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HIERARCHICAL, INTERMINGLED AND SEGMENTED:
THE ORGANIZATION OF NEURAL ACTIVITY IN MOUSE SENSORIMOTOR CORTEX

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BY
SOHRAB SALIMIAN

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For my Mom and Dad.

‘In the face of overwhelming odds, I am left with only one option. I’m going to have to science
the shit out of this’ – Mark Watney

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ABSTRACT

Cortical control of movement is a distributed computation spanning multiple, densely interconnected regions. Although we have rich anatomical atlases and a coarse understanding of how function maps to these areas and subareas, we lack a detailed account of how behaviorally relevant activity is organized across the cortical sheet. Progress requires two key factors: (i) a challenging sensorimotor task with many conditions to engage sensorimotor circuits, and (ii) large-scale, single-neuron-resolution sampling across the full set of involved areas.

In this dissertation, I address these gaps with two studies that pair a motorically demanding behavior with dense sampling of mouse sensorimotor cortex. First, we trained mice to perform a challenging reach-to-grasp task while recording neural activity in primary motor cortex (M1) and forelimb somatosensory cortex (S1-fl). Detailed kinematic information was similarly present in both regions; however, movement-related signals emerged earlier and were more persistent in M1, whereas S1-fl responses were more time-varying. These findings support a distributed control framework and are consistent with somatosensory areas receiving and transforming efference-copy-like information.

Second, we expanded the task to fifteen target locations and performed cortex-wide, single-neuron sampling across much of sensorimotor cortex. Using statistical models on tuning features, we identified interleaved subpopulations with distinct response profiles. This large and rich dataset reveals a parallel-processing architecture where local specializations sit inside a distributed, flexibly routed network.

CHAPTER 1

GENERAL INTRODUCTION

1.1. Introduction

The work presented in this thesis explains how cortical activity is structured across the mouse sensorimotor cortex during a reach-to-grasp behavior. To date, there has been no dense, single-neuron sampling of the mouse sensorimotor cortex during behavior. This General Introduction provides a concise background on the motor system, outlines why the mouse is an attractive model species to answer this question, and identifies key gaps in mouse motor neuroscience. Chapter Two introduces a kinematically challenging task and examines the distribution of kinematic information across primary motor cortex (M1) and the somatosensory forelimb cortex (S1-fl). Chapter Three extends that work by presenting a more challenging version of the task and sampling a much larger fraction of the sensorimotor cortex while mice perform it, to map the spatial footprint of activity patterns. Chapter Four situates the findings in the broader literature, discusses remaining questions, and outlines next steps.

1.2. Macaque motor cortex: Establishing motor neuroscience

A central question in motor neuroscience is straightforward to pose and difficult to answer: how does the brain drive muscles to produce voluntary movement? Early work approached this from the periphery—muscles and motoneurons—back toward the brain, seeking a mechanistic chain that runs from cortical spikes to spinal α -motoneuron activation and force production (Adrian and Bronk 1928; Cheney and Fetz 1980; Eccles et al. 1957; Evarts 1968;

Fetz 1969; Georgopoulos et al. 1982; Henneman 1957; Porter and Lemon 1995; Sherrington 1906; Somjen et al. 1965). The macaque became the natural testbed for this problem because primates, unlike many other mammals, possess a particularly strong corticospinal system with monosynaptic corticomotoneuronal (CM) connections onto spinal motoneurons, especially for distal hand musculature (Cheney and Fetz 1980; Heffner and Masterton 1975; Lemon and Griffiths 2005; Porter and Lemon 1995; Rathelot and Strick 2009). Those direct links provide an experimentally accessible bridge between cortical discharge and muscle activity, enabling inferences that are more difficult in species lacking such connections (Lemon 2008; Porter and Lemon 1995). Against this background, macaque work set out not merely to correlate cortex with kinematics, but to identify which cortical neurons provide the descending command, how they are organized, and how their activity relates to the timing and patterning of muscle contraction.

Within this agenda, the first major step was to look at the output pathway most likely to influence spinal circuits: pyramidal tract (PT) neurons. Seminal experiments by Evarts (1968) recorded PT neurons—corticospinal cells projecting to the spinal cord—during the production of voluntary movement. These recordings uncovered both phasic and tonic-like response profiles across neurons with different conduction velocities, immediately demonstrating that single units in the descending pathway exhibit task-related, heterogeneous tuning. This was critical: the earliest evidence tying identified corticofugal neurons to movement already showed diversity, suggesting that motor cortical neurons possess a mixed-tuning profile.

The next step was to show that individual cortical spikes can, in fact, be linked to muscle activation. Fetz (1969) demonstrated operant conditioning of single cortical neurons in awake

monkeys, proving that the activity of identified units could be systematically related to behavior. Building on that foundation, Cheney & Fetz (1980) used spike-triggered averaging of EMG to isolate CM cells—cortical neurons whose spikes produce short-latency postspike facilitation in specific muscles. By focusing exclusively on such corticomotoneuronal cells, they cataloged a diverse set of temporal motifs (phasic, tonic, ramping, phasic-tonic, phasic-ramp) during a wrist movement task. This established a concrete physiological chain from cortical spike to muscle response and explained why PT/CM neurons became a focal point: they are the most proximal, identifiable cortical handle on the descending command to muscle. Parallel lines of work strengthened the link from cortex to muscle by relating cortical activity to detailed EMG/kinematic structure (London and Miller 2013; Morrow and Miller 2003) and by decoding muscle activity and movement from motor cortex (Pohlmeyer et al. 2007), reinforcing the idea that M1 signals can be transformed into muscle-level outputs.

Having shown that cortical spikes can influence muscles, the field then asked when and how cortical output is engaged. Introducing an instruction (delay) epoch before movement, Evarts & Tanji (1976) observed anticipatory activity in motor cortex—especially among PT neurons—before movement onset. This was an early indication that descending pathways are preparatory, not merely reactive: the motor system appears to set up the required state for upcoming action before muscles are recruited. That framing naturally motivated questions about where in cortex different movement components live and how those components are organized spatially.

To localize function, macaque studies turned to stimulation mapping. Short-train intracortical microstimulation identified sites whose activation produces specific muscular

outputs, revealing a somatotopic organization in which cortical location relates systematically to body part (Graziano et al. 2002; Hatanaka et al. 2001; Qi et al. 2010; Raos et al. 2003). Recognizing the limitations of brief stimulation (often under anesthesia), Graziano et al. (2002) applied longer (~500 ms) stimulation in awake monkeys, evoking coordinated, ethologically relevant actions that varied with stimulation site. This suggested that these fields jointly map the reachable workspace and can coordinate multi-joint patterns, not merely twitch isolated muscles. Crucially, however, CM/PT physiology and anatomy imply extensive divergence and convergence. A single corticomotoneuronal cell can facilitate multiple motoneuron pools, and each muscle receives input from many cortical cells (Cheney and Fetz 1980; Porter and Lemon 1995; Rathelot and Strick 2009). That many-to-many mapping means single-site stimulation or single-neuron analyses, while invaluable for where outputs can be elicited, cannot by themselves explain how graded, multi-joint forces and trajectories are constructed from distributed cortical activity. This realization naturally pushed the field toward population descriptions that could capture combination rules across neurons.

A complementary approach, therefore, asked a normative question: how might populations of M1 neurons specify movement variables? In center-out reaching, Georgopoulos et al. (1982) reported that most M1 neurons exhibit preferred-direction tuning with a bell-shaped dependence on reach direction, motivating the population-vector concept in which direction is read out as a weighted average across neurons. Critically, pooling across neurons improved predictions of limb kinematics and muscle activity, reinforcing the idea that the many-to-many mapping between cortex and muscle is best captured at the population level (London and Miller 2013; Morrow and Miller 2003; Pohlmeier et al. 2007; Salinas and Abbott 1994). However, direction alone proved insufficient: Moran & Schwartz (1999) showed speed sensitivity, and

Churchland et al. (2006) demonstrated that tuning for distance, direction, and speed is time-varying across task epochs. Together with the known anatomical divergence and convergence of CM/PT projections, and dense intracortical connectivity, these findings implied that single neurons operate as part of covarying groups whose joint activity evolves over time—pushing the field beyond static tuning toward methods that explicitly model population covariance and its temporal structure (Churchland and Shenoy 2007).

Given this shift, the next step was to analyze population activity as a dynamical process. During reaching, M1/PMd populations trace low-dimensional trajectories that include conserved rotational dynamics across conditions (Churchland et al. 2012), suggesting that motor cortex implements a dynamical system that generates time-evolving commands rather than a bank of fixed encoders. Within this perspective, planning and execution occupy partially distinct subspaces, reducing interference between preparing and producing movement (Elsayed et al. 2016). A mechanistic account emerged from Kaufman et al. (2014): preparatory activity can evolve within the output-null subspace of a downstream readout (another cortical area or muscles), so plans do not prematurely drive movement. Building on this logic, recent work argues that cortex also uses selective “communication subspaces” to route information between areas, so that only particular population patterns are shared while others remain local—supporting flexible, context-dependent interactions (Perich et al. 2024; Semedo et al. 2019). These insights dovetail with optimal feedback control accounts in which commands are continuously shaped by predictions, sensory errors, and task constraints (Scott 2004; Todorov and Jordan 2002), and they motivate analyses that explicitly relate within-area dynamics to across-area communication. Seen through that lens, population dynamics point directly to circuit mechanism: if preparation unfolds in an output-null subspace (Kaufman et al. 2014) and inter-

areal interactions are routed through restricted communication subspaces (Perich et al. 2024; Semedo et al. 2019), then the wiring diagram—which projections exist, which cell types participate, and which laminar targets they reach—must constrain which population patterns are shared, gated, or kept local. Because premotor, motor, and somatosensory cortices exchange signals continuously and are embedded within parietal, cerebellar, and basal ganglia loops for goals, state estimation, timing, and vigor (Cisek and Kalaska 2005; Diedrichsen et al. 2010; Hatanaka et al. 2001; Kalaska et al. 1997; Raos et al. 2003), explaining behavior now requires circuit-level accounts that link subspaces to identified pathways and cell classes. Yet in macaques, routine cell-type-specific access, large-scale projection-defined recordings, cortex-wide single-cell imaging, and precise ensemble perturbations remain limited—constraining causal tests of how subspaces are implemented and communicated. These practical limits do not diminish the primate insights; rather, they motivate a complementary move to a model with deeper circuit access. In the next section, we motivate the mouse—with genetic targeting, projection-specific labeling, cellular-resolution imaging, and optical perturbations—as the platform to test how identifiable subpopulations implement the population dynamics and communication subspaces inferred from primate work.

1.3. The mouse as a model to study the neural underpinnings of the motor system

The mouse is emerging as a promising animal model for understanding the neural basis of motor control. The promise stems from access: genetics, viral tools, and optical methods enable researchers to identify cell types, mark projection targets, and observe or perturb activity across large cortical territories. On the anatomy side, this access has been matched by standardized atlases that anchor measurements across labs. Leveraging the genetic tools

available, multiple groups have undertaken large segmentation efforts to determine the anatomical organization of the mouse brain (Harris et al. 2019; Muñoz-Castañeda et al. 2021; Oh et al. 2014; Wang et al. 2020; Yao et al. 2023; Zingg et al. 2014). Of particular use is the comprehensive atlas by Wang et al. (2020), which leveraged more than 1600 mice to produce a 3D, 10 μ m resolution reference. The segmentation drew on serial two-photon tomography, axonal projection patterns, immunohistochemistry, cytoarchitecture, and in situ hybridization, integrated with expert curation. Within the sensorimotor strip, this work delineated secondary motor cortex (M2), primary motor cortex (M1), somatosensory forelimb (S1-fl), somatosensory hindlimb (S1-hl), and somatosensory trunk (S1-tr), providing a reproducible scaffold for cross-animal registration and for mapping functional signals onto anatomy. These atlas efforts lay the foundation for the parcellation of cortex, while complementary studies zoom in on specific sectors. For instance, Muñoz-Castañeda et al. (2021) targeted M1 to chart its projection classes and revealed extensive intracortical connectivity, including strong links with S1-fl and M2, consistent with a network in which motor, sensory, and frontal regions communicate densely. Extending beyond cortex, Oh et al. (2014) combined viral tracers with serial two-photon tomography to trace parallel routing between cortex and thalamus, while analyses incorporating laminar specificity showed that M2 preferentially innervates deep output layers (L5B) of other cortical areas (Harris et al. 2019). Together, these studies supply a structural map that can be coupled with physiology to ask where, and through which pathways, motor-relevant signals are routed during behavior.

With that scaffold in place, the field has begun to leverage projection neurons to measure what information is transferred between areas. The mouse's genetic toolbox makes it feasible to label, record from, and manipulate neurons based on their downstream targets, revealing how

information flows across cortex (Carmona et al. 2024; Chen et al. 2013; Currie et al. 2022; Hira et al. 2013b; Kim et al. 2018; Manita et al. 2015; Maurer et al. 2023; Park et al. 2022; Terada et al. 2022; Ueno et al. 2018; Yamawaki et al. 2021; Zingg et al. 2014). A straightforward classification separates PT-like corticofugal neurons from IT-like corticocortical neurons (Currie et al. 2022; Park et al. 2022). Currie et al. (2022) examined layer 5B neurons in M1 and compared the information carried by PT neurons that project to the spinal cord versus IT neurons that project primarily within cortex. They found that PT neurons carry more movement-invariant signals, whereas IT neurons more often encode movement-varying signals such as “push” vs “pull.” Moreover, movement type could be decoded before movement onset in IT neurons but only after onset in PT neurons. The implication is a parallel-processing architecture where distinct projection classes perform distinct computational roles along different routes of the motor hierarchy. This idea naturally raises the question of whether similar specializations appear within a single cortical region when its neurons fan out to different targets. Chen et al. (2013) addressed precisely that point. By tracing long-range projections from whisker S1 to M1 or S2, and recording while mice performed either texture discrimination or object localization, they showed that the relative strength of touch-related signals and choice decoding depends jointly on task and target. In other words, the circuit does not merely broadcast a common message; it routes different information along different axonal paths in a task-dependent manner (Chen et al. 2013; Terada et al. 2022).

Projection-defined analyses also illuminate putative hierarchical relationships. Areas that project to deep output layers (L5B) of a target can exert stronger control over corticofugal output, whereas inputs to superficial layers may provide modulatory or context-setting influence (Hira et al. 2013b; Manita et al. 2015; Mao et al. 2011; Yamawaki et al. 2021). Consistent with

this idea, M2 sends strong projections to L5B in M1, while M1 projects more heavily to superficial L2/3 in M2 (Hira et al. 2013b). Manita et al. (2015) further demonstrated reciprocal M2–S1 connectivity and showed that optical stimulation of M2→S1 projections produced persistent firing in S1 L5B, consistent with a top-down, potentially instructive influence. Converging findings indicate that M1 receives dense sensory inputs superficially and frontal inputs (from M2) that reach deeply into L5B (Hira et al. 2013b; Mao et al. 2011; Yamawaki et al. 2021). This arrangement positions M1 as an integrator that blends sensory evidence with higher-order signals, sitting between the sensory cortices and M2 in a functional hierarchy. Nevertheless, anatomical and projection tracing, while essential, are static snapshots. They show who connects to whom and at what laminar depth, but they do not reveal how signals are timed, combined, and transformed during ongoing behavior. To bridge that gap, the field has continued to borrow from primate work and interrogate the system with stimulation and inactivation while also measuring natural activity during behavior.

Stimulus-driven and loss-of-function studies in the mouse have played a central role in testing regional contributions. Thanks to genetic access, researchers have performed intracortical microstimulation (ICMS), optogenetic stimulation, and focal inactivation to map function across sensorimotor cortex (Alonso et al. 2023; Brown et al. 2023; Brown and Teskey 2014; Khanal et al. 2024; Mathis et al. 2017; Morandell and Huber 2017; Tennant et al. 2011). On the mapping side, ICMS studies relate stimulation sites to muscle activations or movements (Brown et al. 2023; Brown and Teskey 2014; Khanal et al. 2024; Resta et al. 2021; Tennant et al. 2011). While there is broad agreement that the map is somatotopic-like, details differ across methods. Tennant et al. (2011), using microstimulation, found stronger digit representation in M1 and more proximal wrist/elbow representation in M2. In contrast, Resta et al. (2021) used long-duration

optical stimulation in awake mice and reported grasping movements from M2 and tapping-like movements from M1. Methodology likely explains some of the discrepancies. Moreover, the mouse corticospinal system differs from primate systems, with mice lacking direct corticomotoneuronal projections onto spinal motoneurons, and much descending control is routed through polysynaptic spinal and brainstem circuits (Carmona et al. 2024; Peirs et al. 2015; Ueno et al. 2018). This architecture can smear stimulation effects and blur the interpretation of site-specific causal roles.

Complementary inactivation studies have probed necessity. Morandell & Huber (2017) asked how M1 and M2 contribute to a delayed joystick push–pull behavior. They found that only dual inactivation consistently biased choice, whereas M1 inactivation alone was sufficient to produce motor deficits; M2 inactivation alone often spared execution. Other work shows that M1 is necessary for learning multisensory contingencies and for integrating sensory information during ongoing behavior (Heindorf et al. 2018; Mathis et al. 2017). As with stimulation, inactivation must be interpreted cautiously: optical approaches may spread widely, affect fibers of passage, and drive the system into non-physiological states, so observed performance changes may not map cleanly onto a single, local computation. These caveats reinforce the value of experiments that record neural activity during behavior and relate it quantitatively to kinematics, choices, and task events.

This logic has motivated a large body of work that records neural activity while mice perform quantified behaviors, then relates the activity to behavioral variables using modern computational methods (Chen et al. 2013; Costa et al. 2025; Guo et al. 2014; Kristl et al. 2025; Miri et al. 2017; Musall et al. 2019; Pinto et al. 2019; Saiki-Ishikawa et al. 2025; Wang et al.

2023b). The recording toolkit spans two-photon imaging for cellular resolution, widefield imaging for cortex-wide coverage, and multi-electrode arrays for high-temporal-resolution spiking. Analytically, studies use decoding (predicting behavior from neural activity), encoding (predicting neural activity from behavioral regressors), and dimensionality reduction approaches to identify shared components and subspaces. An example is Musall, Kaufman et al. (2019), who used widefield imaging during multisensory licking and found that movement-related signals pervade the cortical sheet and that a large share of variance is explained by uninstructed movements. This result reframed interpretations of “task signals,” emphasizing that the behavioral state—including subtle, spontaneous movements—must be modeled to avoid confounds. Single-neuron work complements and extends this picture. Chen et al. (2017) trained mice on whisker-based object localization while imaging ALM, demonstrating spatially localized encoding of choice, sensory, and motor variables. Such results show that even at cellular resolution, population activity often contains a mixture of behaviorally relevant signals. At the same time, Wang et al. (2023) reported that although motor information is broadly present across regions, it is most prominent in areas closer to the periphery—the sensory and motor sectors most directly linked to the limb and whiskers.

These observations pose an apparent paradox. On the one hand, the anatomy looks parcellated and hierarchical, with identifiable areas and laminar routing (Harris et al. 2019; Muñoz-Castañeda et al. 2021; Oh et al. 2014; Wang et al. 2020; Yao et al. 2023; Zingg et al. 2014). Some projection neurons carry task- and behavior-specific signals, and their routing is target- and task-dependent (Currie et al. 2022; Terada et al. 2022; Wang et al. 2023b). On the other hand, movement-related signals appear everywhere at the population level (Musall et al. 2019), and multiple regions participate in the computations that shape action. How can both be

true? One promising reconciliation is that distributed signals are organized into distinct subspaces that align partially with anatomical modules and projection classes—an idea that fits with the projection and laminar data summarized above (Currie et al. 2022; Perich et al. 2024). Another is that task demands matter profoundly: if the task is too simple, shared components and global state factors can dominate, obscuring area-specific computations. Supporting this view, Pinto et al. (2019) trained mice on increasingly cognitively demanding tasks and found that as difficulty rose, cortical activity across regions became more decorrelated, and inactivations produced more diverse (area-specific) behavioral deficits. These results were further supported by recent work showing greater engagement of cortex in more demanding tasks (Arlt et al. 2022). In other words, rich behavior can unmask the functional parcellation potentially implied by the wiring diagram.

1.4. Expanding beyond simple kinematics and anatomy

The work outlined in the general introduction of this thesis highlights some of the major advances made in understanding the structure of neural activity across the sensorimotor cortex. Anatomically, the results from multiple groups complement and agree with each other. These works paint a parcellated, densely connected system with some hierarchical arrangement across areas. Functionally, however, we still lack a clear consensus. In this portion of the thesis, I will highlight the challenges that have led to a lack of consensus and discuss how the works discussed in Chapters Two and Three address these challenges.

A key challenge in our current understanding of the cortical control of motor output in the mouse is the lack of challenging and motorically interesting behaviors. This behavior must produce interesting kinematic outputs while being constrained enough to allow for dense tracking of the animal. In mouse motor research, tasks are often either too kinematically

unconstrained or too kinematically impoverished. Once this desired motor behavior is achieved, it is imperative to then densely track the animal, quantifying the behavior. Modern techniques such as DeepLabCut (Mathis et al. 2018), LightningPose (Biderman et al. 2024), and BehaveNet (Batty et al. 2019) provide dense, markerless tracking of multiple keypoints throughout the animal's body, enabling the capture of interesting kinematic data. At the neural level, a similar issue is present; groups have either focused on coarsely imaging the entire cortex or densely sampling a small region of sensorimotor cortex. These efforts have provided some foundational knowledge, but without a dense sampling of the entire sensorimotor cortex at the single-neuron level, we really can't infer how cortical activity patterns are organized across these areas. In the following section of this thesis, I will discuss how the works in Chapters Two and Three aim to address the identified gaps and expand our understanding of the structure of cortical activity patterns across mouse sensorimotor cortex.

In Chapter Two, we introduce our behavioral task called WaterGrab. Here, we aim to strike a fine balance between fully unconstrained kinematic behavior and a kinematically impoverished task. We trained head-fixed mice to produce forelimb reaches to two fixed targets positioned at the limits of their forelimb reaching distance. This challenging target positioning produced motorically interesting kinematic behavior. While the mice performed this task, we captured high-framerate video, which we then used to train DeepLabCut (Mathis et al. 2018), providing us with detailed forelimb kinematics. These behavioral data were then leveraged to build an understanding of motor information in the forelimb region of mouse primary motor cortex (CFA) and somatosensory forelimb areas. These results showed that kinematic details could be decoded from both CFA and S1-fl, with the onset of such information arriving in CFA before S1-fl.

In Chapter Three, we expand on our previous behavioral paradigm by leveraging a movable spout to get our mice to produce forelimb reaches to 15 targets. While the mice produced this behavior, we wanted to expand beyond just M1 and S1-fl, and so we sequentially imaged (through two-photon microscopy) a large swath of mouse sensorimotor cortex spanning from anterior M2 to posterior somatosensory trunk. This dense sampling at the single neural level was then used to build feature vectors of tuning profiles. These tuning features were then used as inputs into statistical models aimed at creating maps of how tuning features are distributed across sensorimotor cortex. Along with other findings, we show interleaved subpopulations of neurons with unique tuning profiles spanning anatomical boundaries. These results show that mouse sensorimotor cortex contains parallel channels carrying information about a distinct, behaviorally relevant aspect of motor control. Thus, Chapter Two orients the reader to our behavioral workflow and shows that through a thorough analysis of kinematics, we are only then able to parse the roles of areas in the sensorimotor cortex. Chapter Three then expands on this understanding by first creating an even more cognitively challenging task and leveraging a dense sampling of the cortical medium to show how cortical activity is organized across the entire sensorimotor cortex of the mouse.

CHAPTER 2

MOUSE SENSORIMOTOR CORTEX REFLECTS COMPLEX KINEMATIC DETAILS DURING REACHING AND GRASPING

This chapter originally appeared, in modified form, as the following publication:

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Author Contributions: The author of this dissertation developed the aperture metric and target decoding analysis and designed and wrote the initial version of the neural modulation screening code. The author also produced the respective figures (2.1, 2.2, 2.4, 2.6). In addition, the author of this dissertation labelled half of the kinematic data and trained some of the animals used in this paper.

2.1. Introduction

Primary motor cortex (M1) and primary somatosensory cortex (S1) are the neocortical structures most strongly and directly connected to the sensorimotor periphery. Accordingly, signals related to movement are strongly present in M1 (Evarts 1973; Galiñanes et al. 2018; Georgopoulos et al. 1982; Moran and Schwartz 1999; Park et al. 2022; Saleh et al. 2012; Sauerbrei et al. 2020), and signals related to both cutaneous and proprioceptive somatosensation are strongly present in S1 (Alonso et al. 2023; Chowdhury et al. 2020; Costanzo and Gardner 1981; Fromm and Evarts 1982; Gardner and Costanzo 1981; Pei et al. 2009; Prsa et al. 2019;

Umeda et al. 2019). Yet despite being among the longest-studied brain structures, our understanding of these areas' exact roles in movement production remains incomplete (Omrani et al. 2017).

M1 and S1 are strongly interconnected (Yamawaki et al. 2021) and work closely together (Nelson 1996): sensory signals are often strongly present in M1 (Lucier et al. 1975; Lemon et al. 1976; Evarts and Fromm 1977; Wong et al. 1978; Fetz et al. 1980; Fromm et al. 1984; Alonso et al. 2023; Asanuma 1975), and movement related signals are often present in S1 (Chowdhury et al. 2020; Cohen et al. 1994; Goodman et al. 2019; Nelson 1987; O'Connor et al. 2021; Prud'homme and Kalaska 1994; Tanji and Wise 1981; Umeda et al. 2019; Weber et al. 2011). Nevertheless, stimulation in M1 produces movement much more readily than in S1 (Penfield and Boldrey 1937), and lesions (Lawrence and Kuypers 1968; Whishaw et al. 1993; Kawai et al. 2015; Mizes et al. 2023, 2024a; Nicholas and Yttri 2024) and inactivations (Galiñanes et al. 2018; Guo et al. 2015; Morandell and Huber 2017; Miri et al. 2017; Hwang et al. 2019a; Sauerbrei et al. 2020; Hwang et al. 2021; Park et al. 2022) of M1 produce strong deficits in movement production, especially for movements that are kinematically complex (Bollu et al. 2024), flexible (Heindorf et al. 2018; Mizes et al. 2023), or coordinated across the hand and arm (Guo et al. 2015).

In monkeys, motor cortical activity has been argued to relate to high-level signals, such as movement endpoint (Georgopoulos et al. 1986) or hand velocity (Ashe and Georgopoulos 1994; Kalaska et al. 1983; Wang et al. 2007); or, low-level signals such as joint kinematics (Vargas-Irwin et al. 2010; Saleh et al. 2012, 2010; Goodman et al. 2019; Omlor et al. 2019; Okorokova et al. 2020) or muscle forces (Kakei et al. 1999; Lemon et al. 1986; Miri et al. 2017; Morrow and Miller 2003; Warriner et al. 2022); or even very detailed control previously thought

to be reserved for the spinal cord, such as selecting types of motor pools (Marshall et al. 2022). The presence of signals at all of these levels suggests that monkey M1 may play a role in multiple levels of control. However, it is challenging to tease these roles apart in a model system where fine-grained causal perturbations and circuit tools are less developed.

The mouse has emerged recently as a model of interest for dissecting motor control (Warriner et al. 2020). As a model system, it offers high throughput, detailed atlases through both anatomical and functional tracing (BRAIN Initiative Cell Census Network (BICCN) et al. 2021; Muñoz-Castañeda et al. 2021; Winnubst et al. 2019; Zingg et al. 2014), and powerful techniques for large-scale recording (Manley et al. 2024; Steinmetz et al. 2021; Yu et al. 2021) and perturbation (Guo et al. 2015, 2021; Sauerbrei et al. 2020). However, the study of systems-level motor control in actively behaving animals is less mature in the mouse than in primates.

Extensive motor mapping experiments in rodents have revealed that activating different parts of the sensorimotor cortex evokes movements of different body parts or different kinds of movements of the same body part, as it does in primates (for review, see (Harrison and Murphy 2014)). Yet it is unclear how the topography of stimulation-evoked movements relates to the roles of these areas during volitional actions. Behavioral tasks in mice involving forelimb lever or reaching movements have provided a coarse-level understanding of how these areas contribute during behavior. Inactivations and lesions of M1 and S1 have shown that M1 is required for the execution of dexterous reach-to-grasp movements (Guo et al. 2015; Sauerbrei et al. 2020) and that S1 is essential for adapting learned movements to external perturbations of a joystick (Mathis et al. 2017). However, stimulation of brainstem nuclei alone can also evoke naturalistic forelimb actions, including realistic reaching movements involving coordinated flexion and extension of the proximal and distal limb (Esposito et al. 2014; Ruder et al. 2021; Yang et al.

2023). A key question is therefore what role M1 and S1 play in forelimb movement control. Namely, are these areas dominated by coarse, high-level signals while brainstem structures control the movement details? Or, is mouse sensorimotor cortex involved in controlling fine-grained movement features as in the monkey?

Here we characterize what kinematic information is present in M1 and S1 as a first step towards answering this question. To do so we combine a reach-to-grasp-to-drink paradigm (based on (Galiñanes et al. 2018; Galiñanes and Huber 2023)) with detailed high-speed behavior tracking and two-photon calcium imaging to ask what movement-related signals are present in mouse M1 and S1. Decoding models revealed that both M1 and S1 encode fine details of movement in the mouse, enabling decoding of all 24 joint angles we reconstructed from the shoulder to digits – even features such as digit splay and opposition within the paw. Differences between the areas emerged only in the decoding of higher-level aspects of the task: M1 reflected target location earlier than S1 and S1 reflected movement initiation time more accurately than M1. Our results link M1 and S1 activity to the execution of reach-to-grasp movements and highlight their distinct yet complementary roles in encoding low-level kinematic details and higher-order movement features.

2.2. Results

2.2.1 Complex movement kinematics during mouse reaching and grasping movements

Assessing the relationship between neural activity and the details of movement requires striking a balance between achieving repeatable behavior and evoking sufficient trial-to-trial variability to avoid model overfitting. To achieve this balance, we modified a previously-developed water-reaching task (Galiñanes et al. 2018) to evoke challenging reaching and

grasping movements to two locations (Fig. 2.1A). Importantly, the task setup was made compatible with high-speed stereo camera imaging and modern markerless tracking techniques for tracking putative joint centers, allowing for a detailed quantification of reaching trajectories and grasp attempts. Using a custom 3D-aware frame-labeling GUI and large numbers of labeled frames, we achieved high-quality tracking on nearly every frame (99% of lift-locked frames passed quality assurance for all 24 joint angles; 94.5% of trials were tracked usably; see Methods). This allowed us to quantify the kinematics at a fine level of detail and to obtain a more comprehensive description of these movements through joint angle kinematics (see Fig. 2.3).

Our task involved two water spout targets fixed in place on either side of the mouse's head at distances near the extent of the mouse's reach. After an initial hold period, the animals were instructed to reach to a specific spout by the pitch of a cue tone, which occurred simultaneously with water delivery at that spout. The probability of each spout being cued was 50% on each trial, so the animal had no information as to where they should reach until the cue was played. After the cue, they performed rapid reaching and grasping movements to retrieve the water reward from the spout (Fig. 2.1B). The mice produced reach trajectories with substantial variability around the basic reach shapes (Fig. 2.1C), likely resulting from small differences in initial posture causing execution errors and corrections. Three design choices increased this movement complexity: (1) the water spouts were located near the maximal range of motion of each animal, (2) the water spouts were designed so that water would cling to them, and (3) we required good contact between the paw and the spout using touch sensors. As a result, the mice often displayed repeated grasp attempts to contact the spout and retrieve the entire water droplet (Fig 2.1G). This variability across trials, spout targets, and mice was also seen in trial events like

lift reaction time, reach duration, time-to-first-contact, and the number of contact events that occurred within 1 second of the cue on each trial (Fig. 2.1D-G).

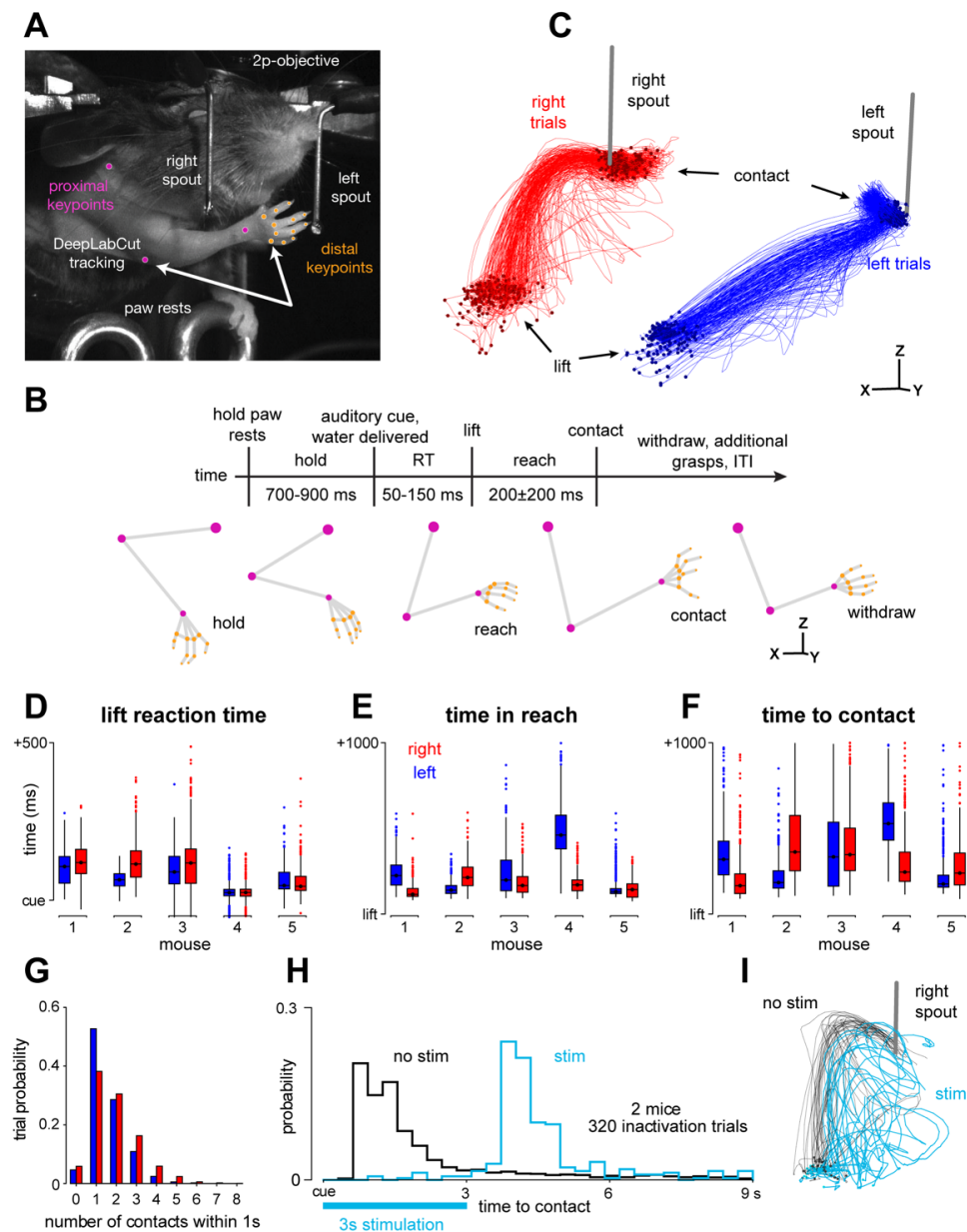


Figure 2.1: Mice make complex reaching and grasping movements to retrieve water rewards during head-fixation (A) Task setup: a head-fixed animal mid-reach with representative markerless tracking. (B) Task timeline, with representative 3D triangulated forelimb “skeletons”. (C) Distal digit centroid trajectories from 100 ms before lift to 100 ms after

Figure 2.1, continued. Mice make complex reaching and grasping movements to retrieve water rewards during head-fixation.

contact, left and right trials offset for visualization. One example session. (D) Lift reaction time distributions, left and right trials depicted separately. (E) Time from lift to paw centroid arriving within 5 mm of the spout target tip. (F) Time between lift and spout contact. Box plots in D–F show the quartiles (box and central line), whiskers extend 1.5 times the interquartile range past the quartile boundary, and more distant points (outliers) are plotted individually. (G) Number of contact events within 1 second of the water cue per trial. Data were pooled across sessions for D–G. (H) Time between cue and spout contact during optogenetic experiments. (I) Representative digit centroid trajectories for a subset of right trials during optogenetic experiments, from –100 ms before lift to 1000 ms after lift. Data from one example session.

In similar tasks, optogenetic inactivation of mouse M1 can halt the ongoing execution of reaching and grasping movements (Galiñanes et al. 2018; Guo et al. 2015). However, given that many of our mice performed the task for upward of 8 weeks and evidence that motor cortex disengages after extensive practice with lever pressing (Hwang et al. 2019a, 2021; Kawai et al. 2015), it was not clear whether motor cortex would remain necessary in our task. To determine this, we performed optogenetic inactivations of the Caudal Forelimb Area (CFA) in M1 (Muñoz-Castañeda et al. 2021; Tennant et al. 2011) by activating inhibitory neurons in two VGAT-ChR2 mice. These inactivations blocked the execution of the reach to grasp sequence, preventing the animal from making contact with the spout during the 3-second laser stimulation period (Fig. 2.1F; 86.5% control trials with contact within 3 seconds of cue, 5.1% inactivation trials with contact, $P < 10^{-191}$, Mann-Whitney U test, 2 mice, 495 stimulation trials). Interestingly, inactivation at the time of cue often did not prevent reach initiation (mouse 1: 54.7%, mouse 2: 34.2% of inactivation trials with lift within 3 seconds; 93.5%, 86.2% control trials). Yet the movement stalled once the paw and digits extended towards the spout, producing uncoordinated and unsuccessful reaching trajectories (Fig. 2.1I, two representative datasets).

2.2.2 Stereotyped aperture kinematics across idiosyncratic reach-to-grasp movements

Rodent forelimb movements have been described qualitatively as a set of sequential stereotyped postures when reaching to grasp food or water rewards (Whishaw and Pellis 1990) (Fig. 2.1B). We identified these features in our data using our high precision quantification of the mouse's proximal and distal forelimb during reaching. First, we defined a simple geometric measure of paw aperture that captured the strongest features of paw opening and closing, including the splay of the digits and flexion of the metacarpophalangeal (MCP) and proximal interphalangeal joints (PIP) (Fig. 2.2A). The first aperture minimum corresponded tightly with the peak of vertical paw velocity after lifting, with the paw reaching maximum vertical velocity shortly before the paw and digits became maximally flexed (Fig. 2.2B) ($r=0.6-0.95$ across mice, Pearson correlation; maximum velocity preceded minimum aperture by 27.2 ± 10.5 ms across mice). When aligning single-trial aperture traces to the peak vertical velocity (max Z velocity), we observed clear, stereotyped structure in how paw aperture evolved during the movements, across both spout targets for all five mice (Fig. 2.2E). This stereotyped shape was surprising given the heterogeneity in reach trajectories in this task and the strong differences in reaching strategies adopted for each target.

We termed the first aperture minimum after the lift to be the time of “collect,” following (Whishaw et al. 2010), and termed the next maximum after collect “extend” (Fig. 2.2B). These events structured the elaboration of the reach-to-grasp movement over time: after lifting, the paw closed to its minimum aperture (collect) before the digits were maximally opened (extend) as the paw was moved towards the spout. Extend occurred more heterogeneously in time after collect (Fig. 2.2C), perhaps reflecting single-trial variability in initial posture. These grasp-related events corresponded strongly with the reach's progress as well. Regardless of the initial paw

location on the paw rest, lifting the arm brought the flexing paw to a consistent location at the moment of collect, from which the mouse could prepare an accurate extension movement towards the spout (Fig. 2.2D). These observations serve as quantitative confirmation of previous qualitative details obtained from high speed video and confirm rich structure in the mouse reach-to-grasp movement.

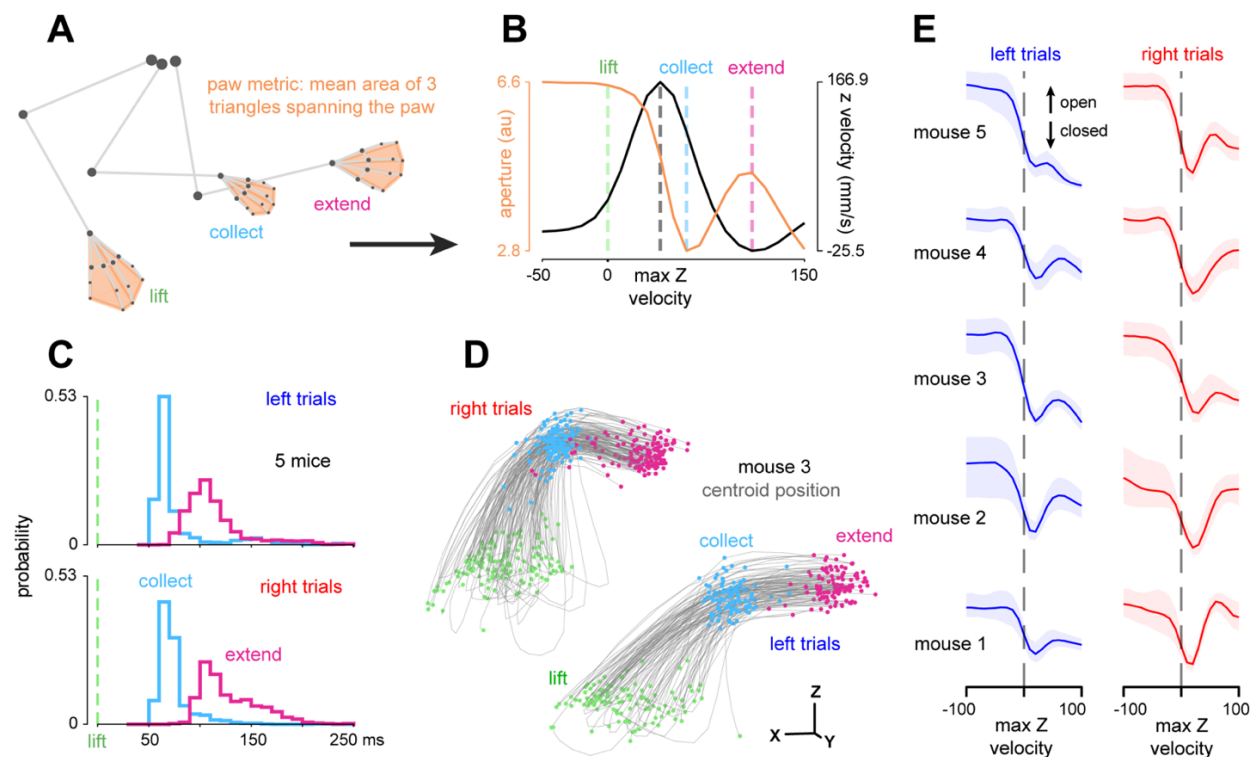


Figure 2.2: Paw aperture reveals stereotyped kinematics across idiosyncratic single trial reach-to-grasp movements (A) Paw aperture was computed from the paw markers using a simple geometric measure. (B) Aperture time series overlaid on the Z velocity of the paw centroid. The collect and extend events are defined by the aperture minimum and its subsequent local maximum. Representative trial. (C) Histograms of collect and extend event times for all trials, left and right trials separated. (D) Lift, collect and extend event times depicted on digit centroid trajectories for one example mouse. (E) Median over trials of aperture time series aligned to the moment of maximum centroid Z velocity.

2.2.3 Proximal and distal joints are coordinated during mouse reach-to-grasp

To obtain a high fidelity representation of the reach-to-grasp kinematics, we constructed a 3D “skeletal” representation of the forelimb by video-tracking keypoints at 15 putative joint centers along the proximal and distal axis (Methods). We then used axis-angle geometries to approximate 24 joint angles (7 proximal, 17 distal), including a number of intrapaw angles that captured deformations of the paw itself (Fig. 2.3A). These joint angles displayed rich temporal heterogeneity when reaching and grasping to both targets (Fig. 2.3B). Joint angles were strongly correlated across the entire limb, consistent with the expectation that the forelimb is a system of joints that are controlled cooperatively and, in some cases, are mechanically linked (Fig. 2.3C). The strongest correlations occurred for angles describing the same joint (e.g., the 3 angles describing the shoulder) and for the flexion of MCP and PIP joints. The block-like structure of the joint angle correlation matrices indicated that control of the mouse distal limb is tightly linked to control of the proximal limb, consistent with previous observations of the strong stereotypy in mouse forelimb movements across behaviors (Naghizadeh et al. 2020; Whishaw 1996; Whishaw et al. 2010; Whishaw and Pellis 1990).

Despite the strong coupling between the proximal and distal joint angles, rich variation remained in the action of different joints over time. To assess the number of linearly independent degrees of freedom amongst the 24 joint angles and velocities, we used double-cross-validated PCA (Yu et al. 2009); Methods; Fig. 2.3D), finding intermediate dimensionalities of 7 (median for joint angles) and 10 (velocities; Fig. 2.3E). This is consistent with the idea that joint angles across the limb are coordinated instead of controlled independently, and that this coordination is flexible enough over time to enable accurately performing reaching and grasping to different targets.

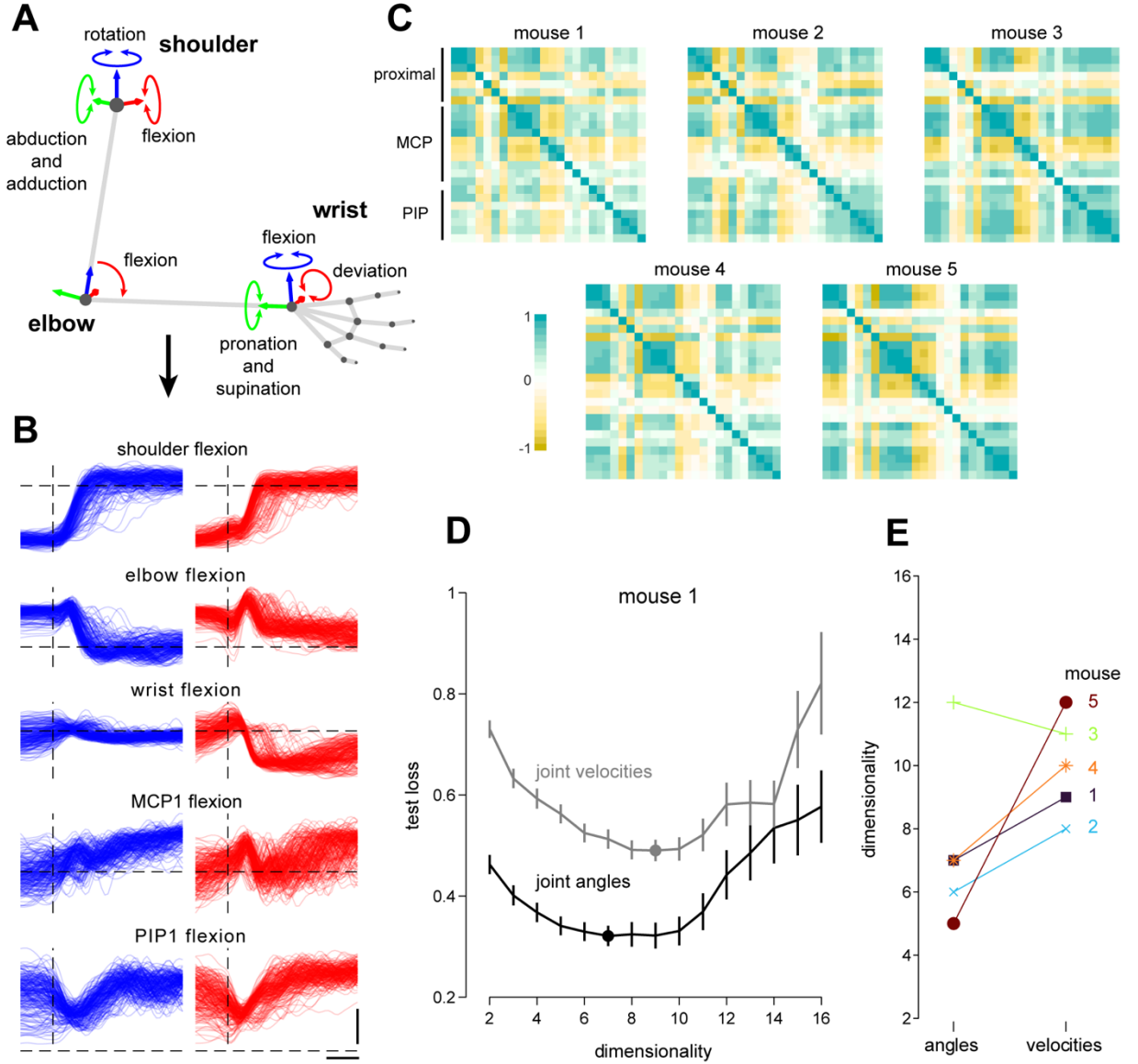


Figure 2.3. Mouse reaching and grasping coordinate proximal and distal joints. (A) Inverse kinematics using Euler angles: seven proximal joint degrees of freedom are shown; 17 distal angles (digits) omitted for clarity. (B) Time series for five representative joints from -100 to +400 ms around lift onset (vertical dashed line). Left trials in blue, right trials in red. Horizontal scale bar, 100 ms; vertical scale bar, 30°. Dashed horizontal lines indicate 0°. (C) Correlation matrices of joint angles across all recorded time points. Rows ordered proximal→distal (shoulder to MCP to PIP). (D) Example cross-validated PCA loss curve fit to joint-angle and joint-velocity time series. (E) Estimated dimensionalities of joint-angle and joint-velocity kinematics across mice.

2.2.4 Layer 2/3 cells in CFA and fS1 are strongly modulated and heterogeneously tuned

Having characterized the structure of the mouse forelimb reaching and grasping movements, we next sought to determine how the kinematic details of these movements were distributed across the sensorimotor cortex. To do so, we recorded neural activity from neurons in layer 2/3 of the caudal forelimb area of M1 (CFA) extending into the immediately adjacent secondary motor cortex (M2), and the forelimb region of S1 (fS1) using two-photon calcium imaging. A slight majority of recorded neurons in both areas (51.8% in CFA and 52.2% in fS1, $p < 0.05$ with Bonferroni correction at $n=6$) were statistically modulated to at least one event during the task: cue, paw lift, and/or first spout contact (Fig. 2.4B; see Methods, “ZETA”). Cells showed strong modulation locked to different task events, such as the cue onset (51.7% / 52.7% in CFA / fS1, left or right p -value < 0.05 corrected at $n=2$), paw lifting from the rest (51.6% / 54.8% in CFA / fS1), and time of first contact with the spout (45.2% / 46.4% in CFA / fS1). 7

Cells were also modulated by the time taken to complete the reach: many cells exhibited different firing rates on trials with movement duration briefer versus longer than the median (Fig. 2.4C-D, see ROIs 35 and 116). Finally, many cells showed temporal heterogeneity in their activity beyond that of simple transient bumps. This included cells (such as ROI 54) that were strongly modulated by movement onset and remained active late into the trial. This activity may reflect the details of movement as the animals made repeated grasping attempts with a fully extended arm to retrieve the entire water reward from the spout.

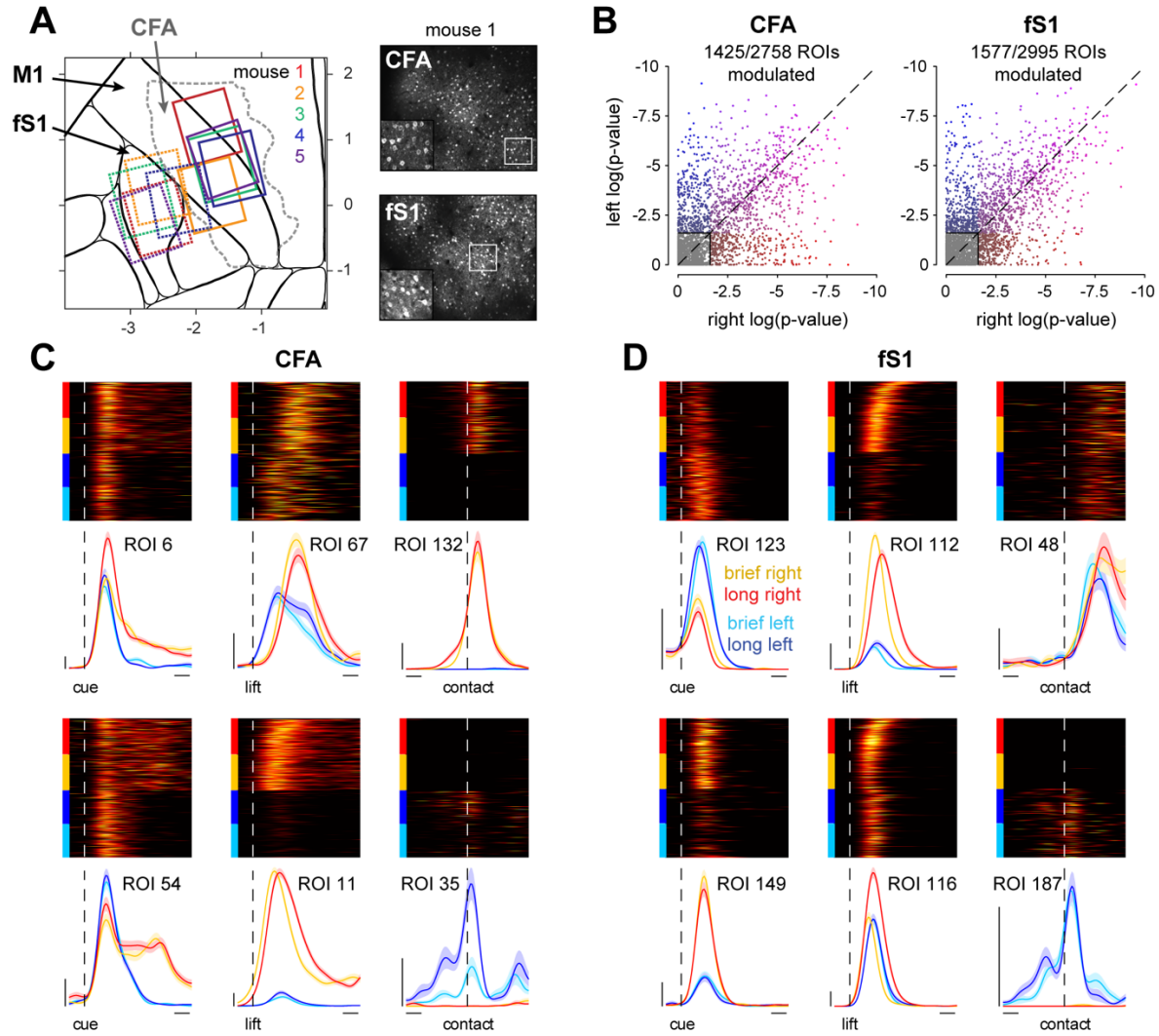


Figure 2.4: CFA and fS1 cells are heterogeneously tuned to specific movement features (A) 2-photon imaging fields of view for all mice aligned to the Allen Atlas common coordinate framework (CCF). fS1 corresponds to SSp-ul and M1 to MOp in the CCF. The dashed gray line indicates the outline of the forelimb primary motor cortex (MOp-ul, referred to here as CFA) as described in Muñoz-Castañeda et al 2021. See Figure 4S1 and Methods for field of view alignment details. Right, example max-projection images for CFA and fS1 for mouse 1 with magnified insets. M1 and fS1 correspond to MOp and SSp-fl in the Allen CCF nomenclature. (B) Modulation metric (see Methods) of cue-locked data for individual cells in CFA and fS1. Values displayed are the logarithm of the computed p-value, where each point represents a cell. All cells with p-values < 0.025 are shown colored: left-only modulated cells are blue, right-only modulated cells are red, and cells modulated to both conditions are purple. (C) Peri-event histograms and rasters of single trial activity from individual cells in CFA. Each column displays two cells locked to each trial event: cue, lift and first-spout-contact. Trials grouped within each condition by time-in-reach. Vertical scale bar: 10 events per second (arbitrary units). Horizontal scale bar: 100 ms. (D) As in C but for fS1.

2.2.5 Proximal and distal joint kinematics can be decoded from CFA and fS1 population activity

To assess how movement related signals were distributed across CFA and fS1, we decoded the time series of each joint angle from neural population activity in each area. A simple linear regression model related the single-trial joint angles at all time points to single-trial neural activity at the corresponding moments. We found that every measured proximal and distal joint angle could be decoded from CFA activity at above-chance levels during reaching and grasping (Fig. 2.5A-B). Interestingly, fS1 decoding exhibited similar decoding performance as CFA across all mice (Fig. 2.5E-F).

Across both areas we found that proximal joints were better decoded than distal joints (Fig. 2.5D,H). A number of reasons for this difference are possible: this may reflect the time scale of 2p-recorded neural activity, overall smaller total variance of the most distal joint angles (see Fig. 2.5S2E-F), or measurement noise present in the markerless tracking for small features like the fingertips. Nevertheless, we were able to explain substantial variance in the most distal intrapaw joints (see joints MCPf, MCPa, MCPo, PIPs and PIPf).

Additionally, we reconstructed the skeletal representation of the forelimb from the decoded joint angles and found that the reconstructed postures generally closely tracked the true trajectories, even of “minor” angles like cylindrical paw deformation or digit splay (Fig. 2.5C,G). This suggests that movement-related signals that could be used to actuate forelimb joints are present in both CFA and fS1 populations.

Finally, we tested whether the ability to decode these many joint angles was a direct consequence of inter-joint correlations, and might not be indicative of the presence of “real” information about some of these joints. To do so, we fit partial correlation models that removed correlations between proximal and distal joints, or removed correlations of the joint angles with a

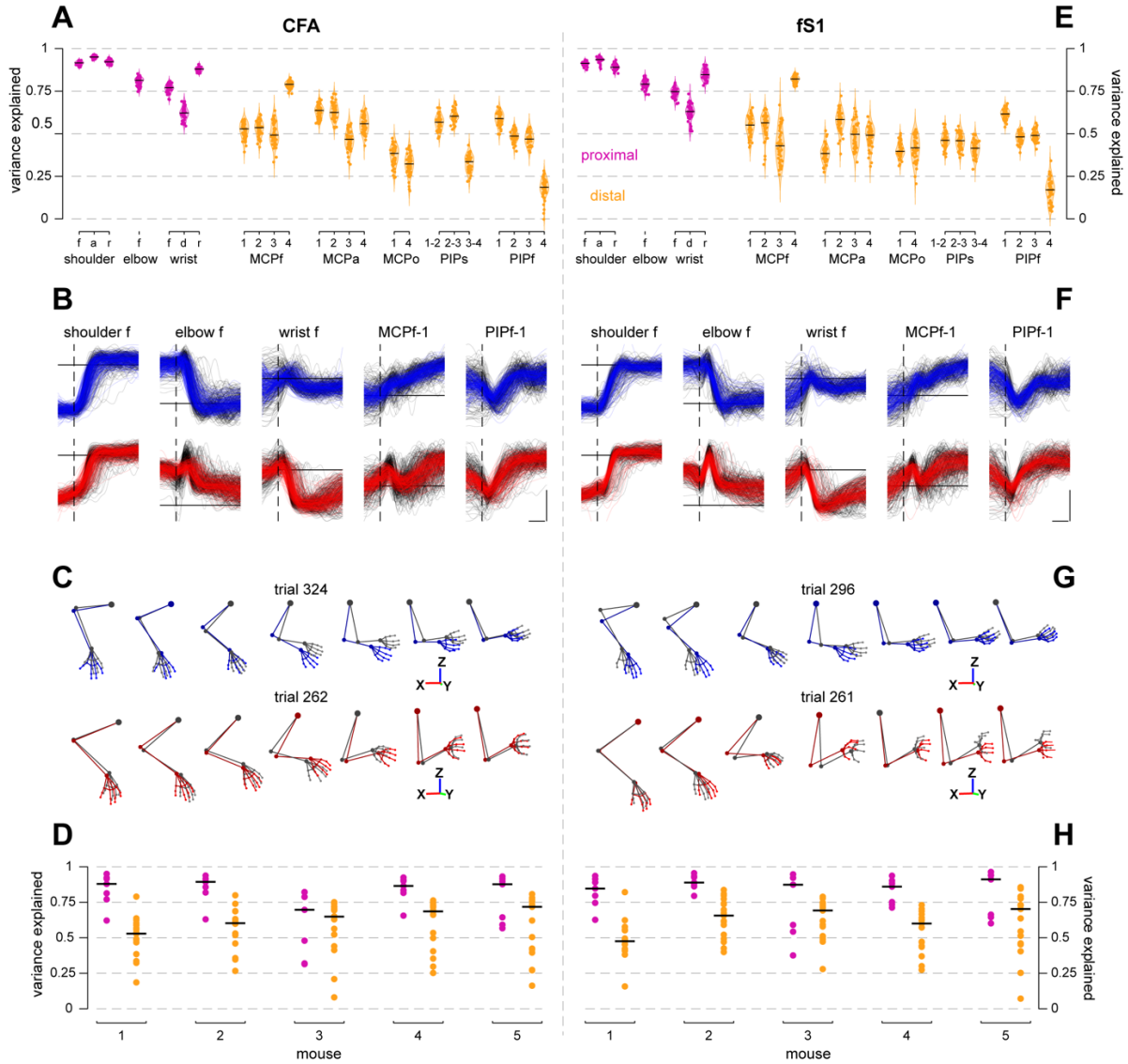


Figure 2.5: Kinematic details of proximal and distal joints during reaching and grasping can be decoded from CFA and fs1 population activity (A) Performance of decoding models predicting joint angle kinematics from RADICaL-inferred CFA rates. Violins depict the distribution of variance explained over cross-validation folds for each individual joint angle. (f: flexion, a: abduction/adduction, r: rotation, d: deviation, o: opposition, s: splay, MCP: metacarpal-phalanges, PIP: proximal-interphalanges; digits indicated by number). (B) Reconstructed joint angle time series for 5 representative joint angles. Black traces are original and colored are reconstructed; left trials are blue and right trials are red. Vertical dashed line indicates lift time, horizontal line indicates 0 degrees. Vertical scale bar is 30 degrees, horizontal scale bar is 100 ms. (C) Forelimb skeletal postures reconstructed from decoded joint angles for representative left and right trials (trials having median variance explained). 7 representative

Figure 2.5, continued. Kinematic details of proximal and distal joints during reaching and grasping can be decoded from CFA and fS1 population activity.

time points from lift time to grab time are shown. Gray postures are original data, colored postures are reconstructed as in B. Decoded joint angles in each time step were applied to the neutralized posture of the original data, preserving the original lengths of inter-marker lines. (D) Performance of decoding models for all 5 mice. Each point is the median of the distribution of variance explained over cross-validation folds, with proximal (magenta) and distal (goldenrod) angles considered separately. The black lines indicate the median variance explained over all proximal or distal joints for an individual mouse. (E–H) As in A–D but for fS1 data.

high-level parameter, the overall distance of the paw centroid to the spout. Despite substantially lowering the behavioral variance, the residuals could still be decoded from neural activity (Fig 2.5S2A-D). Overall, these results suggest that instantaneous movement-related signals are similarly distributed across CFA and fS1.

2.2.6 CFA reflects target-specific information earlier and more persistently than fS1

To assess whether higher-level target-related signals were present in each area, we analyzed how well target identity could be decoded from neural activity over time. For each session, a linear decoder was trained to predict whether the neural activity from a particular window of time came from a left-target or right-target trial. This decoder was then applied to held-out trials from the same time window. This analysis revealed that CFA activity reflected the target location slightly more faithfully than did fS1, as shown by its higher overall performance (93.2% CFA vs. 89.1% fS1 median over cross-validation folds and time bins; $p = 4.1 \times 10^{-4}$, Wilcoxon rank sum test). To examine single trials, we then projected each trial's population activity onto the identified decoding axis (the “target-specific dimension”). In this projection, CFA activity predicted the target sooner after the cue than did fS1 activity (59.9 ± 34.7 ms earlier) and displayed a stronger, more consistent, and longer-lasting prediction throughout the trial (Fig. 2.6A,B).

To determine whether the target-specific dimension was conserved or whether it changed over the course of a movement, we then tested how well a decoder trained during one time

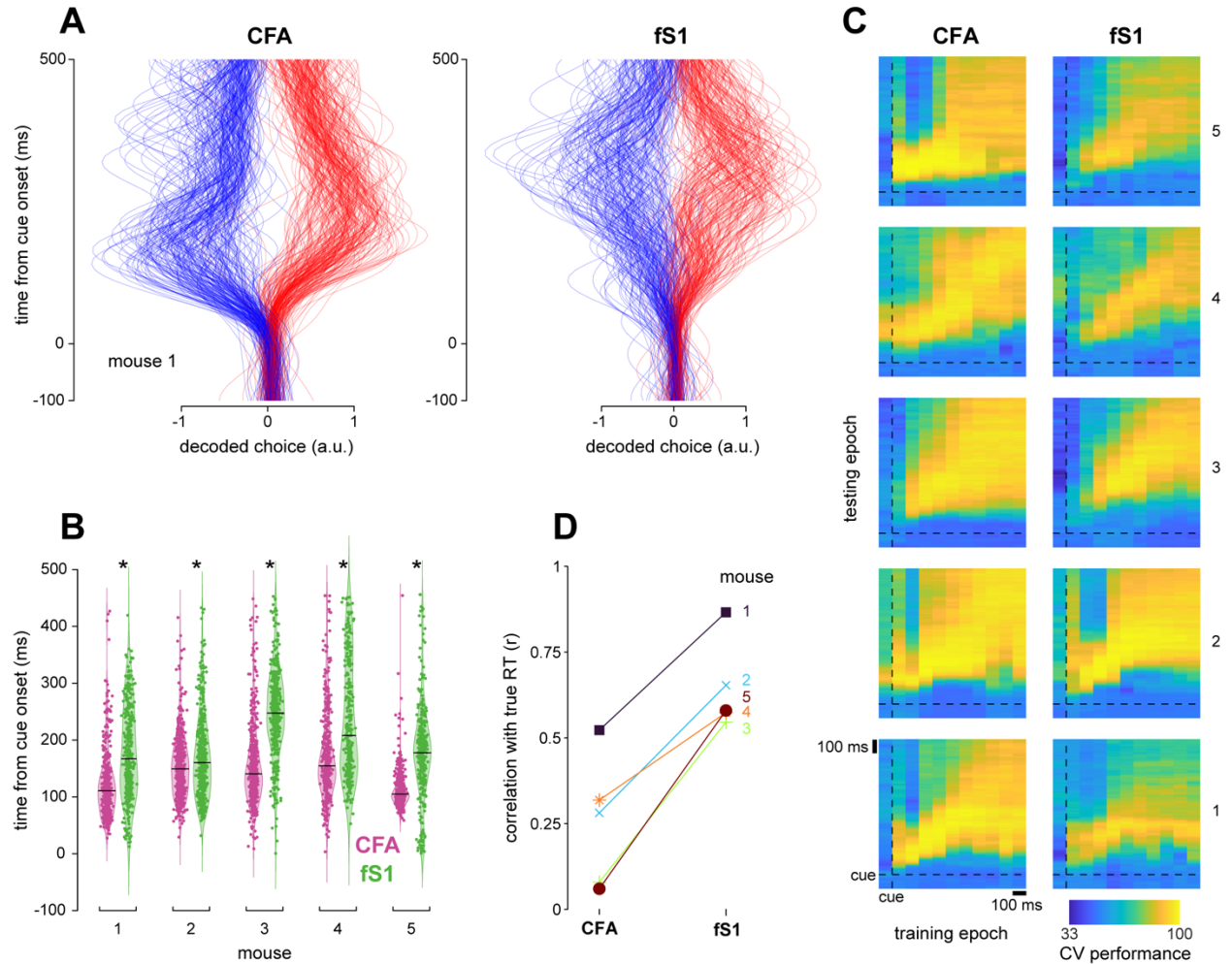


Figure 2.6: CFA reflects target-specific information earlier and more persistently than fS1
 (A) Projections of population activity from held-out single trials onto the target decoding dimension for CFA and fS1. Right trials are red and left trials are blue. Traces were normalized by the 90th percentile value across trials for left and right trials separately. (B) Distribution of threshold crossing times for projections shown in A. Black lines indicate medians. Star indicates significantly different medians at $p < 0.05$, Wilcoxon ranksum. (C) Performance of time-generalized target decoding analysis. Each heat map depicts the performance over all model training windows (columns) and test time points (rows). Time in trial runs vertically as in A. Both the training and testing epochs were -100 ms (leftmost column training, bottommost row test) to $+1000$ ms (rightmost column training, top row test) relative to cue. Training epochs were 100 ms non-overlapping windows and testing epochs were 10 ms bins.

window generalized to data from other time windows. CFA decoders were more stable over the trial, and performed better when generalizing to far-away time points than did fS1 decoders (Fig. 2.6C). These results suggest that the encoding of target-specific information is more consistent over time in CFA than fS1.

Finally, we asked how strongly each area encoded a different high-level parameter, resting versus reaching. To do so, we trained a decoder to predict whether neural activity was from a time point pre- or post-lift, then computed a movement onset time from when the decoder switched from pre- to post- (Methods; Fig. 2.6D). Unlike our results for predicting target identity, fS1 activity more strongly predicted lift time than did CFA.

2.3 Discussion

The extent to which activity in sensorimotor cortex reflects the details of movement has remained unclear in the mouse. Here we show that in a challenging reach-to-grasp task evoking variable movements, both CFA and fS1 relate tightly to all the joint angles we could track throughout the arm, paw and digits. Using high-fidelity markerless tracking, we replicated prior, coarser results that the reach-to-grasp kinematics have stereotyped structure and involve strong coordination across proximal and distal joints. Using two-photon calcium imaging, we revealed that single neuron activity in CFA and fS1 is strongly modulated and tuned to fine movement features. Decoding models then demonstrated that CFA and fS1 both possess rich representations of low-level joint angle information at similar levels of fidelity. Despite these similarities, target decoders revealed that movement information emerges more quickly at the population level after the cue and persists longer in CFA than it does in fS1. Activity in fS1 is thus more transient and more closely linked to lift onset than in CFA.

The strength of movement-related activity in these areas is surprising given the results of loss-of-function experiments in rodents, which have suggested that sensorimotor cortex can become disengaged from forelimb control with extensive training (Hwang et al. 2019a, 2021; Kawai et al. 2015), and that subcortical structures like the medulla or basal ganglia can independently control coordinated forelimb movements (Esposito et al. 2014; Ruder et al. 2021; Yang et al. 2023). Our finding of low-level kinematic information in sensorimotor cortex suggests a role for cortex beyond simply providing high-level commands to these subcortical areas.

There are a number of possible roles for this movement-related cortical activity. One is that motor cortex may dominate control when the behavior is complex enough in some way – like when a cue is needed to identify the target (Mizes et al. 2024) or when sensory feedback is necessary to coordinate dexterous grasps in the presence of errors (Guo et al. 2015; Perich et al. 2024; Sauerbrei et al. 2020). Despite the overtraining of our behavior, these task features might preclude use of the presumably less-flexible subcortical circuits alone. Alternatively, subcortical areas like the medulla or cerebellum may initiate the movement (Dacre et al. 2021; Esposito et al. 2014; Inagaki et al. 2022; Ruder et al. 2021) while the cortex elaborates the details of otherwise-generic commands (Kaufman et al. 2016). This might permit the previously-observed disengagement of cortex with learning of simple behaviors, could explain our observations of partially executed reach-to-grasp movements during CFA inactivation, and would explain the weak representation of lift-onset in our CFA data. A third alternative is that medulla alone executes low-level control, but low-level information is maintained in sensorimotor cortex for some other function, such as to enable learning (Kawai et al. 2015; Makino et al. 2016; Wolff et al. 2022). These hypotheses are not mutually exclusive.

Our observation of detailed kinematic signals in the mouse forelimb somatosensory cortex raises the possibility of a central role for fS1 in dexterous movements, and may implicate a more broadly distributed circuit in forelimb control than previously considered. Indeed, the magnitudes of representation in CFA and fS1 were nearly identical. This may simply be a consequence of widely distributed representations of movement across mouse cortex (Musall et al. 2019; Steinmetz et al. 2019; Stringer et al. 2019), including forelimb somatosensory areas. However, the strength of detailed kinematic information in fS1 suggests a role beyond simply receiving a broad corollary discharge signal.

One possibility is that proprioceptive signals are strong enough to carry these movement details into fS1 from subcortical structures, as is true in primate forelimb S1 (Umeda et al. 2019). Our lift time decoding results are consistent with this view and align with recent observations characterizing mouse proprioceptive forelimb cortex spanning both S1 and M1 (Alonso et al. 2023). Further, our task evoked repetitive grasping attempts, which may engage fS1 strongly to inform motor areas of the sensory consequences of movement (Perich et al. 2024). Extensive circuit tracing has shown that forelimb M1 and forelimb S1 are highly interconnected in the mouse (Muñoz-Castañeda et al. 2021; Yamawaki et al. 2021), suggesting that detailed movement signals in fS1 may reflect not only sensory feedback but also efference copies of motor commands generated by CFA. Rodent fS1 is known to influence interneuron populations in the spinal cord through direct and indirect projections (Ueno et al. 2018), and thus these signals may also reflect fS1's important role in modulating reflex circuits in cooperation with movement generation (Moreno-Lopez et al. 2021; Moreno-López et al. 2016; Seki et al. 2003).

Several limitations of the present work should be noted. Pose estimation was performed with state-of-the-art neural networks (Mathis et al. 2018) and extensive hand-labeling of

keypoints together with quality assurance metrics, but behavior tracking is inevitably imperfect. In particular, a noise floor remains that particularly impacts small bones such as the distal phalanges, and markers estimated at the skin surface cannot perfectly reflect underlying joint and muscle kinematics. Additionally, calcium imaging has limited temporal resolution and is a nonlinear transformation of spiking activity (Stringer and Pachitariu 2019; Wei et al. 2020). Although we used leading-edge deconvolution methods (Friedrich et al. 2017) and cutting-edge neural network approaches for sub-frame event estimation (Zhu et al. 2022), these limitations cannot be entirely overcome. These slow temporal dynamics limited our ability to disambiguate whether joint angles or joint velocities are more linearly encoded in these areas. Nevertheless, our ability to decode substantial variance from small joint angles and fast changes within the paw supports the claim that movement signals are represented in CFA and fS1 activity. Finally, all recordings included here were taken from layer 2/3 of both CFA and fS1. Activity may differ in layer 5, where the primary subcortical outputs originate.

Overall our results suggest that mouse sensorimotor cortex is intimately involved in the control of dexterous forelimb movements, and support use of the mouse as a first-class model system for studying the cellular and circuit basis of motor control. Further work will be required to map the roles of other sensorimotor cortical areas, understand their interactions, and differentiate between ballistic reaching and feedback-driven grasping and withdrawal.

2.4 Methods

2.4.1. Animal subjects

All procedures were approved by the University of Chicago Institutional Animal Care and Use Committee. A portion of the data presented here were analyzed in a previous study

(mice 1 and 2; (Zhu et al. 2022)). For two-photon imaging, five male Ai148D transgenic mice (TIT2L-GC6f-ICL-tTA2, stock 030328; Jackson Laboratory) aged 8-12 weeks were used. Four of the mice carried the GCaMP6f transgene heterozygously and one homozygously (mouse 3). For optogenetic experiments, 2 VGAT-ChR2 (stock 014548; Jackson Laboratory) transgenic mice aged 8-12 weeks were used. Each animal underwent a single surgery. Mice were then individually housed in a reverse 12-h light/dark cycle, with an ambient temperature of 71.5 °F and a humidity of 58%. Experiments were conducted in the afternoon, during the animal's dark cycle.

2.4.2. Surgical procedures

Mice were injected subcutaneously with dexamethasone (8 mg kg⁻¹) 24h and 1h before surgery to reduce inflammation. Mice were anesthetized with 2–3% inhaled isoflurane gas and then injected intraperitoneally with a ketamine–medetomidine solution (60 mg kg⁻¹ ketamine and 0.25 mg kg⁻¹ medetomidine). Mice were given supplemental low level isoflurane (0–1%) if they showed any signs that the depth of anesthesia was insufficient or began emerging from anesthesia towards the end of the surgery. Animals received a subcutaneous injection of meloxicam (2 mg kg⁻¹) at the beginning of the surgery and once daily for 2 days post-operation. The scalp was shaved, cleaned and resected, then the skull was cleaned and the wound margins glued to the skull with tissue glue (VetBond, 3M). A circular craniotomy was then made with a 3-mm or 4-mm biopsy punch centered over the left CFA / fS1 border. The coordinates for the center of CFA were taken to be 0.4 mm anterior and 1.5 mm lateral of bregma. The craniotomy

was cleaned with SurgiFoam (Ethicon) soaked in phosphate-buffered solution (PBS). If the dura remained intact, a durotomy was performed.

Virus (AAV9-CaMKII-Cre, diluted to 1×10^{13} particles per mL in PBS, Addgene stock 105558-AAV9) was then pressure injected (NanoJect III, Drummond Scientific) at 2-6 sites near the target site, with 140 nL injected at each of two depths per site (250 and 500 μ m below the pia) over 5 min each. The injection pipette was kept in place for another 3-6 minutes to ensure viral dispersion before being removed slowly over 2-3 minutes. In mice numbers 3-5 an additional virus injection (AAVretro-tdTomato, diluted to 1×10^{13} particles per mL in PBS, Addgene stock 59462-AAVrg) of 60-100 nL was made targeting the bulk of forelimb somatosensory cortex at -0.73 ± 0.08 mm posterior and $\sim 2.69 \pm 0.15$ mm lateral of bregma. This injection retrogradely labeled cell bodies in CFA that sent projections to fS1. In this paper the labeling was used solely for stabilizing the imaging plane (see below) in both areas. In mice 1 and 2, a small craniotomy was made using a dental drill over the right CFA at 0.4 mm anterior and 1.6 mm lateral of bregma. 140 nL of AAVretro-tdTomato (1×10^{13} particles per mL, Addgene) was then injected at 300 μ m below the pia. This injection labeled cells in the left CFA projecting to the contralateral CFA. As above, this labeling was used solely for stabilizing the imaging plane.

The injection craniotomy was sealed with a drop of Kwik-Cast (World Precision Instruments). For mice 1-3 and the two VGAT-ChR2 mice the cranial window craniotomy was sealed with a custom cylindrical glass plug (3 mm in diameter, 660 μ m in depth; Tower Optical) bonded (Norland Optical Adhesive 61, Norland) to a 4-mm number 1 round coverslip (Harvard Apparatus) that was glued to the skull surface with tissue glue. For mice 4 and 5 the craniotomy was sealed with a custom metal and glass cannula created from a small stainless steel ring (3.96

mm outer diameter, 3.45 mm inner diameter, 0.76 mm length, MicroGroup) bonded to a 4 mm number 1 round coverslip using cyanoacrylate glue (ZAP-A-GAP, Robart). All window implants were glued in place first with tissue glue (VetBond) and then with cyanoacrylate glue (Krazy Glue) mixed with dental acrylic powder (Ortho Jet; Lang Dental). In all mice two layers of MetaBond (Parkell) were applied, and then a custom laser-cut titanium head bar was affixed to the skull with black dental acrylic. Animals were awoken by administering atipamezole via intraperitoneal injection and allowed to recover at least 3 days before water restriction.

2.4.3. Behavioral task

As previously reported (Zhu et al. 2022), the behavioral task (Fig. 1) was a variant of the water-reaching task of (Galiñanes et al. 2018) that we term the ‘water grab’ task. This task was performed by water-restricted, head-fixed mice, with the forepaws beginning on paw rests (eyelet screws) and the hindpaws and body supported by a custom, 3D-printed, tapered, clear acrylic tube enclosure. After holding the paw rests for 700–900 ms, a tone was played by stereo speakers and a 2- to 3- μ l droplet of water appeared at one of two water spouts (22 gauge, 90-degree bent, 1-in blunt dispensing needles, McMaster) positioned on either side of the snout. The pitch of the tone indicated the location of the water, with a 4 kHz tone indicating left and a 7 kHz tone indicating right. The cue tone lasted 500 ms or until the mouse made contact with the correct water spout, whichever came first. The mouse could grab the water droplet and bring it to its mouth to drink any time after the tone began. Both the paw rests and spouts were wired with capacitive touch sensors (Teensy 3.2, PJRC). Good contact with the correct spout produced an intertrial interval of 3–6 s, while failure to make contact (or insufficiently strong contact) with the spout produced an intertrial interval of 20 s. Because the touch sensors required good contact

from the paw, this setup encouraged complex contacts with the spouts. Control software was custom written in MATLAB R2018a using PsychToolbox 3.0.14. Touch event monitoring and task control were performed at 60 Hz using the Teensy.

2.4.4. Behavioral task training

The mice were trained to make all reaches with the right paw and to keep the left paw on the paw rest during reaching. For the first 1-3 days, mice were head-fixed and provided water directly to their mouths to acclimate them to head-fixation and reward collection. During this period the animals were encouraged to make consistent contact with both paw rests to trigger water delivery at their mouths. After acclimation and consistent trial initiation the spout was withdrawn to a position on the right side of their face intended to elicit forelimb reaching with the right arm. If animals were reluctant to reach, small droplets of water were placed on their right whisker pad with a blunt syringe to induce grooming and water collection. After animals reliably performed reaches toward the rightward spout location (>80-90% success), another spout was installed near the initial location (the “left” spout). Water was then solely delivered through the left spout and its position was shifted across the midline to the ventral contralateral whisker pad over the course of 7-10 days. Once the left spout was in a nearly-equidistant location from the midline as the right spout, the animal was cued to reach to both spouts in blocks of 10-50 trials. After reaching >90% in blocked trials, trials were cued randomly. This training procedure took 2-4 weeks, although the behavior continued to solidify for at least two more weeks. Data presented here were collected after 6–8 weeks’ experience with the task.

2.4.5. Optogenetic inhibition

Two VGAT-ChR2 mice were trained to perform the task. After 6-8 weeks of training, inactivations were performed using the epifluorescence path of our 2-photon microscope, centered over coordinates 1.5L 0.5A from bregma. The stimulation protocol was designed to approximately replicate the experiments of (Guo et al. 2015). Pulses were 17 mW peak power, 12.5 ms long and delivered at 40 Hz (50% duty cycle, 8.5 mW average power). The spot of light was approximately 1.3 mm in diameter at the focal plane. Stimulation was delivered beginning 150 ms before the cue and then pulsed continuously for 3 seconds after the cue. After an initial behavioral warm-up period of 50 control trials, stimulation was delivered with 25% probability on subsequent trials. To compensate for visible blue light due to leak around the light-sealing cone or through the brain, two bright blue LED strips were placed in front of the animal and illuminated constantly throughout the session.

2.4.6. High speed videography and stereo triangulation

As previously reported (Zhu et al. 2022), high-speed video data was recorded using a pair of cameras (BFS-U316S2M-CS, FLIR; varifocal lenses COZ2813CSIR2, Computar) mounted 150 mm from the right paw rest at 10° apart. Infrared illuminators enabled behavioral imaging while performing 2p imaging in a darkened microscope enclosure. Cameras were synchronized and recorded at 150 frames per second with real-time image cropping and JPEG compression and streamed to one HDF5 file per camera (areaDetector module of EPICS, CARS). DeepLabCut (DLC) (Mathis et al. 2018) was used to track 15 keypoints in each image (see next section). The MATLAB Stereo Camera Calibration toolbox was used for estimation of stereo camera parameters to triangulate paired keypoints into 3D.

2.4.7. *Markerless tracking*

3-5 days before recording, the right forelimb of each mouse was shaved and depilated using Nair while the animal was under light anesthesia (1-2% isoflurane). We did not observe any obvious impacts of this depilation on the behavior. DLC was then used to identify 15 keypoints associated with bony landmarks spanning the proximal and distal forelimb. These keypoints included: the tip (#1-4), proximal interphalangeal joints (#5-8) (PIP) and metacarpal phalangeal joints (#9-12) (MCP) of each digit, the center of the wrist (#13), distal edge of the elbow joint (#14), and proximal tip of the lesser tubercle of the humerus (#15). We developed a custom GUI in Python to improve the quality of manually labeled training frames. This GUI allowed simultaneous labeling in both images and visualized the epipolar lines of each keypoint in each image to improve stereo correspondence. We then employed an active learning approach to create a dataset of manually labeled images. First, 20-80 frames paired between cameras were randomly selected from all 12 datasets. These images were manually labeled for all 15 markers and an initial DLC model was trained to identify keypoint locations for both cameras. We then performed 3 rounds of active learning, selecting 15-35 additional paired frames per dataset per round. We selected frames for inclusion in each round by identifying 3D postures in each video that strongly differed from those already contained in the training set (Feng et al. 2023). We assessed the similarity of candidate frames in a window 300-1000 ms around the water cue to the training set by computing the sum of Euclidean distances between vectorized 3D keypoint positions. We then sorted candidate frames by this distance and manually selected additional frames to label from the 10% of candidate frames with the greatest total distance. At the end of this process we labeled a total of 1455 paired images across 12 datasets (100-165 images per dataset) for a total of 2910 high quality labeled images.

2.4.8. Joint angle kinematics

To extract joint angle kinematics from the 3D triangulated keypoints, we first obtained vectors connecting adjacent keypoints in the unsmoothed data. Because we chose keypoint locations to closely align with putative joint centers across the forelimb, the angles between vectors connecting keypoints were taken to approximate the angles associated with the joint.

For the shoulder joint, we identified the “humerus” with the vector pointing from the shoulder keypoint to the elbow keypoint. For the elbow joint we identified the “radius/ulna” with the vector from elbow to wrist. The vector used to compute wrist angles pointed from wrist to the mean of the second and third MCP keypoints (virtual MC or vMC). For each digit, we computed the vector connecting the corresponding MCP to PIP keypoints, and finally the PIP to DIP keypoints. The vectors defined in this way were used to compute extrinsic Euler angles around each of the putative joint centers in a proximal-to-distal fashion. After each joint angle was computed, the corresponding vector and all of its children vectors were rotated so as to neutralize the rotation around that joint.

We chose a coordinate system appropriate for our task in mice. Starting with the shoulder joint, we computed the abduction/adduction of the humerus as the rotation around the external (world) Y axis (green vector in Fig. 2.3A) necessary to bring the humerus into the ZY plane; abduction was chosen to be negative and adduction was positive. Next we computed the flexion/extension as the rotation around the X axis (red vector in Fig. 2.3A) required to align the humerus with the Z axis (blue vector in Fig 2.3A); flexion was positive. Finally, we computed the shoulder rotation as the angle around the Z axis required to bring the radius/ulna into the YZ plane; internal rotation was positive. At the elbow we computed flexion/extension as the angle around the X axis required to make the radius/ulna parallel to the Y axis; flexion was positive.

We followed a similar procedure for the wrist as the shoulder; abduction/adduction was computed as the angle around the X axis required to bring the vMC into the XY plane; radial deviation or adduction was positive. We computed the wrist flexion/extension as the angle around the Z axis that brought the vMC into the YZ plane; flexion was positive. Finally, to compute the wrist rotation we first obtained the vector normal to the plane spanned by the second and third MCP vectors (nMCP). Wrist “rotation” (torsion of the radius around the ulna) was computed as the angle around the Y axis required to bring the nMCP into the YZ plane; pronation was positive. We then computed two intermetacarpal angles, quantifying how “opposed” MCP1 or MCP4 was to MCP2 or MCP3 respectively. This measure quantifies the conical deformation of the paw. This was done by computing the angle around the Y axis required to bring the MCP1 or MCP4 vectors into the YZ plane so that they were coplanar with MCP2 and MCP3. We calculated paw splay for the metacarpal 1-2, 2-3, and 3-4 vectors as the angle around the Z axis required to bring them into the XY plane. We then computed two angles around each MCP joint: abduction/adduction and flexion/extension. MCP abduction/adduction was computed as the angle around the X axis required to bring the proximal phalanx into the XY plane; abduction from the wrist midline was positive. We computed the metacarpal flexion/extension as the angle around the Z axis required to bring the proximal phalanx vector into the YZ plane; flexion was positive. Finally, we computed the PIP flexion as the angle between the distal phalanx and the proximal phalanx for each digit.

2.4.9. Trial screening

To identify trials with problematic keypoint tracking, we used the extracted joint angle kinematics. We first binned joint angles for each trial at 10 ms in a window from -100 ms to 400

ms locked to lift onset and smoothed with a 15 ms Gaussian kernel. Left and right trials were considered separately. For each joint angle we found the 2.5 and 97.5 percentile values (pctile1 and pctile2) across all trials and time points. We then computed threshold values for each joint angle as $(\text{pctile1} + \text{pctile2}) / 2 \pm \text{abs}(\text{pctile1} - \text{pctile2})$. Across all trials, we identified individual joint angle values that exceeded the bounds of these two thresholds. Next, for each trial we computed the number of time bins for which any joint angle was outside the bounds. If a trial had more than 5 such time bins it was excluded from further analysis. Across all mice this procedure identified 4719/4993 trials with high quality tracking, which were further confirmed by visual inspection. After screening, no substantial tracking errors were detected even on single frames, and thus no further cleaning was performed.

2.4.10. Joint angle correlations and dimensionality

As in the screening procedure we first binned at 10 ms, locked joint angles from -100 ms to 400 ms relative to lift onset, and smoothed each angle within trials with a 15 ms Gaussian kernel. We then concatenated all trials across time. To exclude unusual joint angle configurations due to contact forces, we removed time points during which the paw made contact with the spout target. We then computed the correlation matrix shown in Figure 3 using the *corrcoef* MATLAB function on this concatenated matrix. To compute the dimensionality of the joint angles and joint velocities, we performed double cross-validated principal component analysis (PCA) on the same concatenated matrix (Yu et al. 2009). To perform this procedure, we first held out a subset of observations and performed PCA. Then, for each held-out observation, we held out one joint, used the values of the other joints together with the loadings to estimate the low-D coordinates on that observation via regression, and finally estimated the value of held-out joint in the held-

out observations from the low-D coordinates and the loading for that joint. After cycling over all variables and 5 random observation partitions for each candidate dimensionality, we identified the optimal dimensionality as the one that minimized the reconstruction error of the held-out variable in the held-out observations. This procedure was performed separately for joint angles and joint velocities.

2.4.11. Wide-field imaging for area identification

To identify the forelimb primary somatosensory cortex, we performed widefield imaging while providing sensory input to the contralateral paw using a vibration motor (SparkFun, part ROB-08449) glued to a paw rest (Alonso et al. 2023). This provided vibrotactile stimulation of the entire forelimb. The mouse was in the type of behavioral setup they were accustomed to except for the paw rest, and did not have to perform any task. Vibration was delivered for 250 ms once the mouse's paws had been on the paw rests for at least 2 seconds.

The widefield macroscope was identical to the setup of (Musall et al. 2019), except that a pco.panda 4.2 sCMOS camera (Excelitas) was substituted and LED triggers were delivered by a Teensy 3.2. Imaging was performed at 30 frames/s, alternating blue and violet illumination. Violet frames were used for hemodynamic correction.

We used the locus of stimulation-evoked activity to align each cranial window to the Allen Common Coordinate Framework v3. The location of strongest activation was aligned with the center of the widest part of the CCF area named S1-fl (roughly 0 AP 2.6 ML relative to bregma), where proprioceptive and tactile responses most strongly overlap. We targeted our S1 imaging fields of view to this location and referred to the recorded region as forelimb S1 or fS1 as done in (Alonso et al. 2023). With the window aligned to the atlas, we identified CFA as a

compromise between this alignment and the coordinates of 0.4 AP and 1.6 ML relative to bregma. We targeted our CFA imaging field of views to this region. In some cases, thick vasculature or weaker indicator expression prevented high-quality imaging of the ideal location. In these few cases, we imaged as close as possible to the target. Results were similar between all datasets.

2.4.12. Two-photon imaging

Calcium imaging procedures have been described previously. Briefly, imaging was performed with a NeuroLabware 2p microscope running Scanbox 4.1 and a pulsed Ti:sapphire laser (Vision II, Coherent). Depth stability of the imaging plane was maintained using a custom plugin that made automatic movements of the objective up to every ~10s based on comparing acquired red-channel data with an initially-acquired image stack. Offline, images were run through Suite2p to perform motion correction, region-of-interest (ROI) detection and fluorescence extraction. ROIs were manually curated using the Suite2p GUI. As previously described (Zhu et al. 2022), fluorescence was neuropil-corrected, detrended, and passed through OASIS using the ‘thresholded’ method, AR1 event model, and limiting the tau parameter to be between 300 and 800 ms. Neurons were discarded if they did not meet a minimum SNR criterion. To put events on a more useful scaling, for each ROI, we found the distribution of event sizes, smoothed the distribution (ksdensity in MATLAB, with an Epanechnikov kernel and log transform), found the peak of the smoothed distribution and divided all event sizes by this value. This rescales the peak of the distribution to have a value of unity. When time-locking the data for visualization, modulation testing, or for decoding analyses, the deconvolved events were resampled into 10-ms bins. This resampling was performed by

assigning a fraction of each event into the new 10-ms bins proportionally to how much the 10-ms bins overlapped the original ~32.2 ms frames, and taking into account the exact time each ROI centroid was sampled based on its position within the field of view (FOV). Alignment for RADICaL has been described previously (Zhu et al. 2022).

2.4.13. Modulation index

To test whether regions of interest (ROIs) were task responsive, we adapted the “ZETA” method from (Montijn et al. 2021) to time series data, closely related to the approach used in (Heimel et al. 2023). The goal of this procedure was to determine whether the activity of each ROI was reliably modulated over time relative to some task event, such as cue or paw lift. Left and right trials were considered separately, and Bonferroni correction for multiple comparisons was performed at the end. First, neural data for all trials were resampled to 10 ms as described in the previous section. For each ROI, we averaged activity over trials and computed its fractional cumulative sum over time bins. This cumulative sum was compared to the linear baseline that would be expected from an ROI that fired uniformly at a matched rate in the same time window. The deviation of the cumulative sum from the linear baseline (i.e., the residuals) was computed, then this deviation trace was mean centered and its maximum absolute value was found. This value was the data maximum deviation. We then generated a null distribution for this statistic. To do so, we shuffled by circularly permuting the event-locked data independently for each trial before averaging across trials, mean-centering, and computing the shuffle’s maximum deviation as above. This procedure was repeated 100 times to produce the distribution for the maximum deviation under the null hypothesis that the cell fired uniformly over time. We then computed the p-value as the probability of observing the data maximum deviation under a Gumbel distribution

parameterized by the mean and variance of the distribution of maximum deviations over shuffles. Compared to the ZETA procedure in (Heimel et al. 2023), our procedure may be slightly less sensitive for small time windows because of the disruption of temporal correlation at the circular permutation wraparound point. However, it uses data only from within the time window of interest, so is less sensitive to unrelated modulation due to events occurring just outside the time window of interest; and it is much faster to compute. We performed this procedure for cue-locked (-200 ms to +400 ms), lift-locked (-200 ms to +400 ms), and first-contact-locked (-300 ms to 300 ms) data, for left and right trials separately.

2.4.14. RADICaL modeling

RADICaL models were fit using the same procedures developed in (Zhu et al. 2022) except we trained these models using the NeuroCAAS implementation (Abe et al. 2022). As in (Zhu et al. 2022), we used lift-locked data binned at 10 ms in a window from -100 to +800 ms around lift time with 100 ms padding. After models were fit, the padding was removed and RADICaL-inferred rates for each ROI were handled identically to sub-frame aligned smoothed and deconvolved data. Note that all results were qualitatively similar without RADICaL, but as expected decoding quality and correlations were generally somewhat lower.

2.4.15. Trial grouping

We observed considerable heterogeneity in the time from lift to spout contact (movement time) for both conditions for all mice. We reasoned that this could follow from single trial heterogeneity in either reach shape or accuracy. We therefore separated trials within each condition into two groups, determined by whether their movement time was smaller or greater

than the median movement time for that condition. This procedure yielded 4 groups of trials, “brief” and “long” for left and right conditions.

2.4.16. Peri-event time histograms

PETHs were computed for three different locking events: cue, lift and first contact. Neural data for all trials were binned at 10 ms and smoothed with a Gaussian (35 ms s.d.). Cue and lift-locked PETHs were locked in a window of -100 to 700 ms relative to the corresponding event. Contact-locked PETHs were locked in a window of -400 to 400 ms relative to first contact. Trials were then averaged within the groups described above.

2.4.17. Joint angle and velocity decoding

Joint angle or velocity kinematics were linearly interpolated from their original 6.66 ms to 10 ms and smoothed with a Gaussian (15 ms s.d.). We then screened for modulated neurons. Neurons deemed significant at a ZETA $p < 0.0083$ threshold for any behavioral events to either target were included. For decoding, neural and kinematic data were locked to -100 ms before and 400 ms lift onset. Deconvolved data were smoothed with a Gaussian (35 ms s.d.); RADICaL-inferred rates were not smoothed. We did not include a lag between neural activity and kinematics for simplicity; tests on selected sessions indicated that adding lags did not improve decoding. We applied PCA to the neural activity and reduced its dimensionality to be 90% of the number of included neurons. The decoder was trained and tested using cross-validated ridge regression. We partitioned the trials into 10 non-overlapping training (90%) and test (10%) set splits and fit the regularization parameter on the training set using a heuristic described in (Karabatsos 2018) with code developed in (Musall et al. 2019). We then applied the trained

decoder to the test set and computed the fraction variance explained for each joint angle or velocity independently. We then repeated this same procedure for four additional 10-fold partitions for a total of 50 cross-validated folds. We report the variance explained across joints for all folds. To visualize reconstructions of the joint angles using the fit decoders, for each trial we “bagged” the decoder weights: we averaged across the 5 cross-validation folds where that trial was in the test set. This produced a denoised decoder that was used to predict the joint kinematics for that trial.

2.4.18. Target decoding analysis

We used logistic regression to classify individual time points as belonging to rightward or leftward targets from population activity. Neurons with significant modulation at a ZETA $p < 0.01$ threshold for any locking event and either target were included. Neural data from each trial were binned at 10 ms, locked to a window of -100 ms to 500 ms centered on cue onset, and smoothed with a Gaussian (35 ms s.d.). We partitioned the trials into five non-overlapping training (80%) and test (20%) set splits, stratifying left and right trials. We then trained a logistic regression classifier using the MATLAB *fitclinear* function on all post-cue time points across training set trials. We then projected individual trials onto the classifier dimension. We normalized each trial’s projection according to the 90th percentile value over all time points of the same trial type. We report the classifier performance on all time points across test set trials. Our procedure for computing the threshold crossing times for individual trials was similar to the one used in (Kaufman et al. 2015). We computed the threshold value by first taking the median of all projections across trials at each time point, finding the maximum and minimum values of this trace, then computing their midpoint; this procedure was done separately for left and right

trials. We found the threshold crossing time as the first time bin after which the projection stayed above the threshold for at least five consecutive bins (50 ms). If trials never crossed the threshold, or did not stay above threshold for more than five bins, they were discarded. We then interpolated between 10 ms bins to find the exact time that the projection crossed the threshold.

2.4.19. Time-generalized target decoding analysis

To assess the stability of the target-classifier dimension over time, we employed a similar logistic regression approach as above. Neural data from each trial were first interpolated as usual to 10 ms bins from -100 to 1000 ms relative to the cue, then smoothed with a Gaussian (35 ms s.d.). Time bins were then grouped into 11 non-overlapping 100 ms epochs. For each epoch, logistic regression was used to classify individual 10 ms time bins as belonging to left or right trials. A separate classifier was trained for each epoch, and thus 11 models were fit in total. This procedure was performed using 5-fold cross validation, with left and right trials stratified. The trained models for each epoch were used to predict the target identity on time points across all epochs for the held out 20% of trials from each fold. We reported the mean performance of the classifier over folds for each prediction epoch.

2.4.20. Reaction time decoding analysis

Deconvolved neural data were used to predict the lift reaction time (RT) on single trials. Our approach follows closely the procedure used in (Zhu et al. 2022) based on (Kaufman et al. 2016). A cross-validated logistic regression classifier was trained to discriminate pre-lift neural data (a bin -100 to 0 ms relative to lift onset) from post-lift neural data (a bin 0 to 100 ms relative to lift onset). Training was performed with *fitclinear* in Matlab, stratifying left and right trials.

Neural data from the held-out trials was interpolated at 10 ms as usual, locked -200 to 600 ms relative to the lift, smoothed with a Gaussian (10 ms s.d.), then projected onto this dimension. The lift RT was calculated as the time that the projection crossed a threshold. To compute the threshold, we calculated the median across trials, then took the midpoint of the maximum and minimum. We summed the predicted time (which was effectively an offset from the true RT) with the ground truth lift RTs and correlated these with the ground truth lift RTs.

CHAPTER 3

ACTIVITY FEATURES REVEAL INTERMINGLED SUBPOPULATIONS SPANNING AREA BORDERS IN MOUSE SENSORIMOTOR CORTEX

A modified version of this chapter has been submitted to *eLife* as a Research Article.

Author contributions: The author of this dissertation was involved at every stage in developing the scientific plan, helped train the mice, collected all the neural imaging data, computed all analyses related to figures 3.3 – 3.9, produced most of the figures (3.1, 3.3-3.9, all supplementary) and in collaboration with co-authors wrote the manuscript.

Co-authors: Grier HA, Kaufman MT

3.1 Introduction

Controlling movement is a critical function of the brain, and a sizable fraction of mammalian neocortex is dedicated to this task (Currie et al. 2022; Li et al. 2024; Muñoz-Castañeda et al. 2021; Musall et al. 2019; Steinmetz et al. 2019; Stringer et al. 2019). Executing adaptive movements in dynamic environments relies on diverse processes such as trajectory planning, error monitoring, feedback integration and pattern generation. A diversity of approaches have been used to understand how these processes are distributed across different cortical areas (Brown and Teskey 2014; Goard et al. 2016; Guo et al. 2014; Harrison et al. 2012; Hira et al. 2013a; Inagaki et al. 2018; Mathis et al. 2017; Morandell and Huber 2017; Sauerbrei et al. 2020; Tanji and Evarts 1976; Tennant et al. 2011; Vyas et al. 2020). Yet these approaches have suggested a variety of potentially dominant patterns of organization, complicating our view of how cortical structure and function are related. Further, these approaches have rarely attempted to relate coarse anatomical or stimulation-based maps to the firing patterns of

individual neurons during movement production. Here, we aim to complement these prior approaches, performing large-scale cellular-resolution imaging across several regions of mouse sensorimotor cortex to determine the organization of neurons' response properties.

Anatomical mapping has classically leveraged cytoarchitecture and projection tracing to describe how structure varies across the cortical sheet. . These approaches support the view that cortex is divided into many areas that serve different functions, while remaining strongly interconnected (Brodmann 2005; Majka et al. 2021; Passarelli et al. 2011; Yeterian et al. 2012) especially in the mouse (Gămănuț et al., 2018; Harris et al., 2019; Oh et al., 2014; Winnubst et al., 2019; Zingg et al., 2014). More recent genetic tools have complemented these methods with finer-grained insight into neuron-type distributions and connectivity, providing additional distinctions between parts of the brain (DeNardo et al. 2015; Muñoz-Castañeda et al. 2021; Wang et al. 2020) and revealing substantial circuit diversity within cortical areas (Carmona et al. 2024; Park et al. 2022; Ueno et al. 2018). Subpopulations with distinct projection patterns and layer-specific connectivity support the existence of parallel circuits in both somatosensory (Kerr et al. 2007) and motor areas (Carmona et al. 2024; Hausmann et al. 2022; Hira et al. 2013b; Muñoz-Castañeda et al. 2021; Zingg et al. 2014). Cortex further sends strong projections to numerous subcortical, thalamic, and spinal targets, which originate from portions of these cortical areas in patterns that both do (Carmona et al. 2024; Jeong et al. 2016) and do not (Hausmann et al. 2022; Ueno et al. 2018) respect anatomical boundaries determined by other methods.

Mapping the motor cortices using stimulation and inactivation (“functional mapping”) has produced a still more complicated picture. Short-train electrical microstimulation produces twitches in specific body parts in monkey (Neafsey et al. 1983; Van Acker et al. 2013) and

human (Fried et al. 1991; Penfield and Boldrey 1937); long-train microstimulation produces coordinated movements of portions of the body in both monkey (Graziano et al. 2002) and mouse (Brown et al. 2023); and long-train optogenetic stimulation evokes moderately coordinated movements in the mouse as well (Khanal et al. 2024). Stimulation thus makes clear that there is a coarse somatotopy (Kwon et al. 2018; Mitz and Wise 1987; Qi et al. 2010; Shoham and Grinvald 2001) and biases toward movement types in different cortical locations (Brown et al. 2023; Brown and Teskey 2014; Graziano et al. 2002). Inactivations of sensorimotor regions in rodents sometimes interfere with large-scale movement (Grier et al. 2025; Guo et al. 2016), yet often only prevent the animal from refining movements, integrating sensory information, and correcting its motor output (Bollu et al. 2024; Heindorf et al. 2018; Mathis et al. 2017). Secondary motor cortex inactivations can also impact high-level movement planning in the form of choices (Chen et al. 2024; Li et al. 2016; Morandell and Huber 2017). Taken together it is unclear how cortical maps obtained from stimulation or inactivation relate to the spatial distribution of activity during behavior.

Correlational approaches have instead related neural activity to behavior, but have produced mixed views of the system. Large-scale recordings with simple behaviors have suggested that motor signals are widespread across cortex (Musall et al. 2019; Steinmetz et al. 2019; Stringer et al. 2019; Wang et al. 2023b), while efforts with slightly more complex behaviors have shown a greater proportion of motor signals in areas closer to the motor or sensory periphery (Li et al. 2024; Wang et al. 2023b)). However, even in a more challenging motor task, M1 and primary somatosensory forelimb (S1-fl) neural populations yielded similarly strong kinematic decoding of many tracked joint angles (Grier et al. 2025). These findings describe a distributed system, where signals are densely shared but where area distinctions

remain unclear despite their sharp differences in connectivity with the periphery. A key question then remains: how are cortical activity patterns organized in sensorimotor cortex during behavior, and are single neurons' tuning properties organized in line with anatomy or with a different plan?

Here, at a single-cell resolution, we densely mapped the activity of mouse sensorimotor cortex during a reach-to-grasp behavior to capture the spatial distribution of tuning properties. We used two-photon calcium imaging to record from over 39,000 neurons as mice performed a 15-target reach-to-grasp task. We focused on five areas involved in this behavior: secondary motor cortex (M2), primary motor cortex (M1), and the primary sensory areas corresponding to the forelimb (S1-fl), hindlimb (S1-hl), and trunk (S1-tr). Some tuning properties related to response onsets and peaks closely followed anatomical borders. Other properties were more closely related to somatotopy, such as tuning sharpness, response duration, or the linearity of responses with respect to target location. Using data-driven approaches, we were also able to identify subpopulations that shared response features, which were intermingled within areas but had characteristic distributions that spanned anatomically-defined areas. Together, these results begin the systematic characterization of cortical activity patterns across the sensorimotor cortices during behavior.

3.2 Results

3.2.1. Dense sampling of sensorimotor cortex reveals heterogeneous tuning

We implanted cranial windows covering sensorimotor cortex (Fig. 3.1B) in six male mice expressing GCaMP8s in most cortical pyramidal cells (Zhang et al. 2023). Dense sampling of layer 2/3 neurons was achieved using two-photon calcium imaging across 98 fields of view

(FOVs) spanning the forelimb, hindlimb, and trunk representations (Fig. 3.1C). FOVs were aligned to the Allen atlas common coordinate framework (Wang et al. 2020) using widefield imaging and paw vibration mapping (Methods; Fig. 3.1B). A total of 39,398 neurons were extracted across the five relevant regions in the sensorimotor cortex (Fig. 3.1D). A larger number of neurons were imaged in M2 and M1 (31,453 neurons) than in somatosensory areas (7,945); (Fig. 3.1E).

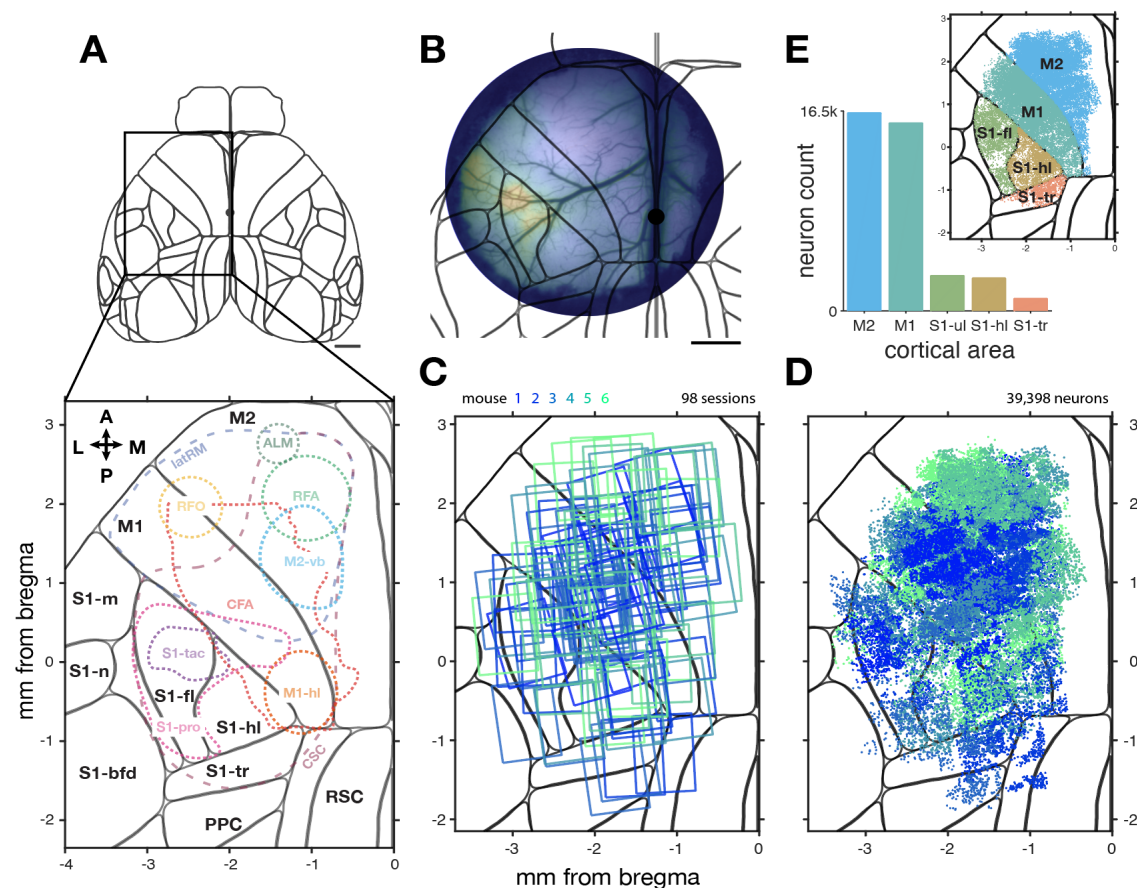


Figure 3.1. A dataset of densely sampled activity across mouse sensorimotor cortex. (A) *Top*, dorsal view of the Allen atlas, with the area investigated here indicated by a box. *Bottom*, summary of previously-identified subareas of mouse sensorimotor cortex (Methods). (B) Widefield calcium imaging in a 5 mm cranial window superimposed on the Allen CCF to show location. Activation elicited by paw vibration (Methods). (C) Counts of neurons imaged for each area, with identities colored in inset. (D) Field of view locations of the 98 sessions of two-photon imaging collected in layer 2/3 for 6 mice (colors). (E) Locations of the 39,398 neurons extracted from all sessions. Mouse identity colored as in D.

Behavior. While neural activity was acquired the mice performed a trained, motorically-challenging, delayed reach-to-grasp-to-drink task (Methods). The task was a modified version of the Galiñanes and Huber (2023) multi-target reaching task. In our behavior mice were head-fixed and held two paw rests to initiate a trial (Fig. 3.2A). A trial began with a spout moving into one of 15 positions. After a variable delay period an auditory cue was given and a water droplet released, and the mouse could use its right paw to reach toward the target and retrieve its reward (Fig. 3.2B). Stereo high speed camera data enabled markerless tracking (DeepLabCut; Mathis et al. (2018)) offline.

As a basic characterization of the behavioral kinematics, single trial paw centroid trajectories were plotted colored by their corresponding target position (Fig. 3.2C). Three relevant features were readily apparent. First, the position of the paw on the paw rest was not target-specific. This implies that the animal did not position its paw in target-specific ways during the delay. Second, the paw centroid location was strongly target-specific near the spout, demonstrating that reaches were in fact targeted. Finally, a looping trajectory near the spout was typically present, which corresponds with repeated reaches to collect all the water from the spout. This was due to a combination of our custom-shaped spouts, which were designed to cause the water to cling, and our use of touch sensors which encouraged strong contact with the spout itself (Methods). Although variability was present in both reaction time (Fig. 3.2D) and movement time (Fig. 3.2E), as is typical in reaching data, both intervals were generally fast and indicated that animals were highly engaged in the task. A fuller characterization of how neural activity relates to the kinematic output will be detailed in forthcoming work.

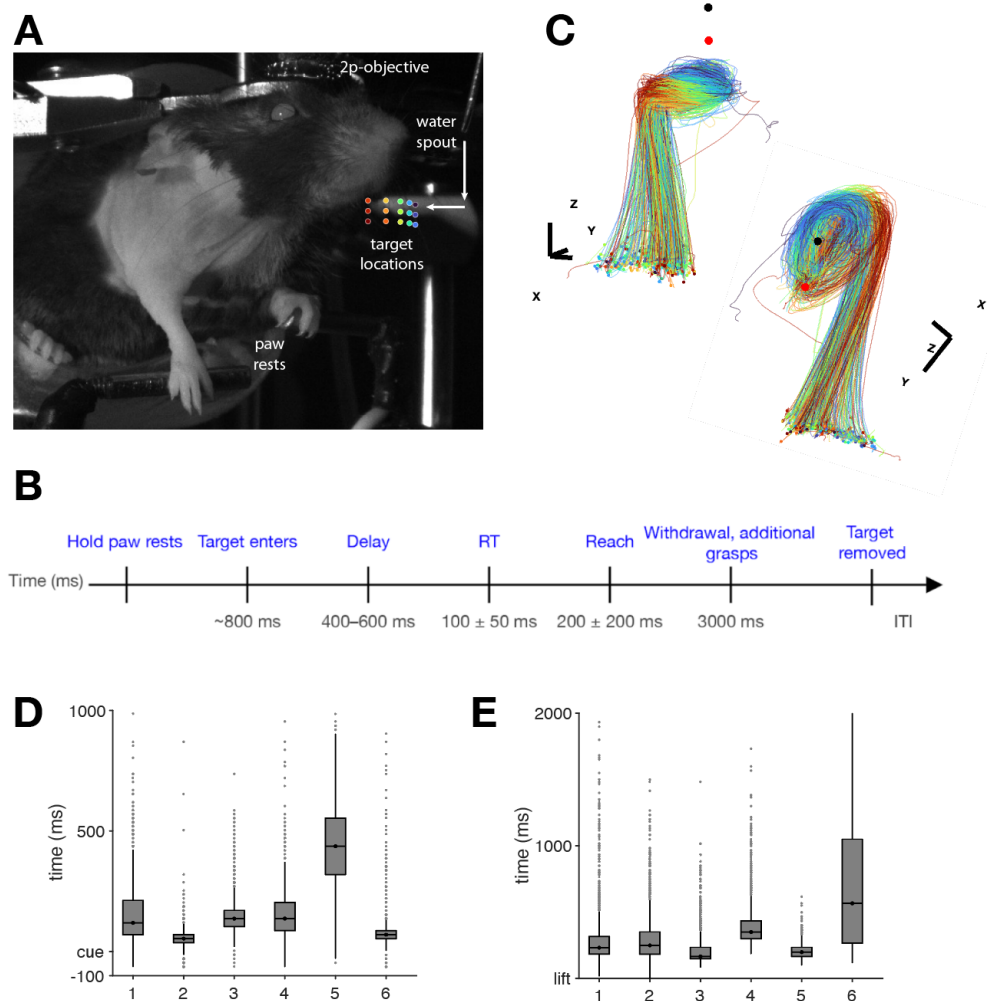


Figure 3.2. Mice performed delayed reach-to-grasp-to-drink movements to 15 distinct targets. (A) Infrared image of the mouse during the inter-trial interval from one of the high-speed cameras. The 15 possible target locations illustrated with colored dots. (B) Task timeline. (C) Paw centroid trajectories from a frontal and dorsal view, illustrating kinematic separation. (D) Box plots of lift reaction times for each mouse. Box shows interquartile range (IQR) with a line for median, whiskers show 1.5 times the IQR, dots show outliers. (E) Time between lift and spout contact across mice. Three sequential sessions per animal shown for both D and E.

Neural data visualization. To begin investigating the neural activity, we identified which neurons modulated their firing rates during the task. To identify modulated neurons, we computed a modulation score for all cells using the ZETA method (Methods; Montijn et al. (2021b)), which asks whether the activity for a neuron changes over time in relation to each

target. Modulated neurons were common and scattered across all five regions of interest in the sensorimotor cortex (Fig. 3.3A). To quantify their spatial distribution, we computed the fraction of neurons that were modulated across the cortical sheet. The five areas of interest varied in the fraction of neurons that were modulated: M2 had 14%, M1 had 23%, S1-fl had 30%, S1-hl had 25%, and S1-tr had 27% (Methods). Given that these areas had spatial organization within them and structure was apparent by eye in the spatial scatterplot of modulated neurons (Fig. 3.3A), we then produced a high-resolution map of the fraction of neurons that were modulated in small neighborhoods (Fig. 3.3B). Neurons in two zones were more often modulated than elsewhere: 1) a wide strip along the border between S1 and M1 and 2) an anteromedial patch of M2. Modulation was the highest around the border between M1 and S1-fl, corresponding to the center of the forelimb portion of M1 and the proprioceptive region of S1-fl (Alonso et al. 2023). Modulation was substantially lower in a wide strip along the border between M1 and M2.

Modulation data are shown here computed with neural activity aligned to the cue. When aligning data to the paw lift (“Lift”) or spout contact (“Grab”), a mostly-overlapping set of cells were identified as modulated (Fig. 3.3C, Fig. 3.3S1). Although more cells were identified as modulated when using the Lift or Grab event alignments, all results were similar across all three possible alignments. Results are shown through the rest of this work aligned to Cue because that best preserved some unique activity structure in M2 (noted below) while preserving all other findings.

We next visualized the tuning profiles of individual neurons through peri-event time histograms (PETHs; Fig. 3.3D-H). PETHs of five example neurons for each of the five regions of interest were chosen to represent the diversity of responses in each area, with a mild emphasis on showing response types that were more prevalent in that area. The diversity observed in the

PETHs of neurons can be organized into the following four buckets: 1) Temporal, 2) target tuning, 3) response persistence, and 4) spatial acuity. Neurons exhibited temporal diversity through multi-peaked and temporally offset bumps of activity (Fig. 3.3G,H). Neurons in S1-tr and S1-hl exhibited the most temporal diversity across conditions. Neurons in M1 and M2 contained temporal diversity within a trial-averaged but exhibited less temporal diversity across conditions. Interestingly, target tuning varied drastically across cortical regions. Neurons in M2 and M1 produced spatial tuning profiles that closely resembled the physical organization of target positions (Fig. 3.3D,E). Conversely, neurons in sensory areas exhibited dense tuning but lacked the spatial organization found in the motor areas (Fig. 3.3F-H). In all five cortical regions, neurons exhibiting persistent response profiles were present. These cells maintained a robust firing rate over a wide temporal window spanning periods before the cue to well after (Fig. 3.3D-H). Interestingly, neurons in M2 and M1 not only had persistent responses but also maintained their tuning profile across time segments. Conversely, in the sensory regions, neurons with persistent responses contained time-varying tuning profiles. PETHs of single neurons build a multidimensional tuning picture where each cell has a unique combination of features.

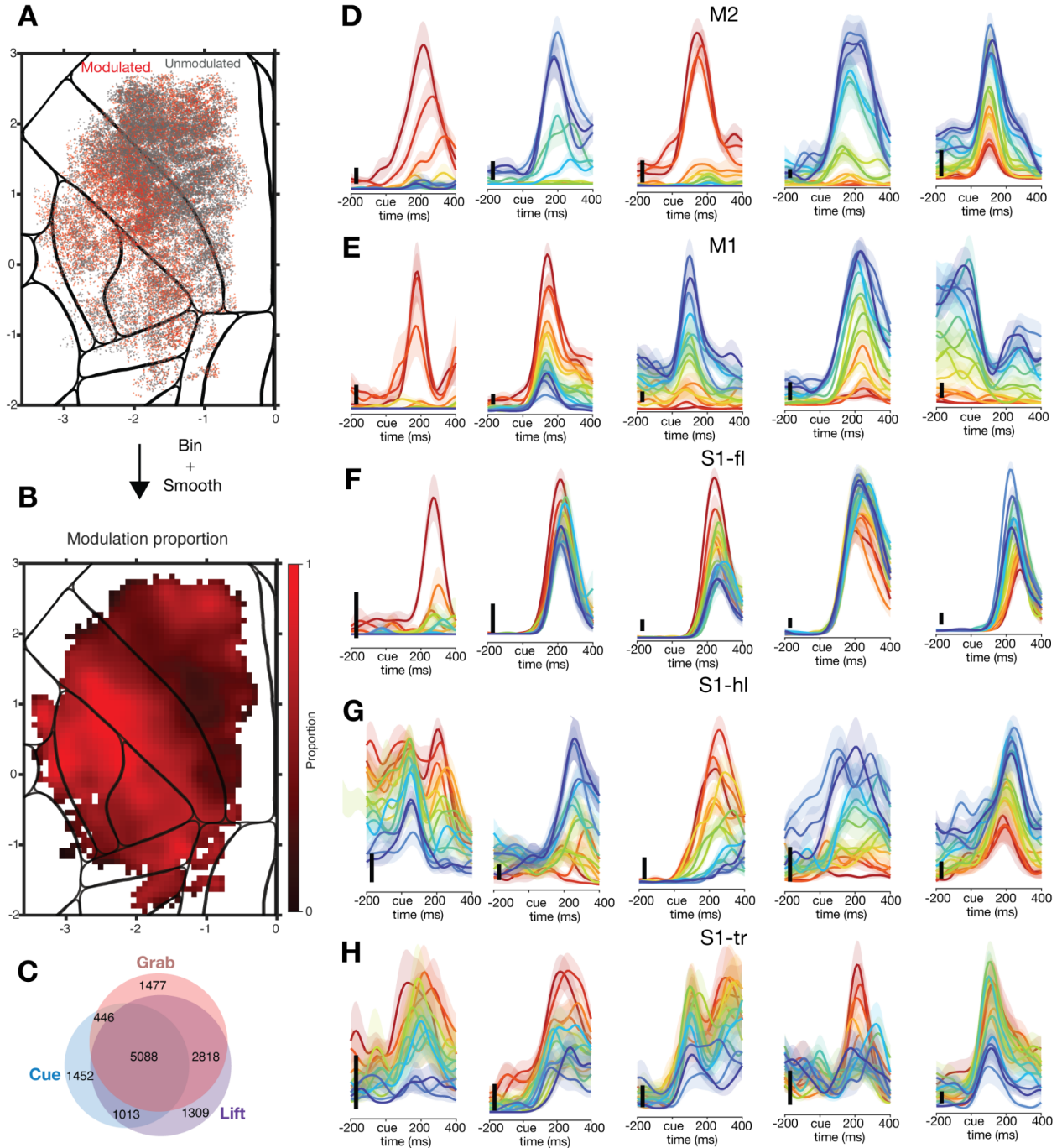


Figure 3.3. Neurons in mouse sensorimotor cortex exhibited heterogeneous tuning profiles. (A) Extracted neuron locations superimposed on the Allen CCF. Red indicates a neuron that was modulated according to our statistical test (Methods), gray indicates a neuron that was not. (B) Binned and Gaussian-smoothed (s.d. 150 μ m) map derived from A. (C) Venn diagram depicting the number of modulated cells for each event alignment. (D-H) Peri-event time histograms (PETHs) of five example neurons in each area. Successful trials to each target were averaged and smoothed. Colors as in Figure 2A. Shaded regions, SEMs; scale bars, 10 events/second. Neurons were chosen to be clear and representative examples of response profiles observed in each area.

3.2.2. *Onset of neural activity varied with somatotopy and subregion*

To begin systematically assessing the tuning heterogeneity of single neurons, we first looked at the onset of neural activity ramping up. To compute the onset times, we first averaged the activity for each neuron across all trials, creating a single grand-mean trace, then found the time to half-max for every neuron. We first asked whether onset times differed by anatomical area (Fig. 3.4A). This revealed clear differences in the overall profile of activation: the earliest onsets were present in S1 trunk and hindlimb regions, perhaps due to the animal stabilizing itself; then M2, and finally S1-forelimb and M1.

Next, we leveraged our dense spatial sampling to build a higher-resolution map of the time of activation across cortex. The temporal onset metric was computed for each neuron, neurons were binned into pixels; then this map was lightly smoothed (Methods). The resulting temporal onset map revealed a number of sub-area features (Fig. 3.4B). The earliest activations, occurring after the target had moved in but well before the Go cue, were in S1 at the juncture of the trunk, hindlimb, and forelimb regions; and in the portions of M1 and M2 that correspond somatotopically to the hindlimb. As suggested by the area-level result above, these zones presumably reflect the animal preparing its body to support the reach. Shortly after, there was activation in a small zone of S1 that corresponded closely with the tactile subregion of the forelimb region, and in the vibrissal subregion of M2 (see Fig. 3.1A, Esmaili et al. (2021) ; Mayrhofer et al. (2019)). This latter activation may relate to the animal whisking to find the target. Next, activation spread through much of M2, part of the forelimb subregion of M1, and into other parts of S1; and then finally to more lateral parts of M1 and M2 that relate to the face and oromaneal feeding (Fig. 3.1A; An et al. (2025)).

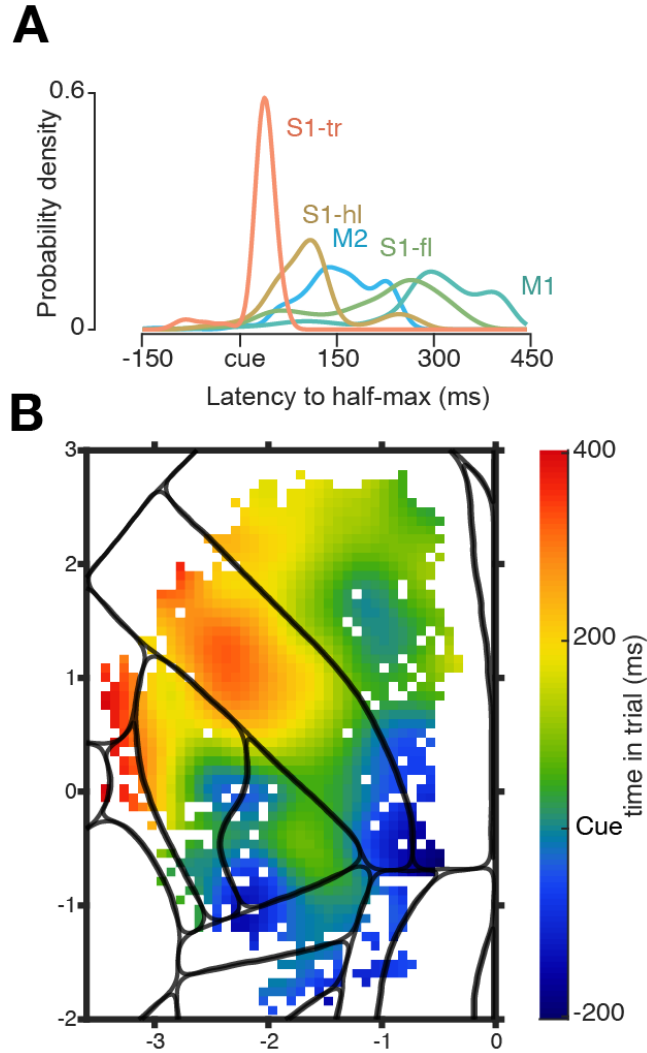


Figure 3.4. Onset of neural activity across sensorimotor cortex followed a somatotopic organization. (A) Smoothed histogram of latency to half-max activation for neurons by anatomical area (Methods). (B) Latency to half-max activation metric map (Methods). The metric was computed on individual neurons, neurons were binned into pixels, then the map was Gaussian smoothed (s.d. 150 μ m).

3.2.3. Activity profiles of single neurons varied systematically across sensorimotor cortex

To characterize the heterogeneity of tuning profiles across single neurons, we developed several summary metrics that captured the most salient variations in properties that we observed in our inspection of thousands of PETHs. This provides greater interpretability than using unsupervised methods such as Principal Component Analysis (PCA), and is consistent with the

long practice of feature engineering to extract known-meaningful structure from data where more generic methods fail to capture this structure (e.g., spikesorting; Caro-Martin et al. (2018) , Lee et al. (2021), Lewicki (1994)). Importantly, note that all key results were replicated using PCA as well. Brief intuition is given for each metric below, with complete explanations of each in the Methods.

Response duration. To capture whether a neuron was active only briefly or for a more extended time, we measured the width of the autocorrelation for each neuron. These values were averaged across all targets for which the neuron was modulated (Methods). Larger values indicate that a neuron remained active for longer. This metric was highest in the posterior regions S1-tr, S1-hl, and the portions of M1 and M2 corresponding to the hindlimb (Fig. 3.5A).

Tuning sparsity. To ask whether a neuron was active for only a few targets or many, we computed a metric we refer to as PETH tuning sparsity (Fig. 3.5B). Neurons attained values near zero if they were similarly active for most targets (i.e., active but minimally tuned), while neurons attained values near unity if they were strongly active for few targets (i.e., sharply tuned). This metric showed that neurons in forelimb-related subregions were sparser than neurons in hindlimb-related regions, with M2 neurons being particularly sparse.

Spatial tuning linearity. To quantify how linearly a neuron's activity related to target location in physical space, we correlated the mean activity of the neuron with the targets' locations. Note that this metric primarily captures azimuth, because we observed that only a few neurons were tuned to elevation. The resultant metric map shows that the motor areas were much more linearly tuned with respect to target location than were the somatosensory areas, with M2 even more linear than M1 (Fig. 3.5C).

Tuning persistence. To capture whether a neuron's tuning profile was similar across time points or differed at different time points, we computed a tuning persistence metric. Neurons with high persistence maintained the same pattern of which targets elicited the highest activity across longer windows of time than neurons with low values. Tuning persistence was highest in the anterior of M2, fell off smoothly through the posterior of M2 and into M1, and was low in the sensory regions (Fig. 3.5D).

Peak time variation. From our inspection of the PETHs, we noticed that for some neurons, their firing rate peaked at the same time for every target (e.g., Fig 3.3E,F), while for other neurons, the firing rate peaked at different times for different targets (e.g., Fig. 3.3G). To quantify this for each neuron, we found the peak firing rate of the response to each target, then computed the standard deviation of these peak times across targets. This value is therefore higher if the peak time varied and nearly zero if the timing was consistent. This metric was quite low in M1, with the highest values in the posteromedial part of M2 (presumably corresponding to the hindlimb representation) and the posterior tip of S1-hl (Fig. 3.5E).

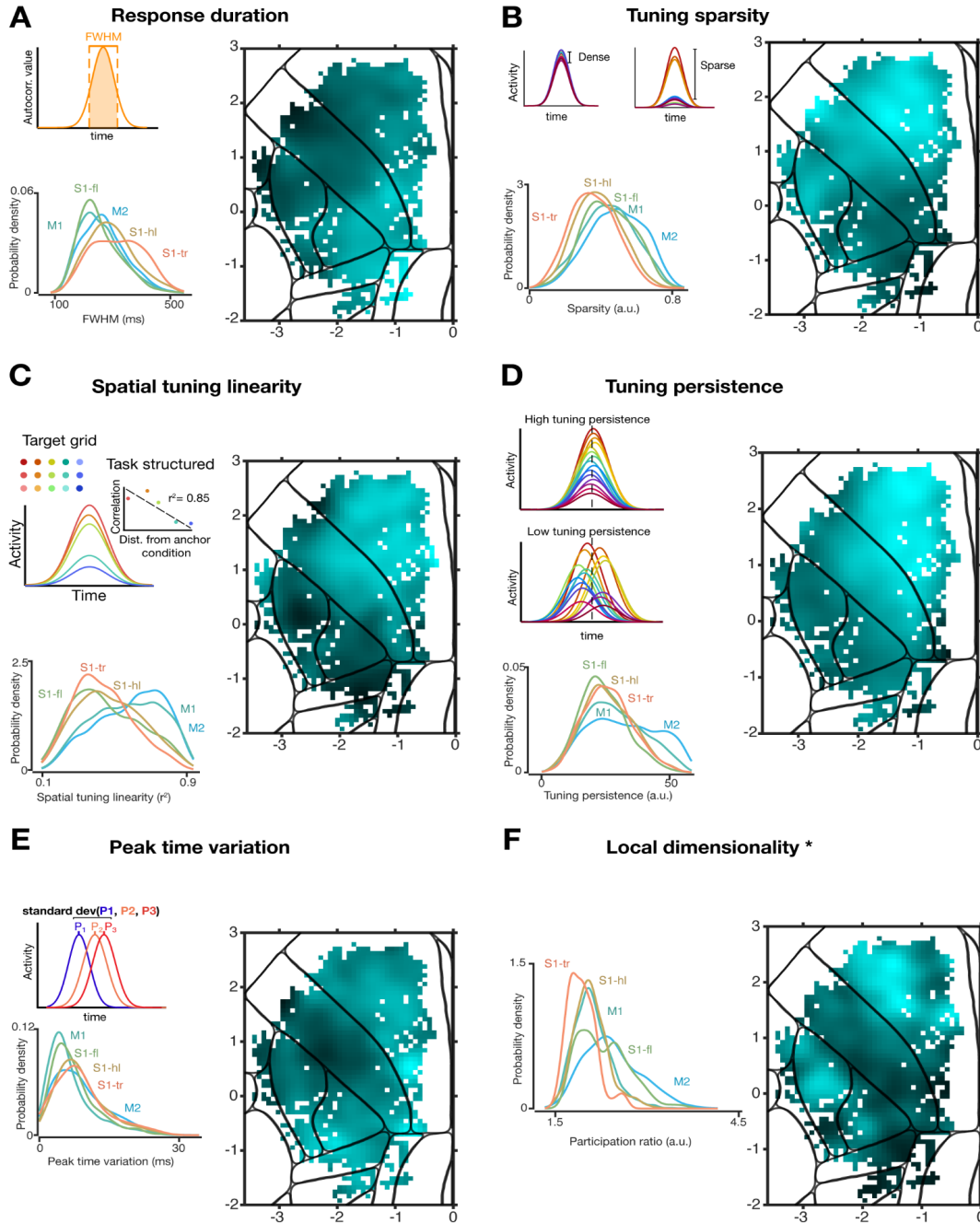


Figure 3.5. PETH tuning metrics captured distinct spatial organization patterns in sensorimotor cortex. (A) *Top left*, schematic for the response duration metric, which measures the autocorrelation width of the trial-averaged trace for each neuron and target. *Bottom left*, histogram of response duration values for all modulated neurons grouped based on anatomical region. *Right*, metric map of the response duration values where bright regions correspond to higher response duration values and darker regions to lower. (B-F) Analogous to A for other metrics. (B) Tuning sparsity, computed as one minus the ratio of the average peak response (excluding the strongest response condition) normalized by the strongest peak response. (C) Spatial tuning linearity, the coefficient of determination of the peak response with physical

Figure 3.5 continued. PETH tuning metrics captured distinct spatial organization patterns in sensorimotor cortex.

distance of the target from the target that evoked the strongest response. (D) Tuning persistence, corresponding to how consistent the tuning was across targets at all lags from the peak (Methods). (E) Peak-time variation, computed as the standard deviation of peak times across targets. Only statistically-modulated targets were included for each neuron. (F) “Local dimensionality”, the participation ratio of the 25 nearest neighbors to each neuron. Note that this metric was not used for analyses below, because it is not independent for each neuron.

Local dimensionality. To ask how heterogeneous small neighborhoods of neurons were, we computed a ‘local dimensionality’ metric: for each neuron, we computed the participation ratio (a simple though imperfect measure of dimensionality) of its 30 nearest neighbors. Higher local dimensionality reflects locations where nearby neurons exhibited more heterogeneity in their tuning profiles. This local dimensionality was highest in a patchy portion of anterior M2, anterior-lateral S1-fl (possibly corresponding to the tactile region (Alonso et al. (2023), see Fig. 3.1A), and perhaps posterior S1-hl (Fig. 3.5F). Note that because this metric involved neighborhoods instead of single neurons, it was not included in subsequent analyses.

We also compared our metric maps with simply using the top 5 PCs, rotated using VARIMAX to identify a sparser basis (Musall et al. 2019). As expected, when compared with our bespoke features created using knowledge of the problem domain, these maps were less spatially structured (Fig. 3.5S1).

3.2.4. Neuron tuning properties were shared with neighboring sensorimotor regions

Given that our PETH metrics differed systematically across areas, we next asked how distinct each area’s neural responses were. As a first attempt to do so, we fit a generative model to each area’s population of neurons. Specifically, we summarized each neuron’s response with a five-dimensional feature vector comprising the five PETH metrics described above. We then

modeled the neurons' feature vectors as arising from a Gaussian mixture model (GMM), with one mixture model for each area. We then computed a likelihood for each neuron as arising from each area's distribution (Fig. 3.6A). Intuitively, this produces a measure of how similar each neuron is to the population of neurons in an anatomically defined area. Finally, we binned and smoothed over neurons (Methods) to produce maps as above.

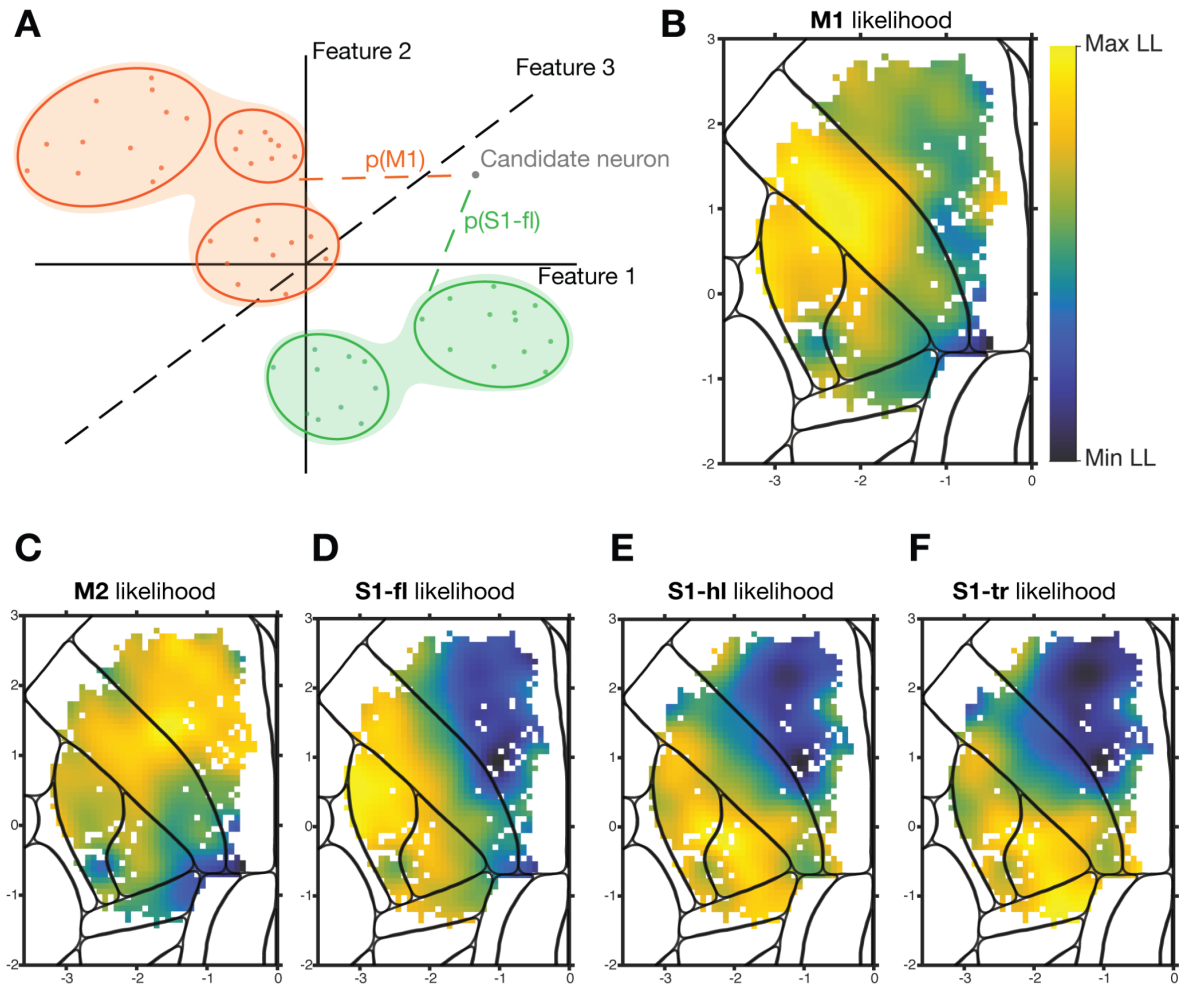


Figure 3.6. Similarity of neurons across sensorimotor cortex to neurons from each area. (A) Schematic of analysis. A Gaussian Mixture Model (GMM) was fit to the distribution of feature values for neurons belonging to a single anatomically defined area, then a likelihood was computed for all neurons from all areas using the fit. (B) The resulting likelihood map for M1, where bright yellow pixels correspond to high likelihood regions and dark blue pixels to low likelihood regions. (C-F) Same as B, where the seed regions were M2, S1-fl, S1-hl, and S1-tr, respectively.

When computing this likelihood map using neurons in M1 to define the distribution (Fig. 3.6B), the highest likelihood zone is, as expected, in M1. However, two additional features are apparent. First, the anterior portion of S1-fl is also strongly similar to M1, in line with previous results (Grier et al. 2025). Second, the posteromedial portion of M1 differs strongly from the rest of the neurons in M1. If we instead defined the distribution using M2 neurons (Fig. 3.6C), we find a narrow strip of M1 is strongly similar. Similarity to S1-fl bleeds over the borders into adjacent M1 and S1-hl (Fig. 3.6D), while S1-hl and S1-tr share a similar spatial layout and bleed over mostly into the other S1 subregions (Fig. 3.6E,F).

3.2.5. Areas exhibited distinct activity profiles

The top-down method used above reveals interesting similarities between neurons in parts of different areas, but because a mixture model was used to accommodate oddly-shaped distributions, that analysis may lump across subpopulations of dissimilar neurons within an area. The maps themselves hint that subpopulations are likely present; for example, even when defining the distribution using M1 neurons, there is a large low-likelihood zone within M1 (Fig. 3.6B).

To better understand the distribution of neurons' properties across not just areas but subpopulations, we complemented the method above with a bottom-up approach. We applied nonlinear dimensionality reduction (t-distributed Stochastic Neighbor Embedding, t-SNE; Hinton and Roweis (2002)) to the full neural population spanning all five brain areas (Fig. 3.7A), then found where the neurons from each area fell in the t-SNE space (Fig. 3.7B). The two-dimensional t-SNE projection led to three findings. First, single neurons in different anatomical

regions spanned the entire t-SNE space (Fig. 3.7B). This makes clear that the full diversity of neuron activity patterns

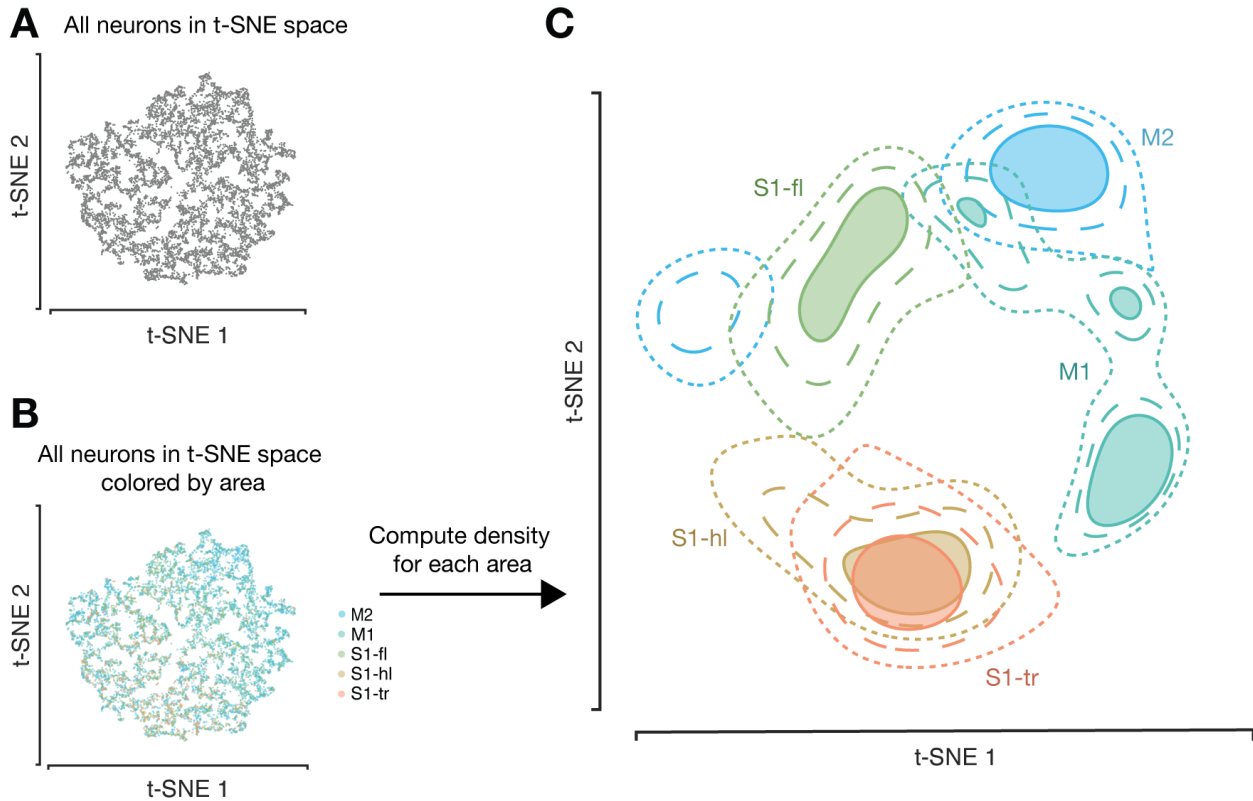


Figure 3.7. Areas had distinct and multimodal distributions of tuning features. (A) t-SNE of the five-dimensional neuron tuning features for all neurons. Each gray point corresponds to a neuron. (B) Same embedding as in A, with neurons (points) colored by anatomical area. (C) Contour plots of the embedding from B, using two-dimensional Kernel Density Estimation to produce a smooth density estimate. Three contour levels (98%, 95%, 90%) are shown for each of the five anatomical regions of interest.

was observed in each area. Second, when looking at the higher-density regions of t-SNE space for each area, the areas were well separated, and the largest differences appear to be between motor and somatosensory areas (Fig. 3.7C). Moreover, S1-fl is somewhat intermediate between the other S1 subregions and M1. This makes clear that the distributions of properties are distinct across areas, but also form an interpretable gradient across areas. Third, the distributions for several of the areas were strongly multimodal. This indicates that there were, in fact,

subpopulations of neurons in several areas: M2, M1, and S1-fl. This multimodality motivated further analysis targeted at understanding the spatial arrangement of the neurons in these neural subpopulations in each area.

3.2.6. Subpopulations spatially overlapped and spanned anatomical areas

To better understand the subpopulations, we used the same GMMs we previously fit to produce our area-based likelihood maps (Fig. 3.6), but considered the component Gaussian in the mixture model separately. This approach explicitly models the different subpopulations as arising from different Gaussian distributions, and retains probabilistic interpretability. In agreement with the t-SNE results, M1, M2, and S1-forelimb were best modeled by the GMM as having multiple components: four for M2 (Fig. 3.8B), three for M1 (Fig. 3.8C), and two for S1-fl (Fig. 3.8D; Fig. 3.8S1 shows all components for all five areas).

The likelihood maps from Figure 3.6B-F suggested that similar populations of neurons were not always confined to the anatomically-defined areas. For example, portions of M1 and S1-fl were strongly similar (Fig. 3.6B,D). If this was due to a subpopulation that was similar across these two areas, we expected that the M1 GMM would contain a component (corresponding to a subpopulation) with similar characteristics to a component in the S1-fl GMM. We therefore computed a similarity index (Methods) between each pair of GMM components (Fig. 3.8E). This analysis revealed that many components found separately in different areas were indeed highly similar, and fell into four groups. Within each group, the components produced similar likelihood maps, which were distinct from those in the other groups (Fig. 3.8S2). We therefore combined components within each of the four groups (Methods) to identify the subpopulations.

These subpopulations differed in two interesting ways. First, by construction, different subpopulations were characterized by distinct PETH features (Fig. 3.8J). Second, their spatial likelihood maps differed strongly as well (Fig. 3.8F-I). One subpopulation, which we refer to as the higher-order M2 subpopulation, was almost unique to M2 (Fig. 3.8F). It had uniquely high tuning persistence and tuning sparsity (sharp tuning) along with highly linear target tuning, arguing for a relatively abstract target representation. A second subpopulation, the “forelimb subpopulation”, spanned M2 and the forelimb portion of M1 (Fig. 3.8G). These neurons were also strongly linear in their target tuning, but less sparse (less sharply tuned) and with tuning that was somewhat less persistent (i.e., changed more over the course of the movement). The “sensorimotor subpopulation” spanned M1 forelimb and orofacial zones (Fig. 3.8H), and was active for most targets, less linearly tuned to target location, and responded very briefly but still at a characteristic time in the movement. Finally, a “hindlimb subpopulation” (Fig. 3.8I) was centered on S1-hl and spilled into S1-tr and the posterior portion of S1-fl. This subpopulation was similar to the sensorimotor subpopulation, but each neuron no longer fired at a specific time point in the movement, instead firing at different times for different targets.

Notably, the different subpopulations spatially overlapped. The higher-order M2 and the forelimb motor subpopulations overlapped throughout M2; the forelimb and the sensorimotor subpopulations overlapped in the forelimb portion of M1; and the sensorimotor and the hindlimb subpopulations overlapped in a band along the S1-fl and S1-hl border. Thus, the neurons that were members of these subpopulations were spatially intermingled (Fig. 3.8S3).

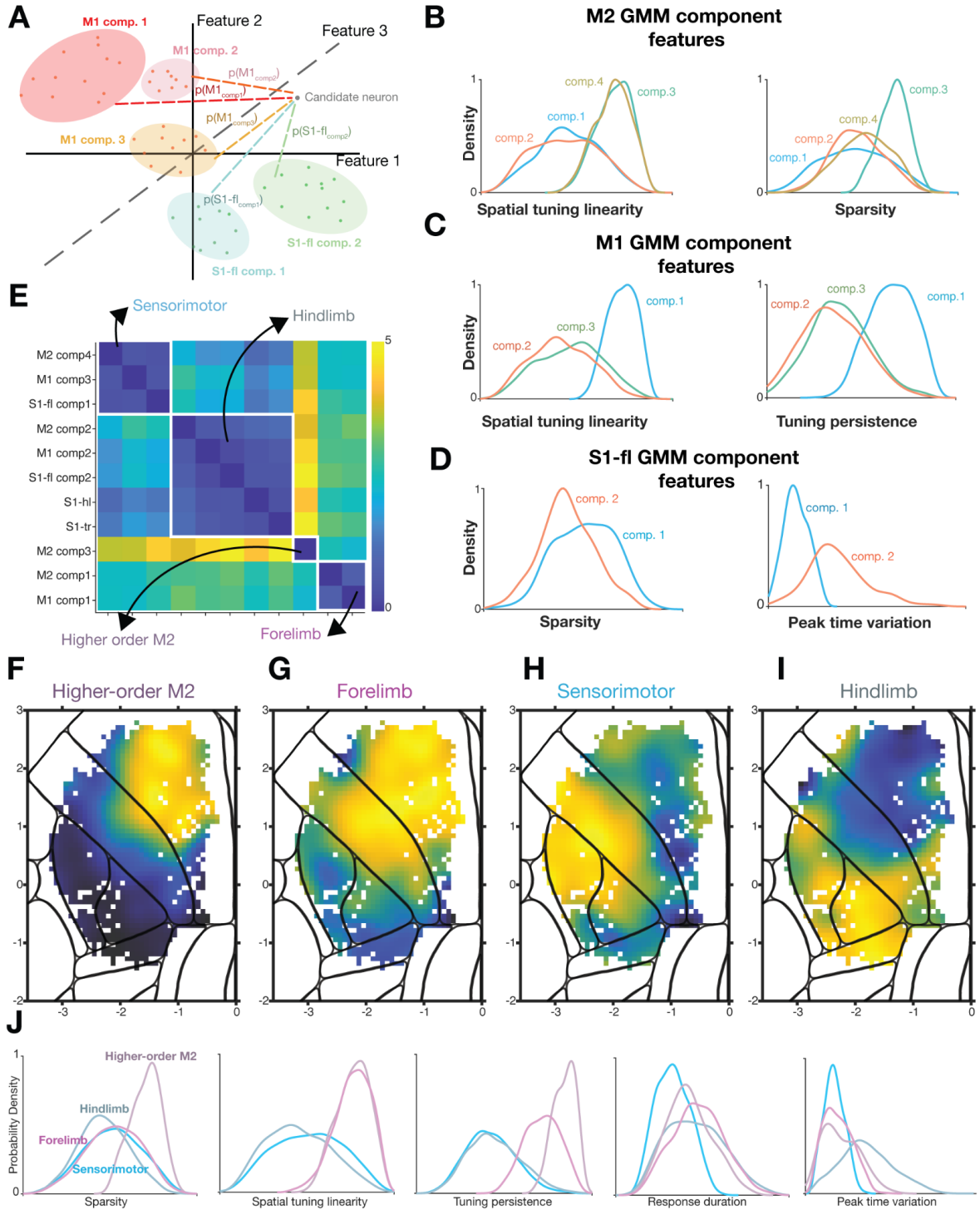


Figure 3.8. GMM component clustering identifies four spatially and functionally distinct subpopulations. (A) Schematic of GMM component-based modeling. Each area's neurons were fit separately with a GMM and the number of components was optimized. The likelihood of each neuron arising from each component was then computed, and neurons were assigned to the most

Figure 3.8 continued. GMM component clustering identifies four spatially and functionally distinct subpopulations.

likely component. Cue-locked data, modulated neurons only. (B) Example histograms of feature values for M2 neurons assigned to each component. (C-D) Same as B for M1 (C) and S1-fl (D) with different example features. All features for all areas shown in Fig. 8S1. (E) Pairwise Mahalanobis distance calculated between all GMM components across all anatomical regions. The matrix is organized based on cluster identity from hierarchical clustering of the pairwise distances. Each cluster is referred to as a subpopulation. (F) Higher-order M2 subpopulation likelihood map. Plotting as in the other likelihood maps. (G-I) Same as F but for forelimb motor (G), sensorimotor (H), and hindlimb (I) subpopulations. (J) Distributions of the five feature values (subpanels) for each of the four subpopulations (traces).

This spatial intermingling was surprising, because it implies that there are “categories” of neurons in these overlap zones. This result agrees with one previous finding (Hirokawa et al. 2017) but contrasts with others (Posani et al. 2025). There are a vast number of possible sources of this difference, given that almost every detail of the experiments and analyses differed between these pieces of work. To determine whether this result was solely due to our use of hand-designed features, we repeated key analyses with an alternate method.

To first determine whether our distinct subpopulations required recordings from a large area, we examined a smaller patch of M1 in an overlap zone (Fig. 3.8S4A). Applying t-SNE to either our metrics or to the top 20 PCs of the PETHs, as is more common in the field, yielded multimodality (Fig. 3.8S4A). We then expanded back to the full dataset, fit a GMM to the PCA scores of the neurons in each area, and generated likelihood maps (Fig. 3.8S4B-D) as in Figure 6. Results were consistent with our feature-based likelihood maps, just less distinct. This indicates that it is not our choice of features that permitted detecting the subpopulations, but that the features did sharpen the subpopulation distinctions.

3.2.7. Distinct spatial boundaries between subpopulations

Each of our likelihood maps contained regions of high likelihood that spanned anatomical borders, and these regions appeared to have distinct boundaries. We wished to ask whether there were true boundaries or whether our method had simply cut a continuum into discrete pieces. To do so, we computed the gradient of each likelihood map and took the magnitude of this gradient at each point (Methods; Fig. 3.9A). Intuitively, this simply asks how quickly the likelihood is changing at each point on the map. Each map revealed a ridge at the edge of the high-likelihood region (Fig. 3.9A-D), suggesting that subpopulations were genuinely localized and did not form a smooth gradient. In the forelimb subpopulation's gradient map (Fig. 3.9A), this boundary was strongest along the border between M1 and S1-fl, cut through the anterior horn of S1-hl (consistent with projection neuron patterns; Ueno et al. (2018a)), and separated what is presumably the forelimb representation from both the hindlimb representation posteromedial and the orofacial representation anterolateral. The hindlimb subpopulation's gradient map was similar (Fig. 3.9D), though the orofacial boundary was weaker. The higher-order M2 subpopulation's gradient traced the boundaries between M1 and M2, and between M1 and S1-fl (Fig. 3.9B). Finally, in the sensorimotor subpopulation's gradient map (Fig. 3.9C), the boundary traced the anatomical border between M1 and M2, and also weakly separated forelimb and hindlimb representations. Each of these candidate functional boundaries was present in at least two of the gradient maps. This gradient approach, therefore, identified boundaries in sensorimotor cortex where single-cell tuning features change over short spatial distances.

The approach above has the advantage of producing a smooth map of how rapidly PETH features change. However, it required many processing steps. We therefore used two other methods to validate this result. First, we used the same gradient (derivative) method on the 5D

feature space directly, instead of on the likelihood maps; results were similar (Fig. 3.9S1; Methods). Second, we used a decoding approach. For each of 10,000 randomly-placed candidate boundaries, we attempted to classify which side of the boundary nearby neurons were on (Fig. 3.9E; Methods). We expected that classification would be better when the random boundary happened to match places where PETH features were changing more rapidly. The top-performing 2.5% of random boundaries are plotted in Figure 3.9E (right). They aligned well with the boundaries revealed by the gradient methods.

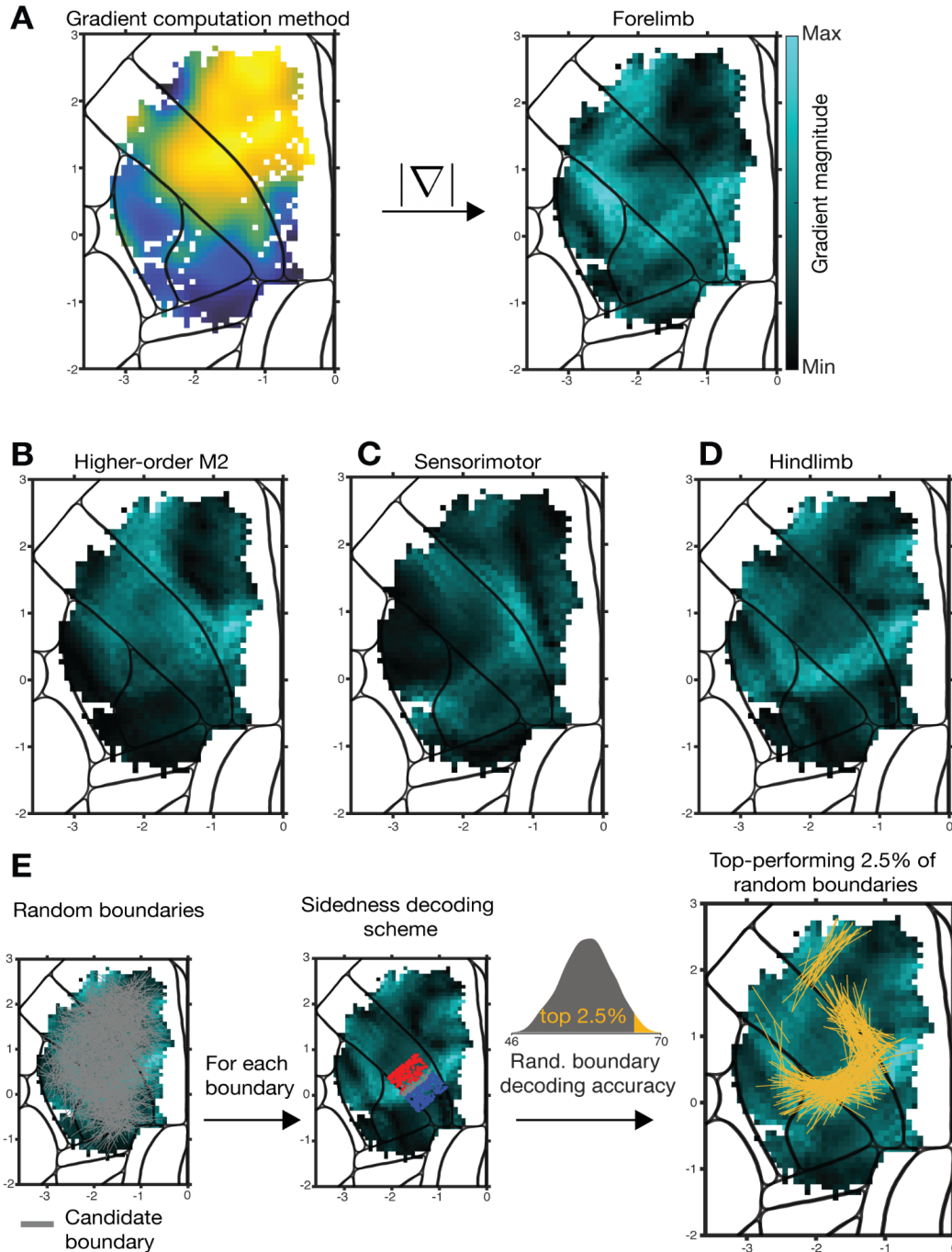


Figure 3.9. Derivatives of likelihood maps produce response-property borders aligned with anatomy and somatotopy. (A) Side-by-side comparison of a likelihood map and its gradient magnitude map. Forelimb motor subpopulation map shown. Bright blue values represent large gradient magnitudes and dark values represent small gradients. (B-D) Same as A *right*, but for the higher-order M2, sensorimotor, and hindlimb subpopulations. (E) *Left*, 10,000 1-mm random boundaries are plotted in gray on top of the average gradient map. *Middle*, for each random boundary, neurons that were within 500 μm above and below the boundary were localized; neurons within 50 μm of the boundary were excluded. An SVM was trained to whether neurons were above

Figure 3.9 continued. Derivates of likelihood maps produce response-property borders aligned with anatomy and somatotopy.

or below the boundary using their feature vectors. *Right*, the top-performing 2.5% of random boundaries (assessed via cross-validation) are plotted in yellow on top of the average gradient map.

3.3. Discussion

By densely sampling mouse sensorimotor cortex during a 15-target reach-to-grasp behavior, we mapped how single cell response characteristics were distributed across five areas. Neurons responded heterogeneously within all imaged areas, and within M2 and M1 in particular, but the average response properties within each area were distinct. Nevertheless, we identified four subpopulations of neurons with unique activity profiles, which were spatially intermingled with one another within-area but also spanned multiple areas. One subpopulation was unique to M2, one spanned M2 and the forelimb portion of M1, one was centered in S1-fl but extended into S1-hl and the forelimb and orofacial portions of M1, and one spanned the likely hindlimb representations of S1, M1, and M2. These activity-based maps thus reveal new structure in sensorimotor cortex and form a complement to a variety of existing mapping methods.

The maps produced here, using response properties of neurons during task performance, aligned with anatomical and stimulation-based maps in some respects but differed in others. We identified response-property boundaries that aligned closely with the anatomical borders between areas M2, M1, and S1. We also identified response-property boundaries consistent with somatotopy, separating forelimb from hindlimb and forelimb from orofacial representations. Interestingly, these somatotopic boundaries were generally stronger than those corresponding to “true” area borders, despite the known coarseness of somatotopic maps in primate motor areas (Mitz and Wise 1987; Neafsey et al. 1983; Qi et al. 2010; Van Acker et al. 2013); whether the

maps are similarly coarse in mouse remains to be tested. Some of our maps also distinguished the tactile portion of S1-fl from the remainder of the area, and grouped the proprioception-receiving portions of S1-fl and M1 (Alonso et al. 2023). We could not, however, readily identify boundaries between other known subareas of M2, such as the Rostral Forelimb Area (RFA; Li et al. (2024); Morandell and Huber (2017); Tennant et al. (2011a)), Anterolateral Motor area (ALM; Chen et al. (2024); Inagaki et al. (2018); Li et al. (2016)), or the LAC/MAC distinction (Yang et al. 2023). This may be due to our relatively shallow recordings; it is possible these distinctions would be apparent in deeper layers.

Some details of our boundaries disagreed with some anatomical maps; in particular, the M1-S1 response-property boundary dipped into S1-hl as it is defined in the gold-standard Allen Common Coordinate Framework (Wang et al. 2020). However, different approaches to anatomical and stimulation-based mapping often produce similar levels of disagreement. Cytoarchitectonic and genetic cell type mapping (Muñoz-Castañeda et al. 2021; Wang et al. 2020) often yield a somewhat different picture than mapping of projection patterns (e.g., (Alonso et al. 2023; An et al. 2025; Maurer et al. 2023; Tennant et al. 2011); see Fig. 3.1A). For example, the deviation of our response-property boundary from the classic M1-S1 border instead agrees with a prior cervical-spinal projection map (Ueno et al. 2018).

Our approach of using intuition to guide the creation of feature metrics has a long history in machine learning. These feature metrics provided interpretability of the identified subpopulations (e.g., Figs. 3.5 and 3.8J), and sharpened the distributions of subpopulation properties. Nevertheless, creating additional features or using neural network approaches to generate other kinds of informative features might enable the discovery of additional structure. Notably, our central findings were similar when using PCA, including that subpopulations

spatially overlapped in some regions. The reason our findings differed from “category-free” findings of prior work (Kaufman et al. 2022; Posani et al. 2025) might stem from any number of differences in these experiments, including task, mouse strain, use of calcium imaging vs. electrophysiology, neuron sampling density, total neuron count, or other factors. Future reconciliation of these findings are likely to prove important to our understanding of network function.

At least two related approaches to using neural response properties have been previously tried in other systems. Tseng et al. (2022) used an encoder/decoder approach to identify how neurons across mouse visual cortex represented different kinds of information in a virtual maze task. With this approach, they were able to identify three broad gradients of encoding, with soft boundaries that aligned closely to anatomical boundaries. Kiani et al. (2015) used a noise-correlation method to perform unsupervised area border-finding in Utah arrays spanning monkey oculomotor cortical areas. This method leveraged the simultaneity of recordings, which could in the future be tested in our data in FOVs that happened to span area borders. In both cases, the primary findings were of response-property boundaries that recapitulated anatomical boundaries, and in the case of Tseng, wider groupings as well. In our approach, we chose to try to capture the variety of structure we observed in the PETHs, some of which did not correspond to task-related meanings that we could readily interpret. This allowed us to expand the number of properties we considered in our boundary-finding and clustering, and enabled us to tease apart the subpopulations discovered here.

Our findings of larger-scale structure, in the form of area-spanning subpopulations, may intersect with three prior findings. First, several widefield calcium imaging studies have found that S1-fl activity groups with activity in portions of M1 and S1-hl as found here (Costa et al.

2025; Musall et al. 2019; Zatzka-Haas et al. 2021). Relatedly, orofacial representations share similar relationships with behavior across M2, M1, and S1 (An et al. 2025; Musall et al. 2019). These previous findings of larger-scale grouping agree closely with our results. Second, S1 populations with different projection targets have known functional differences (Chen et al. 2013; Currie et al. 2022; Hwang et al. 2019b; Kim et al. 2018). However, these specific populations are exclusive to layer 5, while imaging here was performed superficially. Third, the existence of subpopulations of neurons in sensorimotor cortices have been found independently by using genetic identification of excitatory neuron subtypes, and these subpopulations exhibit distinct activity patterns (Hira et al. 2013a; Li et al. 2024; Mohan et al. 2023; Musall et al. 2023). Whether those subpopulations correspond to the ones found here cannot yet be reconciled. However, many of the genetically-identified neuron subtypes in those studies are present only in deeper cortical layers, while the imaging performed here was exclusively superficial. This suggests that the subpopulations we have identified are unlikely to correspond with the subtypes found with genetic tools.

The idea of intermingled subpopulations, though not previously discovered in the present way, is known in the context of visual areas. For example, the field has long known of the existence of color vs. non-color pathways (Tanigawa et al. 2010; Wang et al. 2024) and form vs. motion pathways (Goodale and Milner 1992) coexisting within visual areas. In the case of primate vision, the existence of nearly-complete retinotopic maps in each area has made the definition of areas relatively clear; while in the mouse, the incomplete retinotopy of higher-order visual areas (which may reflect differences in the processing most needed for different parts of the visual field Sit and Goard (2020)) has made area definition somewhat more controversial (Zhuang et al. 2017).

Different parts of the body, unlike the visual field, perform distinct functions. Some obvious examples are that the hindlimbs serve mostly locomotion while the forelimbs enable more flexible behaviors; there are many and various requirements for control of the whiskers, lips and tongue; and the stability requirements of the trunk differ markedly from the dexterity requirements of the digits. Moreover, all these body parts need to be coordinated with one another in behavior-specific ways (Kristl et al. 2025). These requirements for distinct kinds of control with flexible coordination patterns may help explain why the sensorimotor system seems to have so many co-existing kinds of organization.

Several limitations of the present work should be noted. First, the data here include only superficial layer 2/3. Activity patterns in other layers, or even in the deeper portion of layer 2/3, may differ (Currie et al. 2022; Heindorf et al. 2018; Masamizu et al. 2014). Second, while we took great care in aligning our FOVs to the Allen atlas (Methods), we did not analyze the anatomy of fixed tissue for each mouse. Limitations in our atlas alignment, differences between the anatomy of the strain of mice used here from the strain used for building the atlas, and possible mouse-to-mouse variation in the borders of areas all introduce jitter. The sharpness of the boundaries we identified therefore serves as a lower bound. Third, although we achieved coverage of a large swath of sensorimotor cortex, we sampled motor areas more densely than sensory and not every area was densely sampled in every mouse. We therefore may have underestimated heterogeneity in S1, which was sampled less. Fourth, although we obtained high-speed video of the behavior, this work does not consider the kinematics. The relationship of these neural data to the detailed kinematics will be treated fully in future work. Fifth, this work considered only trial-averaged responses, not single trials. This choice enabled us to compare much larger areas of cortex by pooling across sessions, but precluded many types of analysis.

Future work could leverage simultaneous recording of multiple areas to investigate single-trial co-fluctuations in neural activity within subpopulations and across areas.

3.4. Methods

3.4.1. Subjects and surgical procedures

All procedures were approved by the University of Chicago Institutional Animal Care and Use Committee. For two-photon imaging, six male mice aged 8-12 weeks were used. Mice were crosses of Slc17a7-IRES2-Cre-D (JAX strain 037512; Harris et al. (2014)) and TIGRE2-jGCaMP8s-IRES-tTA2 (JAX strain 037719; Sweeney et al. (2025)) which express GCaMP8s in most glutamatergic neurons (Wang et al. 2023a). Each animal underwent a single surgery, then were individually housed on a reverse 12-h light/dark cycle, with an ambient temperature of 71.5 °F and a humidity of 58%. Experiments were conducted in the afternoon, during the animal's dark cycle.

Anesthesia and basic surgical preparation were as described in Grier et al. (2025). A circular craniotomy was outlined with a 5-mm biopsy punch and completed with a dental drill, centered over the left forelimb M1 at approximately 0.4 mm anterior and 1.5 mm lateral of bregma. The craniotomy was cleaned with SurgiFoam (Ethicon) soaked in phosphate-buffered solution (PBS). If the dura remained intact, a durotomy was performed with a needle.

A retrograde virus carrying a tdTomato construct (AAVretro-tdTomato, diluted to 1×10^{13} particles per mL in PBS, Addgene stock 59462-AAVrg) was injected in S1-fl (mice 1-3; 0.0 mm A, 2.65 mm L relative to Bregma) or the center of the forelimb representation of M1 (mice 4-6; 0.4 mm A, 1.5 mm L). 100 nL of virus was pressure injected (NanoJect III, Drummond Scientific) at a depth of 300 μ m from the cortical surface over 5 min. The injection pipette was

kept in place for another 3-6 minutes to ensure viral dispersion before being removed slowly over 2-3 minutes. This injection retrogradely labeled cell bodies in other areas that sent projections to the injected area. In this paper the labeling was used solely for stabilizing the imaging plane (see below) throughout the cortex.

The craniotomy was sealed with a 5 mm #1 round cover glass (Thomas Scientific) glued in place first with tissue glue (VetBond, 3M) and then with cyanoacrylate glue (Krazy Glue) mixed with black dental acrylic powder (Ortho Jet; Lang Dental). In all mice two layers of MetaBond (Parkell) were applied, and then a custom water jet-cut stainless steel head bar was affixed to the skull with black dental acrylic. Animals were awoken by administering atipamezole via intraperitoneal injection and allowed to recover at least 3 days before water restriction.

3.4.2. Behavior and training

The behavioral task (Fig. 2A) was a variant of the multi-target water-reaching task of Galiñanes and Huber (2023). Water-restricted mice were head-fixed with their forepaws on paw rests (flattened eyelet screws) and the hindpaws and body supported by a custom 3D-printed translucent seat. Trials were triggered after the animal held both paw rests for 100 ms. After an additional 100 ms hold period a water spout (22 gauge, modified 90-degree bent, 1-in blunt dispensing needle, McMaster) was moved into one of 15 positions in front of the mouse's face via a set of three motors (LSM050B-T4A, Zaber). The animals were then required to hold the paw rests continuously for an additional 400-600 ms delay period to trigger the delivery of a water droplet on the spout. If the animals released the paw rests during this time the delay was reset; this occurred at variable rates across animals (20-85% of trials). Animals were allowed a

maximum of 2000 ms to achieve paw rest contact for the entire delay; if this did not occur the spout was removed to the neutral location and a 5000 ms time-out was triggered (<3% of all trials across animals). Once the paw rests were held continuously for the variable delay time, a 4000 Hz tone was played by stereo speakers and a 2- to 3- μ l droplet of water appeared on the spout. The cue tone lasted 500 ms or until the mouse made contact with the water spout, whichever came first. The mouse could grab the water droplet and bring it to its mouth to drink anytime after the tone began. The spout was removed after 4000 ms, triggering a variable ITI of 3000-7000 ms. Both the paw rests and spouts were wired with capacitive touch sensors (Teensy 3.2, PJRC). Control software was custom-written in MATLAB R2018a using PsychToolbox 3.0.14. Touch event monitoring and task control were performed at 60 Hz using the Teensy.

The mice were trained to make all reaches with the right paw and to keep the left paw on the paw rest during reaching. For the first 1-3 days, mice were head-fixed and provided water directly to their mouths to acclimate them to head-fixation and reward collection. During this period, the animals were encouraged to make consistent contact with both paw rests to trigger water delivery to their mouths. After acclimation and consistent trial initiation, the spout was withdrawn to a position on the right side of their face intended to elicit forelimb reaching with the right arm. If animals were reluctant to reach, small droplets of water were placed on their right whisker pad with a blunt syringe to induce grooming and water collection. After animals reliably performed reaches toward the rightward spout location (80-90% success), its position was moved away from their cheek along the A-P axis. Once mice could reliably reach towards this new spout position, two new target locations were introduced in azimuth. Once in a multi-target training paradigm, each target was cued in blocks. The size of the blocks was reduced as the mice progressed in their training. This process was repeated until all five targets were reliably

reached along the full azimuth range. The final set of targets was then added along the D-V axis, and the mouse was trained until he could reliably reach towards all 15 target positions. This training procedure took 2-3 weeks, although the behavior continued to solidify for at least two more weeks. Data presented here were collected after 4-6 weeks' experience with the task.

3.4.3. Two-photon calcium imaging

Calcium imaging procedures were similar to those described previously (Grier et al. 2025). Briefly, imaging was performed with a Neurolabware 2p microscope running Scanbox 4.1 and a pulsed Ti:sapphire laser (Vision II, Coherent). Depth stability of the imaging plane was maintained using a custom plugin that made automatic movements of the objective up to every ~10s based on comparing red-channel data with an initially-acquired image stack. Offline, images were run through Suite2p to perform motion correction, region-of-interest (ROI) detection, and fluorescence extraction. ROIs were manually curated using the Suite2p GUI. Fluorescence was neuropil-corrected using a coefficient optimized by minimizing the mutual information between the corrected trace and the neuropil trace (Allen 2025). As previously described (Grier et al. 2025; Zhu et al. 2021), fluorescence was then detrended and passed through OASIS using the 'thresholded' method, AR1 event model, and limiting the tau parameter to be between 300 and 800 ms. To put events on a more useful scaling, for each ROI, we found the distribution of event sizes, smoothed the distribution (ksdensity in MATLAB, with an Epanechnikov kernel and log transform), found the peak of the smoothed distribution, and divided all event sizes by this value. This rescales the peak of the event size distribution to have a value of unity.

As previously described, when time-locking the data for visualization, modulation testing, or decoding analyses, the deconvolved events were resampled into 10-ms bins. This resampling was performed by assigning a fraction of each event into the new 10-ms bins proportionally to how much the 10-ms bins overlapped the original ~32.2 ms frames, and taking into account the exact time each ROI centroid was sampled based on its position within the field of view (FOV).

3.4.4. Measuring modulation

To determine whether a neuron was modulated in the task, we used an adapted ZETA method (Montijn et al. 2021) as described in Grier et al. (2025). Briefly, trial-averaged responses were compared to shuffled trial-averages computed by circularly permuting each trial by a random offset. Modulation was computed as the maximum difference between the real and shuffled data. This procedure was performed separately for each target and for locking data to the cue (-300 ms to +300 ms), lift (-200 ms to +400 ms), and first contact (-300 ms to 300 ms).. For each locking event (cue, lift, and first contact), neurons with p-values ≤ 0.01 were considered modulated. For all analyses, only neurons modulated by the relevant locking event were included.

3.4.5. Mapping cell positions onto a common coordinate frame (Allen atlas)

Each 2P FOV was aligned based on the vasculature to an image of the entire window captured via widefield imaging. S1-fl was identified by paw vibration (Alonso et al. 2023), with imaging and preprocessing performed exactly as described in Grier et al. (2025) except that the camera was upgraded to a pco.edge 5.5 CLHS sCMOS camera (Excelitas). The vibration map

was aligned to the widefield vasculature image via shared vasculature landmarks. This composite image was then scaled and translated to best align the S1-fl activation shape to its CCF boundary, while aligning the central sinus vasculature to the midline overlapping bregma. See Figure 1S1 for vibration map to Allen CCF alignment across animals. 2P images from the cortical surface from each session were then scaled to 720 μm width by 980 μm height and aligned to the registered vasculature via rigid transformations. This series of transformations produced global coordinates for each ROI from its location within the 2P FOV.

3.4.6. Computing map images from single-cell properties

To quantify and visualize the spatial distribution of properties of imaged neurons, we used a binning procedure to prevent biases due to uneven sampling. We binned the neurons into 70 μm -wide square pixels, then averaged the property of interest over all neurons within the pixel. Pixels that contained zero neurons were excluded from further analysis. The images were then Gaussian smoothed (s.d. 150 μm). To Gaussian-smooth the images while excluding empty pixels and accommodating irregular map edges, the final normalization for each pixel was the sum of the weights of contributing pixels, excluding empty pixels. For modulation images, each neuron was assigned a zero if not statistically modulated or a one if modulated. For metric images, each neuron was assigned its metric value. The one-dimensional pixel values were then mapped onto a perceptually-linear colormap (Kovesi 2015).

3.4.7. Metric computations

Tuning sparsity. Tuning sparsity measures how selective each neuron is to some targets over others. For each neuron, we obtained the peak response $v(c)$ for each condition (i.e., target) c . The tuning sparsity then computes the average response as a fraction of the max response, and subtracts this from one

$$\text{Tuning sparsity} = 1 - \frac{1}{14} \sum_{c \neq c_{\max}} \frac{v(c)}{v(c_{\max})}$$

This metric ranges from near one, meaning a neuron is highly selective for a single condition, to near zero, indicating a neuron achieves a similar peak firing rate for all targets.

Spatial tuning linearity. To assess how linearly related a neuron's response profile was to the target locations in physical space, we performed Representational Similarity Analysis (RSA; (Kriegeskorte et al. 2008)). That is, we computed a matrix containing the Euclidean distances between all pairs of targets, and a matrix of the 'distances' between the firing rates for each pair of target responses (over the time window from -200 ms prior to the cue to 400 ms post-cue), then vectorized the two matrices and correlated the vectors. Values near one indicate a neuron whose firing scaled perfectly linearly with target location.

Response duration. We quantified the response duration of single neurons by analyzing the autocorrelation structure of their responses over time. For each neuron, we first computed the autocorrelation function $r(\tau)$ of the trial-averaged response over time $x(t)$ to each target separately:

$$r(\tau) = \frac{\sum_t (x(t) - \bar{x})(x(t + \tau) - \bar{x})}{\sum_t (x(t) - \bar{x})^2}$$

where τ represents time lag, and $\bar{x}$ is the mean of the target-specific response over time. Only targets for which the neuron was statistically significantly modulated were included. From this autocorrelation, we determined the full width at half maximum (FWHM). The mean FWHM was calculated across all the targets for which the neuron was modulated (up to 15), resulting in a scalar measure of response duration for each neuron. Higher values of mean FWHM indicate neuronal responses that are more sustained (with a broader peak), whereas lower values indicate a more transient response.

Peak time variation. Some neurons exhibited firing rate peaks that were very similarly timed for all targets, while other neurons did not. To quantify this, each neuron's PETH was first computed as described above, then for each condition (target) c , the peak response time t_c^{peak} was identified as the time bin at which the trial-averaged trace reached its maximum amplitude. To prevent the inclusion of conditions with minimal modulation, we restricted this analysis to conditions whose peak amplitudes were at least 25% of the maximum peak amplitude across all 15 conditions for each neuron. The variability in peak timing was then quantified as the standard deviation of the selected peak times:

$$\sigma_{\text{peak}} = \sqrt{\frac{1}{n} \sum_{c=1}^n (t_c^{\text{peak}} - \bar{t}^{\text{peak}})^2}$$

where n is the number of conditions meeting our inclusion criterion and $\bar{t}^{\text{peak}}$ is the mean peak time across conditions above the threshold. A higher σ_{peak} indicates greater variability in the timing of peak responses across targets.

Local dimensionality. We estimated the effective dimensionality of local population activity by computing a common and simple metric, the participation ratio (PR), in the spatial neighborhood of each recorded neuron. This metric is limited to only considering the singular value spectrum of the local activity, but when applied exclusively to modulated neurons it provides at least a coarse idea of the variety of responses. For each neuron, we identified its $K = 30$ nearest neighbors (including the anchor neuron) by Euclidean distance in anatomical (x, y) coordinates. For each neighbor, the trial-averaged PETH was vectorized; stacking these vectors formed a data matrix X whose columns correspond to neurons. We then computed the eigenvalues λ of the covariance matrix of X . Finally, the participation ratio for that neighborhood was taken as:

$$\text{PR} = \frac{\left(\sum_{j=1}^K \lambda_j \right)^2}{\sum_{j=1}^K \lambda_j^2}$$

PR increases as variance is distributed more evenly across orthogonal components (higher local dimensionality), approaching 1 when variability is dominated by a single component (neuron) and approaching K (number of neighbors) when contributions are comparable across all components. The procedure was repeated with each neuron serving as the anchor, yielding one PR value per neuron.

Tuning persistence. For each neuron, we first computed the PETH as described above. Then, for each neuron i , an anchor tuning vector $\mathbf{v}_i(t_{rmax})$ was computed at the time point of greatest response for any target, whose entries are the PETH amplitudes across conditions. At

every time point t , we measured the similarity of the instantaneous tuning profile vector $\mathbf{v}_i(t)$ to the anchor vector by the Pearson correlation

$$r_i(t) = \text{corr}(\mathbf{v}_i(t), \mathbf{v}_i(t_{rmax}))$$

This yields a “tuning-distance” curve $r_i(t)$ that falls off as the neuron’s PETH tuning profile deviates from that at $t = t_{rmax}$. To summarize these correlation plots into a single value, we computed the area under the tuning-distance curve

$$\text{AUC}_i = \int r_i(t) dt$$

using trapezoidal integration over the sampled timepoints. Neurons with slowly decaying correlations (large AUC) achieved higher tuning persistence, whereas rapidly decaying neurons (small AUC) received low tuning persistence.

3.4.8. Smoothing metric distributions

In Figure 3.4A and Figure 3.5, distributions of metrics were smoothed with Kernel Density Estimation (KDE). Specifically, for data A, we computed

$$\hat{f}_h(x) = \frac{1}{nh} \sum_{i=1}^n K\left(\frac{x - x_i}{h}\right)$$

where K is the Gaussian kernel, and the bandwidth h was set by Silverman’s rule (Läuter 1988)

$$h = 1.06 \frac{A}{n^{1/5}}, \quad A = \min\left(s, \frac{\text{IQR}(x)}{1.34}\right)$$

With n being the number of observations and s being the sample standard deviation.

3.4.9. Nonlinear dimensionality reduction

We used t-SNE to visualize the structure of our neural population's feature space. Each neuron was represented by a five-dimensional feature vector comprising the metric values described above. To improve the representation of large-scale structure, we seeded the t-SNE optimization with the top two principal components of the feature matrix (Kobak and Linderman 2021). The affinity metric was chosen to be the Pearson correlation. t-SNE perplexity hyperparameter was chosen according to the $N/100$ rule of thumb (Kobak and Berens 2019). The t-SNE space produced a two-dimensional projection of our neural data with coordinates of $Y_i = (y_{i,1}, y_{i,2})$ for each neuron i .

To produce contours in the t-SNE space for each area, we first selected the points for a given area and performed two-dimensional Gaussian KDE on these points to produce an estimate of probability density. We then drew contours (*contour* in Matlab) at the 90nd, 95th, and 98th percentiles of the peak.

3.4.10. Gaussian Mixture Modeling and cluster selection

For the analyses in Figures 3.6 and 3.8, we modeled the neuronal population of each area using Gaussian Mixture Models (GMMs) fit to the same neural feature matrices described above. For each candidate number of clusters k , we fit a GMM with full covariance matrices using the Expectation-Maximization algorithm (*fitgmdist* in Matlab). To select the optimal number of mixture components, we used the Integrated Completed Likelihood (ICL) criterion (Bertoletti et al. 2015), computed as:

$$\text{ICL}(k) = -\text{BIC}(k) - \sum_{i,j} P_{ij} \log(P_{ij} + \epsilon)$$

where P_{ij} denotes the posterior probability that neuron i belongs to cluster j , BIC represents the Bayesian Information Criterion (Schwarz 1978), and ϵ was chosen as 10^{-4} . For each area, we fitted GMMs with one to ten components, and selected the fit that produced the maximal ICL.

3.4.11. Likelihood computations

Following model selection for each region, we computed the log likelihood of every neuron according to the GMM of each anatomical region. The log-likelihood $\mathcal{L}_i$ of neuron i given the full model for a single area was defined as:

$$\mathcal{L}_i = \log \left(\sum_{j=1}^K \pi_j \mathcal{N}(X_i | \mu_j, \Sigma_j) + \epsilon \right)$$

where K is the number of components in the GMM and π_j is the mixing weight for component j . In addition to the full-mixture model, log-likelihoods were calculated for each component within an anatomical area's full mixture model to quantify the spatial footprint of each Gaussian component and to compare this spatial map across components of different anatomical areas. These log likelihoods provide a computational approach for understanding the structure of single neuron feature properties across the sensorimotor cortex.

3.4.12. GMM component distances and clustering

In Figure 3.8E, we computed the distance between each pair of GMM components found in different areas. To do so, we computed pairwise Mahalanobis distances between the Gaussians.

Each Gaussian k was represented by its mean vector $\boldsymbol{\mu}_k \in \mathbb{R}^D$ and covariance matrix $\Sigma_k \in \mathbb{R}^{D \times D}$, where D is the feature dimensionality (5). The distance between components i and j was defined as:

$$d_{ij} = \sqrt{(\boldsymbol{\mu}_i - \boldsymbol{\mu}_j)^T S^{-1} (\boldsymbol{\mu}_i - \boldsymbol{\mu}_j)}, \quad S = \frac{1}{2}(\Sigma_i + \Sigma_j)$$

The distance matrix was then passed in as input into a hierarchical clustering algorithm, where the optimal number of clusters (“subpopulations”) was determined by maximizing the Calinski–Harabasz criterion over possible numbers of clusters (two through eight), yielding four clusters of components. Each cluster includes GMM components with heavily overlapping distributions in the feature space. Clusters were further validated both by inspection of the distance matrix, and by comparing the spatial distribution of the member neurons on the cortical sheet (Fig. 3.8S2), which were very similar for the components grouped in each cluster.

As the simplest way to fairly represent the components that were grouped to form subpopulations, for further analysis we modeled each subpopulation as arising from a GMM comprising the Gaussian components that were grouped together, weighted equally.

3.4.13. PCA-based alternative to feature-based analyses

As an alternative to our feature extraction method for summarizing population structure in trial-averaged activity, we applied PCA to the vectorized PETHs of all modulated neurons and used the first 20 scores as a feature space. For increased interpretability, we performed a VARIMAX rotation on the scores to sparsify them without distorting the space. The rotated scores' spatial structure was visualized in Figure 3.4S1 and their likelihood maps in Figure 3.6S1.

3.4.14. Gradient calculation

In Figure 3.9, we asked whether neurons' response profiles changed more over the same spatial distance in some locations than others. This corresponds to a spatial derivative of the metric field. To compute this for each map in Figure 3.9, we first smoothed the spatial distribution of the log likelihoods using the irregular Gaussian smooth function as described in the section "Computing map images from single-cell properties". We then gridded the smoothed log likelihood values into square bins as described above, and filled missing interior values with linear interpolations of their neighbors. At each pixel, we then computed the 2D gradient magnitude MATLAB's centered finite-difference method. Specifically, if $M(x, y)$ denotes the gridded log likelihood map, then the gradient magnitude $|\nabla M(x, y)|$ was calculated as:

$$|\nabla M(x, y)| = \sqrt{\left(\frac{\partial M(x, y)}{\partial x}\right)^2 + \left(\frac{\partial M(x, y)}{\partial y}\right)^2}$$

This produced a scalar map of local changes in the log likelihood map. For the version in Figure 3.9S1, the two partial derivatives (x and y) were computed for each of the five features. The quadratic mean was then calculated as above over all 10 partial derivatives instead of just two.

3.4.15. Random boundary analysis

In Figure 3.9E, we generated random boundaries through rejection sampling. Potential midpoint coordinates were randomly selected uniformly within the bounding box of the map, and an orientation was selected from a uniform distribution of 0-360°. We then computed the endpoints for a 1 mm line segment with that center and orientation. This boundary candidate was accepted if two criteria were met. First, both endpoints must have been within a non-empty pixel. Second, at least 200 neurons must have been in the valid zones on each side of the boundary (between 50 μm and 500 μm from the boundary). To account for non-uniform distributions of neurons, we downsampled neurons on the denser side of each random boundary to match the count on the sparser side.

To assess how well we could classify neurons as above or below a random boundary using the neurons' feature vectors, we trained a binary SVM with a radial basis function kernel and parameter selection via Bayesian hyperparameter search. Decoding performance was evaluated using 5-fold cross-validation.

CHAPTER 4

GENERAL DISCUSSION

In the preceding chapters of this thesis, I outlined the central question of how motor cortical activity is organized across the mouse sensorimotor cortex and discussed two studies addressing this question. In Chapter Two, I described work aimed at training mice to perform a challenging reach-to-grasp behavior, during which we densely tracked kinematics and examined how S1-fl and M1 neural populations related to these movements. In Chapter Three, we built upon the earlier task by introducing a moveable spout, a delay period, and dense sampling across the entire sensorimotor cortex. These data allowed us, at single-neuron resolution, to develop a comprehensive understanding of how tuning features are organized across the sensorimotor cortex and to define groupings of cortical sub-regions based on single-neuron activity patterns. To conclude, I will discuss the broader implications of these results and highlight questions and future directions that emerge from this work.

4.1. Role of sensorimotor cortex in control during a motorically challenging behavior

We examined the role of M1 and S1-fl in controlling forelimb reaching movements in the mouse by capturing large-scale neural population data with two-photon microscopy and relating it to densely measured forelimb kinematics during the reach-to-grasp behavior. Our findings revealed that kinematic information was similarly present in both M1 and S1-fl. Interestingly, S1-fl neural activity more accurately decoded lift onset time, whereas M1 contained information

about movement type earlier and more persistently. One explanation for this similarity in kinematic decoding across M1 and S1-fl is that cortical control of motor output becomes more dominant when the behavior is sufficiently complex. Prior studies found this in tasks requiring sensory integration, such as identifying a target based on a cue (Mizes et al. 2024) or when sensory input was essential for coordinating movement phases (Guo et al. 2016; Sauerbrei et al. 2020). Our task required mice to time and coordinate movements toward two distinct targets, necessitating integration of peripheral sensory signals with ongoing motor commands. This demanding nature could explain the strong kinematic representation in our data. Another possibility is that motor cortical control is more widely distributed than previously appreciated. Given that these regions are densely interconnected (Muñoz-Castañeda et al. 2021; Wang et al. 2020; Zingg et al. 2014), observing signals across cortical regions is not unexpected. Furthermore, proprioceptive signals span both S1-fl and M1, indicating that such signals may not adhere strictly to anatomical boundaries (Alonso et al. 2023). Additionally, our task required mice to make multiple grasp attempts to obtain water rewards, potentially increasing the importance of sensory feedback for informing motor cortex about movement consequences. This could explain the presence of kinematic signals in S1-fl as an efference copy, enabling somatosensory cortex to modulate reflex circuits alongside movement generation (Moreno-López et al. 2016). Overall, these results motivate deeper exploration of sensorimotor cortex beyond M1 and S1-fl. As other groups have shown (An et al. 2025; Musall et al. 2019; Wang et al. 2023b), motor information is present across the cortical sheet. At the single-neuron level, whether in terms of encoding or tuning features, the key question is: how is this activity organized across the entire sensorimotor cortex? Do functional boundaries align with cortical boundaries, and if not, what does this reveal about the basis of cortical motor control?

Beyond the immediate scope of our experiments, these findings have implications for how we conceptualize cortical control in closed-loop systems. In computational terms, the broad presence of kinematic signals across M1 and S1-fl shows redundancy in representation that could enhance robustness to noise, damage, or variability in sensory feedback. Such redundancy aligns with theories of optimal feedback control (Scott 2004; Todorov and Jordan 2002), where multiple cortical regions maintain overlapping movement representations to allow flexible re-routing of control signals in the face of perturbations. This is not simply duplication—redundant encoding across areas with different connectivity profiles can enable context-dependent weighting of sensory versus motor information streams. This property can be especially important when movements must adapt to changing task demands (Krakauer et al. 2019).

The cross-area similarity we observed also resonates with work in primates showing that motor, premotor, and somatosensory cortices all contain mixed sensory and motor signals that can be decoded to reconstruct limb kinematics (Gallego et al. 2017; Schieber and Hibbard 1993; Vargas-Irwin et al. 2010). This may suggest a conserved computational strategy across species, despite substantial differences in cortical architecture. In the context of human applications, such as brain–computer interfaces (BCIs), this redundancy could mean that high-performance decoders might be constructed from a variety of cortical sources, not just primary motor cortex, potentially expanding surgical targets in clinical settings (Chestek et al. 2011; Sadtler et al. 2014).

Finally, the finding that S1-fl could better decode lift onset while M1 showed earlier and more persistent movement-type coding suggests a division of temporal labor in motor control. S1-fl may be more tuned to discrete, event-related kinematic changes, whereas M1 may maintain

a sustained movement context. This parallels findings in other sensory–motor loops, such as the integration of visual motion signals in parietal cortex and frontal eye fields during eye movements (Gold and Shadlen 2007), where distinct regions track transient versus sustained components of behavior. Understanding this division could guide both modeling efforts—e.g., constructing state–space models with separate transient and sustained channels (Kao et al. 2021)—and experiments that target specific phases of movement execution.

4.2. Parallel processing pathways of interleaved sub-populations in sensorimotor cortex

In Chapter Three, we expanded on the behavioral task of Chapter Two, using three motors to increase the complexity from two static targets to a fifteen-target reach-to-grasp task. We densely sampled mouse sensorimotor cortex at single-neuron resolution while mice performed the task, then applied statistical models to identify interleaved sub-populations based on tuning features. A major takeaway is that functional boundaries largely matched anatomical ones, indicating that anatomy strongly shapes neuronal tuning properties. Connectivity patterns best explained the functional maps we observed, aligning with findings from Zingg et al. (2014). Our work thus captures shared signals across cortical regions through single-neuron tuning features. The identification of tuning-feature-defined interleaved sub-populations provides insight into how cortical control may operate, suggesting parallel channels of unique information traversing simultaneously across cortex. This is reminiscent of parallel pathways in the visual system (Goodale and Milner 1992), though in motor cortex, the functional context differs. For example, controlling digits of the hand requires a very different strategy from controlling the hamstring muscles of the leg, motivating interleaved sub-populations with specialized tuning.

Each body part needs distinct neural control “levers” to coordinate movement. Notably, one subpopulation was located entirely in secondary motor area (M2), with tuning properties enabling a linear mapping of task organization at the single-neuron level. This group best encoded task layout, showed persistent responses, and maintained tuning throughout the trial; features consistent with higher-order encoding of behavior. This supports the role of M2 as a higher-order motor area maintaining movement type and condition information, while other neurons control forelimb execution (Hira et al. 2013b; Manita et al. 2015). M2 also contained the most sub-components, suggesting multiplexed neural activity patterns. In contrast, M1 included subpopulations linked to both sensory areas and M2, positioning M1 as an integrator of sensory and higher-order signals. This is consistent with findings that disrupting M1 during multisensory tasks prevents learning (Bollu et al. 2024), impairs sensory integration (Mathis et al. 2017), and degrades motor refinement (Heindorf et al. 2018). Together, these results support a hierarchical circuit in which M2 formulates motor plans that M1 executes while integrating time-varying sensory input.

From a computational perspective, the existence of interleaved sub-populations can be viewed as a form of mixed selectivity (Fusi et al. 2016; Rigotti et al. 2013), where neurons participate in multiple representational subspaces simultaneously. This arrangement can dramatically increase the dimensionality and flexibility of neural population codes, allowing the same cortical volume to support many different behaviors without requiring dedicated, segregated channels for each. In the motor domain, this could underlie the brain’s ability to rapidly switch between effectors or control policies without a complete reconfiguration of the circuit (Elsayed et al. 2016; Raposo et al. 2014).

This organization also aligns with network-level theories of recurrent population dynamics in motor cortex, where behavior is supported by distributed trajectories through neural state space (Churchland et al. 2012; Sussillo et al. 2015). In such frameworks, parallel sub-populations could represent partially independent dynamical modes—one subspace maintaining higher-order task context (as in M2), while another implements lower-level execution dynamics (as in M1). This division could improve computational efficiency by reducing interference between control signals for different effectors or phases of movement, while still allowing shared information exchange through interleaved connections.

The fact that some sub-populations were confined to specific anatomical regions while others were distributed suggests a hybrid organizational scheme: local specialization for high-priority computations (e.g., higher-order planning in M2) embedded within a distributed mesh of multi-area pathways that can flexibly route information. This hybrid scheme balances efficiency (short, local processing loops) with robustness and adaptability (long-range distributed loops), reminiscent of modular–distributed architectures in artificial neural networks (Yamins and DiCarlo 2016), and consistent with principles from control theory, where parallel controllers with overlapping influence can improve stability and responsiveness (Todorov 2004).

Looking beyond motor cortex, similar hybrid architectures appear in the visual system (parallel dorsal and ventral streams), in hippocampal–entorhinal circuits (parallel spatial and non-spatial channels), and in prefrontal cortex, where sub-populations support both working memory and rule encoding (Aronov et al. 2017; Busch et al. 2024; Maass et al. 2015; Spaak et al. 2017). This suggests that parallel, interleaved processing may be a canonical cortical computation, adapted to the specific demands of each domain.

These findings also have direct translational implications. In closed-loop BCIs, selectively targeting sub-populations with stable, persistent tuning (as in our M2 finding) could improve decoder stability, while leveraging distributed, sensory-linked sub-populations could enhance adaptability to novel movement contexts (Pandarinath et al. 2017; Willett et al. 2021). In neurorehabilitation, interventions could aim to differentially modulate higher-order planning subspaces and execution subspaces, depending on the patient’s deficits. In basic science, mapping communication between these sub-populations during learning or perturbation could reveal how the motor system flexibly reallocates resources between parallel pathways based on behavioral demands, potentially informing models of adaptive motor learning (Vyas et al. 2020).

4.3. Future directions: Identifying kinematic information, cortico-cortical communication, and holographic stimulation

Finally, here I will discuss questions and future directions inspired by this work. Our dense sampling of sensorimotor cortex revealed intermingled subpopulations spanning anatomical areas, defined solely by tuning features from trial-averaged single-neuron responses. In Chapter Three, we did not examine kinematics or forelimb relationships. A natural next step is to, as in Chapter Two, relate neural population activity to tracked motor output. Beyond decoding, it would be valuable to build encoding models that rebuild single neuron activity from kinematic variables. Such encoding maps could confirm the somatotopic structure observed in our temporal response timing maps. Is kinematic information somatotopically organized or more “salt and pepper”? Chapter Three results suggest strong somatotopy, but only fitting encoding models can confirm this.

Another question concerns projection neurons. Our dataset includes projection neurons labeled to M1 and S1-fl. Do these neurons encode kinematic signals differently? Do their responses resemble source-area activity or that of their target area? For example, if M2 is higher-order, do its projection neurons transmit these signals or adopt target-area properties? Examining projection neurons could explain the functional organization seen in Chapter Three, as they convey signals underlying cortical organization.

To fully characterize inter-areal communication, future work should expand our dense sampling to simultaneously image two or more regions. This would enable single-trial analyses linking behavior to communication dynamics—overcoming our current limitation of trial-averaged analysis due to sequential data acquisition. Single-trial imaging is critical to: 1) validate trial-averaged results, 2) relate trial-specific behavioral variations to cortical communication, and 3) develop computational models of cortico-cortical signaling. These advances would build directly on Chapter Three and deepen understanding of single-trial motor control.

Computationally, our results also motivate deeper modeling work to formalize the principles underlying the observed organizational patterns. Building recurrent neural network (RNN) models trained to perform our behavioral task could help identify whether mixed selectivity and parallel subspace organization naturally emerge from task demands, and which network architectures best reproduce the division of labor between M1, M2, and S1-fl. Comparisons between biological and model dynamics could further refine theories of motor cortical computation (Sussillo et al. 2015; Vyas et al. 2020).

Finally, spatially patterned optogenetic stimulation presents an exciting future direction. Holographic stimulation of specific cortical subpopulations offers a causal, single-trial approach

to linking neural patterns to behavior. However, the preceding steps are necessary before pursuing this, as holography requires precise knowledge of circuit structure to be effective. Once these steps are completed, the field will be well-positioned to manipulate subpopulations and measure behavioral effects—particularly through projection cell manipulation to “trick” the brain into perceiving tactile input from a sensory area and triggering programmed activity sequences. This future work will move systems neuroscience toward fully mapping causal, behaviorally relevant cortical computations across the sensorimotor cortex.

Appendix A: Chapter 2 Supplementary Figures

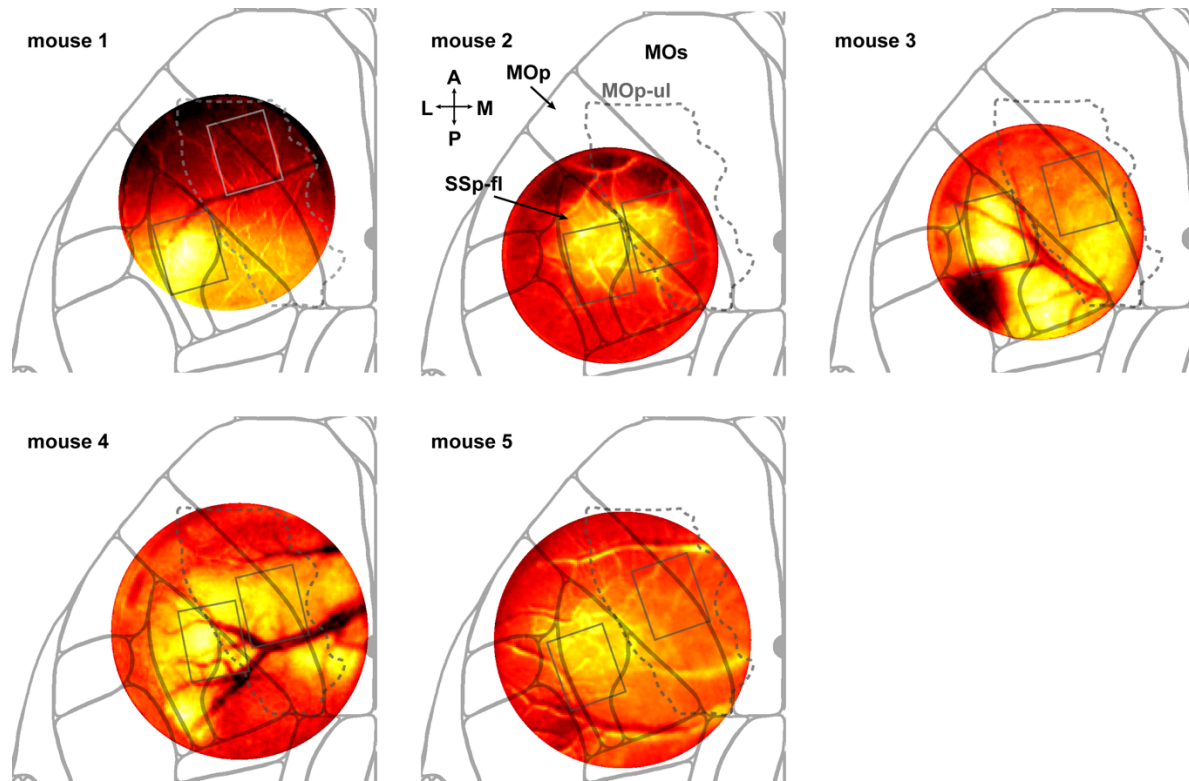


Figure 2.4S1: Vibration mapping for each individual mouse. Each image shows the widefield calcium response +250 ms after a vibrational stimulus applied to the contralateral paw. Each image was aligned with the underlying Allen CCF by placing the broad fS1 (SSp-fl in the Allen nomenclature) shaped activity profile within the corresponding region. Placement was then verified against veridical injection coordinates obtained during surgery (not shown). The dashed line in each map indicates the outline of the forelimb primary motor cortex (MOp-ul) as described in Muñoz-Castañeda et al 2021. Each grey box indicates the placement of the imaging fields of view in CFA and fS1 for each animal. Note that the window for mouse 1 was tilted anterior-posterior during widefield imaging, reducing the measured response in the anterior half of the window. This was accounted for in estimating atlas alignment.

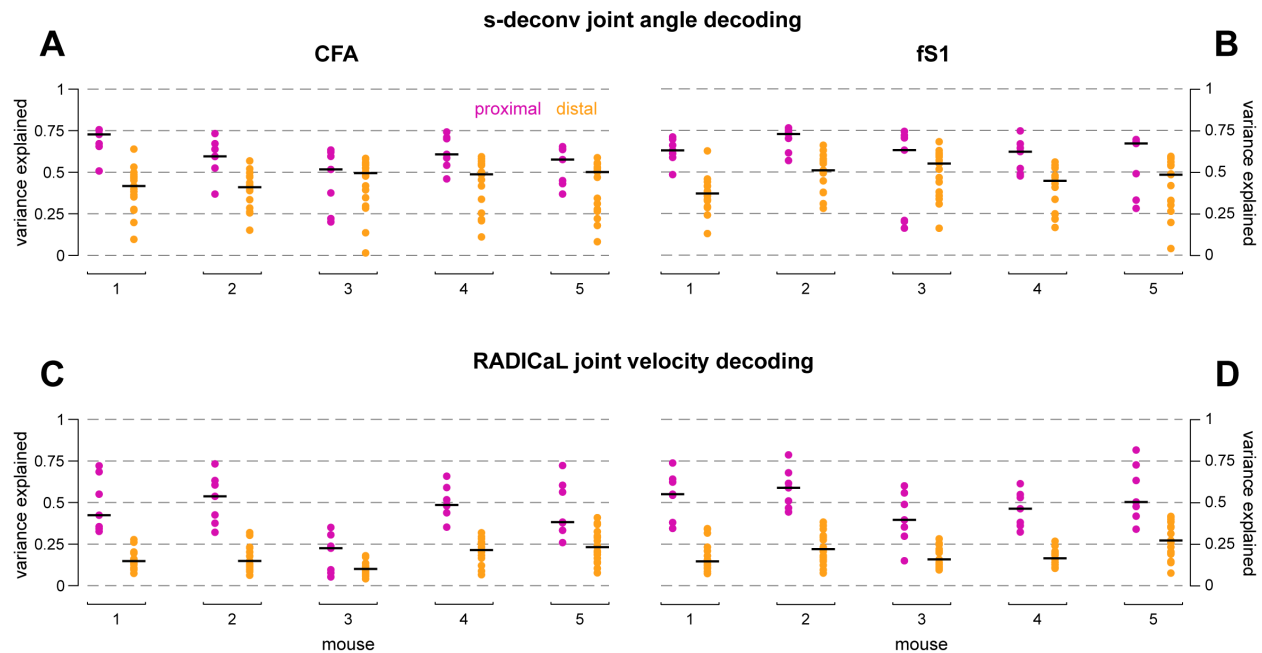


Figure 2.5S1: (A) Joint angle decoding as in Figure 2.5, but from CFA deconvolved calcium transients smoothed with a 35 ms Gaussian kernel. Data is presented as in Figure 2.5D and 2.5H. (B) as in A but for fS1. (C) Joint velocity decoding using RADICaL rates from CFA. (D) Same as C but for fS1.

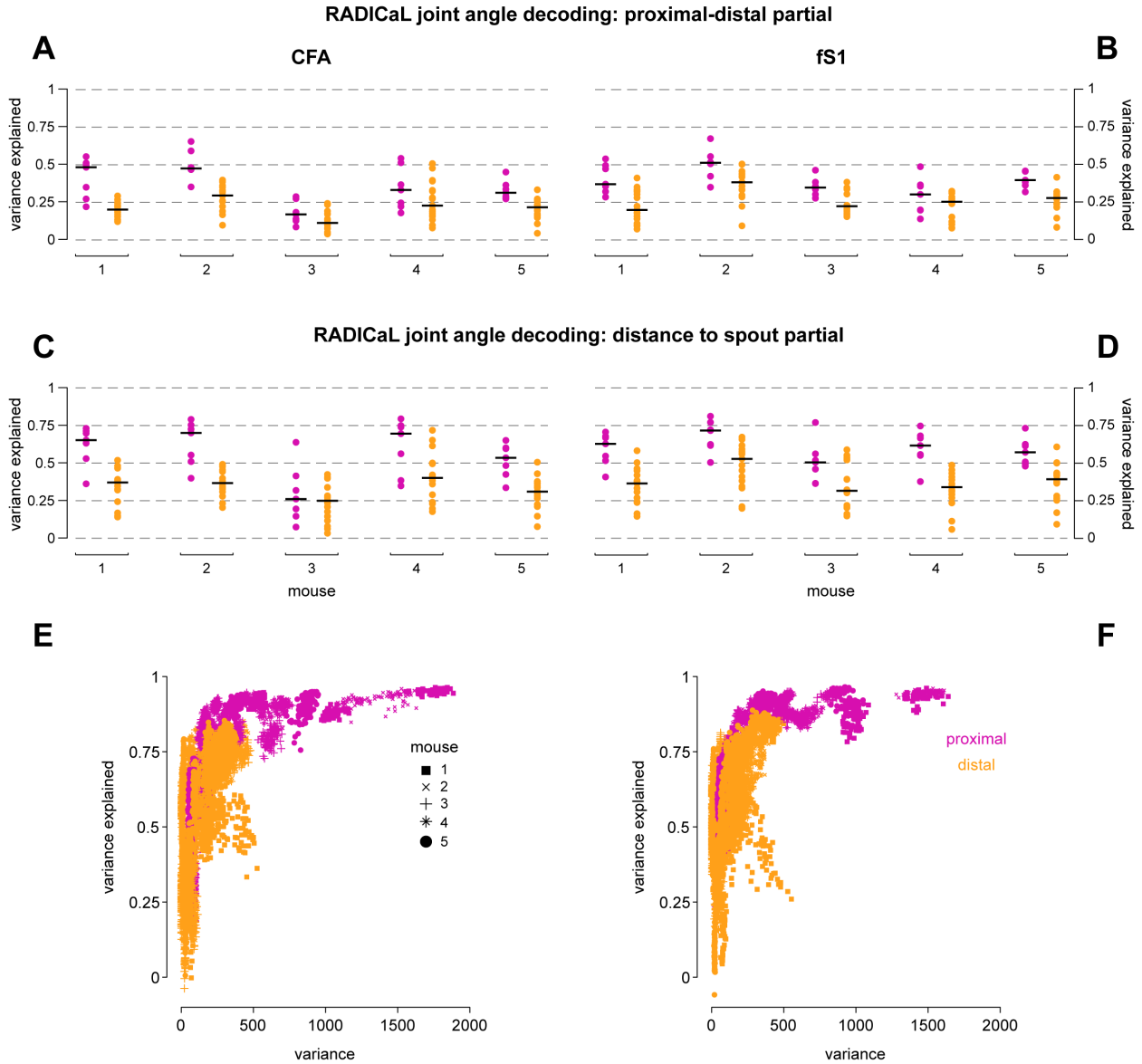


Figure 2.5S2: (A) Joint angle decoding as in Figure 2.5 but with proximal joint angle time series regressed off of distal joints and distal joint angle time series regressed off of proximal joints. Decoding performed with RADICaL rates. Data is presented as in Figure 2.5D and 2.5H. (B) as in A but for fs1. (C) Joint angle decoding as in Figure 2.5 but with the distance to the spout of the paw centroid regressed off of all joint angles. (D) Same as C but for fs1-fl. (E) Variance of joint angles and their variance explained when decoded using CFA RADICaL rates as in Figure 5. Each point has coordinates of the true kinematic variance and the variance explained for a single joint on a single cross-validation fold. (F) Same as E but for fs1.

Appendix B: Chapter 3 Supplementary Figures

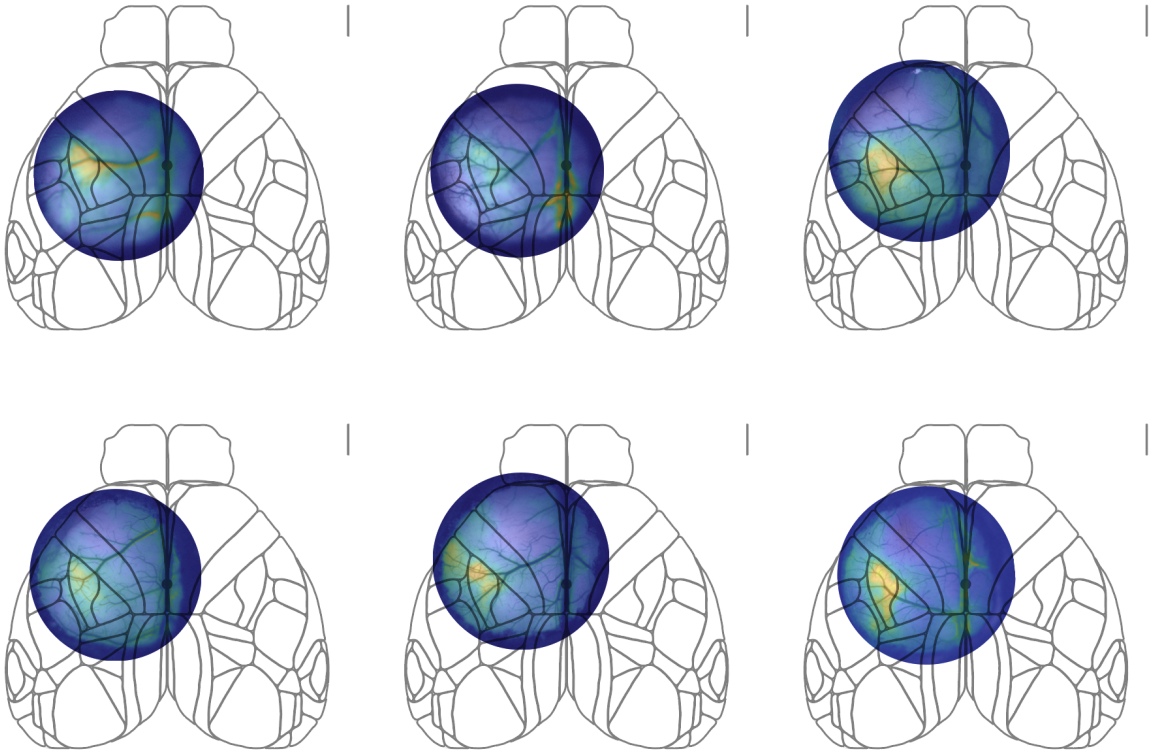


Figure 3.1S1. Widefield window images and vibration maps aligned to the Allen CCF for all mice, as in Figure 3.1B.

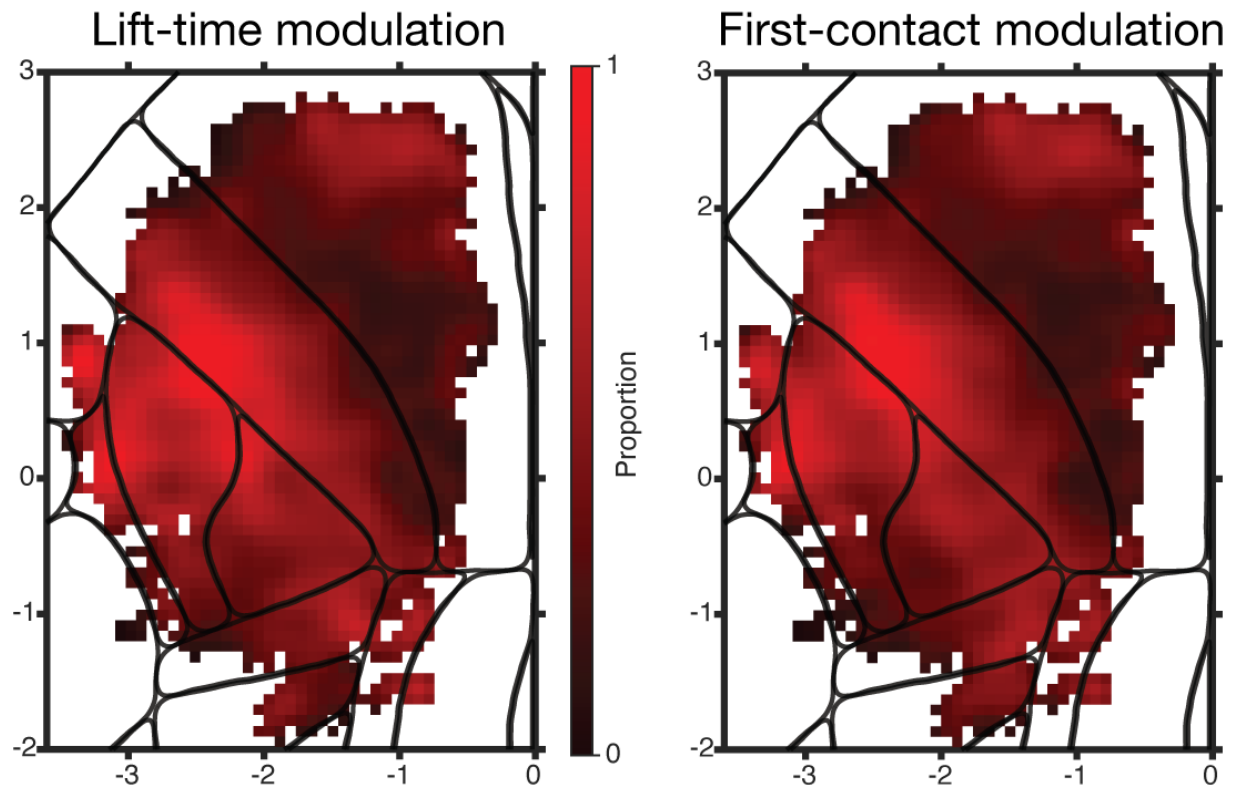


Figure 3.3S1. Binned and smoothed modulation map as in Figure 3.3, but with data aligned to Lift (*left*) or Grab (*right*). Lift alignment used neural data from 200 ms before to 400 ms after lift. Grab alignment used neural data from 300 ms before to 300 ms after first spout contact.

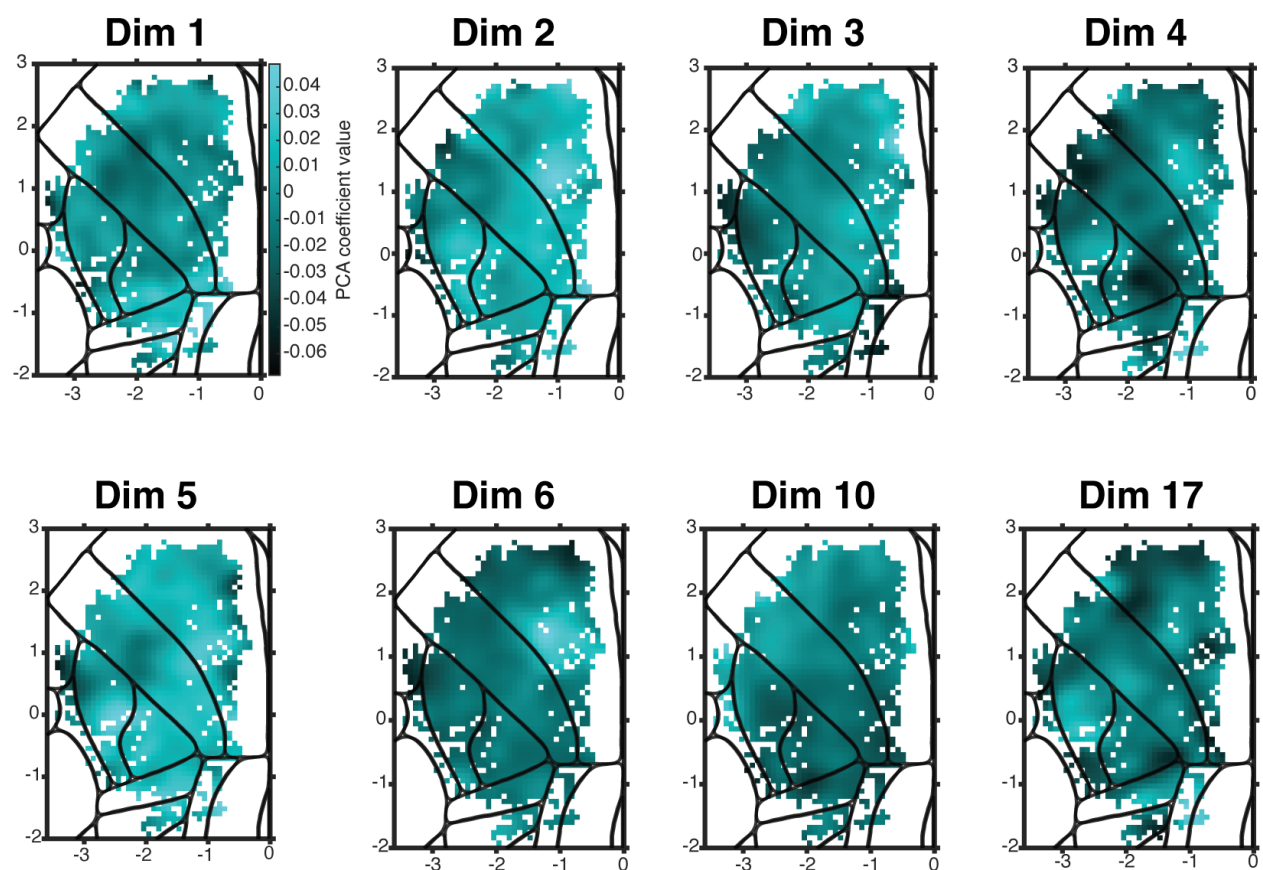


Figure 3.5S1. Principal component scores after VARIMAX rotation for each neuron plotted as feature maps using the same method as Figure 3.5. Most coefficients lack clear global structure, but some (e.g., Dim 6, Dim 4) exhibited distinct local structure.

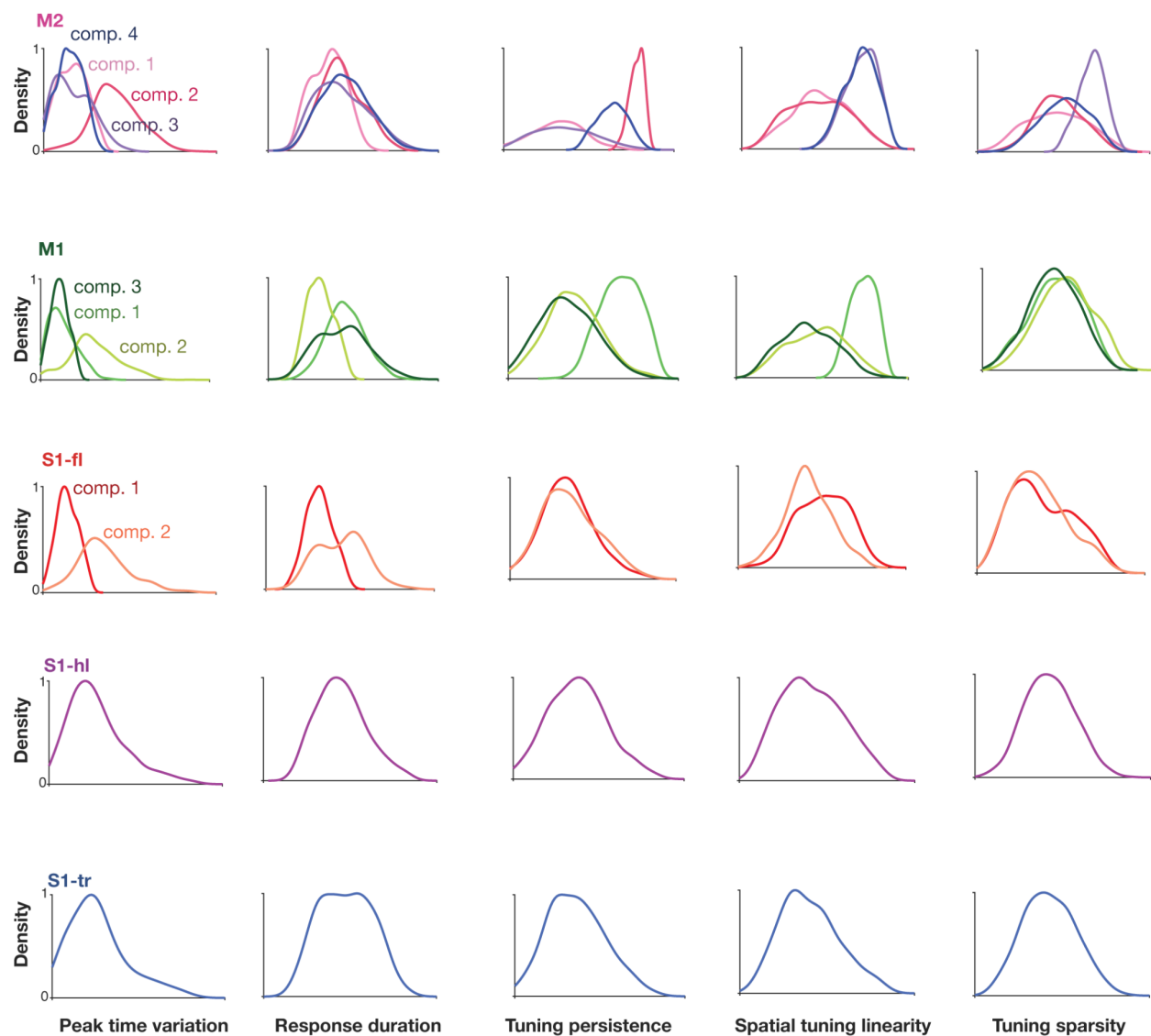


Figure 3.8S1. Feature value histograms as in Figure 3.8B but for all GMM components and all areas.

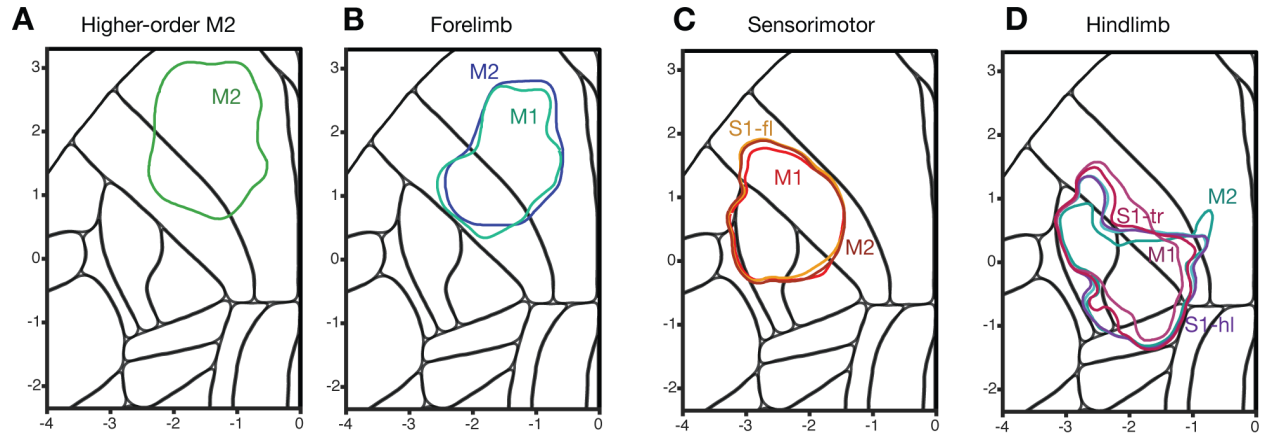


Figure 3.8S2. GMM component contours at 60% level for each subpopulation. For the Forelimb, Sensorimotor and Hindlimb (B-D) subpopulations, component contours made from neurons in different areas largely overlap. This provides validation of the subpopulation grouping done in Figure 3.8.

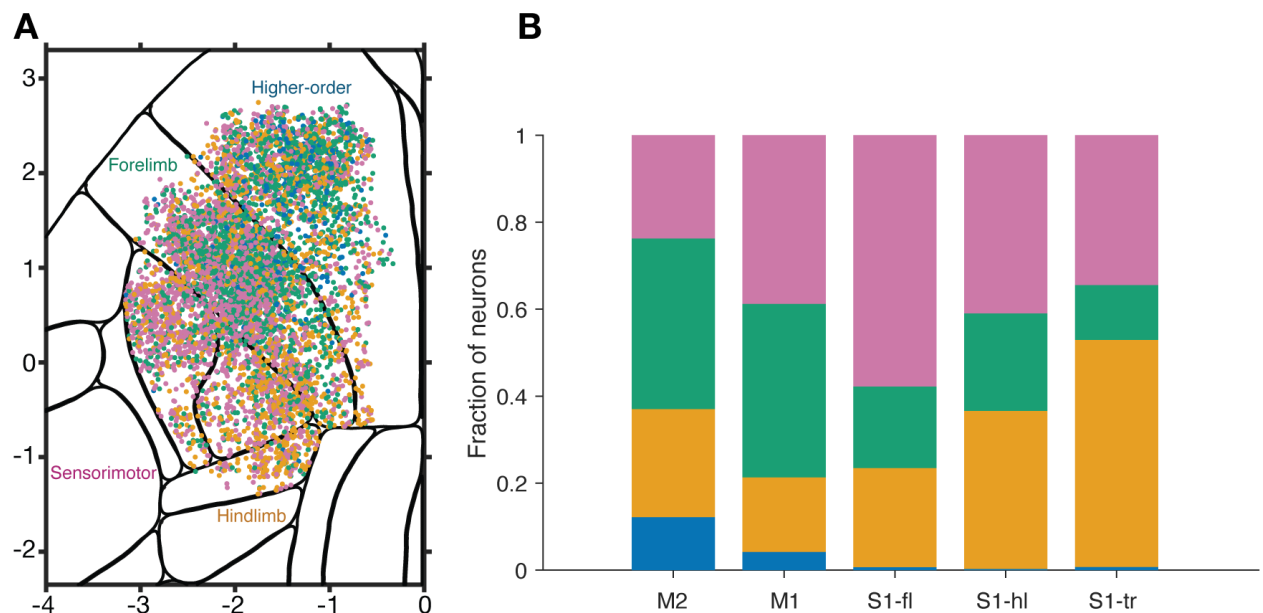


Figure 3.8S3. (A) Neurons colored based on subpopulation membership. Membership was assigned for each neuron as the subpopulation GMM for which the neuron had the greatest likelihood. (B) Proportion of neurons belonging to each subpopulation for each neuron. Colors as in A.

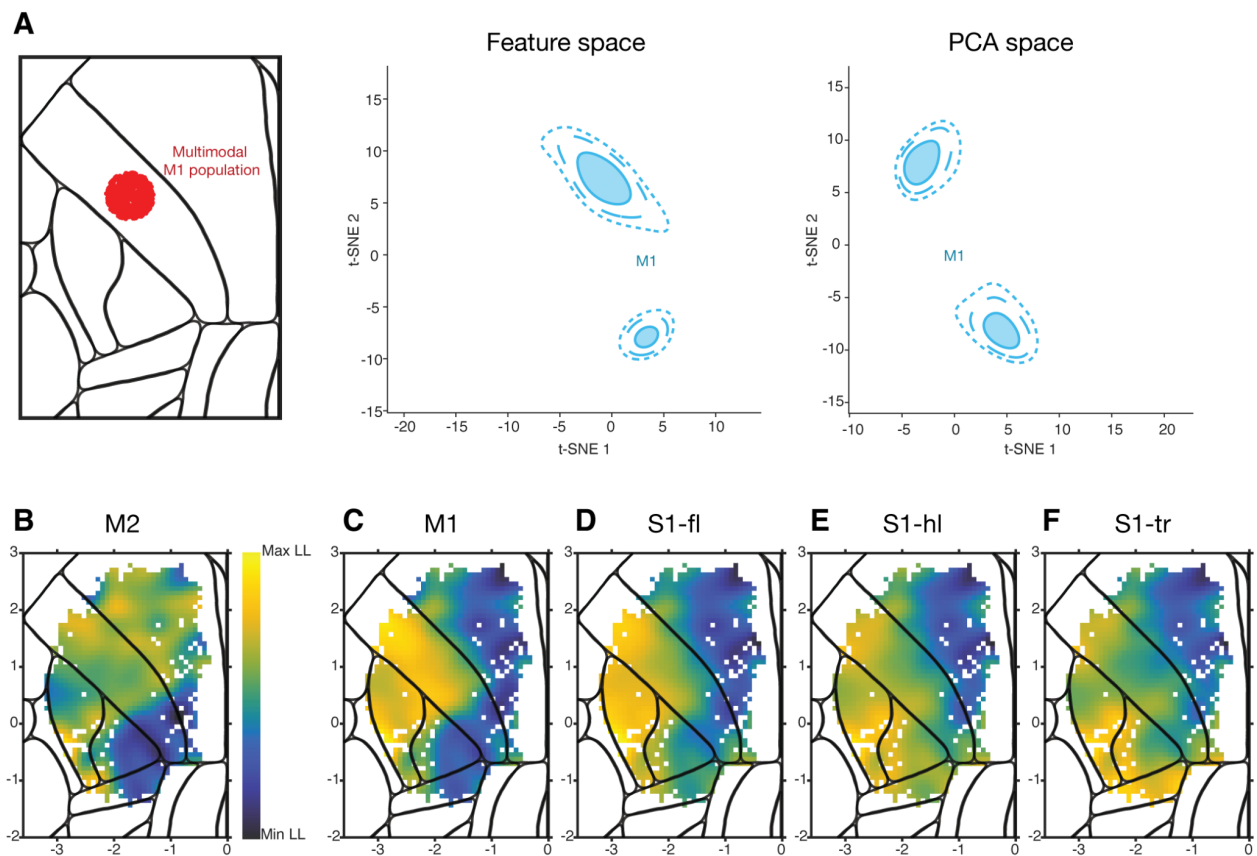


Figure 3.8S4. (A) *Left*, circular population of M1 neurons chosen in the center of the primary motor cortex, to analyze for subpopulations while avoiding spatial somatotopy. *Middle*, t-SNE embedding of this M1 population using the feature space. *Right*, t-SNE embedding of the same M1 population using PCA scores. Both methods yield clear multimodality. B-F) Same as Figure 3.6B-F but using top 20 PC scores instead of features.

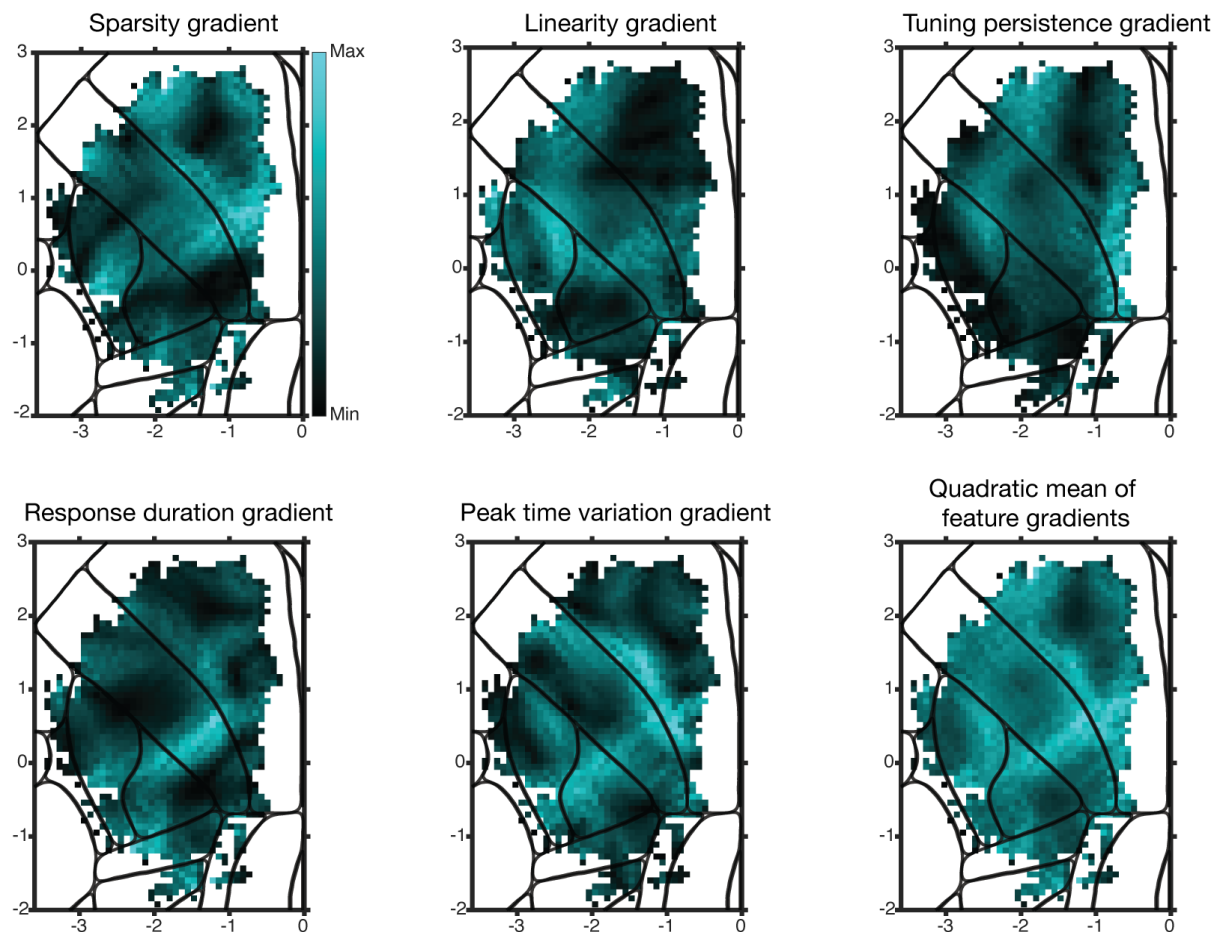


Figure 3.9S1. Gradients as in Figure 9 but calculated using the feature maps depicted in Figure 3.5. Each feature had a distinct gradient with boundaries approximately aligned with borders between anatomical regions, and other boundaries approximately separating somatotopic representations. The *bottom-right* plot depicts the quadratic mean of the five feature gradients. This pooled gradient map closely resembles the pooled map in Figure 3.9E, highlighting the boundaries separating M1-fl from M1-hl, the M1-M2 border, and the forelimb-hindlimb somatotopic border.

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