, 1992). They can provide a trigger signal in the study of lick-synchronous electrophysiological activity: evoked potentials, single and multi-unit activity, electromyographic activity (Chen et al., 2017; Guo et al., 2014b; Komiyama et al., 2010; Weijnen, 1998; Weijnen et al., 1984). Access to the drinking tube or water surface should be restricted to the tongue. Operation of the lick sensor by paws, nose, jaw, lips or teeth should be made impossible. Each lick needs to be detected separately, so maintained contacts of the tongue with the drinking tube or water surface during two or more licks cannot be allowed. A lick sensor only detects part of the tongue trajectory, around the moment of maximal tongue extension.

Because licking is a highly stereotyped rhythmic motor pattern, it is suitable to the study of motor microcircuits underpinning voluntary movements.

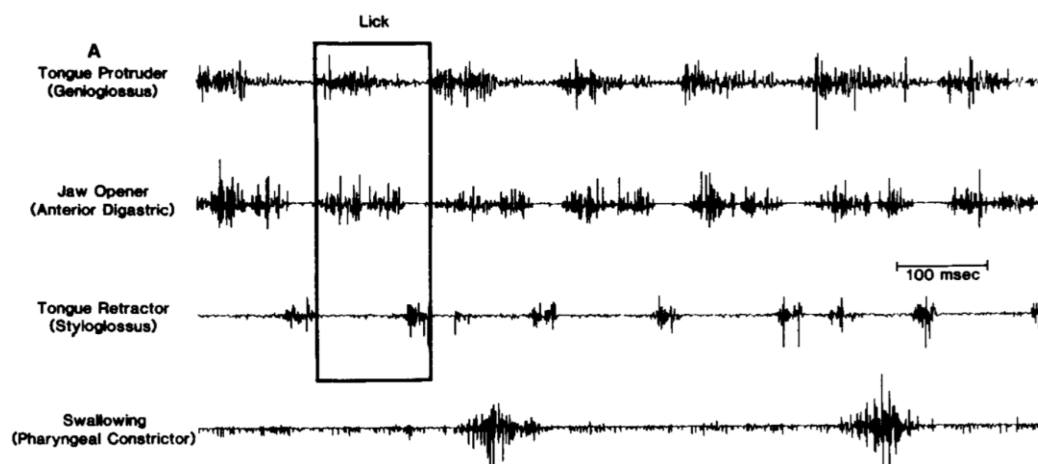

Figure I5. A rat electromyographic activity from four oro-pharyngealmuscles while licking 0.3 M sucrose from a water bottle. There is a rhythmic alternations of muscles at about 6–8 Hz. One of the more salient features of this pattern is the coordinated movement of the jaw with the tongue. In the rectangle a sequence of lingual protruder, jaw opener and tongue retractor is represented. The anterior digastric contraction (jaw-opening) is more or less concomitant with lingual protrusion in voluntary licking. Figure adapted from Travers et al., 1997.

1.4.2 ALM Pathways

ALM could be considered part of a multi-regional loop that produces and maintains preparatory activity (Guo et al., 2017). Indeed, preparatory activity has been observed in structures of the brain that are directly or indirectly coupled to ALM, such as thalamus (Guo et al., 2017; Tanaka, 2007), cerebellum (Ohmae et al., 2013), striatum (Ding and Gold, 2010), and the basal ganglia (Howe et al., 2013).

Diverse patterns of behaviour-related activity, including preparatory activity, seen in motor cortex could be distributed differentially across cell types within a motor cortical area. Evidence for cell-type-specific coding has come from recordings from the two major projection neuron classes in motor cortex (Li et al., 2015; Sommer and Wurtz, 2000; Turner and DeLong, 2000): intratelencephalic (IT) neurons that project to other cortical areas and pyramidal tract (PT) neurons that project out of the cortex, including the superior colliculus, brainstem, and spinal cord (Shepherd, 2013). IT neurons connect to other IT neurons and excite PT neurons, but not viceversa. PT neurons are thus at the output end of the local motor cortex circuit (Brown and Hestrin, 2009; Hooks et al., 2013; Kiritani et al., 2012; Morishima and Kawaguchi, 2006). ALM PT neurons are present specifically in a sublayer of the layer V, named VB; they project to subcortical structures that control facial movements, including the contralateral intermediate nucleus of the reticular formation, which is presynaptic to the hypoglossal nucleus and the intrinsic and extrinsic muscles of the tongue (Komiyama et al., 2010; Stanek et al., 2014; Travers et al., 1997). A comparison of IT and PT neuron activity in ALM suggests that both populations show preparatory activity, but activity consistent with a movement command is specific to PT neurons.

In particular, among PT neurons, there are two types of motor cortex output neurons which have specialized roles in motor control. Both types project to several targets in the basal ganglia and brainstem. Indeed, using single-cell transcriptional profiling and axonal reconstructions, it has been found that one type projects to motor centres in the medulla and mainly produced late preparatory activity and motor commands, and seem to control movement execution. The second type projects to thalamic regions that connect back to motor cortex and avoid motor centres in the medulla; populations of these neurons produced early preparatory activity that persisted until the movement was initiated (Economo et al., 2018). Projecting to the thalamus, they also seem to participate in the maintenance of a persistent neural activity related to motor planning in the ALM that requires direct excitation from the thalamus and vice versa, in a frontal thalamocortical loop. Indeed, similar to ALM neurons, thalamic neurons exhibited selective persistent delay activity that predicted movement direction. Unilateral photoinhibition of delay activity in the thalamus introduce a directional bias towards the ipsilateral side. Photoinhibition of the thalamus caused a short-latency and near-complete collapse of ALM activity. Similarly, photoinhibition of the ALM diminished thalamic activity (Guo et al., 2017).

The segregation of PT neurons into two distinct types persists across the motor cortex and may generalize to other cortical areas (Hattox and Nelson, 2007) and other mammals (Catsman-Berrevoets and Kuypers, 1981; Steriade et al., 1977). Consistent with roles in both

the planning and initiation of movement, PT neurons seem to be structurally heterogeneous (Guo et al., 2017; Kita and Kita, 2012; Wise and Jones, 1977).

The preparatory activity in ALM has been observed across all cortical layers, however, the activity related to future licking direction was first detected in deep layers of the ALM, where PT and IT neurons were intermingled. PT neurons appeared to display stronger and earlier lick direction selectivity than IT neurons (Li et al., 2015). Specifically, cellular imaging in transgenic mice expressing the protein calcium indicator GCaMP6s (Chen et al., 2013; Dana et al., 2014) has allowed to measure the encoding of behaviour-related variables in tens of thousands of neurons distributed across the motor cortex. Neurons showing preparatory activity selective for upcoming licking directions emerged first in the deep layers of ALM. Preparatory activity was largely confined to ALM in the epoch before the lick event and spread to superficial ALM layers and other parts of motor cortex at the time of movement (Chen et al., 2017).

However, several key questions remain unsolved. What circuit mechanisms are responsible for the maintenance of motor plan? How do distinct neuronal classes contribute to this process? In particular, the role of different classes of GABAergic inhibitory neurons, which represent approx. 10-20% of cortical neurons, remain enigmatic.

1.5 The role of Inhibition in the Motor Control

A key challenge in systems neuroscience is to link intracortical mechanism underlying motor-command generation to behaviour. An important role in the generation and shaping of voluntary movement has been attributed to inhibitory circuits.

Inhibitory interneurons constitute a small but crucial neuronal class in the cortex. While these cells comprise only 10%–20% of the total neural population, their connectivity and recruitment are essential in sensation, movement and cognition (Meyer et al., 2011; Ren et al., 1992). One difficulty in synthesizing the role of inhibitory cells lies in their diversity: these neurons express an array of molecular markers and have heterogeneous firing properties as well as distinct synaptic connectivity (Kubota, 2014; Lourenço et al., 2020). However, the diversity of inhibitory neurons allows these cells to provide the appropriate inhibition for a wide variety of stimuli and behaviours. Great strides have been made in identifying clusters of inhibitory interneuron groups based on their varying gene expression (Cauli et al., 2000; Kubota et al., 2011; Paul et al., 2017; Tasic et al., 2016).

In the primary motor and premotor cortex, diverse subtypes of interneurons may play a role in planning and execution of voluntary movement. A major population of nonpyramidal, inhibitory

GABAergic interneurons consists of fast-spiking interneurons (mostly basket cells and chandelier cells), which fire narrow action potentials at a fast rate and usually express parvalbumin (**Figure I6**) (Cauli et al., 1997).

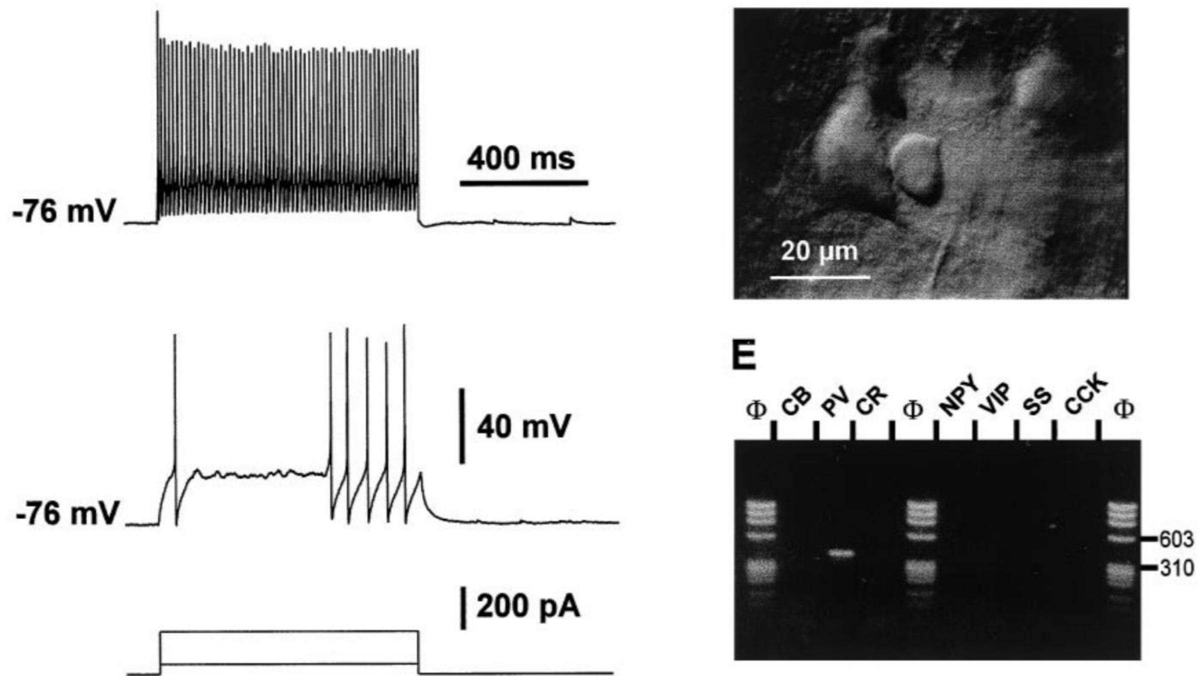

Figure I6. Electrophysiological and biochemical characterization of a layer V fast spiking (FS) cell. Current-clamp recording during injection of depolarizing current pulses. Membrane potential was adjusted to -76 mV by continuous current injection, as indicated on the left of each recording. In response to a near-threshold current pulse (50 pA; bottom trace), this FS cell emitted a single fast action potential with a large AHP followed by a silent period and a late discharge of action potentials. Note the membrane potential oscillations during the silent period. Application of a larger depolarizing current (200 pA; top trace) induced a continuous discharge at high frequency. On the right, a picture of the FS cell. The FS cell is located at the center and has a small round soma (diameter, 10 µm). Note the large layer V pyramidal cell immediately on the left. On the bottom right, agarose gel analysis of the RT-mPCR products of the same FS cell. Figure adapted from Cauli et al., 1997.

Parvalbumin-expressing (PV+) GABAergic interneurons might gate spike outputs from the pyramidal cells with powerful inhibitory synapses on somata or axons (Markram et al., 2004). Indeed, with their extensive somatic targeting of neighbouring excitatory glutamatergic pyramidal neurons (Hu et al., 2014), they are ideally placed to inhibit the activity of M1 output neurons (van brederode et al., 1991) that target downstream motor centres. In mouse sensory and prefrontal cortex, genetically identified GABAergic neurons have been shown to have distinct roles in information processing and discrete firing dynamics (Fu et al., 2014; Gentet et al., 2012; Isaacson and Scanziani, 2011; Kepecs and Fishell, 2014; Kvitsiani et al., 2013; Lagler et al., 2016; Lee et al., 2013, 2012; Pi et al., 2013; Pinto and Dan, 2015; Polack et al., 2013; Sachidhanandam et al., 2016).

In general, inhibitory interneurons are more broadly tuned than pyramidal cells and tend to respond more strongly to inputs throughout many cortical areas. In the sensory system, these increased response properties facilitate feedforward inhibition in response to thalamo-cortical input. Across primary visual cortex (V1) (Gur et al., 1999; Mitchell et al., 2007; Shapley et al., 2003; Swadlow, 1988; Swadlow and Weyand, 1987), somatosensory cortex (S1) (Barthó et al., 2004a; McCormick et al., 1985; Simons, 1978; Swadlow, 1989, 2003), and the primary auditory cortex (Atencio and Schreiner, 2008), for example, fast spiking cells show broader tuning curves and higher response to stimuli and to thalamic stimulation than pyramidal cells. Hence, the inhibition-mediated sculpting of excitatory cortical input is thought to be a key feature of perceptual processing. Furthermore, similar FSNs properties have been found in the inferior temporal cortex (Tamura et al., 2004), the frontal eye field (Cohen et al., 2009), but also in the primary motor cortex (Isomura et al., 2009; Merchant et al., 2008; Murray and Keller, 2011), dorsal premotor cortex (Kaufman et al., 2010), and posterior parietal cortex (Yokoi and Komatsu, 2010).

As in sensory cortices (Lee et al., 2012; Wilson et al., 2012), motor cortex PV+ neurons have been proposed to be a broadly tuned cell type that modulates the tuning properties of adjacent pyramidal neurons (Merchant et al., 2008). The finding that interneurons are more strongly modulated across conditions than pyramidal neurons is consistent with their larger dynamic range (Connors and Gutnick, 1990). More specifically, in rat motor cortex, it has been found that the majority of identified fast-spiking interneurons participate in an on-going modulation of command-like activity of pyramidal cells during the execution of a single voluntary movement (Isomura et al., 2009). It has also been demonstrated that the activity of fast-spiking interneurons was less specific to movement direction than that of pyramidal neurons and was independent of the preferred direction of neighbouring pyramidal neurons (Isomura et al., 2009).

Experimentally induced inhibition of rodent motor cortex typically reduces the ability to perform movements (Guo et al., 2015; Otchy et al., 2015; Sreenivasan et al., 2016), one straightforward interpretation being that the function of motor cortex inhibitory neurons is to suppress voluntary movements. However, the role of fast spiking neurons seems to cover a more complex pattern. Indeed, motor cortex PV+ neurons may also act as an “inhibitory gate” preventing premature movements during preparatory phases with high firing rates that drop transiently to release a correctly timed reach. However, an increase in the amplitude of movements and in the number of false positive movements has also been observed after inhibition of the motor cortex (Ebbesen et al., 2017; Huber et al., 2012; Zagha et al., 2015). Furthermore, electrophysiological recordings suggesting an increase rather than a decrease in firing of fast-spiking cells during movement (Isomura et al., 2009; Kaufman et al., 2013). Another suggestion

is that PV+ neurons track firing rate changes in putative excitatory pyramidal units to maintain a balance between cortical synaptic excitation and inhibition during behaviour (Isomura et al., 2009). The functional significance of the rise in inhibition during movement could be that once the incoming and local excitation rises, the relative rise in internal inhibition may serve to maintain a balance of excitation and inhibition (Shadlen and Newsome, 1998; van Vreeswijk and Sompolinsky, 1996). While task design and off-target effects (Otchy et al., 2015) may play a role on the impact of experimentally altered motor cortex inhibition, another possibility is that different subtypes of GABAergic neurons have distinct functional roles in motor control.

More surprising was the finding that putative interneurons are more likely to have firing rates that rise during movement planning, while putative pyramidal neurons showed firing rate changes only later. This is perhaps remarkable - one usually supposes that overall activity goes up during motor planning - yet the results indicated the average rate of a population of pyramidal neurons rises only slightly. In Merchant et al 2008, an analysis of onset response latencies showed that despite the large overlap in the onset response distributions, the FSNs activity occurred before pyramidal neurons directional responses (Merchant et al., 2008). Although, the onset response analysis is an indirect measure of functional relationships between cell types, these results suggest a potential functional link between FSNs and pyramidal neurons. Moreover, in layer V of mouse primary forelimb motor cortex, GABAergic PV+ neurons rapidly increased their firing rate to stimuli and are positively correlated to the amplitude of reaching. Fast spiking neurons fired with shorter latency than pyramidal neurons at reaching onset and have high rates throughout sensory-triggered and spontaneous reaches. Thus, an early activation of PV+ neurons may play a critical role in the release of reaching (**Figure I5**) (Estebanez et al., 2017).

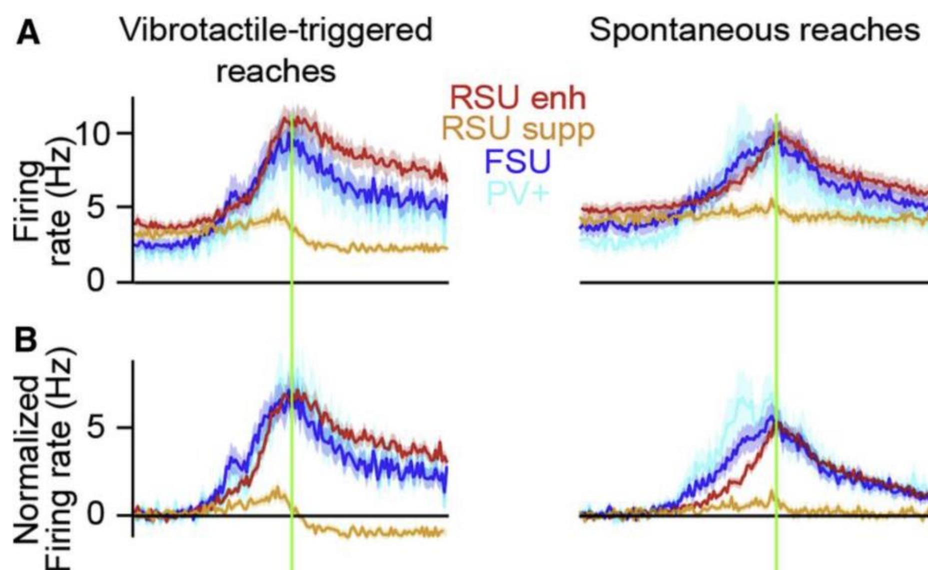

Figure I5. A. Average firing rate of all PV+ (cyan), fast spiking neurons (FSUs, blue), and pyramidal neurons (RSUs enh) with increased activity after reach onset (red, Z score > 0), and pyramidal neurons (RSUs supp) with decreased activity (orange, Z score < 0), aligned to (left) vibrotactile-triggered and (right) spontaneous reach onset. The background represents SEM. **B.** Same as (**a**), but firing rates have been normalized by subtraction of baseline firing −500 ms to −250 ms before reach. Figure adapted from Estebanez et al., 2017.

Therefore, broad and balanced inhibition might work effectively at the surround as well as the center of the tuning curve, presumably to sharpen the feature specificity of pyramidal cells through the so-called iceberg effect, across many areas and behavioural functions (Isaacson and Scanziani, 2011; Wehr and Zador, 2003).

However, it is still unclear whether and how, during action initiation in the premotor cortex - the first node in the downstream pathway to motor neurons, pyramidal and fast-spiking, GABAergic neurons, differently contribute into representing movement or distinguishing specific motor tasks. In particular, little is known regarding how different representations of movements are encoded by PNs and FSNs for building the motor command.

1.6 Electrophysiological Approach: Single-Units Recordings

To investigate the role of distinct neuronal classes, an electrophysiological approach is often suited to that purpose. Electrophysiological recordings from behaving animals provide, indeed, an important view into the functional role of individual neurons. These recordings have yielded fundamental insights into the cellular and circuit mechanisms underlying neural activity during natural behaviours in such areas as sensory perception, motor sequence generation, and spatial navigation, forging a direct link between cellular and systems neuroscience.

The behavioural spectrum of head-restrained mice is limited, but, as mentioned above, the animals will perform spontaneous orofacial behaviours such as whisker movements (Ferezou et al., 2007) and rhythmic fluid licking (Hayar et al., 2006). These behaviours also yield a large number of repetitions, which is an important prerequisite for electrophysiological investigations of the underlying neuronal processes. Head fixation allows the investigator to exert fine control over stimulus presentation, collect high-quality extracellular single-unit recordings, and precisely measure behaviours.

Specifically, *in* vivo single-unit electrophysiological recording techniques provide a unique and powerful window through which to observe the functioning brain. Single-unit recording involves sampling the activity of single neurons, or small clusters of neurons, using a multichannel acute electrode or an array of microelectrodes chronically implanted in the brain. When recordings are conducted during the performance of tasks that engage observable sensory or behavioural

processes, the contribution of the sampled cells to processing task-relevant information can be evaluated.

1.6.1 Spike sorting

Advances in our understanding of how populations of neurons represent and process information has been enabled by tightly packed multi-electrode arrays that allow for isolation of large numbers of simultaneously recorded neurons (Csicsvari et al., 2003; Gray et al., 1995; McNaughton et al., 1983; Mora Lopez et al., 2017). Data collected from these devices comprise multiple channels of continuously sampled extracellular voltages. A key step in making these data interpretable is spike sorting, the process of detecting spiking events and assigning those events to single units corresponding to putative individual neurons (Einevoll et al., 2012; Harris et al., 2016; Lewicki, 1998; Muller, 1996; Quiroga, 2012).

Spike sorting is typically done using dedicated software (e.g., Offline Sorter from Plexon, Boss from Blackrock to name a few). There are three stages between the recording of extracellular unit activity and the identification of spikes representing the activity of a single neuron. The first stage is spike detection, in which the electrical activity measured on the electrodes is used to derive the times corresponding to extracellular spikes. This is usually achieved by high-pass filtering followed by thresholding and may be done by hardware or software. The second stage is feature extraction. During this stage, a feature vector (i.e., an array of quantitative parameters) is calculated for every spike. More advanced methods quantify additional information about the spikes, such as waveshape and discharge pattern. Wave shapes may be quantified by measuring parameters such as spike width, or by principal component analysis (Abeles and Goldstein, 1977). The third stage is “clustering” of spikes. In this stage spikes with similar feature vectors are grouped into clusters, assumed to represent the spikes of a single neuron. The majority of spike sorting algorithms comprise the sequence of steps such as bandpass filtering, spatial whitening, detection of threshold-crossing events, and clustering based on voltage waveform shape in a suitable feature space (such as amplitude on channel 1, peak-to-trough ratio on channel 3, etc.), computed from filtered waveforms (Einevoll et al., 2012; Lewicki, 1998; Marre et al., 2012; Quiroga, 2012). Clustering is automated, but the user must curate the results by selecting which clusters to reject, merge, or even split (Hill et al., 2011; Kadir et al., 2014; Rossant et al., 2016). There also exist post-processing steps that resolve overlapping spikes (Ekanadham et al., 2014; Franke et al., 2015; Pachitariu et al., 2016; Pillow et al., 2013).

A deep understanding of the microcircuits underlying movement planning, using animal models, can lay the foundations for understanding mechanisms of motor control and how they could be modified in pathological conditions.

1.7 Action Understanding: Mirror Neurons System

About 30 years ago, the group of Giacomo Rizzolatti in Parma reported findings on macaques showing the existence of a "surprising new class" of neurons in a particular region of the premotor cortex (F5) of the macaque brain (**Figure I6**) (di Pellegrino et al., 1992). These neurons were active not only when the macaque performed an action – like grabbing an object – but also when the macaque watched the same action being performed by a person or another monkey. Researchers named these neurons as "mirror neurons" and proposed that they provide the basis for what became known as "action understanding", allowing a subject to interpret the intentions or goals of the person whose actions they are observing. They were first characterized in premotor cortex (di Pellegrino et al., 1992; Gallese et al., 1996) then in posterior parietal cortex (PPC) (Fogassi, 2005; Fogassi and Luppino, 2005) in monkeys. Later, these neural circuits, that integrate performed and observed actions, have been found in different species of primates, also humans (Mukamel et al., 2010) and songbirds (Prather et al., 2008).

Based on these properties, mirror neurons have been postulated to enable specific social functions, ranging from selecting appropriate actions in response to observed behaviours (di Pellegrino et al., 1992) to understanding the intentions and imitating the actions of others (Iacoboni, 2009; Rizzolatti et al., 2001). After years of investigation, however, it is still debated whether mirror cells are at the basis of action understanding or if their physiological properties can be better explained by simple, temporally contingent sensory-motor associations (Hickok, 2009). It is also debated whether specific alterations of the mirror neuron system might be at the basis of neurological alterations in brain disorders, such as autism spectrum disorders (Cattaneo et al., 2007; Fabbri-Destro et al., 2009). It is also unclear how the mirror neuron system develops and how this development is related to early experiences. Are mirror neurons the result of sensorimotor learning processes or genetic prewiring? Finding mechanistic resolutions to these questions would benefit tremendously if it will be possible to access cellular networks underlying action understanding in a genetically tractable animal model.

Therefore, a major challenge is to establish whether mirror-like neurons occur in specific areas of the rodent brain.

1.7.1 Searching for Mirror Neurons in Rodents

The first attempt has been made in 2013. Ushakov et al (2013) employed functional mapping of brain activity by using the expression of induced transcription factor c-Fos to determine the spatial localization of the mirror neurons systems in mice of the C57/BL6 line.

This method has been applied in mice performing the supervision task of swimming mice-demonstrators in Morris water maze. The experimental setup is a standard Morris water maze filled with clear water and mice are not inherent in swimming (although they can do it), so this is the expected motivation in mice in the monitoring of the floating-mouse-demonstrator. The behavioural test was aimed to verify the hypothesis that mice sitting in boxes located in the corners of the Morris water maze observe the mice freely swimming in the pool. They showed the presence of c-Fos positive, putative mirror neurons systems, in the motor and premotor cortices, cingular cortex and hippocampus of mice having direct experience of the swimming (Ushakov et al., 2013) and observing the test. However, c-Fos is an indirect marker of neuronal activity, prompting the search for more compelling evidence.

The electrophysiological proof of the mirror neurons system existence in rodents has come a few years later. Recording multi- and single-unit activity using chronically implanted silicon probes, it has been showed that the rat's anterior cingulate cortex (ACC, area 24) contained neurons responding when a rat experiences pain as triggered by a laser and while observing another rat that receives a foot-shock. Most of these neurons do not respond to a mere fear-conditioned sound (CS). Deactivating this region reduces freezing while witnessing footshocks to others but not while hearing the CS. Moreover, deactivating this region reduces socially triggered freezing without compromising freezing to the CS (Carrillo et al., 2019).

Another recent study has attempted, unsuccessfully, to understand whether mirror-like neurons occur in frontal motor areas, specifically in the secondary motor cortex, or posterior parietal cortex in mice. They used a miniaturized, head-mounted fluorescent microscope to image the activity of hundreds of individual neurons at a time while mice performed or observed pellet-reaching and wheel-running tasks. Cell populations in both areas robustly encoded several naturalistic behaviours, showing clear differences in the structure of ensemble responses during performed and observed behaviours. However, neural responses to the same set of observed actions were absent, although, monitoring pupil dynamics, it has been shown that observer animals were attentive to performers and showed reliable neural responses to visual stimuli in the task. The neural coding of observed behaviour was below chance levels in both brain areas, suggesting that the representation of the observed actions occurs outside the parieto-frontal circuit in mice (Tombaz et al., 2020).

However, we are still far from understanding whether the mirror neuron system is actually present also in the rodents' motor cortex and, if present, its meaning.

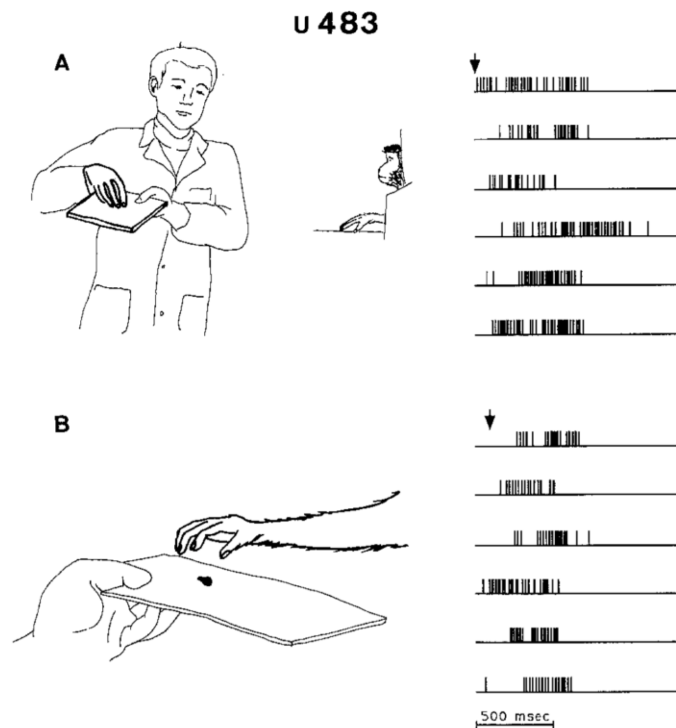

Figure I6. Example of a unit selectively discharging during monkey grasping movements and during monkey observation of grasping movements made by the experimenter. **a.** The experimenter grasps the food; **b.** the monkey grasps the food. Arrows indicate the (approximate) onset of grasping. Figure adapted from di Pellegrino et al., 1992.

1.7.2 Social Facilitation

Rizzolatti and co-workers (Iacoboni, 1999; Rizzolatti et al., 2002, 2001) proposed that the internal motor representation of the observed action can be used for response facilitation, i.e. for triggering the repetition of an observed action that is already in the observer's motor repertoire. According to this view, response facilitation is achieved by means of a "resonance" mechanism (Rizzolatti et al., 2002) in which the motor system of the observer (or of the listener) is activated specifically by observing (or listening) actions.

It is worth to note that action observation recruits mirror areas to the extent that the observed action is represented in the subject's personal motor repertoire, i.e. if the subject has acquired the motor skills to perform such actions. Premotor and parietal cortices of humans show greater activity, in a fMRI study, when dancers watched their own dance style compared to movements they had not been trained to, showing that the response to seeing an action is influenced by the acquired motor skills of the observer; whereas no such differences were detected in subjects not trained in dancing (Calvo-Merino et al., 2005). In addition, pianists showed stronger activations within a fronto–parietal–temporal network while observing piano playing compared to controls, using fMRI. Observation of silent piano playing additionally recruited auditory areas in pianists. Perception of piano sounds coupled with serial finger–

thumb opposition movements evoked increased activation within the sensorimotor network. This indicates specialization of multimodal auditory– sensorimotor systems within a fronto-parieto-temporal network by professional musical training. The transmodal audiovisual network stronger activated in pianists during passive observation of musical motor activity could functionally couple motor observation, auditory feedback, and imitation for learning of musical skills (Haslinger et al., 2005). However, within the context of the adult mirror neuron system, the infant mirror neuron system is characterized by a network circuit not well defined yet. Indeed, exploiting the desynchronization of the mu band (8-10 Hz) occurring during the mirror neuron activity, it has been shown that infants show strong desynchronization to human motion in the mu frequency band in the sensorimotor regions, irrespective of their own motor experience. Infants, who had not yet started to walk, responded equally to motion depicting walking and reaching. In addition, infants showed similar mu desynchronization in the sensorimotor regions to observation of coherent object motion in the form of movement of toy cars or balls (Virji-Babul et al., 2012). Therefore, early in life, infants may display a broadband response to human motion and coherent motion in the form of moving objects (Shimada and Hiraki, 2006). This response may be refined with experience through a process of Hebbian learning (Del Giudice et al., 2009), providing a mechanism for the integration of perceptual-motor learning with a genetic predisposition to motion resulting in the emergence of the mirror neuron system.

However, in adults, at behavioural level, the observation of actions can actually lead, in some cases, to the repetition of those same actions. This phenomenon has been named social facilitation. Indeed, it has been demonstrated that the observation and hearing of eating actions activate the motor programs underlying similar actions, thus facilitating their execution (Ferrari et al., 2005).

Specifically, social facilitation is a psychological phenomenon that an individual's performance improves in frequency and intensity due to the presence of other individuals doing the same action (Wöhr and Scattoni, 2013), and affects behaviours, such like food consumption and cognitive behaviour (Bond and Titus, 1983; Herman, 2017). The underlying mechanism has been mainly explained as Zajonc's drive theory, in which the arousal level is heightened (driven) through the perception of the presence of other individuals and it induces a dominant response of the performance on the task (Zajonc, 1965). Since Zajonc (1965) proposed that the presence of others involved in the same task was enough to facilitate well-learned behaviours or to retard new learning or complex behaviours via the increase in drive or arousal, a number of studies have been conducted on the effect of the mere presence of others on the performance of a task (Guerin and Innes, 1993). Intriguingly, observational learning is learning that occurs through observing the behaviour of others (Taylor and DeQuinzio, 2012). Many

primate species, such as chimpanzee and monkey, can effectively learn from others (Hopper et al., 2013; Horner and Whiten, 2005). However, the phenomenon has been observed in many species, including not only primates, but also birds and insects (Chabaud et al., 2009; Dindo et al., 2009; Jesse and Riebel, 2012).

Mice and rats are social species with a wide repertoire of social behaviours that range from parenting and communal nesting their pups, juvenile play to sexual and aggressive behaviours as adults. Social facilitation is observed also in rats (Zentall and Levine, 1972). Specifically, it has been demonstrated that performance and food intake were modified by the presence of conspecifics (Sekiguchi and Hata, 2019; Takano and Ukezono, 2015). Similar to primate species, rodents also exhibit observational learning of spatial exploration and operant learning (Carlier and Jamon, 2006; Takano et al., 2017).

Takano and Ukezono (Takano and Ukezono, 2015) investigated the performance of rats using a motor task in the presence of another non-competitive observer rat. These authors required a subject rat to perform a skilled reaching task (Metz and Whishaw, 2000) in front of a cage-mate which could not interfere with the subject. In the original task, rats were trained to reach and grasp a pellet placed on the shelf inside of the wall slit. Takano and Ukezono (Takano and Ukezono, 2015) partly modified the original task by adding an observational room opposite the reaching room; moreover rats were trained to spin in front of the wall slit before the reach-to-grasp movement to reduce wasteful motions. They alternately trained rats to reach or observe every other day. Results showed that rats spun faster in the presence of the cage-mate, in comparison to when they performed the task solitarily. Thus, the presence of conspecifics has been shown to commonly accelerate motor behaviours.

2. Aim of the project

Preparatory neural activity related to direct initiation of voluntary movements is critical for action planning and execution (Churchland, 2006; Godschalk et al., 1985; Guo et al., 2014b; Murakami et al., 2014; Weinrich and Wise, 1982; Wise et al., 1986). This activity is more frequently found in premotor cortical areas than in the motor cortex (Afshar et al., 2011). Indeed, premotor areas act as a conductor to orchestrate the network activity of the rest of the motor modules, on a moment-by-moment basis, and exhibit tuning for specific movements (Churchland, 2006; Churchland and Shenoy, 2007; Georgopoulos et al., 1982; Godschalk et al., 1985; Hocherman and Wise, 1991; Messier and Kalaska, 2000; Riehle and Requin, 1993). How do distinct neuronal classes contribute to this process?

While previous studies have examined anticipatory activity in populations of pyramidal neurons (PNs; reviewed in Svoboda and Li, 2018), little is known on the contribution of GABAergic interneurons to these cortical computations (Merchant et al., 2008; Isomura et al., 2009; Kaufman et al., 2013). Specifically, how pyramidal and GABAergic cells in the different layers of the premotor cortex interact and process information to build a voluntary motor command remains enigmatic.

To fill this gap, here I extracellularly recorded single units activity in the premotor area from head-restrained mice, classifying neurons into PNs and FSNs, accordingly to well established waveforms parameters.

In the first set of experiments, I explored PNs and FSNs properties in animals allowed to spontaneously lick a reward delivered at random intervals. In particular, I exploited a 16-channel single shank silicon probe to record neurons from all cortical layers of the ALM, the putative premotor area controlling voluntary licking in mice. Using a custom-made Matlab code, I analyzed peristimulus time histograms for each recorded neuron, built aligning spike events on the start lick, and I extrapolated information about the onset of the response, the peak time of the activity, the duration and the intensity of the activation. Moreover, I compared the firing of sorted units during either single or multiple licks, exploring the difference between an isolated event and a burst of consecutive movements.

Next, I performed a computational analysis to determine the mean information content for all the recorded units, analysing the mutual information between the local firing rate and the behavioural states (i.e., rest, single lick and multiple licks).

In the second set of experiments, we studied the role of FSNs, specifically their interplay with PNs, during the computation of the preparatory activity in both ALM and the premotor area for the forelimb, the RFA. I used chronically implanted microelectrode arrays to cover both areas. As during acute recordings, I evaluated onset and duration of the activity. Moreover, I explored PNs and FSNs selectivity for the two types of movements, i.e. licking and forelimb retraction, and the effect of their interaction on the neuronal firing rate.

Finally, based on this electrophysiological background I started a project aimed at identifying mirror-like neurons in the mouse premotor cortex. Mirror neurons have been characterized in monkeys and other species and they are active not only when animals perform an action but also when they watch the same action being performed by another conspecific (di Pellegrino et al., 1992; Pineda, 2005; Prather et al., 2008). Their discovery in the macaque monkey 3 decades ago prompted the notion that action execution and observation are closely related processes, and indeed that our ability to interpret the actions of others requires the involvement of our own “mirroring” motor system (Kilner and Lemon, 2013; Rizzolatti and Fabbri-Destro, 2009).

Since the discovery of the monkey mirror neuron system, there has been much speculation over the existence of mirror neurons in animal genetically tractable models, allowing the access to cellular networks underlying action understanding. Therefore, a challenge is to establish whether mirror-like neurons occur in rodent models, which could allow a detailed understanding of the properties, connectivity and function of these neurons. However, currently there is no compelling proof that the mirror neuron system is actually present also in the rodents’ motor cortex.

Here, I set up in mice a behavioural paradigm, the social facilitation test, previously used in monkeys (Ferrari et al., 2005), to verify whether the same behaviour was present also in rodents, as a first step to explore the possibility of the presence of mirror neurons in mice premotor cortex.

3. Materials and Methods

3.1 Animals

All experiments were carried out in accordance with the EU Council Directive 2010/63/EU on the protection of animals used for scientific purposes, and were approved by the Italian Ministry of Health. Animals were housed in rooms at 22 °C with a standard 12h light/dark cycle. Food (standard diet, 4RF25 GLP Certificate, Mucedola) and water were available *ad libitum,* except for the experimental period, during which mice were water deprived overnight. Electrophysiological recordings were conducted on 13 subjects. Experiments were carried out on 3–5 months old wild-type mice (C57BL6/J), males. For optogenetic experiments 2 PV-Cre mice (Tanahira et al., 2009) (B6;129P2-Pvalb tm1(cre)Arbr/J, Jackson Laboratories, USA) were used, injected with 600 nl of an AAV vector (AAV1.EF1.dflox.hChR2(H134R)-mCherry.WPRE.hGH, Addgene, USA), into the motor cortex. The AAV vector contains the doublefloxed channelrhodopsin-2 (ChR2) gene, which is thus expressed in parvalbumin interneurons through Cre-mediated recombination (Spalletti et al., 2017; Tantillo et al., 2020). We referred to them as PV+FSNs (**Figure 1d**). 6 B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng (ChR2) were used to map mouth/tongue movements in the ALM.

3.2 Surgery Procedure and Animal Preparation

Mice were deeply anesthetized with an intraperitoneal injection of avertin (0.020 ml/g), and positioned on a stereotaxic frame; the scalp was partially removed, the skull cleaned and dried. A ground screw was implanted above the cerebellum. Mice were implanted with a custom-made lightweight head post, placed on the skull on the left hemisphere, aligned with the sagittal suture and cemented in place with a dental adhesive system (Super-Bond C&B). A thin layer of the dental cement was used to cover the entire exposed skull.

For the acute recordings, a recording chamber was built using a dental cement (resin adhesive cement, Ivoclar Vivadent) and centred on the ALM (1.8 mm lateral and 2.5 mm anterior to Bregma). The skull over the recording area was covered by sterile low melting agarose Type III (A6138, Sigma-Aldrich, Inc.) and sealed with Kwik-Cast (WPI). On the day before the first acute recording session, 6 B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng (ChR2) mice were anaesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) cocktail, the chamber covering removed and cortex was optogenetically stimulated following a grid with nodes

spaced 500 µm. For each site, optogenetic stimulation (3 ms single pulses, 0.2Hz) was delivered by means of PlexBright Optogenetic Stimulation System (PlexonInc, USA) with a PlexBright LD-1 Single Channel LED Driver (PlexonInc, USA) and a 473 nm Table-top LED Module connected to a 200 µm Core 0.39 NA optic fiber (ThorlabsInc, USA). Movements of tongue/mouth were collected by a second experimenter, blinded to the stimulation coordinates. A small craniotomy (diameter, 0.5 mm) was then performed over sites where the larger tongue/mouth movements could be evoked. In wild-type mice, the craniotomy was performed in the same region of Thy1-ChR2 mice. Finally, the chamber was filled with agarose and sealed.

For chronic implants, a craniotomy (diameter, 0.8 mm) was made over the ALM, partially covering the Rostral Forelimb Area (RFA, 1.2 mm lateral and 2 mm anterior to bregma). A planar multi shank 4x4 array (Microprobes for Life Science) was positioned over the craniotomy and microwires were inserted into the cortex, up to ~800 µm depth. Then the craniotomy was covered with low melting agarose and the array fixed and embedded with dental cement (Super-Bond C&B and Paladur). Mice were allowed to awaken and then housed separately.

3.3 Behavioural Training and Data Analysis

After recovering from surgery, mice were water restricted in their home cages, with food still available. Condensed milk was provided as a reward during the tasks and mice were also provided water *ad libitum* for about 1 hour/day, following each recording session.

During the shaping phase, mice were placed in a U-shaped restrainer (3 cm inner diameter), head-fixed through the metal post cemented on their skull and habituated to lick drops provided randomly by the experimenter through a feeding needle mounted on a piezoelectric sensor sensing the movement of the tongue.

Each shaping session lasted from 15 min up to 60 min for at least 3 consecutive days. Digital signals from the licksensor provided information about the licking movements directly to the recording apparatus. The licking behavioral strategy was categorized into two groups: single and multiple licks. The start lick was defined as a movement that was not preceded for at least 0.6 s from any other licking event. The single lick was a start lick not followed by any other lick for at least 0.6 s; multiple licks were defined as start licks followed by at least two other consecutive licks (≤ 0.4 s among consecutive licks). Time intervals lasting for ≥ 1 s and distant at least 0.5 s from the end or the start of licking trials were considered as resting intervals and used as a baseline for the analysis of neural activity.

For identification of PV neurons in PV-cre mice, the site of AAV injection was illuminated with an optic fiber (200µm Core 0.39 NA, Thorlabs, USA). Optogenetic stimulation (50 0.2 s pulses, 0.2 Hz) was delivered by means of PlexBright System (Plexon, USA) with a PlexBright LD-1 Single Channel LED Driver (Plexon, USA) and a 473 nm Table-top LED Module. After spike sorting, PV-positive neurons were defined as neurons increasing their firing rate by 5ms from the beginning of the blue light pulse (i.e. ChR2-positive neurons) and with a sustained activity for the entire stimulation length (i.e. FSNs).

For the chronic recordings, in which forelimb-driven response was also assessed, mice were shaped and trained on a robotic platform, the M-Platform (Spalletti et al., 2014) that comprises a 1-axis load cell, a linear slide connected to a custom-designed handle that was fastened to the left wrist of the animal. During recording sessions, the forepaw, contralateral to the implanted ALM, was maintained in a slightly extended position and the force peaks exerted to attempt retractions were detected by the load-cell and offline aligned with neural signals.

3.4 Single-Unit Recording

The electrophysiological data were continuously sampled at 40 kHz and bandpass filtered (300 Hz to 6 kHz), using a 16-channel Omniplex recording system (Plexon, Dallas, TX).

For the acute recordings, a NeuroNexus Technologies 16-channel linear silicon probe with a single-shank (A1x16-3mm-50-177, 50µm spacing among contacts) was slowly lowered into the ALM; the tip of the probe was placed at about 1000 µm depth using a fine micromanipulator. The recording chamber was filled with sterile saline solution (NaCl 0.9%). Before the beginning of the recording, the electrode was allowed to settle for about 10 min. For each animal, a number of one up to seven extracellular recording sessions were performed.

For chronic recordings, mice were recorded on ~ 10 separate daily sessions over a 15 days period.

3.5 Single Unit Spike Sorting

The extracellular recording data were processed to isolate spike events by a spike sorting software (Offline Sorter™ v3.3.5, Plexon), using principal component analysis; events (spike-detection interval > 1.0 ms) that exceeded a 4 SDs threshold above the background were sorted (**Figure M1**). The spike waveforms were aligned at global minimum and the artifact waveforms were removed. The single-unit clusters were manually defined.

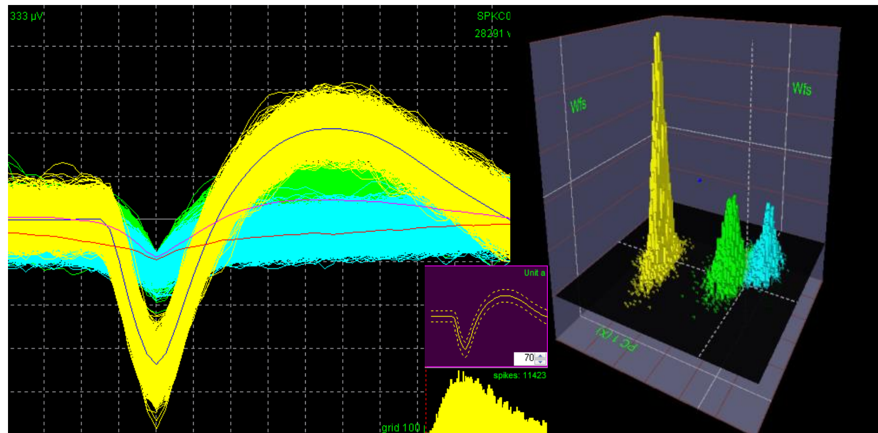

Figure M1. Offline spike sorting was used to extract information from extracellular recordings. Head-restrained preparation allowed recordings of well-isolated single units at multiple sites during repeated experimental sessions. On the left an example of single-unit waveform is represented in yellow. The detected spikes are grouped into different clusters (on the right) and each separated cluster is associated to a single unit (yellow).

3.6 Data Analysis

The recorded units were classified based on their average waveforms into putative pyramidal neurons (PNs) and putative fast-spiking neurons (FSNs). Two waveform parameters were used for the classification: the ratio between the height of the maximum peak and the initial negative trough, and the trough-peak time. A k-means clustering was applied. The clustering was verified by optogenetic tagging of PV-positive neurons.

The relation between single neuron activity and the events of the behavioral task was analyzed using MATLAB (MathWorks). Peristimulus Time Histograms (PSTHs) were built aligning spike events on the start lick, for both single and multiple licks, and on the onset of the force during forelimb pulling evaluation; only intervals with stable unit activity were included and spikes were averaged over 0.05 s with 0.01 s steps. The PSTH covered a time window of 1 s, from 0.6 s before the starting event (lick or force onset) and 0.4 s after it. Neurons were tested for licking or forelimb selectivity comparing spikes/s activity in the PSTHs with the mean firing rate, calculated during resting periods (lasting ≥ 0.5 s, and distant from event trials ≥ 0.5 s) ± an upper or a lower threshold. Bootstrapping was used to estimate the thresholds; lower and upper thresholds were, respectively, the 2.5 and 97.5 percentile of the probability distribution function obtained during the resting intervals. A unit was considered responsive for the licking behaviour or forelimb retraction when, for at least three consecutive bins (0.03 s), its firing rate went over (enhanced neurons) or under (suppressed neurons) the considered thresholds.

The onset of activity was defined as the first bin of the ≥ 3 consecutive bins above/below the upper/lower threshold; the time of the bin in which the firing rate (spk/s) was maximum/minimum was considered as the peak time. Selectivity indices were measured considering the firing rate (spk/s) of the peak time; they were defined as:

$$\frac{[|Peak\ \left(\frac{spk}{s}\right) during\ single\ lick| - |Peak\ \left(\frac{spk}{s}\right) during\ multiple\ licks|]}{[|Peak\ \left(\frac{spk}{s}\right) during\ single\ lick|\ + |Peak\ \left(\frac{spk}{s}\right) during\ multiple\ licks|]}$$

and:

$$\frac{[|Peak\ \left(\frac{spk}{s}\right) during\ multiple\ licks|\ - |Peak\ \left(\frac{spk}{s}\right) during\ forelimb\ retraction|]}{[|Peak\ \left(\frac{spk}{s}\right) during\ multiple\ licks\ + |Peak\ \left(\frac{spk}{s}\right) during\ forelimb\ retraction|]}$$

The duration of the response was the number of bins above/below the upper/lower threshold. The intensity of activation was defined as:

$$\frac{area\ above/below\ the\ upper/lower\ threshold}{duration\ of\ the\ response}$$

The interaction between licking and forelimb pulling was measured only for those neurons that increased their firing rate during both licking and forelimb movements. As a reference of activity, the mean firing rate of each considered neuron, over 11 bins, 150 ms (bin size 50 ms, with a sliding time window of 10 ms width), centered on the expected peak time of licking and pulling (calculating using PSTHs) in isolated (> 0.6 s distant from other movements) single licks events or forelimb pulling respectively, was measured.

After that, single licking events (or force onset event) spontaneously occurring in a - 0.6 s – + 0.6 s interval from a force onset event (or single lick event) were selected. For each couple of co-occurring events the expected distance among the two expected peak times (calculating using PSTHs) was computed.

Finally, only the events (single lick or force onset events) with an expected distance ranging from -0.1 s to 0.1 s were selected; for each considered neuron the mean firing rate was calculated as for the isolated events (over 11 bins, 150 ms (bin size 50 ms, with a sliding time window of 10 ms width)).

Firing rates of different neurons were reported as normalized on their baseline firing rate and were averaged.

3.7 Information Content

We measured the information content (Shannon, 1948), carried out by the mean firing rate of each neuron about two different sets of conditions. Set 1: 0.8 s intervals centred at single licks (see above) vs rest, i.e. randomly selected 0.8 s intervals during which animals were at rest, distant at least 1.5 s from other licking or rest intervals. Set 2: 0.8 s intervals centred at the onset of multiple licks (see above) vs rest.

The mean firing rate mf associated to each trial was measured over the whole window. The mutual information $\mathrm{ISF(E, Mf)}$ between mf and each set of events E was computed as follows:

$$\mathrm{ISF(E, Mf)} = \sum_{\mathrm{e}} \mathrm{P(e)} \sum_{\mathrm{mf}} \mathrm{P}(\mathrm{mf} \mid e) * log2(\frac{\mathrm{P}(\mathrm{mf} \mid e)}{\mathrm{P(mf)}})$$

where $P(e)$ was the probability of the presentation of the specific event e, $P(m)$ the probability over all trials and all conditions of the neuron to have the mean firing rate mf in a given interval, $\mathrm{P}(\mathrm{mf} \mid e)$ the probability of the mean firing rate mf to be associated to the event e. Mean firing rates were binned in N equipopulated bins, where N was the minimum value between the square root of the number total number of trials and the number of unique values in the array of mean firing rates.

To reduce the bias in the estimation of the information due to the limited dataset, a quadratic extrapolation method was used (Panzeri et al., 2007). A statistically significant threshold was obtained bootstrapping 100 times (shuffling the conditions associated to each trial), and, for a major solidity, only neurons with an IC > 95 percentiles of the bootstrapped distribution, in at least one of the two combinations, were included, generating a subset of informative neurons.

We also calculated the information content over time: we considered 0.8 s before and 0.4 s after the licking event, and we computed a local mean firing rate (LMF) over a moving average of 50 ms with steps of 10 ms. Then, for each step we repeat the procedure described above. For this analysis we only used the subset of informative neurons described above.

For each recording session, we computed animal-wise the amount of information carried by the summed firing rate (ISF) of the recorded FSNs and PNs population. Each recording session has a different number of neurons and a different ratio between FSNs and PNs, for this reason, to be able to compare results from different recording sessions, the ISF was computed considering *N* couples of neurons belonging to the same class for each recording. *N* was the minimum value between all the possible combinations of same-class-neurons and 40.

For each couple of neurons, ISF was calculated with the following equation:

$$\mathrm{ISF(E, MF\ 12)} = \sum\nolimits_{e} \mathrm{P(e)} \sum_{\mathrm{mf\ 12}} \mathrm{P(mf\ 12} \mid e) * log2(\frac{\mathrm{P(mf\ 12} \mid e)}{\mathrm{P(mf\ 12\ )}})$$

Where $\mathrm{ISF(E, ISF\ 12)}$ is the information given by the summed firing rate of neuron 1 and 2, $\mathrm{P(e)}$ was the probability of the presentation of the specific event e, $\mathrm{P(mf\ 12\ )}$ the probability that the sum firing rate of the neurons to have the mean firing rate $\mathrm{mf\ 12}$ over all trials of all conditions, $\mathrm{P(mf\ 12} \mid e)$ is the probability of the mean firing rate $\mathrm{mf\ 12}$ to occur during the event e.

We used the same bias correction method and the same statistically significant threshold of the previous analysis. Only couples with a ISF > 95 percentiles of the bootstrapped distribution, in at least one of the two combinations, were considered.

We then normalized the $ISF(\mathrm{E}, ISF\ 12)$ (Nisf) to the sum of the information contained in the mean firing rate of neuron 1 and 2 calculated separately with the following equation:

$$\mathrm{Nisf(12)} = 1 - (\frac{\mathrm{ISF(E, ISF12)}}{\mathrm{I(E, Mf1)} + \mathrm{I(E, Mf2)}})$$

Where $\mathrm{Nisf(12)}$ is the normalized information carried by the sum of the firing rate of neuron 1 and 2, $\mathrm{ISF(E, ISF\ 12)}$ and $\mathrm{I(E, Mf1)}$ are defined above.

When $\mathrm{Nisf(12)}$ is equal to 0 it suggests that the information carried by the means of the two neurons are mostly independent, while higher values suggest that the information are more dependent.

3.8 Social Facilitation Test

Mice were divided into two groups: observers (12 animals) and demonstrators (20 animals).

Observers were deeply anesthetized with an intraperitoneal injection of avertin (0.020 ml/g), and positioned on a stereotaxic frame; the scalp was partially removed, the skull cleaned and dried. Mice were implanted with a custom-made lightweight head post, placed on the skull on the left hemisphere, aligned with the sagittal suture and cemented in place with a dental adhesive system (Super-Bond C&B). A thin layer of the dental cement was used to cover the entire exposed skull. Observers were allowed to recovery from surgery for at least two days. Before the experimental sessions, observers were habituated for at least three days to the head restrained condition, starting from 5 min/day until 20-30 min/day.

One to two days before the first experiment, demonstrators were water deprived in their home cages, with food still available. Water was provided during the tasks and mice were also provided water *ad libitum* for about 1 hour/day, following each session. In contrast, observers were given abundant food and water, in order to satiate them.

The apparatus (**Figure M2**) included a chamber in which a demonstrator can freely move; a head-restrained observer was positioned so that the side of chamber and the A-P axis of the observer formed an angle of 45° (see **Figure M2** below). A drinking spout was always positioned in front of the observer' snout; the tip of the drinking spout was covered with a sponge constantly soaked with water. The observer and the chamber were positioned at a distance of about 10 cm, to allow visual and auditory contact of the demonstrator. Before beginning the session, demonstrators were left in the experimental chamber for at least 1h to familiarize with it.

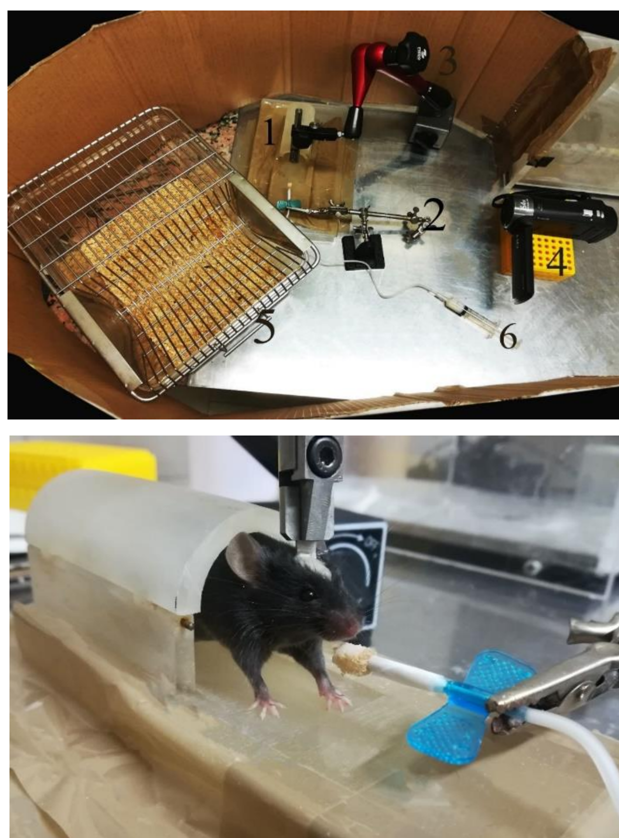

Figure M2. On the tom the social facilitation apparatus is represented: 1. Head-restrained position; 2. Drinking snout; 3. Head-restrain tool; 4. Camera position; 5. Demonstrator’s chamber; 6. Water delivery system. On the bottom a head restrained mouse is represented.

The social facilitation test lasted 15 min and comprised three consecutive periods of 5 min each: (B) baseline period, during which the demonstrator was obscured, (D) a control period, during which the demonstrator was present but not drinking because the bottle was not available in the chamber and (D + B) a testing period, in which the demonstrator was present

and allowed to drink. The baseline period was always administrated as first condition. The order of the two conditions “D” and “D + B” was randomly balanced. The experiment was repeated for 3 weeks for each observer.

In a second set of experiments, a bottle condition (B), lasted 5 min, in which the demonstrator was not present and there was only the bottle in the empty chamber, has been also performed. The order of the two conditions “D”, “D + B” and “B” was randomly balanced.

Observers’ and demonstrators’ licking intervals were measured. Observers’ total amount of time spent to lick and the first lick latency calculated. For a deeper analysis the demonstrator’s licking intervals were scrambled using MATLAB (**Figure M3**) (MathWorks).

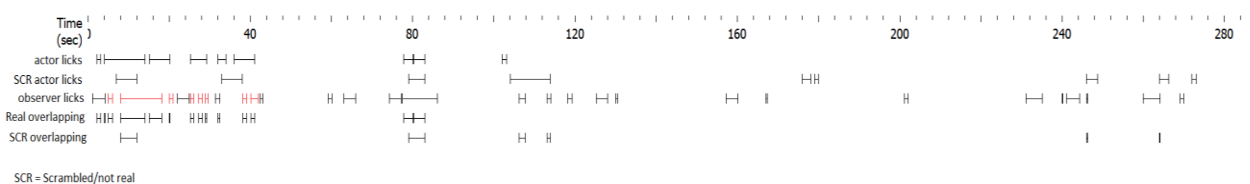

Figure M3. A time scale of a social facilitation analysis. The time segments indicate (from top to bottom): 1. Demonstrator’s licking interval; 2. Demonstrator’s licking intervals scrambled (scr); 3. Observer’s licking intervals; 4. The overlapped licking intervals between the observer’s and demonstrator’s intervals; 5. The overlapped licking intervals between the observer’s licking intervals and demonstrator’s scrambled licking intervals (scr overlapping).

3.9 Statistics and data

All data are expressed as mean ± standard error of the mean (SEM). Statistical tests were performed using Graphpad Prism 8.0 or SigmaPlot 12.0. Statistical significance was assessed using Wilcoxon matched paired Signed Rank Tests, Mann-Whitney Test, Two Proportion Z Test, Paired t-test, Two way Repeated Measure ANOVA, Friedman Test or Chi-square Test, as appropriate. Cumulative distributions were tested using Kolmogorov-Smirnov (K-S) two-sample Test. All statistical analyses were performed on raw data. The level of significance was set at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

4. Results

4.1 Activity of PNs and FSNs in the ALM during licking

To precisely target extracellular recordings within the ALM, we carried out initial optogenetic mapping experiments in 6 Thy1-ChR2 mice (Spalletti et al., 2017) expressing the light-gated cation channel ChR2 in layer V neurons. We confirmed that the stimulation of a cortical area centred 2.5 mm anterior and 1.8 mm lateral to lambda (data not shown), evoked mouth/tongue movements, as expected for the ALM (Svoboda and Li, 2018).

Water-restricted, head-fixed mice were allowed to lick spontaneously drops of condensed milk which were made available through a drinking spout, positioned in front of the animals' snout and equipped with a piezoelectric element to signal licking events. Mice performed rapid, rhythmic and stereotyped protrusions of the tongue at 7-8 Hz, as previously described (Guo et al., 2014b). Offline, we categorized licking events into isolated, “single” licks, or “multiple” licks (defined as isolated licks within -0.4/+0.4s and as clusters of at least 3 consecutive licks respectively; **Figure 1a**).

We extracellularly recorded neuronal activity with either an acutely inserted single shank, 16-channels silicon probe (electrode contacts spaced by 50 µm to span all cortical layers; n = 10 mice, n = 693 units) or a chronic 16-microelectrodes array (n = 3 mice; n = 759 units, up to 10-15 recording sessions per animal) from the ALM (and RFA) of the right hemisphere. Spike detection and sorting were performed offline (see Methods for cluster separation criteria) and individual neurons’ spikes were classified based on their shape, as described previously (Barthó et al., 2004b; Mitchell et al., 2007; Niell and Stryker, 2010). Specifically, we projected in a 2D-feature space the distribution of waveforms according to two parameters: the trough to peak time and the peak-trough ratio (**Figure 1b**). This analysis allowed us to separate neurons with a thin spike shape (i.e., narrow-spiking neurons) from broad-spiking cells (**Figure 1c**).

Peristimulus time histograms (PSTHs) were created by aligning the spiking activity of each neuron to the first event of each licking bout (timed as “0”). For each PSTH, the mean firing rate in a 1s time window (0.6s before and 0.4s after “0”) was compared to thresholds delimiting a range of baseline activity during resting periods (**Figure 1d**; see Methods for details) to identify responsive neurons. Overall, in both acute and chronic recordings, we found n = 624 units (indicated by violet and green squares in **Figure 1b**) which were significantly modulated by movement (n = 828, PNs and FSNs, not modulated by the task are indicated by light violet

and light green squares, respectively; **Figure 1b** and **Table 1**). Responsive neurons with a thin spike shape displayed higher baseline discharge and shorter inter-spike intervals (ISI) than broad-spiking neurons (**Figure 1e, f**), consistent with their classification as fast-spiking, putative GABAergic interneurons (FSNs). Broad-spiking neurons were instead considered as putative pyramidal neurons (PNs).

To further validate the identification of FSNs, we performed extracellular recordings in Parvalbumin (PV)-Cre mice, inoculated with a floxed AAV vector expressing ChR2 to allow expression of the light-gated cation channel selectively in PV-positive, fast-spiking interneurons (Tantillo et al., 2020). FSNs were identified based on their latency and sustained response to a blue light stimulation (0.2s), and their waveforms added to data for PNs/FSNs clustering. Notably, all the optogenetically tagged PV+-FSNs displayed a small trough to peak time and peak-trough ratio, coherently with their identification as putative interneurons, thereby confirming the reliability of our identification method (**Figure 1b**).

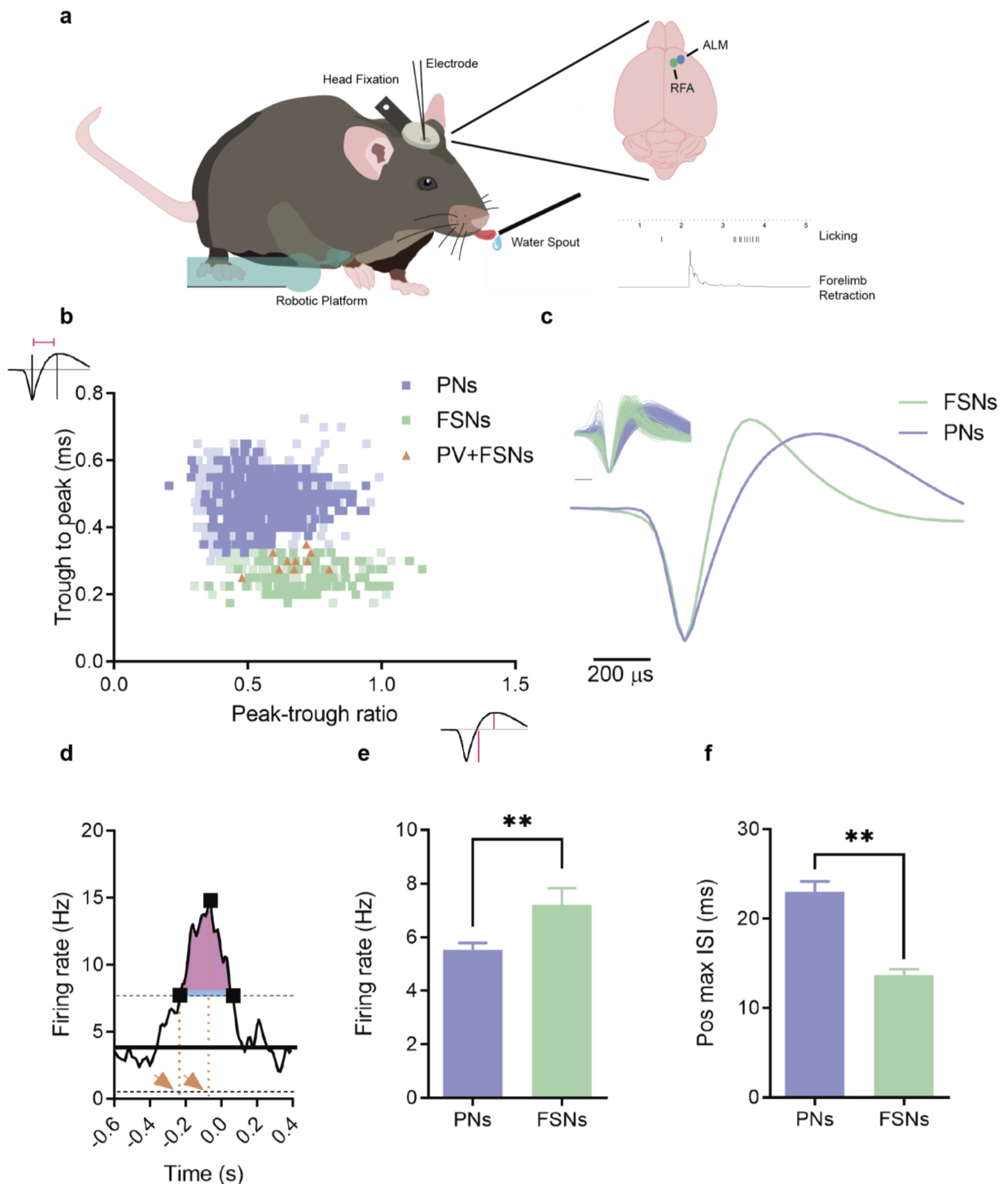

Figure 1. a. Schematic representation of a head-fixed mouse in the behavioral setup. Spontaneous licks of drops of water are tracked using a licksensor. The electrode is positioned into the ALM during acute recordings, while a chronic electrode array covers both ALM and RFA during chronic recordings. The force applied during forelimb pulling is evaluated using a robotic platform. In the bottom right, a scale bar of the licking behaviour and onset force is represented. The single licks are isolated events, neither followed nor preceded by other licks for at least 0.6 s. Multiple licks are clusters of at least 3 consecutive licks (≤ 0.4 s) not preceded by other licks for at least 0.6 s. **b**. Scatter plot of spike waveform parameters for all units recorded (n = 1452). The violet and green filled squares represent individual putative pyramidal neurons (PNs, task related or not, violet and light violet, respectively) and fast-spiking neurons (FSNs, task related or not, green and light green, respectively). The orange filled triangles show spike shapes of individual PV+-tagged FSNs (activated at short latency by light). **c**. Average spike waveforms for all units, PNs and FSNs, aligned to minimum and normalized by trough depth. **d**. A representative peristimulus time histogram. The black line represents the mean firing rate calculated during resting periods, black dotted lines an upper and a lower threshold. The three black squares show the first, the maximum and the last point over the threshold. The orange dotted lines and the orange arrows indicate

the onset of the activity and the peak time, respectively. The blue line shows the duration of the activity, representing the time over the threshold. The pink area is the area above the threshold. The intensity of activation is defined as the pink area divided by duration of the activity. **e, f**. Mean firing rate (**e**) and maximum position of interspike intervals (ISI, **f**) of PNs and FSNs. Baseline firing rate, K-S Test, D = 0.188, p = 0.0043; maximum position ISI, K-S Test, D = 0.206, p = 0.0013, $^{**}p < 0.01$.

	Total Recorded Units	Modulated Units	PNs	FSNs
Acute Exp	693	251	203	48
Chronic Exp	759	373	313	60

Table 1. Total number of recorded units during acute and chronic experiments and the modulated units for both PNs and FSNs are reported.

In the first set of experiments (performed with acutely inserted silicon probes in the ALM), we found that n = 251 neurons (203 putative PNs, 48 putative FSNs) showed a modulation of activity during licking behaviour. The majority of putative PNs showed a firing rate enhanced by licking, and only about 15% of them exhibited a decrease in firing rate during licking epochs (**Figure 2a, b, c**). The proportion of licking-suppressed FSNs tended to be lower (about 6%; **Figure 2d, e, f**; PNs Supp vs FSNs Supp, Two Proportion Z Test, z = 1.65, p = 0.09). In the case of multiple licks, PNs and FSNs showed the maximum response modulation in correspondence with the first licking event. This was demonstrated by building mean PSTHs aligned with the first or second lick in the series. In particular, the alignment with the second lick resulted in a negative shift (about -150 ms) for both onset of the response and peak latency (**Figure 2g, h**). These data suggest that neuronal discharges of both PNs and FSNs in the ALM are mainly related to the planning of rhythmic oral activity (i.e., a licking bout) rather than the execution of each lick of the series.

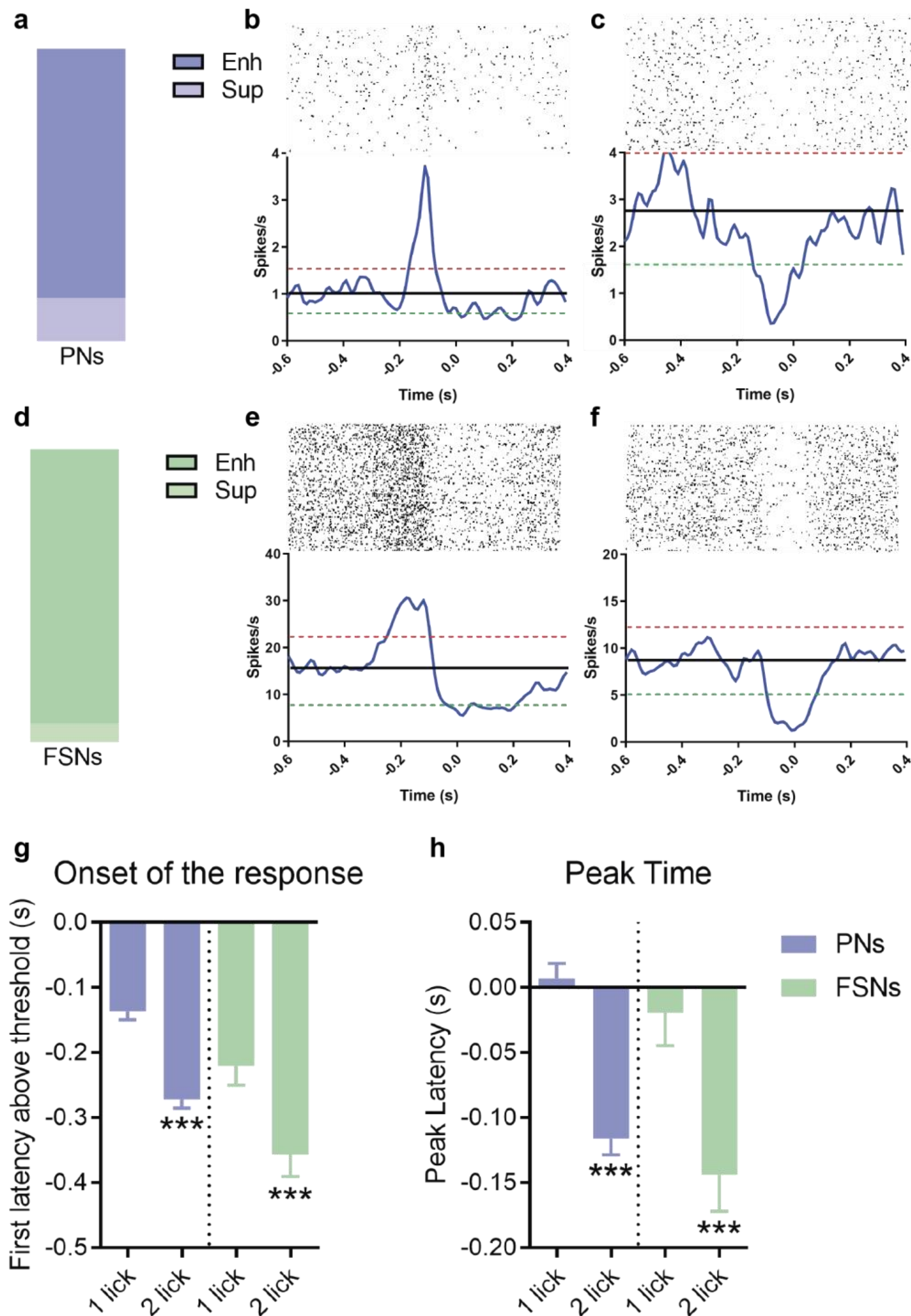

Figure 2. a. Proportion of all responsive putative Pyramidal Neurons (PNs) enhanced (violet) or suppressed (light violet) during the licking activity; the majority of PNs responds increasing their basal firing rate, a small percentage of neurons show a decrease in their basal firing rate in response to the movement. **b, c**. Representative examples of raster plots and corresponding firing-rate-time-histograms showing enhanced (**b**) and suppressed (**c**) PNs, the red dotted lines represent the upper thresholds, the green dotted lines the lower ones, the black line is the mean baseline firing rate. Time = 0 corresponds to the first lick, not preceded by other licks for at least 0.6 s. **d**. Proportion of all responsive putative Fast Spiking Neurons (FSNs) enhanced (green) or suppressed (light green) during the licking activity. **e, f**. Representative examples of raster plots and firing-rate-time-histograms of enhanced (**e**) and suppressed (**f**) FSNs, the red dotted lines represent the upper thresholds, the green dotted line the lower ones, the black line is the mean baseline firing rate. Time = 0 corresponds to the first lick, not preceded by other licks for at least 0.6 s. **g, h**. Histograms of the onset of the response (**g**) and the peak time (**h**) of PNs and FSNs obtained aligning the firing-rate-time-histograms to the first or the second lick of a licking bout: units' activity is related to the beginning of the movement rather than the isolated licking

event. Onset of the response: Wilcoxon matched paired Signed Rank Test, PNs, W = -15010, p = < 0.001; FSNs, W= -914, p = < 0.001; Peak Time: Wilcoxon matched paired Signed Rank Test, PNs, W = -14760, p = < 0.001; FSNs, W= -913, p = < 0.001,****p* < 0.001.

4.2 FSNs display less tuned responses during single vs. multiple licks

We next compared the discharges of PNs and FSNs during either single or multiple licks. To this aim, we computed a selectivity index (see Methods), ranging from “-1” to “1”, based on the peak of neuronal modulation which indicates the degree of tuning of each neuron for the two different licking strategies (**Figure 3a**, “1” indicates exclusive selectivity for single licks, “-1” for multiple licks). The analysis showed that a great proportion (> 70%) of both PNs and FSNs modulated their firing rate during both single and multiple licks. Negative values tended to predominate for both PNs and FSNs, indicating an overall greater activity during the multiple licks (see also below). However, we also found units showing an absolute tuning for either single (+1) or multiple licks (-1). Thus, single and multiple licks are likely to be represented, at least by those neurons, as different motor acts.

To further quantify possible differences in neuronal tuning, we plotted the percentages of PNs and FSNs that responded only during single licks, multiple licks, or during both (**Figure 3b**). The statistical analysis revealed that the distribution was different for PNs vs. FSNs. Specifically, the proportion of neurons responsive for both single and multiple licks was greater for FNs. These data indicate a more specific activity of PNs compared to FSNs, which showed instead a broader tuning.

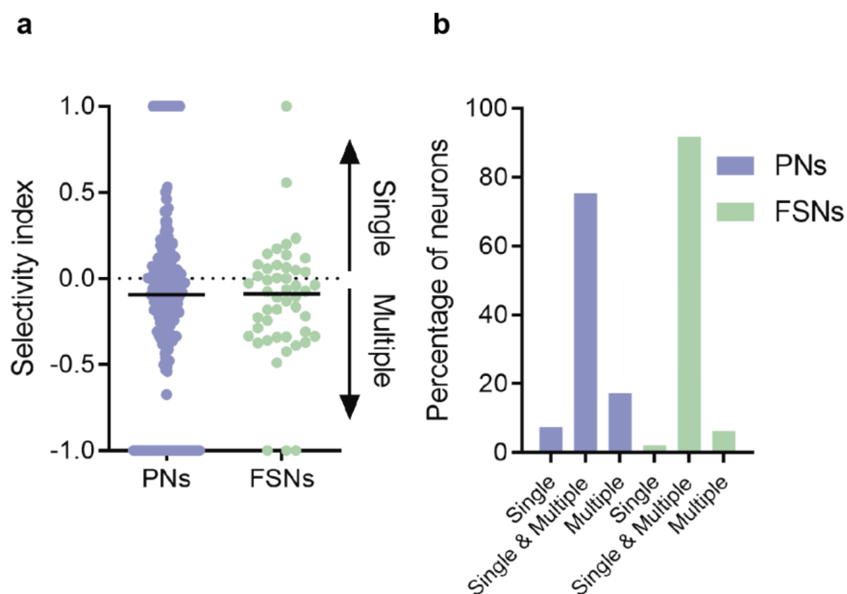

Figure 3. a. The peak of the response (spk/s) is used to calculate a selectivity index (see methods) towards the two identified licking strategies, going from -1 (neurons selective for multiple licks) to +1 (neurons selective for single licks). The black horizontal line corresponds to the median value (PNs,

median = -0.084; FSNs, median = -0.077). **b**. Percentage of neurons selective for: both single and multiple licks, for single or multiple licks individually, Chi-square Test, 6.13, p = 0.046. Single & Multiple PNs vs Single & Multiple FSNs, Z-Test, z = 2.5, p = 0.01.

4.3 FSNs show earlier and more sustained activation than PNs during licking

Based on the observed differences in the neuronal responses during single and multiple licks, we further investigated the changes in firing rates of PNs and FSNs during the two types of licking behaviour.

We first analysed the onset of the (enhanced or suppressed) response (see Methods and **Figure 1d**). The majority of the recorded neurons showed an increase - or a reduction - of their firing rates prior to movement onset, independently from the forthcoming licking behaviour strategy (**Figure 4a**). Onset of PN discharge was slightly earlier in single than in multiple licks (**Figure 4a**). Comparison of the onset of the response between PNs and FSNs showed that FSNs fired ~ 0.1 s earlier than PNs, similarly across the two behavioural conditions. A cumulative distribution curve of the onset for individual neurons demonstrated a clear shift to the left for FSNs vs. PNs (**Figure 4b, c**). This indicates an earlier recruitment of FSNs than PNs, hence suggesting that the modulation of putative GABAergic neurons may be critical for the initiation of the movement.

Then, we examined the timing of the peak of activity (or suppression) for each individual neuron with respect to licking onset. First, we evaluated the difference between the two licking strategies: for the multiple licks, the average peak time was significantly delayed for both PNs and FSNs (**Figure 4d**). Cumulative distributions of the peak latency during single and multiple licks are reported in **Figure 4e, f**. A robust statistical difference between PNs and FSNs was present for multiple licks (**Figure 4f**). Specifically, the cumulative distribution of the peak time for multiple licks showed that about 50% of PNs reached their maximum firing rate before the onset of the licking bout, whereas about 75% of FSNs already reached their peak of activity prior to licking onset (**Figure 4f**). Altogether, these data suggest a more direct and earlier involvement of FSNs in the planning and generation of licking movement than PNs.

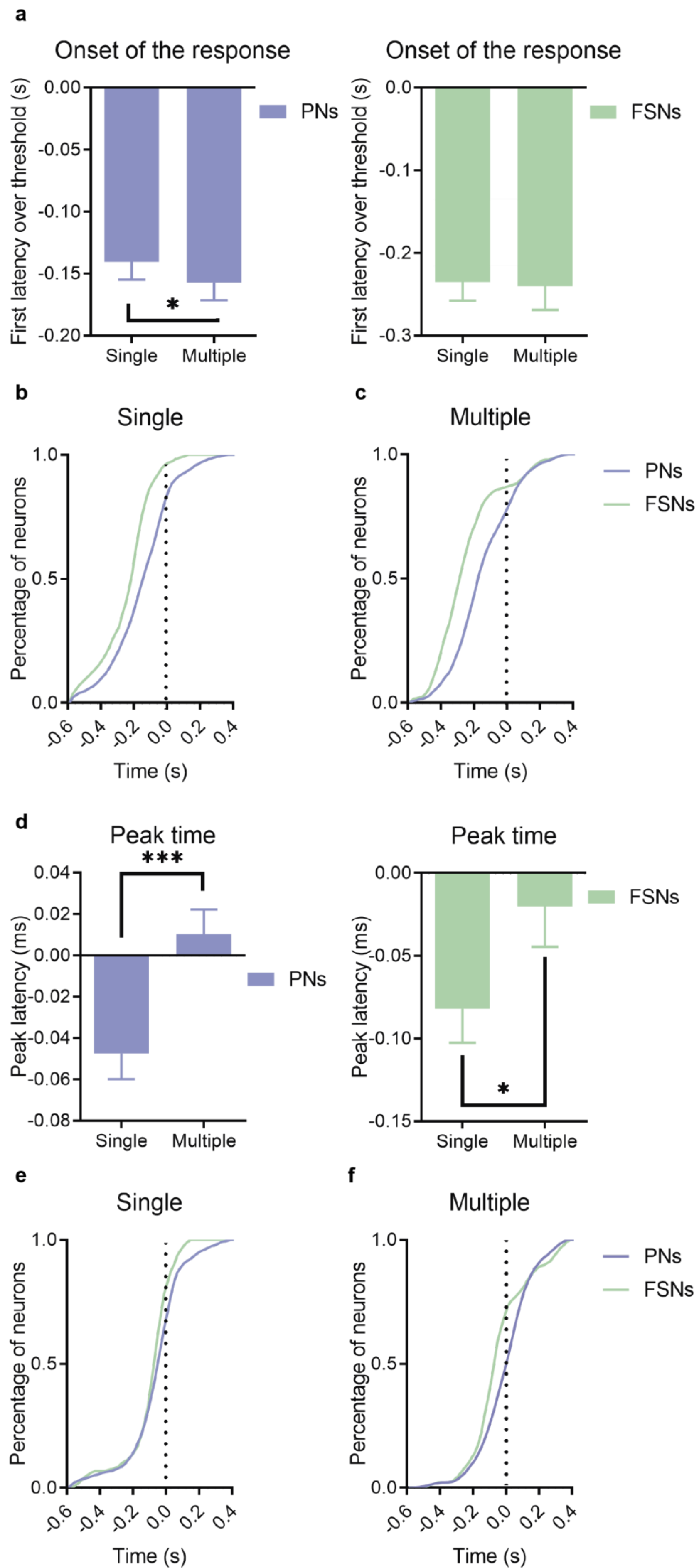
a
Onset of the response
First latency over threshold (s)
0.00
-0.05
-0.10
-0.15
-0.20
PNs
*
Single
Multiple
Onset of the response
0.0
-0.1
-0.2
-0.3
FSNs
Single
Multiple
b
Single
Percentage of neurons
1.0
0.5
0.0
-0.6
-0.4
-0.2
0.0
0.2
0.4
Time (s)
c
Multiple
PNs
FSNs
d
Peak time
***
Peak latency (ms)
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
PNs
Single
Multiple
Peak time
0.00
-0.05
-0.10
-0.15
FSNs
*
e
Single
f
Multiple
PNs
FSNs

Figure 4. a. Histograms of the changes in the onset of the response, defined as the first latency above or below the thresholds on PSTHs, for PNs (left) and FSNs (right), during single and multiple licks. Mean onset: PNs, Single = -0.141 s, Multiple = -0.157 s; FSNs, Single = -0.235 s, Multiple = -0.240 s. Wilcoxon matched paired Signed Rank Test, PNs, W = -2035, p = 0.046; FSNs, W = -158,0, p = 0. 36. $^{*}p < 0.05$. **b, c**. Cumulative distribution of the onset of the response for all the neurons, PNs and FSNs, during the single isolated lick (**b**) or consecutive multiple licks (**c**): FSNs modulated their activity generally earlier than the PNs. K-S Test, Single, D = 0.3163, p = 0.001; Multiple, D = 0.3351, p < 0.001. **d**. Histograms of the changes in the peak time of PNs (left) and FSNs (right) during single and multiple licks: the peak discharge is significantly delayed for both PNs and FSNs. Mean latency: PNs, Single = -0.0475 s, Multiple = 0.0104 s; FSNs: Single = -0.0820 s, Multiple = -0.020 s; Wilcoxon matched paired Signed Rank Test, PNs, W = 4707, p < 0.0001; FSNs, W = 321, p = 0.048, $^{*}p < 0.05$, $^{***}p < 0.001$. **e, f**. Cumulative distribution of the peak time for all the neurons, PNs and FSNs, during the single isolated lick (**e**) or consecutive multiple licks (**f**): FSNs show their peak latency generally earlier than the PNs. K-S Test, Single, D = 0.1933, p = 0.064; Multiple, D = 0.2766, p = 0.0063,.

Next, we explored the neuronal response duration, defined as the time interval during which the activity remained above - or below - the defined thresholds (see **Figure 1d** and Methods for details). The duration of the response was greater when mice performed multiple licks for both subpopulations of neurons (**Figure 5a**). By comparing PNs and the FSNs response duration, we found that the duration of the response was greater in the FSNs during both single and multiple licks compared to PNs (**Figure 5b, c**).

Finally, to compare the magnitude of the activation of the two neuronal classes, we measured the mean firing rate above – or below – the thresholds. During multiple licks, both PNs and FSNs showed greater discharge than during a single lick (**Figure 5d**). Furthermore, the FSNs displayed a higher activity relative to PNs, and this was more evident in multiple than in single licks (**Figure 5e, f**). These findings suggest the idea that the single or multiple licks are coded by the differential activity patterns of both PNs and FSNs, in terms of onset, peak discharge, duration and magnitude of neuronal activity.

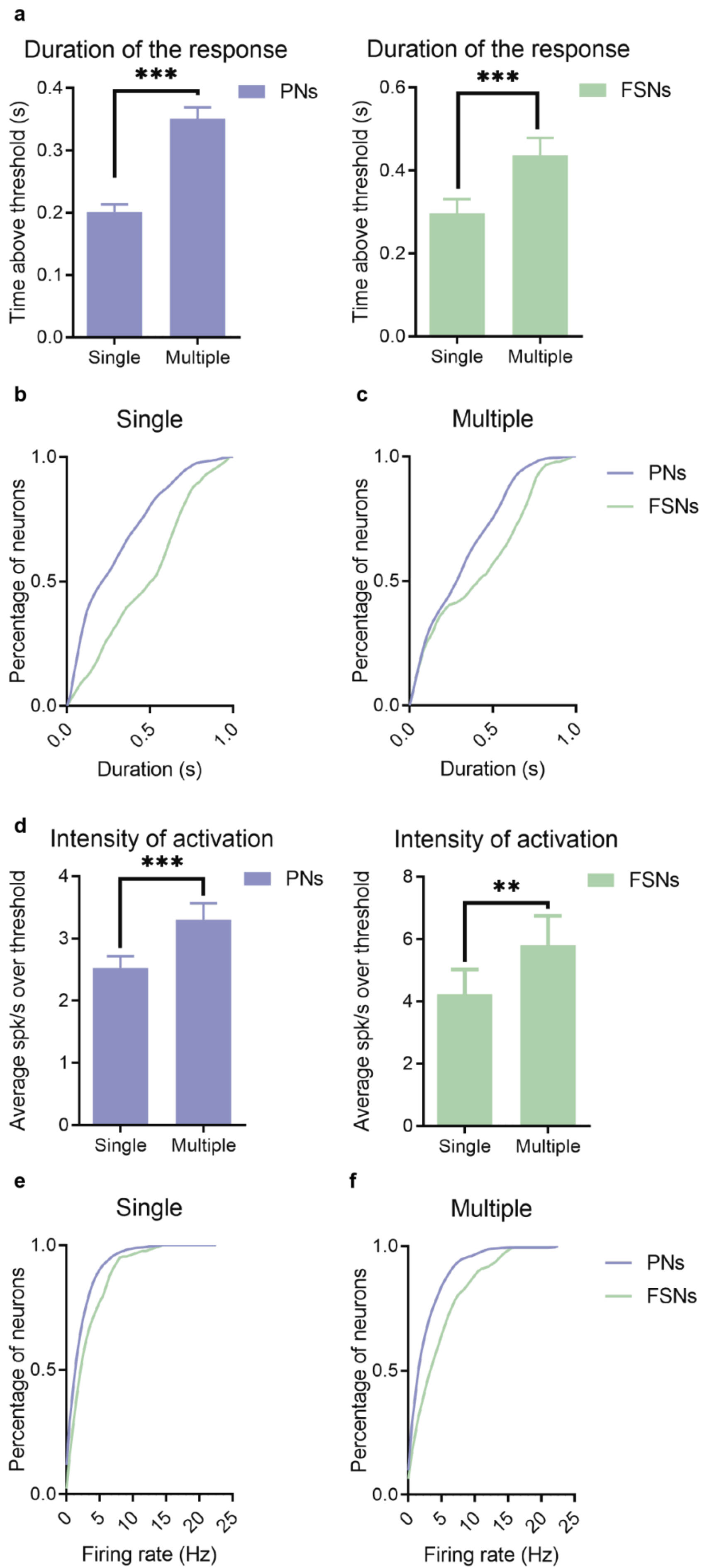
a
Duration of the response
***
PNs
Time above threshold (s)
0.4
0.3
0.2
0.1
0.0
Single
Multiple
Duration of the response
***
FSNs
Time above threshold (s)
0.6
0.4
0.2
0.0
Single
Multiple
b
Single
Percentage of neurons
1.0
0.5
0.0
0.0
0.5
1.0
Duration (s)
c
Multiple
Percentage of neurons
1.0
0.5
0.0
0.0
0.5
1.0
Duration (s)
PNs
FSNs
d
Intensity of activation
***
PNs
Average spk/s over threshold
4
3
2
1
0
Single
Multiple
Intensity of activation
**
FSNs
Average spk/s over threshold
8
6
4
2
0
Single
Multiple
e
Single
Percentage of neurons
1.0
0.5
0.0
0
5
10
15
20
25
Firing rate (Hz)
f
Multiple
Percentage of neurons
1.0
0.5
0.0
0
5
10
15
20
25
Firing rate (Hz)
PNs
FSNs

Figure 5. a. Histograms of the changes in the duration of the response of PNs (left) and FSNs (right), during single and multiple licks: during the multiple licks the time above (or below) the threshold is greater for both PNs and FSNs. Mean duration: PNs, Single = 0.201 s, Multiple = 0.350 s; FSNs, Single = 0.297 s, Multiple = 0.436 s; Wilcoxon matched paired Signed Rank Test, PNs, W = 8645, p < 0.0001; FSNs, W = 606, p = 0.0002, , *** *p* < 0.001. **b, c**. Cumulative distribution of the duration of the response for all the neurons, PNs and FSNs, during the single isolated lick (**b**) and consecutive multiple licks (**c**): a higher percentage of FSNs has a greater duration for both the conditions, single or multiple. K-S Test, Single, D = 0.2611, p = 0.0158; Multiple, D = 0.2394, p = 0. 0269. **d**. Histograms of the intensity of activity of PNs (left) and FSNs (right), during single and multiple licks: during multiple vs single licking events, the intensity of activation is significantly higher for both PNs and FSNs. Wilcoxon matched paired Signed Rank Test, PNs, W = 4917, p < 0.001; FSNs, W = 534, p = 0.0014, ** *p* < 0.01, *** *p* < 0.001. **e, f**. Cumulative distribution of the intensity of activation for all the neurons, PNs and FSNs, during the single isolated lick (**e**) and consecutive multiple licks (**f**): note that FSNs have a greater intensity of activation for both the conditions, single or multiple. K-S Test, Single, D = 0.2194, p = 0.0655; Multiple, D = 0.2819, p = 0. 0051.

4.4 Information content of Firing Rate

The data reported in the previous section demonstrate robust differences in the discharge pattern of PNs and FSNs during single and multiple licks. FSNs were active prior to movement onset and PNs activation, showing a more sustained and intense activity than PNs. The distinct behaviour of PNs and FSNs can be appreciated by building mean PSTHs for the two classes of neurons (**Figure 6a, b**).

We next computed for all the recorded units the mutual information between the local firing rate and the behavioural states (i.e., rest, single lick and multiple licks; see Methods).The fraction of informative neurons was 0.74 for FSNs and 0.63 for PNs. Within the subset of significantly informative neurons, FSNs carried vastly more information than PNs about the onset of both single and multiple licks: 0.074 bits for PNs, 0.130 bits for FSNs (p<0.001, rank test), comparing single lick and rest; 0.140 bits for PNs, 0.221 bits for FSNs, (p<0.001, rank test), comparing multiple licks and rest (**Figure 6c, d**).

Coherently with an earlier onset of the response, FSNs information content ramped up earlier than PNs. Information brought by FSNs became 3 SD larger than baseline (calculating in the interval from -0.8 s to -0.4 s before the event) for, at least, two consecutive bins, 40-50 ms in anticipation of that carried by PNs. When comparing single lick and rest, the information exceeded the previously described threshold 250 ms before onset of the movement in FSNs and 200 ms in PNs. Peak was then reached at 0 ms from the event in FSNs and 30 ms after the event in PNs.

The comparison of multiple licks with rest yielded similar results. The information exceeded the threshold 330 ms before the onset of the movement in FSNs and 270 ms in PNs. Peak was then reached 20 ms after the event in FNS and 50 ms after the event in PNs. This

dynamics was similar to the responsivity illustrated by the PSTHs results (compare **Figure 6c, d** with **Figure 6a, b**).

We then computed animal-wise the amount of information carried by the summed firing rate of the recorded FSNs and PNs population and we found that FSNs carried more redundant information. The Normalized Information carried by the sum of firing rate (Nisf, see methods) is significantly higher for FSNs than for PNs (mean 0.08 for PNs, 0.26 for FSNs, comparing single lick and rest; mean 0.20 for PNs, 0.25 for FSNs, comparing multiple licks and rest) (**Figure 6e**).

Overall, these results suggest that the local firing rate of FSNs conveys a considerable amount of information prior to PNs activation, further supporting the idea that a robust and coherent inhibitory activity might be important during the planning of the movement.

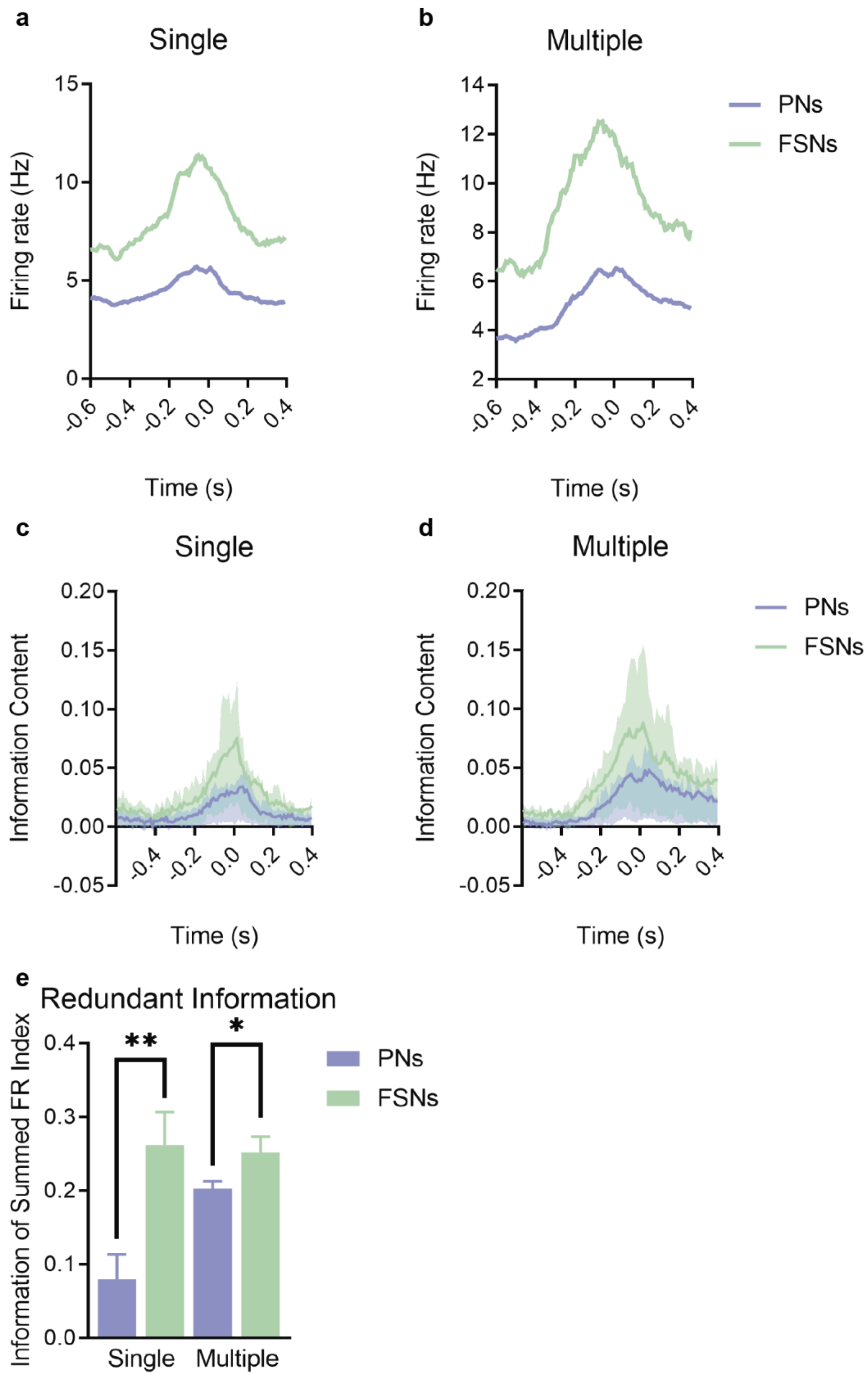
a
Single
Firing rate (Hz)
15
10
5
0
-0.6
-0.4
-0.2
0.0
0.2
0.4
Time (s)
b
Multiple
Firing rate (Hz)
14
12
10
8
6
4
2
-0.6
-0.4
-0.2
0.0
0.2
0.4
Time (s)
PNs
FSNs
c
Single
Information Content
0.20
0.15
0.10
0.05
0.00
-0.05
-0.4
-0.2
0.0
0.2
0.4
Time (s)
d
Multiple
Information Content
0.20
0.15
0.10
0.05
0.00
-0.05
-0.4
-0.2
0.0
0.2
0.4
Time (s)
PNs
FSNs
e
Redundant Information
Information of Summed FR Index
0.4
0.3
0.2
0.1
0.0
**
*
Single
Multiple
PNs
FSNs

Figure 6. a, b. Average PSTHs for all recorded PNs (violet) and FSNs (green) in a 1 s window (0.6 s before and 0.4 s after the licking event) during single (**a**) and multiple (**b**) licks. **c, d**. Information carried by firing rate of PNs (violet) and FSNs (green) about the onset of single (c) and multiple (d) licks. Information is computed over 50 ms bins in a 1 s window (0.6 s before and 0.4 s after the licking event). lower and higher shades represent, respectively, the 25 and 75 percentile. **e**. Information content redundancy for couple of PNs and FSNs of the same recording session for both single and multiple licks. Mann-Whitney Test, Single, U = 6864, p = 0.0058; Multiple, U = 7456, p = 0.044, ** $p < 0.01$ and * $p < 0.05$.

4.5 Layer-specific responses of PNs and FSNs

The use of linear probes allowed us to investigate the laminar distribution of the response properties of the recorded neurons. Specifically, units were classified as superficial (channels 1-8, i.e. recorded at a cortical depth ~ < 400 µm) or deep (channels 9-16, ~ > 400 µm depth) neurons. In our sample, about 25% of PNs and FSNs were recorded from superficial layers. **Figure**s **7a, b** report the onset of activity for each recorded unit as a function of depth (i.e. channel number). From the plot, it is clear that while the average onset of the response of FSNs precedes the one of PNs (consistent with the data reported in **Figure**s **4a-c**), a small proportion of PNs appear to increase their firing rate early in time, simultaneously with FSNs (**Figure 7a, b**).

The early-birds PNs appeared to be more prevalent in deep than in superficial layers. Indeed, by splitting the temporal window before licking onset into two segments of 0.3 s each (-0.6/0.3 s and 0.3/0 s), we found that the percentage of deep PNs was higher in the first vs. the second segment (**Figure 7c, d**).

These results confirm that preparatory activity related to licking begins in deep layers of ALM (Chen et al., 2017), and involves both FSNs and PNs.

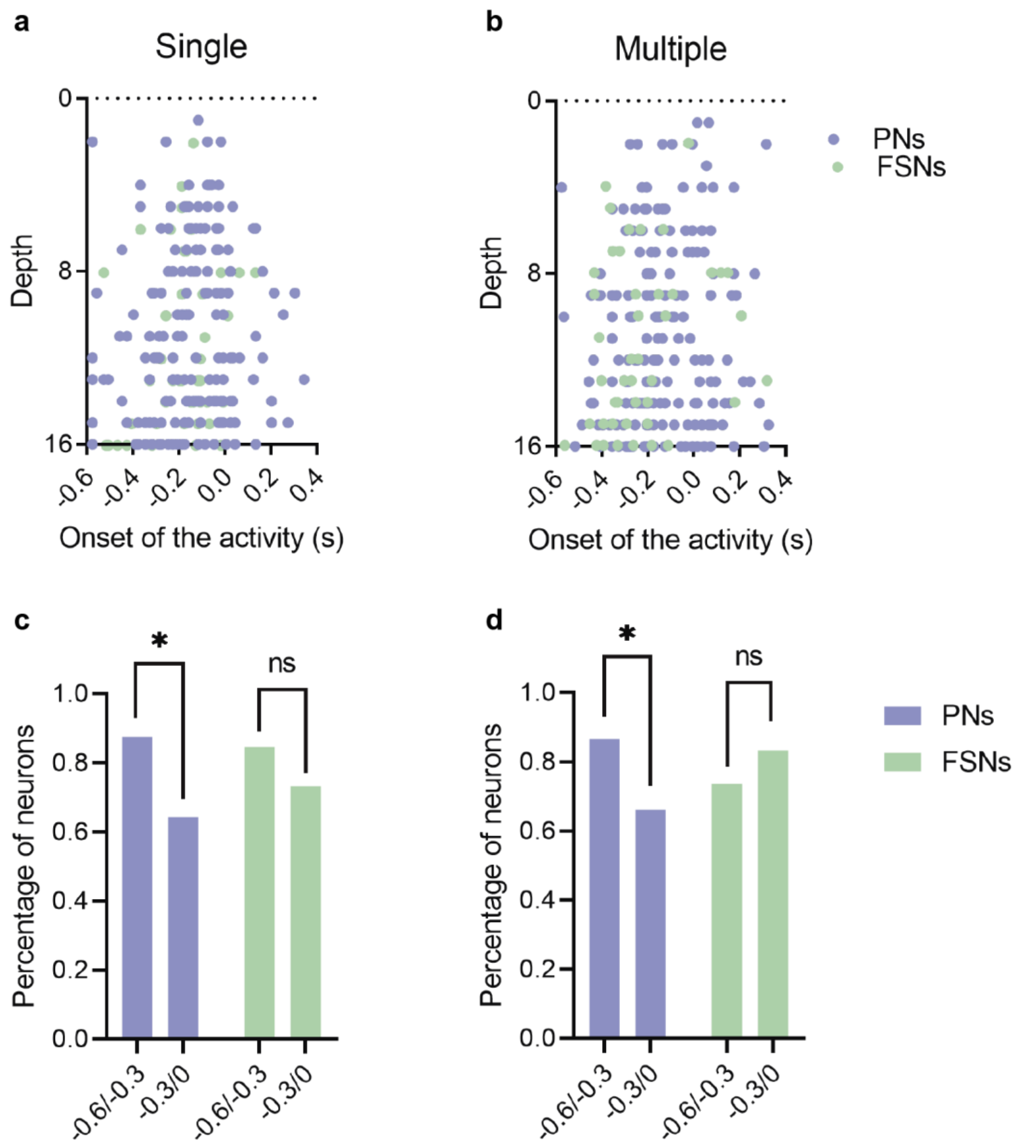

Figure 7. a, b. PNs (violet) and FSNs (green) depth distribution (across sixteen channels probe) of the onset of the activity in a 1 s window (0.6 s before and 0.4 s after the licking event) during single (**a**) and multiple (**b**) licks. **c, d**. Percentage of deep PNs (violet) and FSNs (green) into two time segments before the licking event (- 0.6, - 0.3 and - 0.3, 0.0) during single (**c**) and multiple (**d**) licks. *$p < 0.05$.

4.6 Direct comparison of the neuronal responses of PNs and FSNs during two types of movement

Several hypotheses may explain the precocious and sustained activation of FSNs in preparation of licking. We tested the idea that this early inhibition occurs during action planning, independent of the type of movement. Therefore, we went on to compare FSNs and PNs activity during two types of motor tasks, i.e. licking and forelimb retraction. We took advantage of a robotic platform (M-Platform) (Allegra Mascaro et al., 2019; Spalletti et al., 2017), which allows mice to perform several trials of forelimb pulling. Neuronal discharges were aligned on

the onset of force peaks generated by the forepaw and recorded by a load cell (Pasquini et al., 2018; Spalletti et al., 2014). Animals also performed spontaneous licking within the same experimental session, allowing us to simultaneously probe neural responses to the two different motor tasks. We start by describing the neuronal discharges during “isolated” (i.e. spaced by more than 1 s) pulling and multiple licking events (see below for interactions between the two movements).

For these experiments we employed a chronic microelectrode array (4x4) which was purposefully centered on the ALM but exceeding the boundary with the adjacent RFA. The RFA, a putative premotor area mapping the mouse forelimb (Alia et al., 2016; Tennant et al., 2011), is centered at 2 mm anterior and 1.2 mm lateral to Bregma and partially overlaps with the ALM. Electrode contacts were positioned in deep layers; we isolated n = 373 responsive single units (PNs, n = 313; FSNs, n = 60; mice, n = 3) in 10-15 experimental sessions per animal.

During these chronic recordings, we found a higher proportion of neurons whose discharge was suppressed by movement, with respect to previous data collected in acute recordings. Specifically, 40% of PNs, whose discharge was modulated during forelimb retraction, showed movement-related suppression of their discharge; a similar proportion (37.0%) of PNs responsive for licking behaviour were also suppressed. For FSNs, the percentages of suppressed neurons were similar (39.1%) for forelimb retraction, and lower (20.3%) for licking. These data suggest that corticofugal pyramidal neurons as well as FSNs located in deep layers are particularly susceptible to movement-related suppression. Therefore, we analysed the enhanced and suppressed neurons separately. A summary of the number and properties of isolated units is reported in **Table 2**.

	Lick Enh	Lick Supp	Lick Enh / FP Supp	Lick Enh / FP Enh	Lick Supp / FP Supp	Lick Supp / FP Enh	FP Supp	FP Enh
PNs	52	31	31	96	55	27	6	15
FSNs	7	2	9	31	11	-	-	-

Table 2. Number of neurons in different functional classes. *Lick, licking; FP, forelimb pulling; Enh, enhanced; Supp, suppressed.*

4.7 FSNs exhibit lower selectivity than PNs for licking behaviour and forelimb retraction

Overall, the analysis of single neurons properties revealed no evidence of clear segregation of function in the ALM and the sampled portion of RFA. Indeed, the majority of the

recorded units modulated their firing rate during both licking and limb retraction, confirming partially overlapping neuronal networks for the two movement patterns. The selectivity for each type of movement was quantified based on the amplitude of the discharge modulation by means of an index ranging from “1” to “-1”, with “1” indicating pure selectivity for licking, “-1” for forelimb retraction, and “0” an identical response during both acts (**Figure 8a**). We found that licking was the preferred neuronal response for all the recorded units, consistent with the position of the array which was centered on the ALM. Overall, we found that PNs displayed more variability in their responses to the two different motor acts compared to FSNs. We categorized neurons into either “forelimb retraction-specific”, “licking-specific”, and “forelimb+licking” based on their selectivity indexes. This analysis revealed that FSNs were less tuned than PNs (**Figure 8b**; Chi-square test, 9.13, $p = 0.01$), consistent with the data previously reported for “single” and “multiple” licks (**Figure 3b**).

To further investigate the response properties of PNs and FSNs during the two motor tasks, we subdivided the recorded units into different functional classes, according to the movement-induced modulation of their discharge. Specifically, neurons responsive to only one type of movement were classified as enhanced/suppressed by licking (L+, L-) or forelimb pulling (F+, F-). Neurons responsive to both movements showed either a concordant (L+/P+, L-/P-) or opposite modulation (L+/P-, L-/P+) during each motor task. We found that PNs (violet bars in **Figure 8c**) were distributed across all functional classes. In contrast, the vast majority of FSNs (> 72%) were consistently modulated (i.e., suppressed or enhanced) by the two different movements (i.e., L+/F+, 50% and L-/F-, 20%). The functional distributions of PNs and FSNs were significantly different (**Figure 8c**). These data are in agreement with the previous findings which indicate a broader tuning of FSNs, most of which appear to increase their discharge irrespective of the movement type.

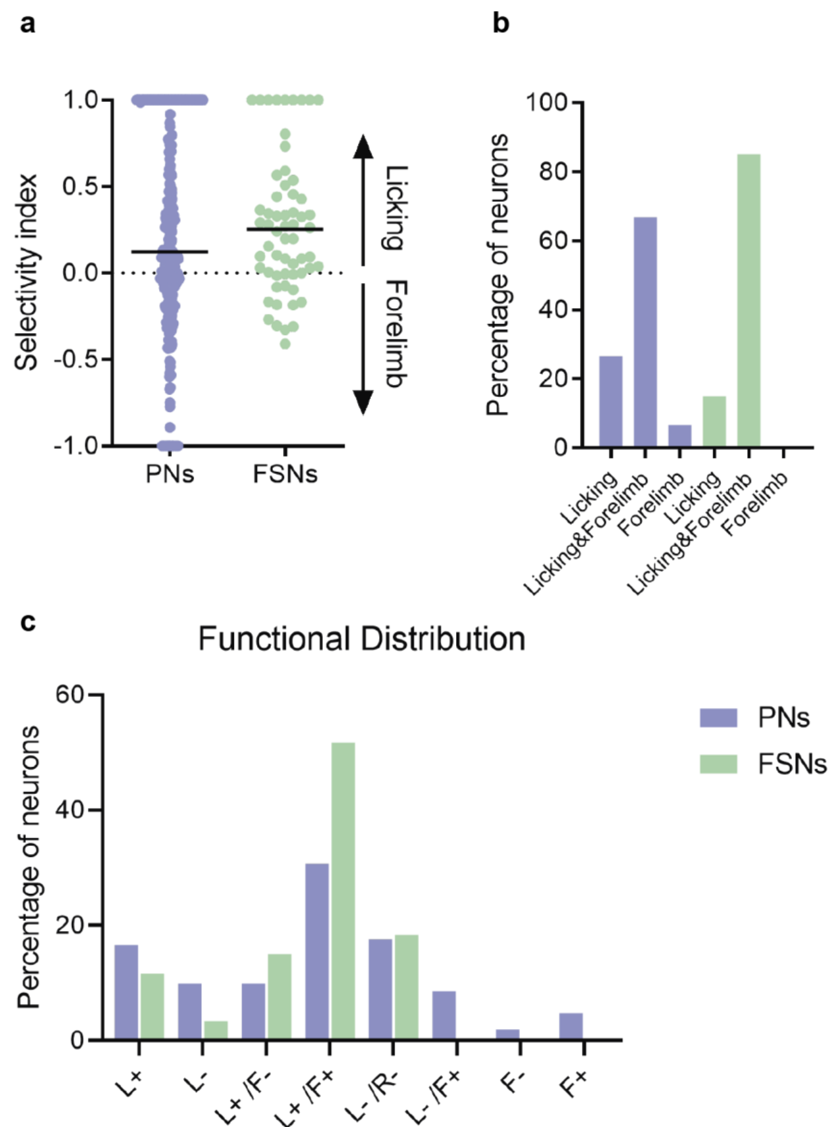

Figure 8. a. The peak of the response (spk/s) is used to calculate a selectivity index (see methods) for the two spontaneous movements, licking vs forelimb, going from -1 (neurons selective for forelimb retraction) to +1 (neurons selective for licking behavior). The black horizontal line corresponds to the median value. **b**. Percentage of neurons selective for: both licking and forelimb retraction, for licking or forelimb pulling individually. **c**. Functional distribution of neurons responsive for licking (L), forelimb pulling (F) or both of them (L/F), classified as enhanced (+) or suppressed (-) by the movement. PNs show a broader functional distribution across classes, while most of the recorded FSNs are enhanced by L and F. Chi-square test, 20.19, p = 0.0052.

4.8 Onset and duration of discharge of PNs and FSNs during licking and forelimb pulling

The majority of FSNs, in keeping with previous results, were facilitated during movement execution and started to discharge before facilitated PNs (**Figure 9a, b**). The onset of the suppressed PNs was even more delayed during licking but not forelimb retraction

(**Figure 9a, b**). Note, however, that, consistent with the laminar recordings (**Figure 7**), a small subset of pyramidal neurons (approx. 15%) modulated their discharges very early, especially during forelimb retraction (**Figure 9a**). Interestingly, the suppressed FSNs showed a delayed discharge onset relative to the enhanced FSNs, especially during licking (**Figure 9a, b**).

In terms of duration of the response, this was significantly greater for the FSNs, specifically those excited, considering both pulling (**Figure 9c**) and licking (**Figure 9d**). The small proportion of suppressed FSNs showed a shorter duration of modulation, although not statistically different from that of enhanced FSNs (**Figure 9c, d**). There was no difference in the discharge duration between enhanced and suppressed PNs (**Figure 9c, d**).

Altogether, these data concur with the previous laminar recordings indicating an early and prolonged discharge of fast-spiking, GABAergic neurons activated by movement. Interestingly, the suppressed neurons were modulated at longer latencies during movement generation.

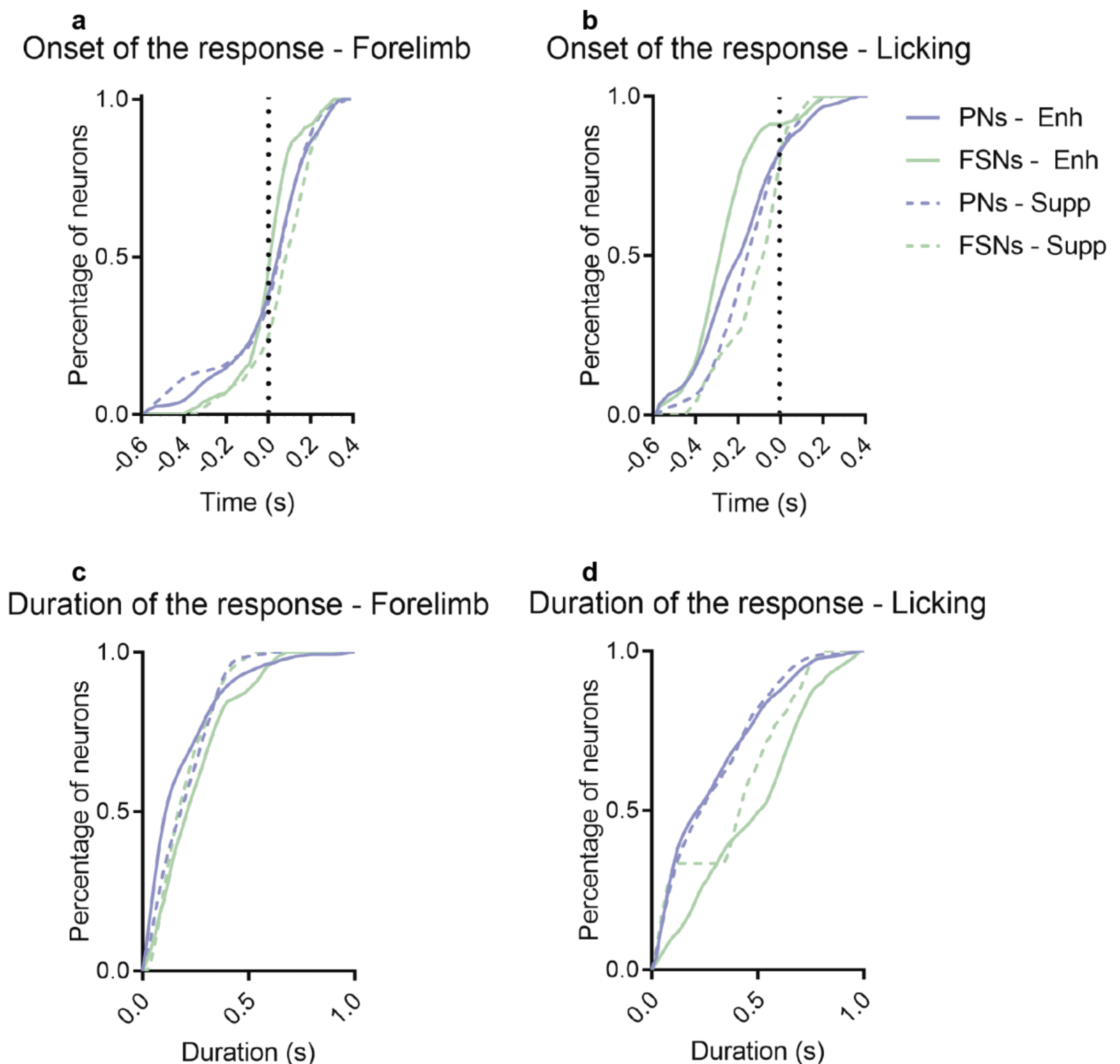

Figure 9. a, b. Cumulative distribution of the onset of the response for all neurons during the forelimb retraction (**a**). Cumulative distribution of the onset of the response for all neurons during a licking bout (**b**).
Enhanced PNs vs suppressed PNs: Licking, K-S Test, D = 0.171, p = 0.0431; Forelimb Retraction, K-S Test, D = 0.0761, p = 0.906.
Enhanced FSNs vs suppressed FSNs: Licking, K-S Test, D = 0.533, p = 0.0090; Forelimb Retraction, K-S Test, D = 0.342, p = 0.116.
Enhanced PNs vs enhanced FSNs: Licking, K-S Test, D = 0.280, p = 0.0069; Forelimb Retraction, K-S Test, D = 0.252, p = 0.081.
c, d. Cumulative distribution of the duration of the response for all neurons during the forelimb retraction (**c**). Cumulative distribution of the duration of the response for all neurons during a licking bout (**d**).
Enhanced PNs vs. enhanced FSNs: Forelimb Retraction, K-S Test, D = 0.288, p = 0.029; Licking, K-S Test, D = 0.326, p = 0.0009.
Enhanced FSNs vs suppressed FSNs: Licking, K-S Test, D = 0.246, p = 0.610; Forelimb Retraction, K-S Test, D = 0.621, p = 0.216.
Enhanced PNs vs suppressed PNs: Licking, K-S Test, D = 0.0558, p = 0.987; Forelimb Retraction, K-S Test, D = 0.156, p = 0. 137.
Enhanced neurons are represented as continuous lines (PNs, violet; FSN, green), dotted lines indicate the suppressed PNs and FSNs (PNs, violet; FSN, green).

4.9 Modulation of neuronal discharges of PNs and FSNs by nearby licking and pulling events

During the experimental sessions, we noted that mice occasionally performed single licks in close temporal proximity with forelimb retractions. This allowed us to evaluate how the interaction of the two movements impacted PNs and FSNs discharges.

In the subset of PNs and FSNs enhanced by both licking and forelimb retraction – since they represented most of the recorded neurons –, we compared the mean firing rate of each neuron during isolated trials, to trials in which the licking and forelimb peaks of activation were in close proximity (distance between two peaks < 0.1 s) (**Figure 10**; see Methods for details). Mean licking (**Figure 10a**) or pulling (**Figure 10b**) firing rates of FSNs did not change when there was a concomitant pulling or licking trial, respectively. Instead, PNs increased both licking- (**Figure 10c**) and forelimb pulling- (**Figure 10d**) induced firing rates in the presence of a nearby movement.

These findings indicate that FSNs firing rates are not influenced by a concomitant movement. In contrast, discharges of PNs appear to be enhanced by nearby pulling/licking events.

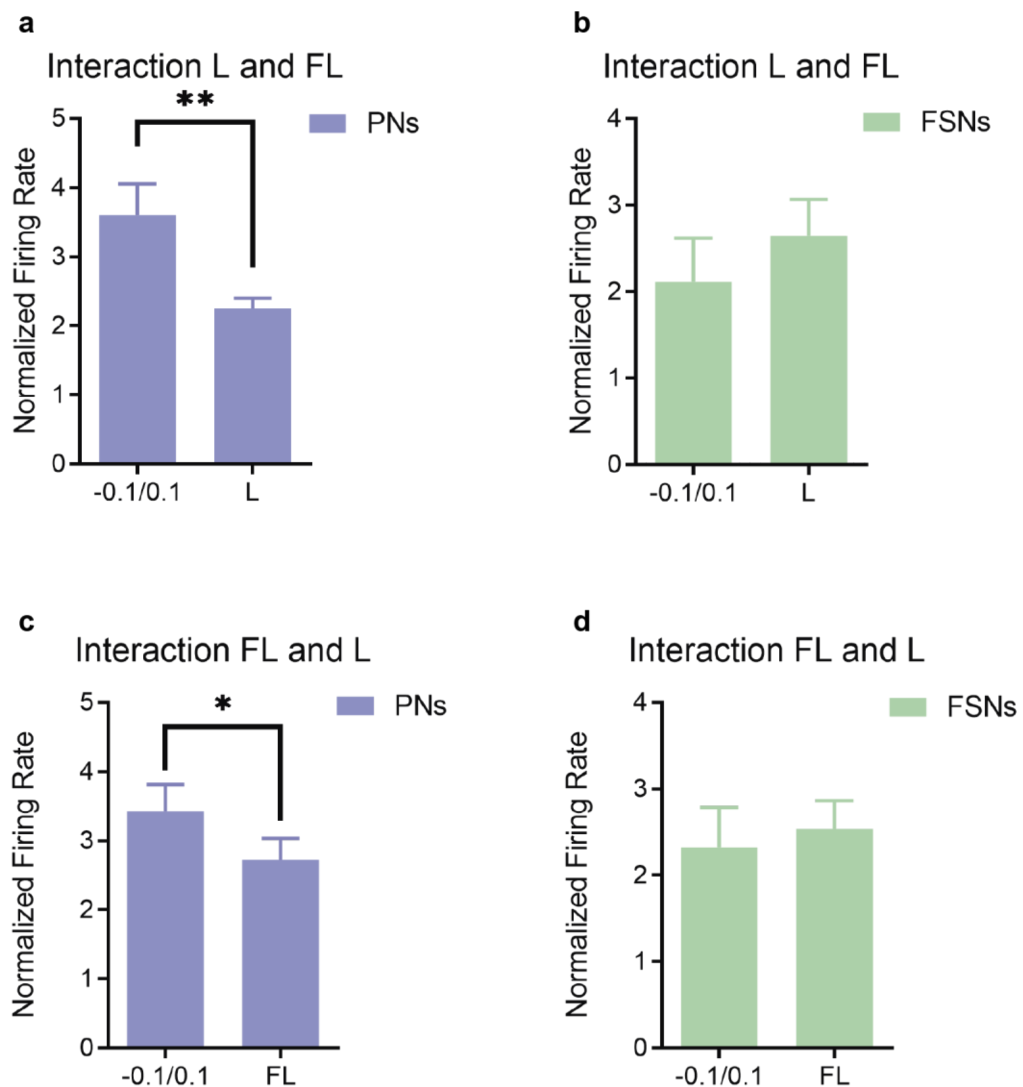

Figure 10. a, b. The mean firing rate during isolated, single licks (L), measured around the expected peak of activity, of enhanced PNs and FSNs, far from other tongue or forelimb movements (> 1 s), compared with the mean firing rate, of the same neurons when a forelimb discharge occurs 0.1 s before or after the lick discharge. Paired t-test, t = 0.91, p = 0.39 (**a**). Paired t-test, t = 0.58, p = 0.57 (**b**). **c, d**. The mean firing rate during isolated, single forelimb pulling (FL), measured around the expected peak of activity, of enhanced PNs and FSNs, far from other tongue or forelimb movements (> 1 s), compared with the mean firing rate, of the same neurons when a lick discharge occurs 0.1 s before or after the forelimb discharge. Wilcoxon matched paired Signed Rank Test, W = 183, p = 0.0007 (**c**). Paired t-test, t = 2.70, p = 0.015 (**d**), * $p < 0.05$, ** $p < 0.01$.

4.10 Social Facilitation during Licking Behaviour

The observation of an action can lead to an increase in the frequency or intensity of behaviours already present in an animal's repertoire, or in the initiation of particular behaviours, recruiting similar motor programs. Since the phenomenon occurs in the presence of others, engaged in the same behaviour, it has been marked as social facilitation. A behaviour particularly sensitive to social input is eating (Clayton, 1978; Fragaszy and Visalberghi, 2004). In fact, social facilitation of eating behaviour has been reported for a variety of animals (Birch

and Marlin, 1982; De Castro, 1990; de Castro and Brewer, 1992; Keeling and Hurnik, 1993). The mechanism allows individuals to recognise other's actions has been recognized as the mirror system. Indeed, observing or hearing actions related to feeding behaviour activate the motor representation of those same actions (Ferrari et al., 2005).

To analyse social facilitation in mice, first we implemented a novel behavioural test based on the one used in monkeys (Ferrari et al., 2005). We took advantage of licking behaviour to set up the social facilitation experiments. Therefore, head-restrained mice were allowed to lick condensed milk from a feeding needle. In order to isolate the effects of action observation on the observer's motor behaviours related to drinking, we compared the observer's drinking behaviour during three periods: (B) baseline period, during which the demonstrator was obscured, (D) a control period, during which the demonstrator was present but not drinking because the bottle was not available and (D + B) a testing period, in which the demonstrator was present and allowed to drink. The drinking spout was always positioned in front of the observer' snout. The baseline period was always the first condition, because it was supposed that at the end of it the mouse observer was satiated. The order of the two conditions "D" and "D + B" was randomly balanced. During testing (condition "D+ B"), all demonstrators provided the input required by the experimental design, i.e. they drank many times from the water bottle available in the cage.

We measured the observer's behaviour and we found that the observer's licking was by far longer in duration and more frequent during the testing period "D + B" and the baseline than during the "D" condition (**Figure 11a, b**). We also evaluated the first time the observers started to lick, showing that during the control period the first latency occurred later (**Figure 11c**). The results were independent from the order of the presentation of "D" and "D + B", as shown in **Figure 11d, e**, where we splitted sessions in which the "D" or the "D + B" were carried out as the second condition, immediately after the baseline period. We did not find any difference for both licking duration (**Figure 11d**) and frequency of licking events (**Figure 11e**).

These findings suggest that, although the mouse was not motivated to drink after baseline, it was facilitated to lick more when another individual was engaged in the same action, supporting the hypothesis of a social facilitation in mouse.

To exclude that the mere presence of the bottle can increase the time and the frequency of licking in the observer, in another cohort of animals, we introduced a bottle condition (B), in which the demonstrator was not present and there was only the bottle in the empty cage. During the test condition ("D + B"), the total duration of the observer's licking behaviour was greater than the two control periods, "D" and "B" (**Figure 11f**). This suggests that the observed increase in licking was not due to the presence of the demonstrator or the bottle per se, rather the time spent to lick was higher when the action was elicited by the demonstrator.

Finally, we focused our analysis during the testing period (condition “D + B”). We took the start and the end time of the demonstrator’s and observer’s licking intervals. The percentage of (i) licking bouts of the observer begun when the demonstrator was licking and (ii) duration of the overlapping demonstrator’s and observer’s licking intervals were calculated. Then, we scrambled the position of the demonstrator’s licking intervals and same percentages were re-measured. In **Figures 11g, h** we compared the real data set with the scrambled data set; we showed that the probability that the observer started to lick was higher when the demonstrator was actually licking (real data) than a randomized data set. (**Figure 11g**). Moreover, the duration of the overlapping intervals was also higher (**Figure 11h**).

Altogether these results indicate that the observers’ behaviour was actually influenced by the demonstrators’ one.

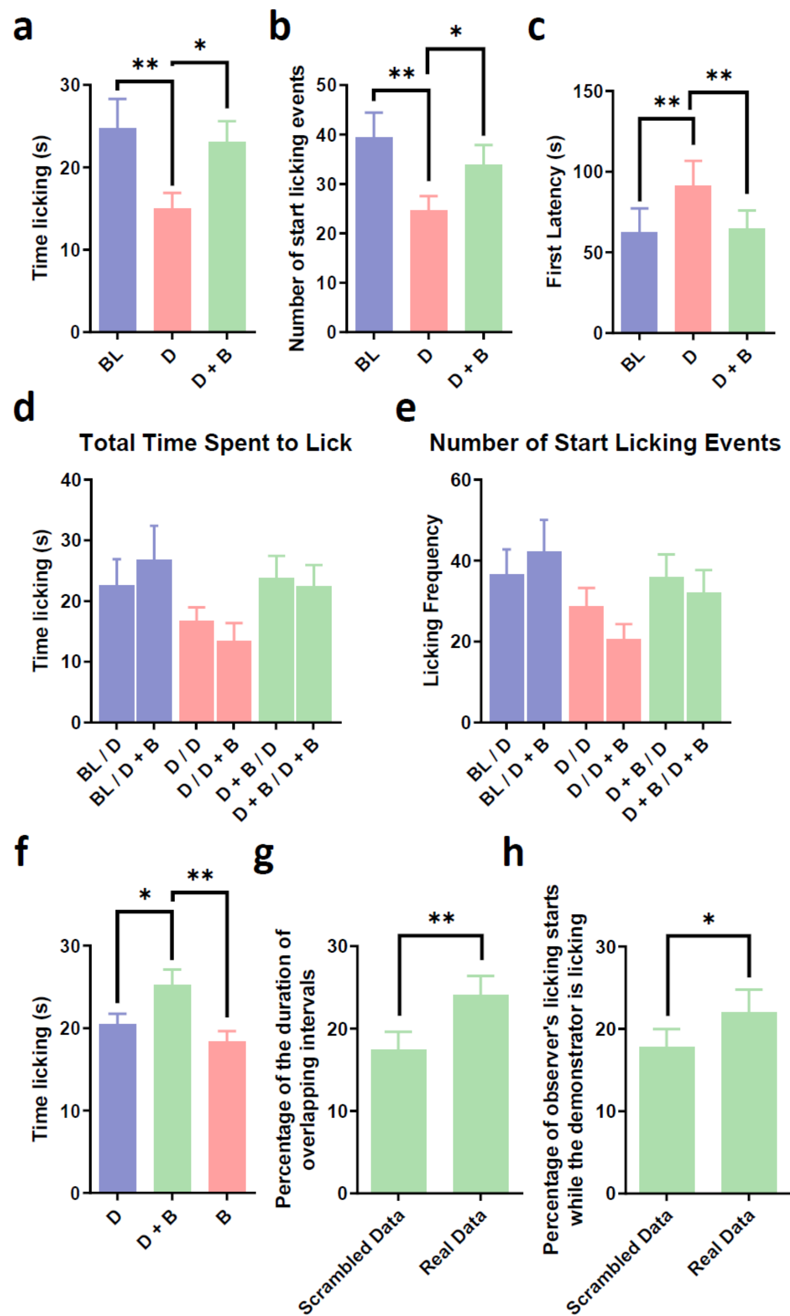

Figure 11. Social facilitation in mouse during the licking behaviour. a, b, c. Total time spent to lick by the observer (**a**), total number of observer start licking events (**b**) and time of the first lick of the trial (**c**) during 3 periods: baseline (BL), demonstrator (D), in which the demonstrator cannot lick, and demonstrator plus bottle (D + B), in which the demonstrator is allowed to lick. Time licking, F = 5.58, p = 0.005; Start Licking Events, F = 5.99, p = 0.005; One Way RM ANOVA, Tukey post-hoc. F = 13.74, p = 0.001; Friedman Test, Dunn's test (**c**). **d, e**. Total time spent to lick by the observer (**d,** Two way RM ANOVA, Interaction, $F_{(2, 86)}$ = 0.76, p = 0.47, Condition, $F_{(2, 86)}$ = 5.48, p = 0.0057, Group, $F_{(1, 43)}$ = 0.002, p = 0.97) and total number of observer start licking events (**e,** Two way RM ANOVA, Interaction, $F_{(2, 86)}$ = 1.35, p = 0.26, Condition, $F_{(2, 86)}$ = 5.92, p = 0.0039, Group, $F_{(1, 43)}$ = 0.11, p = 0.74) during 3 periods, baseline (BL), demonstrator (D), in which the demonstrator cannot lick, and demonstrator plus bottle (D + B), in which the demonstrator is allowed to lick, splitting sessions when the D period or the D + B period are presented as the second condition after BL. **f**. Total time spent to lick by the observer during 3 periods: demonstrator (D), in which the demonstrator cannot lick, demonstrator plus bottle (D + B), in which the demonstrator is allowed to lick, and bottle (B), when there is only the bottle and the demonstrator is not present in the cage, F = 1.99, *p* = 0.0023; One way RM ANOVA, Tukey's Post Hoc. **g, h.** The percentage of the overlapped duration between the demonstrator and the observer licking intervals (**g,** Paired t-test; t = 1.94, p = 0.038) and the percentage of the observer licking start events

when the demonstrator is licking (**h,** Paired t-test; $t = 3.044$, $p = 0.0051$), in the real data and a scrambled data set, in which the demonstrator licking intervals were scrambled in time and the percentage of the overlapping intervals re-measured.

5. Discussion

5.1 Distinct roles of PNs and FSNs during action planning

To directly initiate voluntary movements, preparatory activity is relevant to the upcoming action (Wise at al., 1985). This activity is prevalent in premotor areas, less in the primary motor cortex (Afshar et al., 2011), and it has a predictive role regarding the selection of specific movements (Georgopoulos et al., 1982; Churchland et al., 2006; Godschalk et al., 1985; Hocherman et al., 1991; Messier et al., 2000; Riehle et al., 1993; Churchland et al., 2007). Planning and execution of voluntary movement depend on the mutual contribution of distinct classes of neurons in premotor areas. However, the specific functional role of pyramidal and GABAergic, fast-spiking inhibitory neurons, to these computations, remains only partly understood.

In this thesis, I have directly compared the firing properties of two types of neurons mostly represented in the cortex, i.e. PNs and FSNs – since PV interneurons are more abundant in the motor areas (Whissell et al., 2015) –, during the execution of two distinct motor acts – licking behaviour and forelimb pulling in a robotic device. In particular, *in vivo* extracellular recordings were carried out from the ALM and RFA, which represent possible homologues of mouth and forelimb premotor cortices in primates. While the role played by PNs in the different layers has been elegantly dissected (Guo et al., 2014b; Li et al., 2016, 2015), little is known on the contribution of FSNs within the premotor areas. In these experiments, I demonstrate for the first time a crucial involvement of GABAergic neurons, specifically FSNs, during movement planning.

Altogether, my data reveal that FSNs carry a great amount of information during the planning of the action in the premotor cortex, showing an early and sustained discharge during the preparatory epoch. High inter-neuron redundancy suggests that FSNs act at a population level. Indeed, FSNs display a broad selectivity regarding the type of licking strategy used, i.e. a single lick or a burst of multiple licks, and the type of movement, tongue/mouth or forelimb pulling, compared to PNs. Concurrent execution of two different motor acts is ineffective on FSNs discharge, while it increases the averaged PNs firing rate.

First of all, recorded neurons were distinguished into narrow- and broad-spiking based on their spike waveforms (**Figure 1c, d**). Broad-spiking cells were classified as putative PNs, consistent with previous literature (Niell and Stryker, 2010). Narrow-spiking neurons showed higher baseline firing rates and shorter interspike intervals, consistent with their identification

as FSNs, which was further validated by optogenetic experiments in PV-Cre mice. However, we cannot rule out that a subpopulation of neurons with thin spike shapes actually represents pyramidal neurons, as shown in the monkey (Vigneswaran et al., 2011). On the other hand, our sample of putative PNs may include non-fast- spiking-interneurons (e.g., somatostatin-positive cells), which have spike shapes overlapping with those of PNs.

Our data (**Figure 2g, h**) confirmed that ALM pyramidal neurons show an activity that anticipate movement execution (Guo et al., 2014b; Li et al., 2016, 2015). Evidence for a premotor cortex in rodents has been accumulated since 1982 (Neafsey and Sievert, 1982). Specifically, the first identified area was the premotor area involved in the control of limb movements, defined as the RFA to distinguish it from the CFA in the primary motor cortex. More recently, a series of experiments have elucidated in the mouse the role of the ALM (partly overlapped with the RFA) in the preparation and execution of directional licking (Svoboda and Li, 2018).

On average, we clearly show that putative FSNs fire in anticipation of putative PNs within the same cortical module. Specifically, they start to modulate and reach their peak of activity earlier than broad-spiking cells (**Figure 4**). These findings are in agreement with a previous electrophysiological study examining discharges of FSNs and PNs in mouse primary motor cortex during sensory-triggered as well as voluntary forelimb reaches (Estebanez et al., 2017). Thus, the early involvement of FSNs appears to be a characteristic feature of both primary motor and premotor areas. Interneurons also appear to increase their firing rates more than putative PNs during movement planning and execution.

A novel finding of the present study is that the duration of activation appears to be greater for FSNs as compared to PNs (**Figure 5**). GABAergic interneurons, in addition to inhibit pyramidal neurons, connect strongly with one another. Moreover, these interneurons are coupled by electrical synapses formed by gap-junctions (Connors and Long, 2004; Galarreta and Hestrin, 1999; Gibson et al., 1999). These inhibitory networks do not result from random connections between different interneurons, but rather follow a precise logic (Pfeffer et al., 2013). PV cells, connect preferentially with each other strongly and at very high connectivity rates (Deleuze et al., 2019; Pfeffer et al., 2013; Szabadics et al., 2001; Tamás et al., 2000). Therefore, although sparse, PV cells are strongly and reliably inter- and self-connected and these features could be the basis of FSNs greater intensity of activation and duration of the response during the preparatory activity in premotor areas.

It is worth noting that, our analysis on the onset of the activity across all cortical layers revealed that, although PNs were, on average, involved later than FSNs during motor planning, especially in deep layers, there was a fraction of early-birds neurons, that could be characterized as PNs (**Figure 7**).

Our experiments do not yet determine the origin of preparatory activity. However, the presence of early birds neurons in deep layers is in agreement with the finding that preparatory activity appears first in deep layers of ALM (Chen et al., 2017). Moreover, preparatory/ramping activity in PNs within the ALM has been shown to be maintained by a recurrent excitatory loop that involves both the cortex and the ipsilateral thalamus (Guo et al., 2017). It is worth mentioning that deep layer pyramidal neurons (specifically, those located in layer VI) project to the ipsilateral thalamus (Molnár and Cheung, 2006; Reiner et al., 2003; Saiki et al., 2018; Shepherd, 2013). On the other hand, the thalamus has a powerful functional influence on the ALM, indeed inactivation of thalamus resulted in strong hyperpolarization of ALM PNs (Guo et al., 2017). Therefore, these data lead to the speculation that early birds PNs located in deep layers represent preparatory "master" neurons which are necessary to maintain the cortico-thalamic loop necessary for the preparatory activity, and which subsequently may command downstream, more executive PNs and FSNs.

However, although PNs seem to be involved at the forefront in the generation of this cortico-thalamic loop, it cannot be excluded that long-range excitatory thalamic afferents that directly target FSNs are also present. Indeed, in granular cortex, monosynaptic sensory thalamo-cortical input has been found on layer IV FSNs (Beierlein et al., 2003; Bruno and Simons, 2002; Cruikshank et al., 2010, 2007; Daw et al., 2007; Gabernet et al., 2005; Sun et al., 2013; Wilent, 2004). Thalamic inputs onto FSNs are stronger and faster than those impinging on neighboring layer IV pyramidal neurons (Bagnall et al., 2011; Cruikshank et al., 2010, 2007; Hull et al., 2009; Inoue and Imoto, 2006). Indeed, the short monosynaptic delay creates a tight window of opportunity for the integration of sensory inputs, and strongly increases both spatial and temporal resolution of incoming sensory signals. Therefore, this recurrent cortico-thalamic loop may sustain persistent firing activity in the FSNs.

How the information propagates across cortical layers of the same area is still unknown. However, FSNs receive the strongest glutamatergic input by local principal neurons (Avermann et al., 2012). In cytoarchitectonically less differentiated areas, as in rodent agranular frontal cortex, there is a weak interlaminar inhibitory-to-excitatory connectivity (Kätzel et al., 2011). The study by van Haeften et al. (van Haeften et al., 2003) in medial entorhinal cortex, which reports an absence of inhibitory-to-excitatory synapses from deep to superficial layers, supports the same conclusion. Van Haeften et al. furthermore report that only a small percentage of the observed synapses could potentially be classified as inhibitory-to-inhibitory, thus giving little evidence for such a connection from deep to superficial layers. By contrast, there is abundant evidence for rich intralaminar connectivity including excitatory-to-inhibitory and inhibitory-to-excitatory connections (Barthó et al., 2004b; Fino and Yuste, 2011; Kang and Brorsen, 1995; Kätzel et al., 2011; Kawaguchi, 2002; Otsuka and Kawaguchi,

2009; Somogyi et al., 1998). It might be that the early-birds PNs will excite FSNs, that might be responsible to regulate the appropriate PNs response locally and the response in both PNs and others FSNs also in more superficial cortical layers.

The earlier and stronger activity of FSNs compared to PNs is also accompanied by a reduced selectivity for the type of licking strategy, (i.e. single vs. multiple licks) (**Figure 3**). PNs showed a more specific activity than FSNs, which showed instead a broader tuning. It has been proposed that cortical interneurons inhibit their target without much specificity providing a blanket of inhibition onto PNs, and disinhibitory circuits can open holes in this blanket to allow local computations (Fino et al., 2013; Karnani et al., 2016, 2014). Fast-spiking interneurons are coupled by electrical synapses formed by gap-junctions, which efficiently propagate slow membrane potential fluctuations between FSNs (Connors and Long, 2004; Galarreta and Hestrin, 1999; Gibson et al., 1999). FSNs synchronize themselves as an inhibitory network that is formed exclusively by other FSNs. This property may result in a poor selectivity for the type of motor act, as the discharge of few FSNs may trigger activation of other cells of the network. This is in keeping with the high intra-neuron redundancy that we observed in our computational analyses (**Figure 6**).

In different cortical areas, it has been shown that specific PNs types are differently inhibited by FSNs (Allene et al., 2015; Hilscher et al., 2017; Lee et al., 2014). This highly specific inhibitory diagram ultimately defines the activity of specific subnetworks, layers and cortical areas. Therefore, the specific expression and connectivity of FSNs could likely define the output properties of PNs and the functional characteristics of a specific cortical layer and area.

In the second set of experiments, using chronically implanted silicon microelectrode arrays, we found a higher percentage of suppressed neurons, including both PNs and FSNs. For what concerns FSNs suppressed by movement, the onset data (**Figure 9a, b**) clearly show that they are consistently delayed with respect to other populations. This late involvement of suppressed FSNs is in line with findings obtained during spontaneous forelimb reaching in the mouse (Estebanez et al., 2017). Since parvalbumin-positive FSNs form a highly interconnected set of neurons (Lourenço et al., 2020), it is likely that the suppressed fast-spiking population receive direct synaptic input from the enhanced FSNs.

PNs and FSNs recorded in the present study exhibited robust differences in tuning for the type of movement. Indeed, FSNs were less selective for movement type than PNs, not only for the licking strategy used, i.e. multiple vs single licks (**Figure 3**), as mentioned above, but also for two completely different movements, i.e. tongue vs paw movements.

When forelimb retraction and licking were considered, PNs showed a variety of behaviours and were distributed in several functional classes, with enhancement or suppression of

discharge depending on the specific movement (**Figure 9**). On the contrary, the percentage of suppressed FSNs were lower, and they often displayed increased firing rate relative to baseline during both pulling and licking. This is in line with previous results showing that in the prefrontal cortex of mice performing a sensory discrimination task, parvalbumin-positive interneurons are activated by all task-related events (sensory cues, motor action, and trial outcomes), while responses of PNs are diverse and more selective (Pinto and Dan, 2015). Recordings from the monkey dorsal premotor cortex consistently demonstrate a higher functional heterogeneity of PNs as compared to interneurons (Kaufman et al., 2010). The broad tuning of FSNs is consistent with data in sensory cortices, where inhibitory interneurons are poorly selective for stimulus features, such as orientation selectivity (Hofer et al., 2011; Kerlin et al., 2010).

It has been hypothesized that the activity of interneurons, including FSNs, provides an inhibitory gate that prevents preparatory activity from causing undesired movements. If this were the case, interneuron firing rates should be reduced around the time of movement, which was not observed in the present experiments. Another possibility is that FSN-mediated inhibition may serve to suppress other actions (e.g., movement of other body parts). If FSNs act to prevent adjacent cortical modules from producing other movements, one would predict the existence of distinct licking- and forelimb-related FSNs which reciprocally inhibit the respective PNs. We tested this idea in an experimental setting where the animal is monitored for spontaneous licks and forelimb pulling. However, our data do not provide support for such a model, as more than 50% of FSNs increase their discharge during both licking and forelimb retraction. Thus, a sustained, overall rise in FSN inhibitory activity appears to be required during action planning.

This idea is supported by our analysis of nearby licking/pulling events, performed on neurons excited in both conditions, thus those neurons shared among pulling and licking pathways. Our results show that PN (but not FSN) activity is increased by a nearby (closer than 0.1 s) motor action. Interestingly, a nearby movement triggers the same sustained firing of FSNs observed when the mouse is performing a single action, possibly providing a sufficient inhibitory milieu for correct execution of the subsequent lick/forelimb retraction. Instead, PN activity appears to be dependent on the number of upstream activated motor commands (**Figure 10**).

Several hypotheses may be put forward to explain the early, prolonged and broadly tuned activation of FSNs reported in the present study. (i) The discharge of fast-spiking interneurons may be required to sculpt the response selectivity of nearby pyramidal neurons. In the visual cortex, optogenetic enhancement of the spiking activity of parvalbumin-positive interneurons leads to sharpened orientation tuning and improved direction selectivity of PNs (Lee et al., 2012). In the auditory system, activation of parvalbumin cells increases the signal-to-noise ratio and reduces receptive field bandwidths, indicative of a narrower range of effective stimuli

(Hamilton et al., 2013). In the motor cortex, PNs discharge according to the direction of upcoming movement, with a peak of activity in the preferred direction (Galiñanes et al., 2018; Georgopoulos et al., 1982), and the magnitude of inhibition directly affects tuning of individual PNs before and during movement execution. Specifically, an increase of inhibition is coupled to a decrease in tuning dispersion, i.e. greater selectivity (Merchant et al., 2008). (ii) Activity of FSNs might provide an inhibitory constraint that maintains firing rates of PNs within an “optimal subspace” (Afshar et al., 2011) that allows accurate movement. The dynamics of preparatory activity in the ALM and other motor areas (Svoboda and Li, 2018) can be described in a multidimensional space, where each dimension corresponds to the activity of one neuron. During motor planning, firing rates are brought from their initial state to the optimal subspace which allows to start an effective movement (Churchland et al., 2006), and inhibitory inputs from FSNs may help to stabilize PN firing rates within that subspace. Experimental testing of these and other possibilities requires optogenetic modulation of the activity of FSNs at specific times of motor planning in delayed response tasks (Svoboda and Li, 2018). These manipulations are difficult to implement in the case of spontaneous motor actions such as those studied in the present project.

Altogether, the present data reveal an early and prolonged involvement of FSNs during movement planning in premotor areas. These data will guide future investigations on the key role of inhibition in shaping PNs response properties.

5.2 Social facilitation and identification of mirror neurons in mice: ongoing experiments

In the second part of this project, we took advantage of our experimental tools and acquired knowledge on motor behaviours to evaluate social facilitation in mice. Rodents are commonly considered social species with a wide repertoire of social behaviours, however the social facilitation effect, that was considered to rely on the mirror neuron system in monkeys (Ferrari et al., 2005), has not been extensively explored in mice.

Our experiments demonstrate that licking behaviour is facilitated in mice when they observe the same action performed by another mouse. The licking behaviour is actually triggered by seeing its occurrence in a conspecific. Moreover, the licking increase can be attributed neither to the mere presence of water (since in a control condition the mouse was allowed to see the bottle but the demonstrator was not present), nor to the presence of another individual allowed to freely move in the cage but without water available.

Social facilitation is a concept relating to the tendency for the presence of others to initiate an action or to improve an individual's performance on a task. Observing or hearing actions related to experienced behaviours activate the same neuronal motor representation of those actions in the observer (Ferrari et al., 2005), allowing individuals to recognise other's actions. In humans, thanks to fMRI studies, it has been shown that action observation recruits mirror areas in premotor and parietal cortices if the observed action is represented in the subject's personal motor repertoire (Calvo-Merino et al., 2005; Haslinger et al., 2005).

The spontaneous repetition of the same action is not commonly observed in animals, however, social facilitation for behaviours essential for survival are advantageous for individual survival, promoting group cohesiveness or acceptance of novel food sources (Coussi-Korbel and Fragaszy, 1995). Licking is a spontaneous behaviour in mice and has an ecological and evolutionary significance; therefore we speculate that the neural basis for social facilitation of licking behaviour in mice could be the mirror neuron system through a mechanism of resonance of the observed motor act onto the motor system of the observer representing the same licking behaviour. In monkeys, mirror neurons related to the movement of the mouth/tongue (Fragaszy and Visalberghi, 2004), as well as related to the sound of a specific action (Kohler, 2002), have been found.

Therefore, observing action related to licking behaviour could activate the motor representation of those same actions. This basic mechanism could allow individuals to recognise other's actions. In addition, once the motor system related to eating actions 'resonates', this motor information can be used, subsequently, to reproduce the observed action.

Our results can be considered the first clear-cut evidence in mice of a social facilitation effect that is simply triggered by action observation, leading to the idea that mirror neurons can be involved in facilitating behaviours with essential social meaning, as licking, also in mice. However, one crucial issue would be to directly demonstrate the presence of mirror neurons in premotor and motor areas of mice during the social facilitation paradigm. In an evolutionary perspective, the properties of the mirror neuron system could be suitable for social purposes in rodent species. Future experiments are needed to gain a finer-grained understanding of the observer behaviour.

In view of these considerations, since the listening of a sound could activate mirror neurons response (Kohler, 2002), I plan to improve the social facilitation test, used in this thesis, coupling the licks of the demonstrator with a specific and audible sound, to focalize observer attention to the action performed by the conspecific.

Moreover, it has been demonstrated that the eye's pupil undergoes dynamic changes in diameter associated with cognitive effort and arousal, emotional state and motor activity, and

can be used to index brain state across mammalian species. Since in head-fixed mice pupil dynamics have been associate with licking (Lee and Margolis, 2016), I will evaluate pupil diameter during the execution and the observation of the social facilitation paradigm.

As a last step, I am going to chronically implant electrode arrays in premotor and motor areas, dissecting the neuronal dynamics during the observation and execution of the licking behaviour.

6. Bibliography

Abeles, M., Goldstein, M.H., 1977. Multispike train analysis. Proc. IEEE 65, 762–773. https://doi.org/10.1109/PROC.1977.10559

Afshar, A., Santhanam, G., Yu, B.M., Ryu, S.I., Sahani, M., Shenoy, K.V., 2011. Single-Trial Neural Correlates of Arm Movement Preparation. Neuron 71, 555–564. https://doi.org/10.1016/j.neuron.2011.05.047

Alexander, G.E., Crutcher, M.D., 1990. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends in Neurosciences 13, 266–271. https://doi.org/10.1016/0166-2236(90)90107-L

Alia, C., Spalletti, C., Lai, S., Panarese, A., Micera, S., Caleo, M., 2016. Reducing GABAA-mediated inhibition improves forelimb motor function after focal cortical stroke in mice. Sci Rep 6, 37823. https://doi.org/10.1038/srep37823

Allegra Mascaro, A.L., Conti, E., Lai, S., Di Giovanna, A.P., Spalletti, C., Alia, C., Panarese, A., Scaglione, A., Sacconi, L., Micera, S., Caleo, M., Pavone, F.S., 2019. Combined Rehabilitation Promotes the Recovery of Structural and Functional Features of Healthy Neuronal Networks after Stroke. Cell Reports 28, 3474-3485.e6. https://doi.org/10.1016/j.celrep.2019.08.062

Allene, C., Lourenço, J., Bacci, A., 2015. The neuronal identity bias behind neocortical GABAergic plasticity. Trends in Neurosciences 38, 524–534. https://doi.org/10.1016/j.tins.2015.07.008

Asanuma, H., Sakata, H., 1967. Functional Organization of a Cortical Efferent System Examined with Focal Depth Stimulation in Cats. Journal of Neurophysiology 30, 35–54. https://doi.org/10.1152/jn.1967.30.1.35

Atencio, C.A., Schreiner, C.E., 2008. Spectrotemporal Processing Differences between Auditory Cortical Fast-Spiking and Regular-Spiking Neurons. Journal of Neuroscience 28, 3897–3910. https://doi.org/10.1523/JNEUROSCI.5366-07.2008

Avermann, M., Tomm, C., Mateo, C., Gerstner, W., Petersen, C.C.H., 2012. Microcircuits of excitatory and inhibitory neurons in layer 2/3 of mouse barrel cortex. Journal of Neurophysiology 107, 3116–3134. https://doi.org/10.1152/jn.00917.2011

Bagnall, M.W., Hull, C., Bushong, E.A., Ellisman, M.H., Scanziani, M., 2011. Multiple Clusters of Release Sites Formed by Individual Thalamic Afferents onto Cortical Interneurons Ensure Reliable Transmission. Neuron 71, 180–194. https://doi.org/10.1016/j.neuron.2011.05.032

Baker, S.N., Philbin, N., Spinks, R., Pinches, E.M., Wolpert, D.M., MacManus, D.G., Pauluis, Q., Lemon, R.N., 1999. Multiple single unit recording in the cortex of monkeys using independently moveable microelectrodes. Journal of Neuroscience Methods 94, 5–17. https://doi.org/10.1016/S0165-0270(99)00121-1

Barthó, P., Hirase, H., Monconduit, L., Zugaro, M., Harris, K.D., Buzsáki, G., 2004a. Characterization of neocortical principal cells and interneurons by network interactions

and extracellular features. J Neurophysiol 92, 600–608. https://doi.org/10.1152/jn.01170.2003

Barthó, P., Hirase, H., Monconduit, L., Zugaro, M., Harris, K.D., Buzsáki, G., 2004b. Characterization of neocortical principal cells and interneurons by network interactions and extracellular features. J Neurophysiol 92, 600–608. https://doi.org/10.1152/jn.01170.2003

Bates, C.A., Killackey, H.P., 1984. The emergence of a discretely distributed pattern of corticospinal projection neurons. Brain Res 315, 265–273. https://doi.org/10.1016/0165-3806(84)90161-5

Beierlein, M., Gibson, J.R., Connors, B.W., 2003. Two Dynamically Distinct Inhibitory Networks in Layer 4 of the Neocortex. Journal of Neurophysiology 90, 2987–3000. https://doi.org/10.1152/jn.00283.2003

Birch, L.L., Marlin, D.W., 1982. I don't like it; I never tried it: Effects of exposure on two-year-old children's food preferences. Appetite 3, 353–360. https://doi.org/10.1016/S0195-6663(82)80053-6

Bond, C.F., Titus, L.J., 1983. Social facilitation: A meta-analysis of 241 studies. Psychological Bulletin 94, 265–292. https://doi.org/10.1037/0033-2909.94.2.265

Boughter, J.D., Baird, J.-P., Bryant, J., St John, S.J., Heck, D., 2007. C57BL/6J and DBA/2J mice vary in lick rate and ingestive microstructure. Genes Brain Behav 6, 619–627. https://doi.org/10.1111/j.1601-183X.2006.00293.x

Boughter, J.D., Mulligan, M.K., St John, S.J., Tokita, K., Lu, L., Heck, D.H., Williams, R.W., 2012. Genetic control of a central pattern generator: rhythmic oromotor movement in mice is controlled by a major locus near Atp1a2. PLoS One 7, e38169. https://doi.org/10.1371/journal.pone.0038169

Brecht, M., Krauss, A., Muhammad, S., Sinai-Esfahani, L., Bellanca, S., Margrie, T.W., 2004. Organization of rat vibrissa motor cortex and adjacent areas according to cytoarchitectonics, microstimulation, and intracellular stimulation of identified cells. J Comp Neurol 479, 360–373. https://doi.org/10.1002/cne.20306

Brown, S.P., Hestrin, S., 2009. Intracortical circuits of pyramidal neurons reflect their long-range axonal targets. Nature 457, 1133–1136. https://doi.org/10.1038/nature07658

Bruce, C.J., Goldberg, M.E., 1985. Primate frontal eye fields. I. Single neurons discharging before saccades. Journal of Neurophysiology 53, 603–635. https://doi.org/10.1152/jn.1985.53.3.603

Bruno, R.M., Simons, D.J., 2002. Feedforward mechanisms of excitatory and inhibitory cortical receptive fields. J Neurosci 22, 10966–10975.

Bryant, J.L., Boughter, J.D., Gong, S., LeDoux, M.S., Heck, D.H., 2010. Cerebellar cortical output encodes temporal aspects of rhythmic licking movements and is necessary for normal licking frequency. Eur J Neurosci 32, 41–52. https://doi.org/10.1111/j.1460-9568.2010.07244.x

Bucy, P.C., Fulton, J.F., 1933. IPSILATERAL REPRESENTATION IN THE MOTOR AND PREMOTOR CORTEX OP MONKEYS. Brain 56, 318–342. https://doi.org/10.1093/brain/56.3.318

Calvo-Merino, B., Glaser, D.E., Grèzes, J., Passingham, R.E., Haggard, P., 2005. Action Observation and Acquired Motor Skills: An fMRI Study with Expert Dancers. Cerebral Cortex 15, 1243–1249. https://doi.org/10.1093/cercor/bhi007

Cao, Y., Maran, S.K., Dhamala, M., Jaeger, D., Heck, D.H., 2012. Behavior-related pauses in simple-spike activity of mouse Purkinje cells are linked to spike rate modulation. J Neurosci 32, 8678–8685. https://doi.org/10.1523/JNEUROSCI.4969-11.2012

Carlier, P., Jamon, M., 2006. Observational learning in C57BL/6j mice. Behavioural Brain Research 174, 125–131. https://doi.org/10.1016/j.bbr.2006.07.014

Carrillo, M., Han, Y., Migliorati, F., Liu, M., Gazzola, V., Keysers, C., 2019. Emotional Mirror Neurons in the Rat's Anterior Cingulate Cortex. Current Biology 29, 1301-1312.e6. https://doi.org/10.1016/j.cub.2019.03.024

Catsman-Berrevoets, C.E., Kuypers, H.G., 1981. A search for corticospinal collaterals to thalamus and mesencephalon by means of multiple retrograde fluorescent tracers in cat and rat. Brain Res 218, 15–33. https://doi.org/10.1016/0006-8993(81)90986-0

Cattaneo, L., Fabbri-Destro, M., Boria, S., Pieraccini, C., Monti, A., Cossu, G., Rizzolatti, G., 2007. Impairment of actions chains in autism and its possible role in intention understanding. Proceedings of the National Academy of Sciences 104, 17825–17830. https://doi.org/10.1073/pnas.0706273104

Cauli, B., Audinat, E., Lambolez, B., Angulo, M.C., Ropert, N., Tsuzuki, K., Hestrin, S., Rossier, J., 1997. Molecular and Physiological Diversity of Cortical Nonpyramidal Cells. J. Neurosci. 17, 3894–3906. https://doi.org/10.1523/JNEUROSCI.17-10-03894.1997

Cauli, B., Porter, J.T., Tsuzuki, K., Lambolez, B., Rossier, J., Quenet, B., Audinat, E., 2000. Classification of fusiform neocortical interneurons based on unsupervised clustering. Proceedings of the National Academy of Sciences 97, 6144–6149. https://doi.org/10.1073/pnas.97.11.6144

Caviness, V.S., 1975. Architectonic map of neocortex of the normal mouse. J Comp Neurol 164, 247–263. https://doi.org/10.1002/cne.901640207

Chabaud, M.-A., Isabel, G., Kaiser, L., Preat, T., 2009. Social facilitation of long-lasting memory retrieval in Drosophila. Curr Biol 19, 1654–1659. https://doi.org/10.1016/j.cub.2009.08.017

Chen, C.-C., Gilmore, A., Zuo, Y., 2014. Study motor skill learning by single-pellet reaching tasks in mice. J Vis Exp. https://doi.org/10.3791/51238

Chen, T.-W., Li, N., Daie, K., Svoboda, K., 2017. A Map of Anticipatory Activity in Mouse Motor Cortex. Neuron 94, 866-879.e4. https://doi.org/10.1016/j.neuron.2017.05.005

Chen, T.-W., Wardill, T.J., Sun, Y., Pulver, S.R., Renninger, S.L., Baohan, A., Schreiter, E.R., Kerr, R.A., Orger, M.B., Jayaraman, V., Looger, L.L., Svoboda, K., Kim, D.S., 2013. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499, 295–300. https://doi.org/10.1038/nature12354

Chen, Y.-C., Thaler, D., Nixon, P.D., Stern, C.E., Passingham, R.E., 1995. The functions of the medial premotor cortex: II. The timing and selection of learned movements. Exp Brain Res 102. https://doi.org/10.1007/BF00230650

Chen, Z., Travers, S.P., Travers, J.B., 2001. Muscimol infusions in the brain stem reticular formation reversibly block ingestion in the awake rat. Am J Physiol Regul Integr Comp Physiol 280, R1085-1094. https://doi.org/10.1152/ajpregu.2001.280.4.R1085

Churchland, M.M., 2006. Neural Variability in Premotor Cortex Provides a Signature of Motor Preparation. Journal of Neuroscience 26, 3697–3712. https://doi.org/10.1523/JNEUROSCI.3762-05.2006

Churchland, M.M., Shenoy, K.V., 2007. Temporal Complexity and Heterogeneity of Single-Neuron Activity in Premotor and Motor Cortex. Journal of Neurophysiology 97, 4235–4257. https://doi.org/10.1152/jn.00095.2007

Cisek, P., Crammond, D.J., Kalaska, J.F., 2003. Neural Activity in Primary Motor and Dorsal Premotor Cortex In Reaching Tasks With the Contralateral Versus Ipsilateral Arm. Journal of Neurophysiology 89, 922–942. https://doi.org/10.1152/jn.00607.2002

Clayton, D.A., 1978. Socially Facilitated Behavior. The Quarterly Review of Biology 53, 373–392. https://doi.org/10.1086/410789

Cohen, J.Y., Pouget, P., Heitz, R.P., Woodman, G.F., Schall, J.D., 2009. Biophysical Support for Functionally Distinct Cell Types in the Frontal Eye Field. Journal of Neurophysiology 101, 912–916. https://doi.org/10.1152/jn.90272.2008

Connors, B.W., Gutnick, M.J., 1990. Intrinsic firing patterns of diverse neocortical neurons. Trends Neurosci 13, 99–104. https://doi.org/10.1016/0166-2236(90)90185-d

Connors, B.W., Long, M.A., 2004. Electrical synapses in the mammalian brain. Annu Rev Neurosci 27, 393–418. https://doi.org/10.1146/annurev.neuro.26.041002.131128

Coussi-Korbel, S., Fragaszy, D.M., 1995. On the relation between social dynamics and social learning. Animal Behaviour 50, 1441–1453. https://doi.org/10.1016/0003-3472(95)80001-8

Crammond, D.J., Kalaska, J.F., 2000. Prior Information in Motor and Premotor Cortex: Activity During the Delay Period and Effect on Pre-Movement Activity. Journal of Neurophysiology 84, 986–1005. https://doi.org/10.1152/jn.2000.84.2.986

Cruikshank, S.J., Lewis, T.J., Connors, B.W., 2007. Synaptic basis for intense thalamocortical activation of feedforward inhibitory cells in neocortex. Nat Neurosci 10, 462–468. https://doi.org/10.1038/nn1861

Cruikshank, S.J., Urabe, H., Nurmikko, A.V., Connors, B.W., 2010. Pathway-Specific Feedforward Circuits between Thalamus and Neocortex Revealed by Selective Optical Stimulation of Axons. Neuron 65, 230–245. https://doi.org/10.1016/j.neuron.2009.12.025

Crutcher, M.D., Alexander, G.E., 1990. Movement-related neuronal activity selectively coding either direction or muscle pattern in three motor areas of the monkey. Journal of Neurophysiology 64, 151–163. https://doi.org/10.1152/jn.1990.64.1.151

Csicsvari, J., Henze, D.A., Jamieson, B., Harris, K.D., Sirota, A., Barthó, P., Wise, K.D., Buzsáki, G., 2003. Massively Parallel Recording of Unit and Local Field Potentials With Silicon-Based Electrodes. Journal of Neurophysiology 90, 1314–1323. https://doi.org/10.1152/jn.00116.2003

D'amour, J.A., Froemke, R.C., 2015. Inhibitory and excitatory spike-timing-dependent plasticity in the auditory cortex. Neuron 86, 514–528. https://doi.org/10.1016/j.neuron.2015.03.014

Dana, H., Chen, T.-W., Hu, A., Shields, B.C., Guo, C., Looger, L.L., Kim, D.S., Svoboda, K., 2014. Thy1-GCaMP6 Transgenic Mice for Neuronal Population Imaging In Vivo. PLoS ONE 9, e108697. https://doi.org/10.1371/journal.pone.0108697

Davis, J.D., 1996. Deterministic and probabilistic control of the behavior of rats ingesting liquid diets. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 270, R793–R800. https://doi.org/10.1152/ajpregu.1996.270.4.R793

Davis, J.D., Smith, G.P., 1992. Analysis of the microstructure of the rhythmic tongue movements of rats ingesting maltose and sucrose solutions. Behav Neurosci 106, 217–228.

Daw, M.I., Ashby, M.C., Isaac, J.T.R., 2007. Coordinated developmental recruitment of latent fast spiking interneurons in layer IV barrel cortex. Nat Neurosci 10, 453–461. https://doi.org/10.1038/nn1866

De Castro, J.M., 1990. Social facilitation of duration and size but not rate of the spontaneous meal intake of humans. Physiology & Behavior 47, 1129–1135. https://doi.org/10.1016/0031-9384(90)90363-9

de Castro, J.M., Brewer, E.M., 1992. The amount eaten in meals by humans is a power function of the number of people present. Physiology & Behavior 51, 121–125. https://doi.org/10.1016/0031-9384(92)90212-K

Del Giudice, M., Manera, V., Keysers, C., 2009. Programmed to learn? The ontogeny of mirror neurons. Dev Sci 12, 350–363. https://doi.org/10.1111/j.1467-7687.2008.00783.x

Deleuze, C., Bhumbra, G.S., Pazienti, A., Lourenço, J., Mailhes, C., Aguirre, A., Beato, M., Bacci, A., 2019. Strong preference for autaptic self-connectivity of neocortical PV interneurons facilitates their tuning to γ-oscillations. PLoS Biol 17, e3000419. https://doi.org/10.1371/journal.pbio.3000419

di Pellegrino, G., Fadiga, L., Fogassi, L., Gallese, V., Rizzolatti, G., 1992. Understanding motor events: a neurophysiological study. Exp Brain Res 91, 176–180. https://doi.org/10.1007/BF00230027

Dindo, M., Whiten, A., de Waal, F.B.M., 2009. In-Group Conformity Sustains Different Foraging Traditions in Capuchin Monkeys (Cebus apella). PLoS ONE 4, e7858. https://doi.org/10.1371/journal.pone.0007858

Ding, L., Gold, J.I., 2010. Caudate encodes multiple computations for perceptual decisions. J Neurosci 30, 15747–15759. https://doi.org/10.1523/JNEUROSCI.2894-10.2010

Donoghue, J.P., Wise, S.P., 1982. The motor cortex of the rat: Cytoarchitecture and microstimulation mapping. J. Comp. Neurol. 212, 76–88. https://doi.org/10.1002/cne.902120106

Dorrn, A.L., Yuan, K., Barker, A.J., Schreiner, C.E., Froemke, R.C., 2010. Developmental sensory experience balances cortical excitation and inhibition. Nature 465, 932–936. https://doi.org/10.1038/nature09119

Ebbesen, C.L., Doron, G., Lenschow, C., Brecht, M., 2017. Vibrissa motor cortex activity suppresses contralateral whisking behavior. Nat Neurosci 20, 82–89. https://doi.org/10.1038/nn.4437

Economo, M.N., Viswanathan, S., Tasic, B., Bas, E., Winnubst, J., Menon, V., Graybuck, L.T., Nguyen, T.N., Smith, K.A., Yao, Z., Wang, L., Gerfen, C.R., Chandrashekar, J., Zeng, H., Looger, L.L., Svoboda, K., 2018. Distinct descending motor cortex pathways and their roles in movement. Nature 563, 79–84. https://doi.org/10.1038/s41586-018-0642-9

Einevoll, G.T., Franke, F., Hagen, E., Pouzat, C., Harris, K.D., 2012. Towards reliable spike-train recordings from thousands of neurons with multielectrodes. Curr Opin Neurobiol 22, 11–17. https://doi.org/10.1016/j.conb.2011.10.001

Ekanadham, C., Tranchina, D., Simoncelli, E.P., 2014. A unified framework and method for automatic neural spike identification. Journal of Neuroscience Methods 222, 47–55. https://doi.org/10.1016/j.jneumeth.2013.10.001

Erlich, J.C., Bialek, M., Brody, C.D., 2011. A cortical substrate for memory-guided orienting in the rat. Neuron 72, 330–343. https://doi.org/10.1016/j.neuron.2011.07.010

Estebanez, L., Hoffmann, D., Voigt, B.C., Poulet, J.F.A., 2017. Parvalbumin-Expressing GABAergic Neurons in Primary Motor Cortex Signal Reaching. Cell Reports 20, 308–318. https://doi.org/10.1016/j.celrep.2017.06.044

Fabbri-Destro, M., Cattaneo, L., Boria, S., Rizzolatti, G., 2009. Planning actions in autism. Exp Brain Res 192, 521–525. https://doi.org/10.1007/s00221-008-1578-3

Ferezou, I., Haiss, F., Gentet, L.J., Aronoff, R., Weber, B., Petersen, C.C.H., 2007. Spatiotemporal Dynamics of Cortical Sensorimotor Integration in Behaving Mice. Neuron 56, 907–923. https://doi.org/10.1016/j.neuron.2007.10.007

Ferrari, P.F., Maiolini, C., Addessi, E., Fogassi, L., Visalberghi, E., 2005. The observation and hearing of eating actions activates motor programs related to eating in macaque monkeys. Behav Brain Res 161, 95–101. https://doi.org/10.1016/j.bbr.2005.01.009

Fino, E., Packer, A.M., Yuste, R., 2013. The logic of inhibitory connectivity in the neocortex. Neuroscientist 19, 228–237. https://doi.org/10.1177/1073858412456743

Fino, E., Yuste, R., 2011. Dense inhibitory connectivity in neocortex. Neuron 69, 1188–1203. https://doi.org/10.1016/j.neuron.2011.02.025

Flanders, M., Tillery, S.I.H., Soechting, J.F., 1992. Early stages in a sensorimotor transformation. Behav Brain Sci 15, 309–320. https://doi.org/10.1017/S0140525X00068813

Fogassi, L., 2005. Parietal Lobe: From Action Organization to Intention Understanding. Science 308, 662–667. https://doi.org/10.1126/science.1106138

Fogassi, L., Luppino, G., 2005. Motor functions of the parietal lobe. Current Opinion in Neurobiology 15, 626–631. https://doi.org/10.1016/j.conb.2005.10.015

Fowler, S.C., Mortell, C., 1992. Low doses of haloperidol interfere with rat tongue extensions during licking: A quantitative analysis. Behavioral Neuroscience 106, 386–395. https://doi.org/10.1037/0735-7044.106.2.386

Fragaszy, D., Visalberghi, E., 2004. Socially biased learning in monkeys. Animal Learning & Behavior 32, 24–35. https://doi.org/10.3758/BF03196004

Franke, F., Quian Quiroga, R., Hierlemann, A., Obermayer, K., 2015. Bayes optimal template matching for spike sorting – combining fisher discriminant analysis with optimal filtering. J Comput Neurosci 38, 439–459. https://doi.org/10.1007/s10827-015-0547-7

Fu, Y., Tucciarone, J.M., Espinosa, J.S., Sheng, N., Darcy, D.P., Nicoll, R.A., Huang, Z.J., Stryker, M.P., 2014. A cortical circuit for gain control by behavioral state. Cell 156, 1139–1152. https://doi.org/10.1016/j.cell.2014.01.050

Fulton, J.F., 1937. Spasticity and the Frontal Lobes: A Review. N Engl J Med 217, 1017–1024. https://doi.org/10.1056/NEJM193712232172601

Fulton, J.F., 1935. A NOTE ON THE DEFINITION OF THE "MOTOR" AND "PREMOTOR" AREAS. Brain 58, 311–316. https://doi.org/10.1093/brain/58.2.311

Fulton, J.F., 1932. RELATION OF THE CEREBRUM TO THE CEREBELLUM: I. CEREBELLAR TREMOR IN THE CAT AND ITS ABSENCE AFTER REMOVAL OF THE CEREBRAL HEMISPHERES. Arch NeurPsych 28, 542. https://doi.org/10.1001/archneurpsyc.1932.02240030062003

Funahashi, S., Bruce, C.J., Goldman-Rakic, P.S., 1989. Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. J Neurophysiol 61, 331–349. https://doi.org/10.1152/jn.1989.61.2.331

Gabernet, L., Jadhav, S.P., Feldman, D.E., Carandini, M., Scanziani, M., 2005. Somatosensory Integration Controlled by Dynamic Thalamocortical Feed-Forward Inhibition. Neuron 48, 315–327. https://doi.org/10.1016/j.neuron.2005.09.022

Galarreta, M., Hestrin, S., 1999. A network of fast-spiking cells in the neocortex connected by electrical synapses. Nature 402, 72–75. https://doi.org/10.1038/47029

Galiñanes, G.L., Bonardi, C., Huber, D., 2018. Directional Reaching for Water as a Cortex-Dependent Behavioral Framework for Mice. Cell Rep 22, 2767–2783. https://doi.org/10.1016/j.celrep.2018.02.042

Gallese, V., Fadiga, L., Fogassi, L., Rizzolatti, G., 1996. Action recognition in the premotor cortex. Brain 119, 593–609. https://doi.org/10.1093/brain/119.2.593

Gao, P., Ganguli, S., 2015. On simplicity and complexity in the brave new world of large-scale neuroscience. Curr Opin Neurobiol 32, 148–155. https://doi.org/10.1016/j.conb.2015.04.003

Gentet, L.J., Kremer, Y., Taniguchi, H., Huang, Z.J., Staiger, J.F., Petersen, C.C.H., 2012. Unique functional properties of somatostatin-expressing GABAergic neurons in mouse barrel cortex. Nat Neurosci 15, 607–612. https://doi.org/10.1038/nn.3051

Georgopoulos, A., Kalaska, J., Caminiti, R., Massey, J., 1982. On the relations between the direction of two-dimensional arm movements and cell discharge in primate motor cortex. J. Neurosci. 2, 1527–1537. https://doi.org/10.1523/JNEUROSCI.02-11-01527.1982

Ghez, C., Favilla, M., Ghilardi, M.F., Gordon, J., Bermejo, R., Pullman, S., 1997. Discrete and continuous planning of hand movements and isometric force trajectories: Exp Brain Res 115, 217–233. https://doi.org/10.1007/PL00005692

Gibson, J.R., Beierlein, M., Connors, B.W., 1999. Two networks of electrically coupled inhibitory neurons in neocortex. Nature 402, 75–79. https://doi.org/10.1038/47035

Gnadt, J.W., Andersen, R.A., 1988. Memory related motor planning activity in posterior parietal cortex of macaque. Exp Brain Res 70, 216–220. https://doi.org/10.1007/BF00271862

Godschalk, M., Lemon, R.N., Kuypers, H.G.J.M., Van Der Steen, J., 1985. The involvement of monkey premotor cortex neurones in preparation of visually cued arm movements. Behavioural Brain Research 18, 143–157. https://doi.org/10.1016/0166-4328(85)90070-1

Goldberg, M.E., Wurtz, R.H., 1972. Activity of superior colliculus in behaving monkey. II. Effect of attention on neuronal responses. J Neurophysiol 35, 560–574. https://doi.org/10.1152/jn.1972.35.4.560

Gordon, J., Ghilardi, M.F., Ghez, C., 1994. Accuracy of planar reaching movements: I. Independence of direction and extent variability. Exp Brain Res 99, 97–111. https://doi.org/10.1007/BF00241415

Gray, C.M., Maldonado, P.E., Wilson, M., McNaughton, B., 1995. Tetrodes markedly improve the reliability and yield of multiple single-unit isolation from multi-unit recordings in cat striate cortex. Journal of Neuroscience Methods 63, 43–54. https://doi.org/10.1016/0165-0270(95)00085-2

Guerin, B., Innes, J., 1993. Social Facilitation, 1st ed. Cambridge University Press. https://doi.org/10.1017/CBO9780511628214

Guo, J.-Z., Graves, A.R., Guo, W.W., Zheng, J., Lee, A., Rodríguez-González, J., Li, N., Macklin, J.J., Phillips, J.W., Mensh, B.D., Branson, K., Hantman, A.W., 2015. Cortex commands the performance of skilled movement. Elife 4, e10774. https://doi.org/10.7554/eLife.10774

Guo, Z.V., Hires, S.A., Li, N., O'Connor, D.H., Komiyama, T., Ophir, E., Huber, D., Bonardi, C., Morandell, K., Gutnisky, D., Peron, S., Xu, N., Cox, J., Svoboda, K., 2014a. Procedures for behavioral experiments in head-fixed mice. PLoS One 9, e88678. https://doi.org/10.1371/journal.pone.0088678

Guo, Z.V., Inagaki, H.K., Daie, K., Druckmann, S., Gerfen, C.R., Svoboda, K., 2017. Maintenance of persistent activity in a frontal thalamocortical loop. Nature 545, 181–186. https://doi.org/10.1038/nature22324

Guo, Z.V., Li, N., Huber, D., Ophir, E., Gutnisky, D., Ting, J.T., Feng, G., Svoboda, K., 2014b. Flow of cortical activity underlying a tactile decision in mice. Neuron 81, 179–194. https://doi.org/10.1016/j.neuron.2013.10.020

Gur, M., Beylin, A., Snodderly, D.M., 1999. Physiological properties of macaque V1 neurons are correlated with extracellular spike amplitude, duration, and polarity. J Neurophysiol 82, 1451–1464. https://doi.org/10.1152/jn.1999.82.3.1451

Hall, R.D., Lindholm, E.P., 1974. Organization of motor and somatosensory neocortex in the albino rat. Brain Research 66, 23–38. https://doi.org/10.1016/0006-8993(74)90076-6

Hamilton, L.S., Sohl-Dickstein, J., Huth, A.G., Carels, V.M., Deisseroth, K., Bao, S., 2013. Optogenetic Activation of an Inhibitory Network Enhances Feedforward Functional Connectivity in Auditory Cortex. Neuron 80, 1066–1076. https://doi.org/10.1016/j.neuron.2013.08.017

Hanes, D.P., Schall, J.D., 1996. Neural Control of Voluntary Movement Initiation. Science 274, 427–430. https://doi.org/10.1126/science.274.5286.427

Harms, K.J., Rioult-Pedotti, M.S., Carter, D.R., Dunaevsky, A., 2008. Transient spine expansion and learning-induced plasticity in layer 1 primary motor cortex. J Neurosci 28, 5686–5690. https://doi.org/10.1523/JNEUROSCI.0584-08.2008

Harris, K.D., Quiroga, R.Q., Freeman, J., Smith, S.L., 2016. Improving data quality in neuronal population recordings. Nat Neurosci 19, 1165–1174. https://doi.org/10.1038/nn.4365

Harvey, C.D., Coen, P., Tank, D.W., 2012. Choice-specific sequences in parietal cortex during a virtual-navigation decision task. Nature 484, 62–68. https://doi.org/10.1038/nature10918

Haslinger, B., Erhard, P., Altenmüller, E., Schroeder, U., Boecker, H., Ceballos-Baumann, A.O., 2005. Transmodal sensorimotor networks during action observation in professional pianists. J Cogn Neurosci 17, 282–293. https://doi.org/10.1162/0898929053124893

Hattox, A.M., Nelson, S.B., 2007. Layer V neurons in mouse cortex projecting to different targets have distinct physiological properties. J Neurophysiol 98, 3330–3340. https://doi.org/10.1152/jn.00397.2007

Hayar, A., Bryant, J.L., Boughter, J.D., Heck, D.H., 2006. A low-cost solution to measure mouse licking in an electrophysiological setup with a standard analog-to-digital converter. Journal of Neuroscience Methods 153, 203–207. https://doi.org/10.1016/j.jneumeth.2005.10.023

Herman, C.P., 2017. The social facilitation of eating or the facilitation of social eating? J Eat Disord 5, 16. https://doi.org/10.1186/s40337-017-0146-2

Hickok, G., 2009. Eight problems for the mirror neuron theory of action understanding in monkeys and humans. J Cogn Neurosci 21, 1229–1243. https://doi.org/10.1162/jocn.2009.21189

Hill, D.N., Mehta, S.B., Kleinfeld, D., 2011. Quality Metrics to Accompany Spike Sorting of Extracellular Signals. Journal of Neuroscience 31, 8699–8705. https://doi.org/10.1523/JNEUROSCI.0971-11.2011

Hilscher, M.M., Leão, R.N., Edwards, S.J., Leão, K.E., Kullander, K., 2017. Chrna2-Martinotti Cells Synchronize Layer 5 Type A Pyramidal Cells via Rebound Excitation. PLoS Biol 15, e2001392. https://doi.org/10.1371/journal.pbio.2001392

Hira, R., Ohkubo, F., Ozawa, K., Isomura, Y., Kitamura, K., Kano, M., Kasai, H., Matsuzaki, M., 2013. Spatiotemporal dynamics of functional clusters of neurons in the mouse motor cortex during a voluntary movement. J Neurosci 33, 1377–1390. https://doi.org/10.1523/JNEUROSCI.2550-12.2013

Hocherman, S., Wise, S.P., 1991. Effects of hand movement path on motor cortical activity in awake, behaving rhesus monkeys. Exp Brain Res 83. https://doi.org/10.1007/BF00231153

Hofer, S.B., Ko, H., Pichler, B., Vogelstein, J., Ros, H., Zeng, H., Lein, E., Lesica, N.A., Mrsic-Flogel, T.D., 2011. Differential connectivity and response dynamics of excitatory and inhibitory neurons in visual cortex. Nat Neurosci 14, 1045–1052. https://doi.org/10.1038/nn.2876

Hooks, B.M., Mao, T., Gutnisky, D.A., Yamawaki, N., Svoboda, K., Shepherd, G.M.G., 2013. Organization of Cortical and Thalamic Input to Pyramidal Neurons in Mouse Motor Cortex. Journal of Neuroscience 33, 748–760. https://doi.org/10.1523/JNEUROSCI.4338-12.2013

Hopper, T., Bourgeois, M., Pimentel, J., Qualls, C.D., Hickey, E., Frymark, T., Schooling, T., 2013. An evidence-based systematic review on cognitive interventions for individuals with dementia. Am J Speech Lang Pathol 22, 126–145. https://doi.org/10.1044/1058-0360(2012/11-0137)

Horner, V., Whiten, A., 2005. Causal knowledge and imitation/emulation switching in chimpanzees (Pan troglodytes) and children (Homo sapiens). Anim Cogn 8, 164–181. https://doi.org/10.1007/s10071-004-0239-6

Howe, M.W., Tierney, P.L., Sandberg, S.G., Phillips, P.E.M., Graybiel, A.M., 2013. Prolonged dopamine signalling in striatum signals proximity and value of distant rewards. Nature 500, 575–579. https://doi.org/10.1038/nature12475

Hsiao, S., Fan, R.J., 1993. Additivity of taste-specific effects of sucrose and quinine: Microstructural analysis of ingestive behavior in rats. Behavioral Neuroscience 107, 317–326. https://doi.org/10.1037/0735-7044.107.2.317

Hu, H., Gan, J., Jonas, P., 2014. Fast-spiking, parvalbumin+ GABAergic interneurons: From cellular design to microcircuit function. Science 345, 1255263–1255263. https://doi.org/10.1126/science.1255263

Huber, D., Gutnisky, D.A., Peron, S., O'Connor, D.H., Wiegert, J.S., Tian, L., Oertner, T.G., Looger, L.L., Svoboda, K., 2012. Multiple dynamic representations in the motor cortex during sensorimotor learning. Nature 484, 473–478. https://doi.org/10.1038/nature11039

Hull, C., Isaacson, J.S., Scanziani, M., 2009. Postsynaptic mechanisms govern the differential excitation of cortical neurons by thalamic inputs. J Neurosci 29, 9127–9136. https://doi.org/10.1523/JNEUROSCI.5971-08.2009

Iacoboni, M., 2009. Imitation, Empathy, and Mirror Neurons. Annu. Rev. Psychol. 60, 653–670. https://doi.org/10.1146/annurev.psych.60.110707.163604

Iacoboni, M., 1999. Cortical Mechanisms of Human Imitation. Science 286, 2526–2528. https://doi.org/10.1126/science.286.5449.2526

Inagaki, H.K., Inagaki, M., Romani, S., Svoboda, K., 2018. Low-Dimensional and Monotonic Preparatory Activity in Mouse Anterior Lateral Motor Cortex. J. Neurosci. 38, 4163–4185. https://doi.org/10.1523/JNEUROSCI.3152-17.2018

Inoue, T., Imoto, K., 2006. Feedforward Inhibitory Connections From Multiple Thalamic Cells to Multiple Regular-Spiking Cells in Layer 4 of the Somatosensory Cortex. Journal of Neurophysiology 96, 1746–1754. https://doi.org/10.1152/jn.00301.2006

Isaacson, J.S., Scanziani, M., 2011. How inhibition shapes cortical activity. Neuron 72, 231–243. https://doi.org/10.1016/j.neuron.2011.09.027

Isomura, Y., Harukuni, R., Takekawa, T., Aizawa, H., Fukai, T., 2009. Microcircuitry coordination of cortical motor information in self-initiation of voluntary movements. Nat Neurosci 12, 1586–1593. https://doi.org/10.1038/nn.2431

Jacobsen, C.F., 1935. FUNCTIONS OF FRONTAL ASSOCIATION AREA IN PRIMATES. Arch NeurPsych 33, 558. https://doi.org/10.1001/archneurpsyc.1935.02250150108009

Jerjian, S.J., Sahani, M., Kraskov, A., 2020. Movement initiation and grasp representation in premotor and primary motor cortex mirror neurons. eLife 9, e54139. https://doi.org/10.7554/eLife.54139

Jesse, F., Riebel, K., 2012. Social facilitation of male song by male and female conspecifics in the zebra finch, Taeniopygia guttata. Behav Processes 91, 262–266. https://doi.org/10.1016/j.beproc.2012.09.006

Kadir, S.N., Goodman, D.F.M., Harris, K.D., 2014. High-Dimensional Cluster Analysis with the Masked EM Algorithm. Neural Computation 26, 2379–2394. https://doi.org/10.1162/NECO_a_00661

Kang, T., Brorsen, B.W., 1995. Conditional heteroskedasticity, asymmetry, and option pricing. J. Fut. Mark. 15, 901–928. https://doi.org/10.1002/fut.3990150804

Karnani, M.M., Agetsuma, M., Yuste, R., 2014. A blanket of inhibition: functional inferences from dense inhibitory connectivity. Current Opinion in Neurobiology 26, 96–102. https://doi.org/10.1016/j.conb.2013.12.015

Karnani, M.M., Jackson, J., Ayzenshtat, I., Hamzehei Sichani, A., Manoocheri, K., Kim, S., Yuste, R., 2016. Opening Holes in the Blanket of Inhibition: Localized Lateral Disinhibition by VIP Interneurons. Journal of Neuroscience 36, 3471–3480. https://doi.org/10.1523/JNEUROSCI.3646-15.2016

Kätzel, D., Zemelman, B.V., Buetfering, C., Wölfel, M., Miesenböck, G., 2011. The columnar and laminar organization of inhibitory connections to neocortical excitatory cells. Nat Neurosci 14, 100–107. https://doi.org/10.1038/nn.2687

Kaufman, M.T., Churchland, M.M., Santhanam, G., Yu, B.M., Afshar, A., Ryu, S.I., Shenoy, K.V., 2010. Roles of monkey premotor neuron classes in movement preparation and execution. J Neurophysiol 104, 799–810. https://doi.org/10.1152/jn.00231.2009

Kaufman, M.T., Churchland, M.M., Shenoy, K.V., 2013. The roles of monkey M1 neuron classes in movement preparation and execution. Journal of Neurophysiology 110, 817–825. https://doi.org/10.1152/jn.00892.2011

Kawaguchi, Y., 2002. [No title found]. Journal of Neurocytology 31, 277–287. https://doi.org/10.1023/A:1024126110356

Keeling, L.J., Hurnik, J.F., 1993. Chickens show socially facilitated feeding behaviour in response to a video image of a conspecific. Applied Animal Behaviour Science 36, 223–231. https://doi.org/10.1016/0168-1591(93)90012-E

Kennard, M.A., Viets, H.R., Fulton, J.F., 1934. THE SYNDROME OF THE PREMOTOR CORTEX IN MAN: IMPAIRMENT OF SKILLED MOVEMENTS, FORCED GRASPING, SPASTICITY, AND VASOMOTOR DISTURBANCE. Brain 57, 69–84. https://doi.org/10.1093/brain/57.1.69

Kepecs, A., Fishell, G., 2014. Interneuron cell types are fit to function. Nature 505, 318–326. https://doi.org/10.1038/nature12983

Kerkhoff, G., 2001. Spatial hemineglect in humans. Prog Neurobiol 63, 1–27. https://doi.org/10.1016/s0301-0082(00)00028-9

Kerlin, A.M., Andermann, M.L., Berezovskii, V.K., Reid, R.C., 2010. Broadly Tuned Response Properties of Diverse Inhibitory Neuron Subtypes in Mouse Visual Cortex. Neuron 67, 858–871. https://doi.org/10.1016/j.neuron.2010.08.002

Kilner, J.M., Lemon, R.N., 2013. What We Know Currently about Mirror Neurons. Current Biology 23, R1057–R1062. https://doi.org/10.1016/j.cub.2013.10.051

Kiritani, T., Wickersham, I.R., Seung, H.S., Shepherd, G.M.G., 2012. Hierarchical Connectivity and Connection-Specific Dynamics in the Corticospinal-Corticostriatal Microcircuit in Mouse Motor Cortex. Journal of Neuroscience 32, 4992–5001. https://doi.org/10.1523/JNEUROSCI.4759-11.2012

Kita, T., Kita, H., 2012. The Subthalamic Nucleus Is One of Multiple Innervation Sites for Long-Range Corticofugal Axons: A Single-Axon Tracing Study in the Rat. Journal of Neuroscience 32, 5990–5999. https://doi.org/10.1523/JNEUROSCI.5717-11.2012

Kleim, J.A., Barbay, S., Nudo, R.J., 1998. Functional reorganization of the rat motor cortex following motor skill learning. J Neurophysiol 80, 3321–3325. https://doi.org/10.1152/jn.1998.80.6.3321

Kleim, J.A., Hogg, T.M., VandenBerg, P.M., Cooper, N.R., Bruneau, R., Remple, M., 2004. Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. J Neurosci 24, 628–633. https://doi.org/10.1523/JNEUROSCI.3440-03.2004

Klein, A., Sacrey, L.-A.R., Whishaw, I.Q., Dunnett, S.B., 2012. The use of rodent skilled reaching as a translational model for investigating brain damage and disease. Neurosci Biobehav Rev 36, 1030–1042. https://doi.org/10.1016/j.neubiorev.2011.12.010

Kohler, E., 2002. Hearing Sounds, Understanding Actions: Action Representation in Mirror Neurons. Science 297, 846–848. https://doi.org/10.1126/science.1070311

Komiyama, T., Sato, T.R., O'Connor, D.H., Zhang, Y.-X., Huber, D., Hooks, B.M., Gabitto, M., Svoboda, K., 2010. Learning-related fine-scale specificity imaged in motor cortex circuits of behaving mice. Nature 464, 1182–1186. https://doi.org/10.1038/nature08897

Kubota, Y., 2014. Untangling GABAergic wiring in the cortical microcircuit. Current Opinion in Neurobiology 26, 7–14. https://doi.org/10.1016/j.conb.2013.10.003

Kubota, Y., Shigematsu, N., Karube, F., Sekigawa, A., Kato, S., Yamaguchi, N., Hirai, Y., Morishima, M., Kawaguchi, Y., 2011. Selective coexpression of multiple chemical markers defines discrete populations of neocortical GABAergic neurons. Cereb Cortex 21, 1803–1817. https://doi.org/10.1093/cercor/bhq252

Kvitsiani, D., Ranade, S., Hangya, B., Taniguchi, H., Huang, J.Z., Kepecs, A., 2013. Distinct behavioural and network correlates of two interneuron types in prefrontal cortex. Nature 498, 363–366. https://doi.org/10.1038/nature12176

Lagler, M., Ozdemir, A.T., Lagoun, S., Malagon-Vina, H., Borhegyi, Z., Hauer, R., Jelem, A., Klausberger, T., 2016. Divisions of Identified Parvalbumin-Expressing Basket Cells during Working Memory-Guided Decision Making. Neuron 91, 1390–1401. https://doi.org/10.1016/j.neuron.2016.08.010

Laurent, G., 2002. Olfactory network dynamics and the coding of multidimensional signals. Nat Rev Neurosci 3, 884–895. https://doi.org/10.1038/nrn964

Lecas, J.C., Requin, J., Anger, C., Vitton, N., 1986. Changes in neuronal activity of the monkey precentral cortex during preparation for movement. Journal of Neurophysiology 56, 1680–1702. https://doi.org/10.1152/jn.1986.56.6.1680

Lee, A.T., Gee, S.M., Vogt, D., Patel, T., Rubenstein, J.L., Sohal, V.S., 2014. Pyramidal neurons in prefrontal cortex receive subtype-specific forms of excitation and inhibition. Neuron 81, 61–68. https://doi.org/10.1016/j.neuron.2013.10.031

Lee, C.R., Margolis, D.J., 2016. Pupil Dynamics Reflect Behavioral Choice and Learning in a Go/NoGo Tactile Decision-Making Task in Mice. Front Behav Neurosci 10, 200. https://doi.org/10.3389/fnbeh.2016.00200

Lee, S., Kruglikov, I., Huang, Z.J., Fishell, G., Rudy, B., 2013. A disinhibitory circuit mediates motor integration in the somatosensory cortex. Nat Neurosci 16, 1662–1670. https://doi.org/10.1038/nn.3544

Lee, S.-H., Kwan, A.C., Zhang, S., Phoumthipphavong, V., Flannery, J.G., Masmanidis, S.C., Taniguchi, H., Huang, Z.J., Zhang, F., Boyden, E.S., Deisseroth, K., Dan, Y., 2012. Activation of specific interneurons improves V1 feature selectivity and visual perception. Nature 488, 379–383. https://doi.org/10.1038/nature11312

Lewicki, M.S., 1998. A review of methods for spike sorting: the detection and classification of neural action potentials. Network: Computation in Neural Systems 9, R53–R78. https://doi.org/10.1088/0954-898X_9_4_001

Li, C.-X., Waters, R.S., 1991. Organization of the Mouse Motor Cortex Studied by Retrograde Tracing and Intracortical Microstimulation (ICMS) Mapping. Can. j. neurol. sci. 18, 28–38. https://doi.org/10.1017/S0317167100031267

Li, N., Chen, T.-W., Guo, Z.V., Gerfen, C.R., Svoboda, K., 2015. A motor cortex circuit for motor planning and movement. Nature 519, 51–56. https://doi.org/10.1038/nature14178

Li, N., Daie, K., Svoboda, K., Druckmann, S., 2016. Robust neuronal dynamics in premotor cortex during motor planning. Nature 532, 459–464. https://doi.org/10.1038/nature17643

Li, X.G., Florence, S.L., Kaas, J.H., 1990. Areal distributions of cortical neurons projecting to different levels of the caudal brain stem and spinal cord in rats. Somatosens Mot Res 7, 315–335. https://doi.org/10.3109/08990229009144711

Liang, F., Rouiller, E.M., Wiesendanger, M., 1993. Modulation of Sustained Electromyographic Activity by Single Intracortical Microstimuli: Comparison of Two Forelimb Motor Cortical Areas of the Rat. Somatosensory & Motor Research 10, 51–61. https://doi.org/10.3109/08990229309028823

Lourenço, J., Koukouli, F., Bacci, A., 2020. Synaptic inhibition in the neocortex: Orchestration and computation through canonical circuits and variations on the theme. Cortex 132, 258–280. https://doi.org/10.1016/j.cortex.2020.08.015

Maimon, G., Assad, J.A., 2006. A cognitive signal for the proactive timing of action in macaque LIP. Nat Neurosci 9, 948–955. https://doi.org/10.1038/nn1716

Mamedov, Z., Bureš, J., 1990. Differential control of operant and consummatory licking in rats. Neuroscience Letters 115, 144–148. https://doi.org/10.1016/0304-3940(90)90445-F

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., Wu, C., 2004. Interneurons of the neocortical inhibitory system. Nat Rev Neurosci 5, 793–807. https://doi.org/10.1038/nrn1519

Marre, O., Amodei, D., Deshmukh, N., Sadeghi, K., Soo, F., Holy, T.E., Berry, M.J., 2012. Mapping a Complete Neural Population in the Retina. Journal of Neuroscience 32, 14859–14873. https://doi.org/10.1523/JNEUROSCI.0723-12.2012

McCormick, D.A., Connors, B.W., Lighthall, J.W., Prince, D.A., 1985. Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. J Neurophysiol 54, 782–806. https://doi.org/10.1152/jn.1985.54.4.782

McNaughton, B.L., Barnes, C.A., O'Keefe, J., 1983. The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats. Exp Brain Res 52. https://doi.org/10.1007/BF00237147

Merchant, H., Naselaris, T., Georgopoulos, A.P., 2008. Dynamic Sculpting of Directional Tuning in the Primate Motor Cortex during Three-Dimensional Reaching. Journal of Neuroscience 28, 9164–9172. https://doi.org/10.1523/JNEUROSCI.1898-08.2008

Messier, J., Kalaska, J.F., 2000. Covariation of Primate Dorsal Premotor Cell Activity With Direction and Amplitude During a Memorized-Delay Reaching Task. Journal of Neurophysiology 84, 152–165. https://doi.org/10.1152/jn.2000.84.1.152

Metz, G.A.S., Whishaw, I.Q., 2000. Skilled reaching an action pattern: stability in rat (Rattus norvegicus) grasping movements as a function of changing food pellet size. Behavioural Brain Research 116, 111–122. https://doi.org/10.1016/S0166-4328(00)00245-X

Meyer, H.S., Schwarz, D., Wimmer, V.C., Schmitt, A.C., Kerr, J.N.D., Sakmann, B., Helmstaedter, M., 2011. Inhibitory interneurons in a cortical column form hot zones of inhibition in layers 2 and 5A. Proceedings of the National Academy of Sciences 108, 16807–16812. https://doi.org/10.1073/pnas.1113648108

Mitchell, J.F., Sundberg, K.A., Reynolds, J.H., 2007. Differential Attention-Dependent Response Modulation across Cell Classes in Macaque Visual Area V4. Neuron 55, 131–141. https://doi.org/10.1016/j.neuron.2007.06.018

Molnár, Z., Cheung, A.F.P., 2006. Towards the classification of subpopulations of layer V pyramidal projection neurons. Neuroscience Research 55, 105–115. https://doi.org/10.1016/j.neures.2006.02.008

Mora Lopez, C., Putzeys, J., Raducanu, B.C., Ballini, M., Wang, S., Andrei, A., Rochus, V., Vandebriel, R., Severi, S., Van Hoof, C., Musa, S., Van Helleputte, N., Yazicioglu, R.F., Mitra, S., 2017. A Neural Probe With Up to 966 Electrodes and Up to 384 Configurable Channels in 0.13 μm SOI CMOS. IEEE Trans. Biomed. Circuits Syst. 11, 510–522. https://doi.org/10.1109/TBCAS.2016.2646901

Morandell, K., Huber, D., 2017. The role of forelimb motor cortex areas in goal directed action in mice. Sci Rep 7, 15759. https://doi.org/10.1038/s41598-017-15835-2

Morishima, M., Kawaguchi, Y., 2006. Recurrent connection patterns of corticostriatal pyramidal cells in frontal cortex. J Neurosci 26, 4394–4405. https://doi.org/10.1523/JNEUROSCI.0252-06.2006

Mukamel, R., Ekstrom, A.D., Kaplan, J., Iacoboni, M., Fried, I., 2010. Single-neuron responses in humans during execution and observation of actions. Curr Biol 20, 750–756. https://doi.org/10.1016/j.cub.2010.02.045

Muller, R., 1996. A Quarter of a Century of Place Cells. Neuron 17, 813–822. https://doi.org/10.1016/S0896-6273(00)80214-7

Murakami, M., Mainen, Z.F., 2015. Preparing and selecting actions with neural populations: toward cortical circuit mechanisms. Curr Opin Neurobiol 33, 40–46. https://doi.org/10.1016/j.conb.2015.01.005

Murakami, M., Vicente, M.I., Costa, G.M., Mainen, Z.F., 2014. Neural antecedents of self-initiated actions in secondary motor cortex. Nat Neurosci 17, 1574–1582. https://doi.org/10.1038/nn.3826

Murray, P.D., Keller, A., 2011. Somatosensory response properties of excitatory and inhibitory neurons in rat motor cortex. Journal of Neurophysiology 106, 1355–1362. https://doi.org/10.1152/jn.01089.2010

Nanou, E., Catterall, W.A., 2018. Calcium Channels, Synaptic Plasticity, and Neuropsychiatric Disease. Neuron 98, 466–481. https://doi.org/10.1016/j.neuron.2018.03.017

Neafsey, E.J., Bold, E.L., Haas, G., Hurley-Gius, K.M., Quirk, G., Sievert, C.F., Terreberry, R.R., 1986. The organization of the rat motor cortex: a microstimulation mapping study. Brain Res 396, 77–96. https://doi.org/10.1016/s0006-8993(86)80191-3

Neafsey, E.J., Sievert, C., 1982. A second forelimb motor area exists in rat frontal cortex. Brain Research 232, 151–156. https://doi.org/10.1016/0006-8993(82)90617-5

Niell, C.M., Stryker, M.P., 2010. Modulation of Visual Responses by Behavioral State in Mouse Visual Cortex. Neuron 65, 472–479. https://doi.org/10.1016/j.neuron.2010.01.033

Nudo, R.J., Milliken, G.W., Jenkins, W.M., Merzenich, M.M., 1996. Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. J Neurosci 16, 785–807.

Ohmae, S., Kunimatsu, J., Tanaka, M., 2017. Cerebellar Roles in Self-Timing for Sub- and Supra-Second Intervals. J. Neurosci. 37, 3511–3522. https://doi.org/10.1523/JNEUROSCI.2221-16.2017

Ohmae, S., Uematsu, A., Tanaka, M., 2013. Temporally Specific Sensory Signals for the Detection of Stimulus Omission in the Primate Deep Cerebellar Nuclei. J. Neurosci. 33, 15432–15441. https://doi.org/10.1523/JNEUROSCI.1698-13.2013

Otchy, T.M., Wolff, S.B.E., Rhee, J.Y., Pehlevan, C., Kawai, R., Kempf, A., Gobes, S.M.H., Ölveczky, B.P., 2015. Acute off-target effects of neural circuit manipulations. Nature 528, 358–363. https://doi.org/10.1038/nature16442

Otsuka, T., Kawaguchi, Y., 2009. Cortical Inhibitory Cell Types Differentially Form Intralaminar and Interlaminar Subnetworks withExcitatory Neurons. Journal of Neuroscience 29, 10533–10540. https://doi.org/10.1523/JNEUROSCI.2219-09.2009

Pachitariu, M., Steinmetz, N., Kadir, S., Carandini, M., Kenneth D., H., 2016. Kilosort: realtime spike-sorting for extracellular electrophysiology with hundreds of channels (preprint). Neuroscience. https://doi.org/10.1101/061481

Pasquini, M., Lai, S., Spalletti, C., Cracchiolo, M., Conti, S., Panarese, A., Caleo, M., Micera, S., 2018. A Robotic System for Adaptive Training and Function Assessment of Forelimb Retraction in Mice. IEEE Trans. Neural Syst. Rehabil. Eng. 26, 1803–1812. https://doi.org/10.1109/TNSRE.2018.2864279

Paul, A., Crow, M., Raudales, R., He, M., Gillis, J., Huang, Z.J., 2017. Transcriptional Architecture of Synaptic Communication Delineates GABAergic Neuron Identity. Cell 171, 522-539.e20. https://doi.org/10.1016/j.cell.2017.08.032

Penfield, W., 1951. THE SUPPLEMENTARY MOTOR AREA OF THE CEREBRAL CORTEX: A Clinical and Experimental Study. AMA Arch NeurPsych 66, 289. https://doi.org/10.1001/archneurpsyc.1951.02320090038004

Peters, A.J., Chen, S.X., Komiyama, T., 2014. Emergence of reproducible spatiotemporal activity during motor learning. Nature 510, 263–267. https://doi.org/10.1038/nature13235

Pfeffer, C.K., Xue, M., He, M., Huang, Z.J., Scanziani, M., 2013. Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. Nat Neurosci 16, 1068–1076. https://doi.org/10.1038/nn.3446

Pi, H.-J., Hangya, B., Kvitsiani, D., Sanders, J.I., Huang, Z.J., Kepecs, A., 2013. Cortical interneurons that specialize in disinhibitory control. Nature 503, 521–524. https://doi.org/10.1038/nature12676

Pillow, J.W., Shlens, J., Chichilnisky, E.J., Simoncelli, E.P., 2013. A Model-Based Spike Sorting Algorithm for Removing Correlation Artifacts in Multi-Neuron Recordings. PLoS ONE 8, e62123. https://doi.org/10.1371/journal.pone.0062123

Pineda, J.A., 2005. The functional significance of mu rhythms: Translating “seeing” and “hearing” into “doing.” Brain Research Reviews 50, 57–68. https://doi.org/10.1016/j.brainresrev.2005.04.005

Pinto, L., Dan, Y., 2015. Cell-Type-Specific Activity in Prefrontal Cortex during Goal-Directed Behavior. Neuron 87, 437–450. https://doi.org/10.1016/j.neuron.2015.06.021

Polack, P.-O., Friedman, J., Golshani, P., 2013. Cellular mechanisms of brain state–dependent gain modulation in visual cortex. Nat Neurosci 16, 1331–1339. https://doi.org/10.1038/nn.3464

Prather, J.F., Peters, S., Nowicki, S., Mooney, R., 2008. Precise auditory-vocal mirroring in neurons for learned vocal communication. Nature 451, 305–310. https://doi.org/10.1038/nature06492

Pronichev, I.V., Lenkov, D.N., 1998. Functional mapping of the motor cortex of the white mouse by a microstimulation method. Neurosci Behav Physiol 28, 80–85. https://doi.org/10.1007/BF02461916

Quiroga, R.Q., 2012. Spike sorting. Current Biology 22, R45–R46. https://doi.org/10.1016/j.cub.2011.11.005

Reep, R.L., Corwin, J.V., Hashimoto, A., Watson, R.T., 1987. Efferent connections of the rostral portion of medial agranular cortex in rats. Brain Res Bull 19, 203–221. https://doi.org/10.1016/0361-9230(87)90086-4

Reiner, A., Jiao, Y., Del Mar, N., Laverghetta, A.V., Lei, W.L., 2003. Differential morphology of pyramidal tract-type and intratelencephalically projecting-type corticostriatal neurons and their intrastriatal terminals in rats. J. Comp. Neurol. 457, 420–440. https://doi.org/10.1002/cne.10541

Ren, J.Q., Aika, Y., Heizmann, C.W., Kosaka, T., 1992. Quantitative analysis of neurons and glial cells in the rat somatosensory cortex, with special reference to GABAergic neurons and parvalbumin-containing neurons. Exp Brain Res 92. https://doi.org/10.1007/BF00230378

Requin, J., Riehle, A., Seal, J., 1988. Neuronal activity and information processing in motor control: From stages to continuous flow. Biological Psychology 26, 179–198. https://doi.org/10.1016/0301-0511(88)90019-1

Riehle, A., Requin, J., 1993. The predictive value for performance speed of preparatory changes in neuronal activity of the monkey motor and premotor cortex. Behavioural Brain Research 53, 35–49. https://doi.org/10.1016/S0166-4328(05)80264-5

Riehle, A., Requin, J., 1989. Monkey primary motor and premotor cortex: single-cell activity related to prior information about direction and extent of an intended movement. Journal of Neurophysiology 61, 534–549. https://doi.org/10.1152/jn.1989.61.3.534

Rizzolatti, G., Fabbri-Destro, M., 2009. Premotor Cortex in Primates: Dorsal and Ventral, in: Encyclopedia of Neuroscience. Elsevier, pp. 935–945. https://doi.org/10.1016/B978-008045046-9.01320-6

Rizzolatti, G., Fogassi, L., Gallese, V., 2002. Motor and cognitive functions of the ventral premotor cortex. Current Opinion in Neurobiology 12, 149–154. https://doi.org/10.1016/S0959-4388(02)00308-2

Rizzolatti, G., Fogassi, L., Gallese, V., 2001. Neurophysiological mechanisms underlying the understanding and imitation of action. Nat Rev Neurosci 2, 661–670. https://doi.org/10.1038/35090060

Rizzolatti, G., Matelli, M., Pavesi, G., 1983. Deficits in attention and movement following the removal of postarcuate (area 6) and prearcuate (area 8) cortex in macaque monkeys. Brain 106 (Pt 3), 655–673. https://doi.org/10.1093/brain/106.3.655

Rizzolatti, G., Scandolara, C., Matelli, M., Gentilucci, M., 1981. Afferent properties of periarcuate neurons in macaque monkeys. I. Somatosensory responses. Behavioural Brain Research 2, 125–146. https://doi.org/10.1016/0166-4328(81)90052-8

Roitman, J.D., Shadlen, M.N., 2002. Response of Neurons in the Lateral Intraparietal Area during a Combined Visual Discrimination Reaction Time Task. J. Neurosci. 22, 9475–9489. https://doi.org/10.1523/JNEUROSCI.22-21-09475.2002

Roland, P.E., Larsen, B., Lassen, N.A., Skinhøj, E., 1980. Supplementary motor area and other cortical areas in organization of voluntary movements in man. J Neurophysiol 43, 118–136. https://doi.org/10.1152/jn.1980.43.1.118

Rosenbaum, D.A., Kenny, S.B., Derr, M.A., 1983. Hierarchical control of rapid movement sequences. Journal of Experimental Psychology: Human Perception and Performance 9, 86–102. https://doi.org/10.1037/0096-1523.9.1.86

Rossant, C., Kadir, S.N., Goodman, D.F.M., Schulman, J., Hunter, M.L.D., Saleem, A.B., Grosmark, A., Belluscio, M., Denfield, G.H., Ecker, A.S., Tolias, A.S., Solomon, S.,

Buzsáki, G., Carandini, M., Harris, K.D., 2016. Spike sorting for large, dense electrode arrays. Nat Neurosci 19, 634–641. https://doi.org/10.1038/nn.4268

Rossi, M.A., Li, H.E., Lu, D., Kim, I.H., Bartholomew, R.A., Gaidis, E., Barter, J.W., Kim, N., Cai, M.T., Soderling, S.H., Yin, H.H., 2016. A GABAergic nigrotectal pathway for coordination of drinking behavior. Nat Neurosci 19, 742–748. https://doi.org/10.1038/nn.4285

Rouiller, E.M., Moret, V., Liang, F., 1993. Comparison of the Connectional Properties of the Two Forelimb Areas of the Rat Sensorimotor Cortex: Support for the Presence of a Premotor or Supplementary Motor Cortical Area. Somatosensory & Motor Research 10, 269–289. https://doi.org/10.3109/08990229309028837

Sachidhanandam, S., Sermet, B.S., Petersen, C.C.H., 2016. Parvalbumin-Expressing GABAergic Neurons in Mouse Barrel Cortex Contribute to Gating a Goal-Directed Sensorimotor Transformation. Cell Rep 15, 700–706. https://doi.org/10.1016/j.celrep.2016.03.063

Sacrey, L.-A.R., Alaverdashvili, M., Whishaw, I.Q., 2009. Similar hand shaping in reaching-for-food (skilled reaching) in rats and humans provides evidence of homology in release, collection, and manipulation movements. Behav Brain Res 204, 153–161. https://doi.org/10.1016/j.bbr.2009.05.035

Saiki, A., Sakai, Y., Fukabori, R., Soma, S., Yoshida, J., Kawabata, M., Yawo, H., Kobayashi, K., Kimura, M., Isomura, Y., 2018. In Vivo Spiking Dynamics of Intra- and Extratelencephalic Projection Neurons in Rat Motor Cortex. Cerebral Cortex 28, 1024–1038. https://doi.org/10.1093/cercor/bhx012

Sakai, K., Ramnani, N., Passingham, R.E., 2002. Learning of Sequences of Finger Movements and Timing: Frontal Lobe and Action-Oriented Representation. Journal of Neurophysiology 88, 2035–2046. https://doi.org/10.1152/jn.2002.88.4.2035

Schall, J.D., Thompson, K.G., 1999. Neural selection and control of visually guided eye movements. Annu Rev Neurosci 22, 241–259. https://doi.org/10.1146/annurev.neuro.22.1.241

Sekiguchi, Y., Hata, T., 2019. Effects of the mere presence of conspecifics on the motor performance of rats: Higher speed and lower accuracy. Behavioural Processes 159, 1–8. https://doi.org/10.1016/j.beproc.2018.12.012

Shadlen, M.N., Newsome, W.T., 1998. The Variable Discharge of Cortical Neurons: Implications for Connectivity, Computation, and Information Coding. J. Neurosci. 18, 3870–3896. https://doi.org/10.1523/JNEUROSCI.18-10-03870.1998

Shannon, C.E., 1948. A Mathematical Theory of Communication. Bell System Technical Journal 27, 379–423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x

Shapley, R., Hawken, M., Ringach, D.L., 2003. Dynamics of Orientation Selectivity in the Primary Visual Cortex and the Importance of Cortical Inhibition. Neuron 38, 689–699. https://doi.org/10.1016/S0896-6273(03)00332-5

Shenoy, K.V., Sahani, M., Churchland, M.M., 2013. Cortical control of arm movements: a dynamical systems perspective. Annu Rev Neurosci 36, 337–359. https://doi.org/10.1146/annurev-neuro-062111-150509

Shepherd, G.M.G., 2013. Corticostriatal connectivity and its role in disease. Nat Rev Neurosci 14, 278–291. https://doi.org/10.1038/nrn3469

Shimada, S., Hiraki, K., 2006. Infant's brain responses to live and televised action. Neuroimage 32, 930–939. https://doi.org/10.1016/j.neuroimage.2006.03.044

Simons, D.J., 1978. Response properties of vibrissa units in rat SI somatosensory neocortex. J Neurophysiol 41, 798–820. https://doi.org/10.1152/jn.1978.41.3.798

Sommer, M.A., Wurtz, R.H., 2000. Composition and topographic organization of signals sent from the frontal eye field to the superior colliculus. J Neurophysiol 83, 1979–2001. https://doi.org/10.1152/jn.2000.83.4.1979

Somogyi, P., Tamás, G., Lujan, R., Buhl, E.H., 1998. Salient features of synaptic organisation in the cerebral cortex1Published on the World Wide Web on 3 March 1998.1. Brain Research Reviews 26, 113–135. https://doi.org/10.1016/S0165-0173(97)00061-1

Spalletti, C., Alia, C., Lai, S., Panarese, A., Conti, S., Micera, S., Caleo, M., 2017. Combining robotic training and inactivation of the healthy hemisphere restores pre-stroke motor patterns in mice. Elife 6. https://doi.org/10.7554/eLife.28662

Spalletti, C., Lai, S., Mainardi, M., Panarese, A., Ghionzoli, A., Alia, C., Gianfranceschi, L., Chisari, C., Micera, S., Caleo, M., 2014. A robotic system for quantitative assessment and poststroke training of forelimb retraction in mice. Neurorehabil Neural Repair 28, 188–196. https://doi.org/10.1177/1545968313506520

Spector, A.C., St. John, S.J., 1998. Role of taste in the microstructure of quinine ingestion by rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 274, R1687–R1703. https://doi.org/10.1152/ajpregu.1998.274.6.R1687

Sreenivasan, V., Esmaeili, V., Kiritani, T., Galan, K., Crochet, S., Petersen, C.C.H., 2016. Movement Initiation Signals in Mouse Whisker Motor Cortex. Neuron 92, 1368–1382. https://doi.org/10.1016/j.neuron.2016.12.001

Stanek, E., Cheng, S., Takatoh, J., Han, B.-X., Wang, F., 2014. Monosynaptic premotor circuit tracing reveals neural substrates for oro-motor coordination. Elife 3, e02511. https://doi.org/10.7554/eLife.02511

Steriade, M., Oakson, G., Diallo, A., 1977. Reticular influences on lateralis posterior thalamic neurons. Brain Research 131, 55–71. https://doi.org/10.1016/0006-8993(77)90028-2

Sul, J.H., Jo, S., Lee, D., Jung, M.W., 2011. Role of rodent secondary motor cortex in value-based action selection. Nat Neurosci 14, 1202–1208. https://doi.org/10.1038/nn.2881

Sun, Y.J., Kim, Y.-J., Ibrahim, L.A., Tao, H.W., Zhang, L.I., 2013. Synaptic Mechanisms Underlying Functional Dichotomy between Intrinsic-Bursting and Regular-Spiking Neurons in Auditory Cortical Layer 5. Journal of Neuroscience 33, 5326–5339. https://doi.org/10.1523/JNEUROSCI.4810-12.2013

Svoboda, K., Li, N., 2018. Neural mechanisms of movement planning: motor cortex and beyond. Curr Opin Neurobiol 49, 33–41. https://doi.org/10.1016/j.conb.2017.10.023

Swadlow, H.A., 2003. Fast-spike interneurons and feedforward inhibition in awake sensory neocortex. Cereb Cortex 13, 25–32. https://doi.org/10.1093/cercor/13.1.25

Swadlow, H.A., 1989. Efferent neurons and suspected interneurons in S-1 vibrissa cortex of the awake rabbit: receptive fields and axonal properties. Journal of Neurophysiology 62, 288–308. https://doi.org/10.1152/jn.1989.62.1.288

Swadlow, H.A., 1988. Efferent neurons and suspected interneurons in binocular visual cortex of the awake rabbit: receptive fields and binocular properties. J Neurophysiol 59, 1162–1187. https://doi.org/10.1152/jn.1988.59.4.1162

Swadlow, H.A., Weyand, T.G., 1987. Corticogeniculate neurons, corticotectal neurons, and suspected interneurons in visual cortex of awake rabbits: receptive-field properties, axonal properties, and effects of EEG arousal. Journal of Neurophysiology 57, 977–1001. https://doi.org/10.1152/jn.1987.57.4.977

Szabadics, J., Lorincz, A., Tamás, G., 2001. Beta and gamma frequency synchronization by dendritic gabaergic synapses and gap junctions in a network of cortical interneurons. J Neurosci 21, 5824–5831.

Takano, Y., Ukezono, M., 2015. An experimental task to examine the mirror system in rats. Sci Rep 4, 6652. https://doi.org/10.1038/srep06652

Takano, Y., Ukezono, M., Nakashima, S.F., Takahashi, N., Hironaka, N., 2017. Learning of efficient behaviour in spatial exploration through observation of behaviour of conspecific in laboratory rats. R. Soc. open sci. 4, 170121. https://doi.org/10.1098/rsos.170121

Tamás, G., Buhl, E.H., Lörincz, A., Somogyi, P., 2000. Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. Nat Neurosci 3, 366–371. https://doi.org/10.1038/73936

Tamura, H., Kaneko, H., Kawasaki, K., Fujita, I., 2004. Presumed Inhibitory Neurons in the Macaque Inferior Temporal Cortex: Visual Response Properties and Functional Interactions With Adjacent Neurons. Journal of Neurophysiology 91, 2782–2796. https://doi.org/10.1152/jn.01267.2003

Tanahira, C., Higo, S., Watanabe, K., Tomioka, R., Ebihara, S., Kaneko, T., Tamamaki, N., 2009. Parvalbumin neurons in the forebrain as revealed by parvalbumin-Cre transgenic mice. Neurosci Res 63, 213–223. https://doi.org/10.1016/j.neures.2008.12.007

Tanaka, M., 2007. Cognitive Signals in the Primate Motor Thalamus Predict Saccade Timing. Journal of Neuroscience 27, 12109–12118. https://doi.org/10.1523/JNEUROSCI.1873-07.2007

Tanji, J., Evarts, E.V., 1976. Anticipatory activity of motor cortex neurons in relation to direction of an intended movement. Journal of Neurophysiology 39, 1062–1068. https://doi.org/10.1152/jn.1976.39.5.1062

Tantillo, E., Vannini, E., Cerri, C., Spalletti, C., Colistra, A., Mazzanti, C.M., Costa, M., Caleo, M., 2020. Differential roles of pyramidal and fast-spiking, GABAergic neurons in the control of glioma cell proliferation. Neurobiology of Disease 141, 104942. https://doi.org/10.1016/j.nbd.2020.104942

Tasic, B., Menon, V., Nguyen, T.N., Kim, T.K., Jarsky, T., Yao, Z., Levi, B., Gray, L.T., Sorensen, S.A., Dolbeare, T., Bertagnolli, D., Goldy, J., Shapovalova, N., Parry, S., Lee, C., Smith, K., Bernard, A., Madisen, L., Sunkin, S.M., Hawrylycz, M., Koch, C., Zeng, H., 2016. Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. Nat Neurosci 19, 335–346. https://doi.org/10.1038/nn.4216

Taylor, B.A., DeQuinzio, J.A., 2012. Observational Learning and Children With Autism. Behav Modif 36, 341–360. https://doi.org/10.1177/0145445512443981

Tennant, K.A., Adkins, D.L., Donlan, N.A., Asay, A.L., Thomas, N., Kleim, J.A., Jones, T.A., 2011. The Organization of the Forelimb Representation of the C57BL/6 Mouse Motor Cortex as Defined by Intracortical Microstimulation and Cytoarchitecture. Cerebral Cortex 21, 865–876. https://doi.org/10.1093/cercor/bhq159

Thaler, D., Chen, Y.-C., Nixon, P.D., Stern, C.E., Passingham, R.E., 1995. The functions of the medial premotor cortex: I. Simple learned movements. Exp Brain Res 102. https://doi.org/10.1007/BF00230649

Thexton, A.J., 1992. Mastication and swallowing: an overview. Br Dent J 173, 197–206. https://doi.org/10.1038/sj.bdj.4808002

Thura, D., Cisek, P., 2014. Deliberation and Commitment in the Premotor and Primary Motor Cortex during Dynamic Decision Making. Neuron 81, 1401–1416. https://doi.org/10.1016/j.neuron.2014.01.031

Tombaz, T., Dunn, B.A., Hovde, K., Cubero, R.J., Mimica, B., Mamidanna, P., Roudi, Y., Whitlock, J.R., 2020. Action representation in the mouse parieto-frontal network. Sci Rep 10, 5559. https://doi.org/10.1038/s41598-020-62089-6

Travers, J.B., Dinardo, L.A., Karimnamazi, H., 1997. Motor and Premotor Mechanisms of Licking. Neuroscience & Biobehavioral Reviews 21, 631–647. https://doi.org/10.1016/S0149-7634(96)00045-0

Turner, R.S., DeLong, M.R., 2000. Corticostriatal activity in primary motor cortex of the macaque. J Neurosci 20, 7096–7108.

Ushakov, V.L., Kartashov, S.I., Zavyalova, V.V., Bezverhiy, D.D., Posichanyuk, V.I., Terentev, V.N., Anokhin, K.V., 2013. Network activity of mirror neurons depends on experience. J. Integr. Neurosci. 12, 35–46. https://doi.org/10.1142/S0219635213500040

van brederode, J.F.M., helliesen, M.K., hendrickson, A.E., 1991. Distribution of the calcium-binding proteins parvalbumin and calbindin-D28k in the sensorimotor cortex of the rat. Neuroscience 44, 157–171. https://doi.org/10.1016/0306-4522(91)90258-P

van Haeften, T., Baks-te-Bulte, L., Goede, P.H., Wouterlood, F.G., Witter, M.P., 2003. Morphological and numerical analysis of synaptic interactions between neurons in deep and superficial layers of the entorhinal cortex of the rat. Hippocampus 13, 943–952. https://doi.org/10.1002/hipo.10144

van Vreeswijk, C., Sompolinsky, H., 1996. Chaos in neuronal networks with balanced excitatory and inhibitory activity. Science 274, 1724–1726. https://doi.org/10.1126/science.274.5293.1724

Vigneswaran, G., Kraskov, A., Lemon, R.N., 2011. Large Identified Pyramidal Cells in Macaque Motor and Premotor Cortex Exhibit "Thin Spikes": Implications for Cell Type Classification. Journal of Neuroscience 31, 14235–14242. https://doi.org/10.1523/JNEUROSCI.3142-11.2011

Virji-Babul, N., Rose, A., Moiseeva, N., Makan, N., 2012. Neural correlates of action understanding in infants: influence of motor experience. Brain Behav 2, 237–242. https://doi.org/10.1002/brb3.50

Wehr, M., Zador, A.M., 2003. Balanced inhibition underlies tuning and sharpens spike timing in auditory cortex. Nature 426, 442–446. https://doi.org/10.1038/nature02116

Weijnen, J., 1998. Licking Behavior in the Rat: Measurement and Situational Control of Licking Frequency. Neuroscience & Biobehavioral Reviews 22, 751–760. https://doi.org/10.1016/S0149-7634(98)00003-7

Weijnen, J.A.W.M., Wouters, J., van Hest, J.M.H.H., 1984. Interaction between Licking and Swallowing in the Drinking Rat. Brain Behav Evol 25, 117–127. https://doi.org/10.1159/000118857

Weinrich, M., Wise, S.P., 1982. The premotor cortex of the monkey. J Neurosci 2, 1329–1345.

Welsh, J.P., Lang, E.J., Suglhara, I., Llinás, R., 1995. Dynamic organization of motor control within the olivocerebellar system. Nature 374, 453–457. https://doi.org/10.1038/374453a0

Whishaw, I.Q., Faraji, J., Kuntz, J., Mirza Agha, B., Patel, M., Metz, G.A.S., Mohajerani, M.H., 2017. Organization of the reach and grasp in head-fixed vs freely-moving mice provides support for multiple motor channel theory of neocortical organization. Exp Brain Res 235, 1919–1932. https://doi.org/10.1007/s00221-017-4925-4

Whishaw, I.Q., Pellis, S.M., Gorny, B.P., 1992. Skilled reaching in rats and humans: evidence for parallel development or homology. Behav Brain Res 47, 59–70. https://doi.org/10.1016/s0166-4328(05)80252-9

Whissell, P.D., Cajanding, J.D., Fogel, N., Kim, J.C., 2015. Comparative density of CCK- and PV-GABA cells within the cortex and hippocampus. Front Neuroanat 9, 124. https://doi.org/10.3389/fnana.2015.00124

Wilent, W.B., 2004. Synaptic Responses to Whisker Deflections in Rat Barrel Cortex as a Function of Cortical Layer and Stimulus Intensity. Journal of Neuroscience 24, 3985–3998. https://doi.org/10.1523/JNEUROSCI.5782-03.2004

Wilson, N.R., Runyan, C.A., Wang, F.L., Sur, M., 2012. Division and subtraction by distinct cortical inhibitory networks in vivo. Nature 488, 343–348. https://doi.org/10.1038/nature11347

Wise, S.P., Jones, E.G., 1977. Cells of origin and terminal distribution of descending projections of the rat somatic sensory cortex. J. Comp. Neurol. 175, 129–157. https://doi.org/10.1002/cne.901750202

Wise, S.P., Mauritz, K.H., 1985. Set-related neuronal activity in the premotor cortex of rhesus monkeys: effects of changes in motor set. Proc R Soc Lond B Biol Sci 223, 331–354. https://doi.org/10.1098/rspb.1985.0005

Wise, S.P., Weinrich, M., Mauritz, K.-H., 1986. Movement-related activity in the premotor cortex of rhesus macaques, in: Progress in Brain Research. Elsevier, pp. 117–131. https://doi.org/10.1016/S0079-6123(08)63407-X

Wöhr, M., Scattoni, M.L., 2013. Behavioural methods used in rodent models of autism spectrum disorders: Current standards and new developments. Behavioural Brain Research 251, 5–17. https://doi.org/10.1016/j.bbr.2013.05.047

Woolsey, C.N., Settlage, P.H., Meyer, D.R., Sencer, W., Pinto Hamuy, T., Travis, A.M., 1952. Patterns of localization in precentral and “supplementary” motor areas and their relation to the concept of a premotor area. Res Publ Assoc Res Nerv Ment Dis 30, 238–264.

Xu, T., Yu, X., Perlik, A.J., Tobin, W.F., Zweig, J.A., Tennant, K., Jones, T., Zuo, Y., 2009. Rapid formation and selective stabilization of synapses for enduring motor memories. Nature 462, 915–919. https://doi.org/10.1038/nature08389

Yokoi, I., Komatsu, H., 2010. Putative pyramidal neurons and interneurons in the monkey parietal cortex make different contributions to the performance of a visual grouping task. J Neurophysiol 104, 1603–1611. https://doi.org/10.1152/jn.00160.2010

Zagha, E., Ge, X., McCormick, D.A., 2015. Competing Neural Ensembles in Motor Cortex Gate Goal-Directed Motor Output. Neuron 88, 565–577. https://doi.org/10.1016/j.neuron.2015.09.044

Zajonc, R.B., 1965. Social Facilitation. Science 149, 269–274. https://doi.org/10.1126/science.149.3681.269

Zeigler, H.P., 1991. Drinking in Mammals: Functional Morphology, Orosensory Modulation and Motor Control, in: Ramsay, D.J., Booth, D. (Eds.), Thirst, ILSI Human Nutrition Reviews. Springer London, London, pp. 241–257. https://doi.org/10.1007/978-1-4471-1817-6_15

Zentall, T.R., Levine, J.M., 1972. Observational Learning and Social Facilitation in the Rat. Science 178, 1220–1221. https://doi.org/10.1126/science.178.4066.1220

7. Appendix

During my period of PhD, I have also contributed to other projects with the following publications:

Pathogenic NR2F1 variants cause a developmental non-progressive ocular phenotype recapitulated in a mutant mouse model

Neringa Jurkute[1,2*], Michele Bertacchi[3*], Gavin Arno[1,2], Chiara Tocco[3], Ungsoo Samuel Kim[1,4], Adam M. Kruszewski[5], Robert A Avery[6,7], Emma C. Bedoukian[8], Jinu Han[9], Sung Jun Ahn[10], Nikolas Pontikos[1,2], James Acheson[1,11], Indran Davagnanam[1,12], Richard Bowman[13], Marios Kaliakatsos[14], Alice Gardham[15], Emma Wakeling[16], Ngozi Oluonye[1,17], Ashwin Reddy[1,18], Elaine Clark[19], Elisabeth Rosser[20], Patrizia Amati-Bonneau[21,22], Majida Charif[21,23], Guy Lenaers[21], Isabelle Meunier[24], Sabine Defoort[26], Catherine Vincent-Delorme[27], Anthony G Robson[1,2], Graham E Holder[2,28], Luc Jeanjean[29], Antonio Martinez-Monseny[30], Mariona Vidal Santacana[31], Chloé Dominici[32], Cedric Gaggioli[32], **Nadia Giordano**[33], Matteo Caleo[33], Grant T. Liu[6,7], Genomics England Research Consortium, Andrew R Webster[1,2], Michèle Studer[3#], Patrick Yu-Wai-Man[1,2,34,35#]

[1] Moorfields Eye Hospital NHS Foundation Trust, London, UK
[2] Institute of Ophthalmology, University College London, London, UK
[3] Université Côte d'Azur, CNRS, Inserm, iBV, France
[4] Kim's Eye Hospital, Seoul, South Korea
[5] Department of Neurology, Hospital of the University of Pennsylvania, Perelman School of Medicine, Philadelphia, Pennsylvania, US
[6] Division of Ophthalmology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, US
[7] Departments of Neurology and Ophthalmology, Perelman School of Medicine, Philadelphia, Pennsylvania, US
[8] Roberts Individualized Medical Genetics Center, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, US
[9] Institute of Vision Research, Department of Ophthalmology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea
[10] Department of Radiology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea
[11] National Hospital for Neurology and Neurosurgery, University College London Hospitals NHS trust, London, UK
[12] Department of Brain Repair & Rehabilitation, UCL Queen Square Institute of Neurology, London, UK.
[13] Department of Ophthalmology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
[14]. Paediatric Neurology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
[15] North West Thames Regional Genetics Service, Northwick Park Hospital, Harrow, UK
[16] North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
[17] Wolfson Neurodisability Service, Great Ormond Street Hospital NHS Foundation Trust, London, UK
[18] Royal London Hospital, Barts Health NHS Trust, London, UK

[19] Department of Neuroscience, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
[20] Department of Clinical Genetics, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
[21] MitoLab team, UMR CNRS 6015 - INSERM U1083, Institut MitoVasc, Angers University and Hospital, Angers, France
[22] Departments of Biochemistry and Genetics, University Hospital Angers, Angers, France
[23] Genetics and immuno-cell therapy Team, Mohammed First University, Oujda, Morocco
[24] National center for rare diseases, Inherited sensory disorders, Gui de Chauliac Hospital, Montpellier, France
[25] Institut des Neurosciences de Montpellier, INSERM, Université de Montpellier, Montpellier, France
[26] Service d'exploration de la vision et neuro-ophtalmologie, CHRU de Lille, Lille, France
[27] Service de Génétique médicale, Hôpital Jeanne de Flandre, CHRU de Lille, Lille, France
[28] Yong Loo Lin School of Medicine, Department of Ophthalmology, National University of Singapore, Singapore
[29] Department of Ophthalmology, University Hospital of Nimes, Nimes, France
[30] Genetic and Molecular Medicine Department, Hospital Sant Joan de Déu, Barcelona, Spain
[31] Department of Ophthalmology, Hospital Sant Joan de Déu, Barcelona, Spain
[32] University Côte d'Azur, CNRS UMR7284, INSERM U1081, Institute for Research on Cancer and Aging, Nice, France
[33] Neuroscience Institute-CNR, Pisa, Italy.
[34] Cambridge Eye Unit, Addenbrooke's Hospital, Cambridge University Hospitals, Cambridge, UK
[35] John van Geest Centre for Brain Repair and MRC Mitochondrial Biology Unit, Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK
* These authors contributed equally to this paper
[#] Joint senior authors

Abstract

Pathogenic NR2F1 variants cause a rare autosomal dominant neurodevelopmental disorder referred to as the Bosch-Boonstra-Schaaf Optic Atrophy Syndrome (BBSOAS). Although visual loss is a prominent feature seen in affected individuals, the molecular and cellular mechanisms contributing to visual impairment is still poorly characterized. We conducted a deep phenotyping study on a cohort of 22 individuals carrying pathogenic NR2F1 variants to document the neurodevelopmental and ophthalmological manifestations, in particular the structural and functional changes within the retina and the optic nerve, which have not been detailed previously. The visual impairment became apparent in early childhood and in addition to optic atrophy, small and/or tilted hypoplastic optic nerves were observed in 10 cases. High-resolution optical coherence tomography imaging confirmed significant loss of retinal ganglion cells (RGCs) with thinning of the ganglion cell layer, consistent with electrophysiological evidence of RGC dysfunction. Interestingly, for those individuals with available longitudinal ophthalmological data, there was no significant deterioration in visual function during the period of follow-up. Diffusion tensor imaging tractography studies in one case showed defective connections and disorganisation of the extracortical visual pathways. To further investigate how pathogenic NR2F1 variants impact on retinal and optic nerve development, we took advantage of a Nr2f1 mutant mouse disease model and the availability of non-pathological human foetal samples. Abnormal retinogenesis in early stages of development was observed in Nr2f1 mutant mice with reduction of RGC number and disruption of optic nerve axonal guidance from the neural retina into the optic stalk, accounting for the development of malformations such as optic nerve hypoplasia. Electrophysiological analysis of visual acuity in mice showed reduced visual acuity linked to significant conduction delay and decreased amplitude in superficial layers of the visual cortex. The clinical observations in our study cohort,

supported by the mouse data, suggest an early neurodevelopment origin for the retinal and optic nerve head defects caused by pathogenic variants in NR2F1, ultimately resulting in stable and non-progressive decreased visual acuity, which differs from gradual vision loss found in classical inherited optic neuropathies. We propose NR2F1 as a major gene orchestrating early retinal and optic nerve head development and playing a key role in the maturation of the visual system.

Brain Communications (in revision after review).

Combining robotics with enhanced serotonin-driven cortical plasticity improves post-stroke motor recovery

Conti Sara[1 2], Spalletti Cristina[3], Pasquini Maria[1], **Giordano Nadia**[3], Barsotti Noemi[4], Mainardi Marco[3], Lai Stefano[1], Giorgi Andrea[4], Pasqualetti Massimo[4 5], Micera Silvestro [1 6], Caleo Matteo[3 7]

[1] Translational Neural Engineering Area, The BioRobotics Institute, Scuola Superiore Sant'Anna, Pisa, Italy.
[2] Department of Neuroscience and Movement Science, Platform of Translational Neurosciences, University of Fribourg, Switzerland.
[3] CNR Neuroscience Institute, Pisa, Italy.
[4] Unit of Cell and Developmental Biology, Department of Biology, University of Pisa, Italy.
[5] Center for Neuroscience and Cognitive Systems, Istituto Italiano di Tecnologia, Rovereto, Italy.
[6] Bertarelli Foundation Chair in Translational NeuroEngineering Laboratory, École Polytechnique Fédérale de Lausanne (EPFL), Centre for Neuroprosthetics and Institute of Bioengineering, Lausanne, Switzerland.
[7] Department of Biomedical Sciences, University of Padua, Italy.

Abstract

Despite recent progresses in robotic rehabilitation technologies, their efficacy for post-stroke motor recovery is still limited. These limitations might stem from the insufficient enhancement of plasticity mechanisms crucial for functional recovery. Here, we designed a clinically relevant strategy that combines robotic rehabilitation with chemogenetic stimulation of serotonin release to boost plasticity. Mice treated with our combined therapy showed substantial functional gains that persisted beyond the treatment period and generalized to non-trained tasks. Motor recovery was associated with a reduction in GABAergic markers, suggesting disinhibition in perilesional areas. To unveil the translational potentialities of our approach, we specifically targeted the serotonin 1A receptor by delivering buspirone, a clinically approved drug, in stroke mice undergoing robotic rehabilitation. Administration of buspirone restored motor impairments similarly to what observed with chemogenetic stimulation, showing the immediate translational potential of this combined approach to significantly improve motor recovery after stroke.

Prog Neurobiol. 2021 May 11;102073. doi: 10.1016/j.pneurobio.2021.102073. Online ahead of print.

Dysregulated autophagy as a new aspect of the molecular pathogenesis of Krabbe disease

Ambra Del Grosso [1], Lucia Angella [1], Ilaria Tonazzini [1], Aldo Moscardini [1], **Nadia Giordano** [2], Matteo Caleo [3], Silvia Rocchiccioli [4], Marco Cecchini [5]

[1] NEST, Isituto Nanoscienze-CNR and Scuola Normale Superiore, Piazza San Silvestro 12, 56127 Pisa, Italy.
[2] Scuola Normale Superiore, Piazza dei Cavalieri 7, 56127 Pisa, Italy; CNR Neuroscience Institute, via G. Moruzzi 1, 56124 Pisa, Italy.
[3] CNR Neuroscience Institute, via G. Moruzzi 1, 56124 Pisa, Italy; Department of Biomedical Sciences, University of Padua, 35121 Padova, Italy.
[4] Institute of Clinical Physiology-CNR, Via Giuseppe Moruzzi 1, Pisa 56124, Italy.
[5] NEST, Isituto Nanoscienze-CNR and Scuola Normale Superiore, Piazza San Silvestro 12, 56127 Pisa, Italy. Electronic address: marco.cecchini@nano.cnr.it.

Abstract

Krabbe disease (KD) is a childhood leukodystrophy with no cure currently available. KD is due to a deficiency of a lysosomal enzyme called galactosyl-ceramidase (GALC) and is characterized by the accumulation in the nervous system of the sphingolipid psychosine (PSY), whose cytotoxic molecular mechanism is not fully known yet. Here, we study the expression of some fundamental autophagy markers (LC3, p62, and Beclin-1) in a KD murine model [the twitcher (TWI) mouse] by immunohistochemistry and Western blot. Moreover, the autophagy molecular process is also shown in primary fibroblasts from TWI and WT mice, with and without PSY treatment. Data demonstrate that large p62 cytoplasmic aggregates are present in the brain of both early and late symptomatic TWI mice. p62 expression is also upregulated in TWI sciatic nerves compared to that measured for WT nerves. In vitro data suggest that this effect might not be fully PSY-driven. Finally, we investigate in vitro the capability of autophagy inducers (Rapamycin, RAP and Resveratrol, RESV) to reinstate the WT phenotype in TWI cells. We show that RAP administration can partially restore the autophagy markers levels, while RESV cannot, indicating a line along which new therapeutic approaches can be developed.

Neurobiol Dis. 2019 Sep;129:195-207. doi: 10.1016/j.nbd.2019.05.011. Epub 2019 May 17.

Quantitative Microproteomics Based Characterization of the Central and Peripheral Nervous System of a Mouse Model of Krabbe Disease

Davide Pellegrini [1 2], Ambra Del Grosso [1 3], Lucia Angella [3], **Nadia Giordano** [4], Marialaura Dilillo [2], Ilaria Tonazzini [3], Matteo Caleo [4], Marco Cecchini [1 3], Liam A McDonnell [5 6]

[1] From ‡NEST, Scuola Normale Superiore, Pisa 56127, Italy.
[2] Fondazione Pisana per la Scienza ONLUS, 56107 San Giuliano Terme, Pisa, Italy.
[3] NEST, Istituto Nanoscienze-CNR, Pisa, Italy.
[4] Neuroscience Institute-CNR, Pisa, Italy.
[5] Fondazione Pisana per la Scienza ONLUS, 56107 San Giuliano Terme, Pisa, Italy; l.a.mcdonnell@outlook.com.
[6] Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands.

Abstract

Krabbe disease is a rare, childhood lysosomal storage disorder caused by a deficiency of galactosylceramide beta-galactosidase (GALC). The major effect of GALC deficiency is the accumulation of psychosine in the nervous system and widespread degeneration of oligodendrocytes and Schwann cells, causing rapid demyelination. The molecular mechanisms of Krabbe disease are not yet fully elucidated and a definite cure is still missing. Here we report the first in-depth characterization of the proteome of the Twitcher mouse, a spontaneous mouse model of Krabbe disease, to investigate the proteome changes in the Central and Peripheral Nervous System. We applied a TMT-based workflow to compare the proteomes of the corpus callosum, motor cortex and sciatic nerves of littermate homozygous Twitcher and wild-type mice. More than 400 protein groups exhibited differences in expression and included proteins involved in pathways that can be linked to Krabbe disease, such as inflammatory and defense response, lysosomal proteins accumulation, demyelination, reduced nervous system development and cell adhesion. These findings provide new insights on the molecular mechanisms of Krabbe disease, representing a starting point for future functional experiments to study the molecular pathogenesis of Krabbe disease. Data are available via ProteomeXchange with identifier PXD010594.

Mol Cell Proteomics. 2019 Jun;18(6):1227-1241. doi: 10.1074/mcp.RA118.001267. Epub 2019 Mar 29.

Intravenous infusion of human bone marrow mesenchymal stromal cells promotes functional recovery and neuroplasticity after ischemic stroke in mice

Eliana Sammali [1 2], Claudia Alia [3 4], Gloria Vegliante [1], Valentina Colombo [5 6], **Nadia Giordano** [3 4], Francesca Pischiutta [1], Giorgio B Boncoraglio [2], Mario Barilani [7], Lorenza Lazzari [7], Matteo Caleo [3], Maria-Grazia De Simoni [1], Giuseppe Gaipa [5 6], Giuseppe Citerio [8 9], Elisa R Zanier [10]

[1] Department of Neuroscience, IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Via La Masa,19, 20156, Milano, Italy.
[2] Department of Cerebrovascular Diseases, Fondazione IRCCS - Istituto Neurologico Carlo Besta, Milano, Italy.
[3] Neuroscience Institute, CNR, Pisa, Italy.
[4] Scuola Normale Superiore, Pisa, Italy.
[5] Laboratory for Cell and Gene Therapy "Stefano Verri", ASST-Monza, San Gerardo Hospital, Monza, Italy.
[6] Tettamanti Research Center, Pediatric Department, University of Milano-Bicocca, Monza, Italy.
[7] Cell Factory, Unit of Cell Therapy and Cryobiology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via F. Sforza 35, 20122, Milano, Italy.
[8] School of Medicine and Surgery, University of Milano-Bicocca, Milano, Italy.
[9] Neurointensive Care, ASST-Monza, San Gerardo Hospital, Monza, Italy.
[10] Department of Neuroscience, IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Via La Masa,19, 20156, Milano, Italy. elisa.zanier@marionegri.it.

Abstract

Transplantation of human bone marrow mesenchymal stromal cells (hBM-MSC) promotes functional recovery after stroke in animal models, but the mechanisms underlying these effects remain incompletely understood. We tested the efficacy of Good Manufacturing Practices (GMP) compliant hBM-MSC, injected intravenously 3.5 hours after injury in mice subjected to transient middle cerebral artery occlusion (tMCAo). We addressed whether hBM-MSC are efficacious and if this efficacy is associated with cortical circuit reorganization using neuroanatomical analysis of GABAergic neurons (parvalbumin; PV-positive cells) and perineuronal nets (PNN), a specialized extracellular matrix structure which acts as an inhibitor of neural plasticity. tMCAo mice receiving hBM-MSC, showed early and lasting improvement of sensorimotor and cognitive functions compared to control tMCAo mice. Furthermore, 5 weeks post-tMCAo, hBM-MSC induced a significant rescue of ipsilateral cortical neurons; an increased proportion of PV-positive neurons in the perilesional cortex, suggesting GABAergic interneurons preservation; and a lower percentage of PV-positive cells surrounded by PNN, indicating an enhanced plastic potential of the perilesional cortex. These results show that hBM-MSC improve functional recovery and stimulate neuroprotection after stroke. Moreover, the downregulation of "plasticity brakes" such as PNN suggests that hBM-MSC treatment stimulates plasticity and formation of new connections in the perilesional cortex.

Sci Rep. 2017 Jul 31;7(1):6962. doi: 10.1038/s41598-017-07274-w.