NEUROSCIENCE

Neuroethology of natural actions in freely moving monkeys

Francesca Lanzarini[1]†‡, Monica Maranesi[1]‡, Elena Hilary Rondoni[2]‡, Davide Albertini[1]‡, Elena Ferretti[1], Marco Lanzilotto[1], Silvestro Micera[2,3,4,5], Alberto Mazzoni[2], Luca Bonini[1]*

The current understanding of primate natural action organization derives from laboratory experiments in restrained contexts (RCs) under the assumption that this knowledge generalizes to freely moving contexts (FMCs). In this work, we developed a neurobehavioral platform to enable wireless recording of the same premotor neurons in both RCs and FMCs. Neurons often encoded the same hand and mouth actions differently in RCs and FMCs. Furthermore, in FMCs, we identified cells that selectively encoded actions untestable during RCs and others that displayed mixed selectivity for multiple actions, which is compatible with an organization based on cortical motor synergies at different levels of complexity. Cross-context decoding demonstrated that neural activity in FMCs is richer and more generalizable than in RCs, which suggests that neuroethological approaches are better suited to unveil the neural bases of behavior.

Decades of brain research on the cortical motor system have leveraged nonhuman primates as a model for their marked homologies to humans (*1*), and the findings greatly expanded our knowledge of the anatomical (*2*, *3*) and functional (*4*, *5*) architecture that underlie the cortical control of goal-directed actions. However, the concept of "goal" and the extent to which goal-directedness coincides with the voluntary nature of an action are still widely debated issues.

Some studies that leveraged intracortical microstimulation (ICMS) have revealed that the frontal motor system may have evolved to encode specific final postures of multiple body parts, which resemble those that characterize natural actions (*5*, *6*). Neurophysiological studies have shown that premotor neurons encode the achievement of a specific outcome, such as reaching a specific spatial location (*7*) or obtaining a piece of food (*5*). Often, premotor neurons discharge with considerable independence from the sequence of extension-flexion movements required to accomplish the task, differently from primary motor neurons (*8*) and sometimes even regardless of the effector used (*9*, *10*). Thus, the encoding of action goals has been proposed as a fundamental organizational principle of the premotor cortex (*11*).

[1]Department of Medicine and Surgery, University of Parma, Parma, Italy. [2]The Biorobotics Institute, and Department of Excellence in AI and Robotics, Scuola Superiore Sant'Anna, Pisa, Italy. [3]Interdisciplinary Health Center and Department of Excellence in AI and Robotics, Scuola Superiore Sant'Anna, Pisa, Italy. [4]Modular Implantable Neuroprostheses Laboratory, Università Vita-Salute San Raffaele & Scuola Superiore Sant'Anna, Milan, Italy. [5]Bertarelli Foundation Chair in Translational Neuroengineering, Neuro-X Institute, School of Engineering, Ecole Polytechnique Federale de Lausanne, Genève, Switzerland.
*Corresponding author. Email: luca.bonini@unipr.it
†Present address: Ernst Strüngmann Institute for Neuroscience in Cooperation with Max Planck Society, Frankfurt am Main, Germany.
‡These authors contributed equally to this work.

A common feature of the aforementioned results is that they have been achieved by studying head-restrained monkeys seated on a primate chair. When recording single-neuron activity, the animals are trained to perform formal motor tasks in a highly reproducible and often stereotyped manner under the general assumption that the findings can be generalized to explain how the brain controls natural actions in unconstrained contexts, but this is not necessarily the case (*12*). Furthermore, neuronal recordings during spontaneous behaviors in freely moving contexts (FMCs) have led to important discoveries in small mammals (*13*–*15*) that would not have been possible under restrained contexts (RCs) (*16*). By contrast, studies in which the subjects were freely moving have not taken hold in the investigation of action organization in macaques, mostly because of technological limitations in the tethered recording devices but, undoubtedly, also because "The breadth and complexity of natural behaviors inspires awe" (*17*).

Despite the increasing relevance assigned to the study of natural behaviors (*18*) and the recognized priority of stepwise comparisons of the neural codes under constrained and unconstrained contexts (*19*), an in-depth investigation into the specific neural code for the large repertoire of primate natural actions and their cross-context invariance is lacking.

Stepwise transition from neurophysiology to neuroethology: A neurobehavioral platform

To investigate the cortical mechanisms underlying natural actions and assess their cross-context invariance, we trained two monkeys to spontaneously move from their home cage to a primate chair, which was then moved to the center of an experimental room designed for neuroethological recordings [the NeuroEthoRoom (NER)] (fig. S1) (*20*). In each session, we recorded single-neuron activity and the monkey's behavior in a two-step approach: first, while the monkey sat on a primate chair at the center of the NER in a classical RC in which its head was restrained (Fig. 1A); then, within the same session, while the monkey explored the NER when it was enriched with several items to evoke the largest variety of spontaneous actions in a FMC (Fig. 1A). In each session, off-line frame-by-frame analysis of synchronized videos captured by eight cameras at 50-Hz resolution was performed with well-established ethological coding software (*21*), which allowed us to identify point events related to a variety of forelimb and face and/or mouth actions with high inter-rater reliability and temporal precision (Fig. 1B and fig. S1), likewise in conventional neurophysiological studies (*22*). The alignment events (*20*) of each action defined in the ethogram (fig. S2 and table S1) corresponded to critical transition between distinct motor phases (such as finger or mouth opening and closure) that, in the case of transitive actions, are associated with the contact of a body part (for example, hand or mouth) either with the monkey's own body or with an external object (movie S1). Some of the studied actions occurred in both contexts; others were exclusive to the FMC (Fig. 1C and fig. S2).

We recorded neuronal activity from sets of 128 electrodes (*20*) chronically implanted in the precentral gyrus of two rhesus monkeys (Fig. 1D and fig. S3). During 10 recording sessions, we isolated (*20*, *23*) 424 individual neurons (n = 190 in Mk1 and n = 234 in Mk2), which remained steadily isolated along the entire session (fig. S4) and thus demonstrated the feasibility of recording the same cells in both RCs and FMCs. We attributed to the primary motor cortex those sites in which we could evoke movements with shot trains (50 ms) of ICMS of 25 μA or lower (*20*) and that were located within 2 mm of the putative anatomical border between premotor and primary motor cortices (fig. S3) on the basis of previous anatomo-functional studies (*22*). Most of the recorded neurons (84.7%) fell within the premotor territory encompassing areas F4, F5, and F2 (*22*), whereas the remaining were likely located in the primary motor cortex (Fig. 1D).

By comparing the firing features of individual neurons recorded in the two contexts, we found that they showed a slight increase in their mean firing rate (Fig. 1E) and more marked increase in peak firing rate (Fig. 1F) and firing variability (Fig. 1G) in FMCs relative to RCs, which suggests that the freely moving condition is associated with a greater activity and variability of individual neurons relative to classical primate chair conditions.

Encoding and decoding of natural actions from premotor neurons' activity

In everyday contexts, even the simplest grasping action can be performed on different

objects at varying distances from the body with different speed, force, or trajectory and may be carried out in association with different body postures. No natural action is identical to another. This complexity can account for the greater firing variability we observed in FMCs and could make it challenging to study single-neuron correlates of natural actions in freely moving animals. Furthermore, the statistical analysis to test neuronal tuning during trained laboratory tasks in RCs often depends on comparisons with a stable baseline activity, a concept that hardly applies to FMCs. To overcome these problems, we tested single-neuron activity (*20*) under the null hypothesis that if a neuron is not modulated for a certain action, then its activity should remain unchanged during action unfolding.

Among the 424 isolated cells, we found that 343 neurons (80.9%) were significantly modulated ($P < 0.01$) during at least one action in the FMC. In some cases, neurons exhibited a modulation of their discharge selectively for one ($n = 93$) or two ($n = 72$) actions (Fig. 2A), which indicates a certain degree of sparse coding for natural actions by premotor neurons (*18*). Most of the recorded cells ($n = 178$) encoded three or more different actions (Fig. 2B) and thus exhibited dense coding and mixed selectivity.

This prompted us to investigate the possible relationships between neuronal representations of different natural actions in the primate frontal cortex. To do this, we used a variant of the Sørensen-Dice index (*20*) to measure the similarity of neural responses among any possible pair of actions (Fig. 2C). We found a greater similarity between actions (such as drink and bite) involving the same effector (mouth) or the synergistic control of effectors—such as the forelimb and the mouth—typically linked in foraging behaviors. By contrast, there was a larger separation of actions (such as climb or step) that recruited more complex whole-body components. These results lead us to hypothesize that axio-proximal motor components such as those related to neck and back muscles, which are recruited in many different actions, could represent an important yet previously neglected aspect underlying the premotor coding of natural actions.

To verify this hypothesis, we applied long-train (500-ms) ICMS with fixed parameters (*20*) to the cortical sites where neurons modulated for different actions were recorded. By applying ICMS in head-free conditions, we were able to identify even those axio-proximal components—such as flexion or rotation of the head—that could not be observed, or would be profoundly altered, in head-fixed conditions. We stimulated a total of 205 cortical sites in the four hemispheres of the two monkeys, each of which hosted a variable number of neurons (range one to four, mean neurons per site ± SD: 1.52 ± 0.84) modulated during natural actions (Fig. 2D). The ICMS caused a variety of complex motor patterns (movie S2), which replicated those previously described (*24*, *25*). Neurons responding to yawning were more frequently found in cortical sites where ICMS evoked mouth movements ($P = 1.6 \times 10^{-5}$), whereas neurons modulated during steps were significantly associated with hand-related sites ($P = 9 \times 10^{-4}$), and neurons tuned to climb actions were associated with sites controlling head-axial movements ($P < 0.05$), suggesting the existence of a broad relationship between local neuronal tuning properties and ICMS outcome (fig. S5). A sizeable fraction (from 26 to 40%) of the neurons modulated by each action, except yawning, was recorded in cortical sites contributing to head or axial movements, and these results remain qualitatively similar even when only cells modulated exclusively by one or two actions were considered (fig. S6). Previous studies based on head-restrained experiments in macaques support the existence of

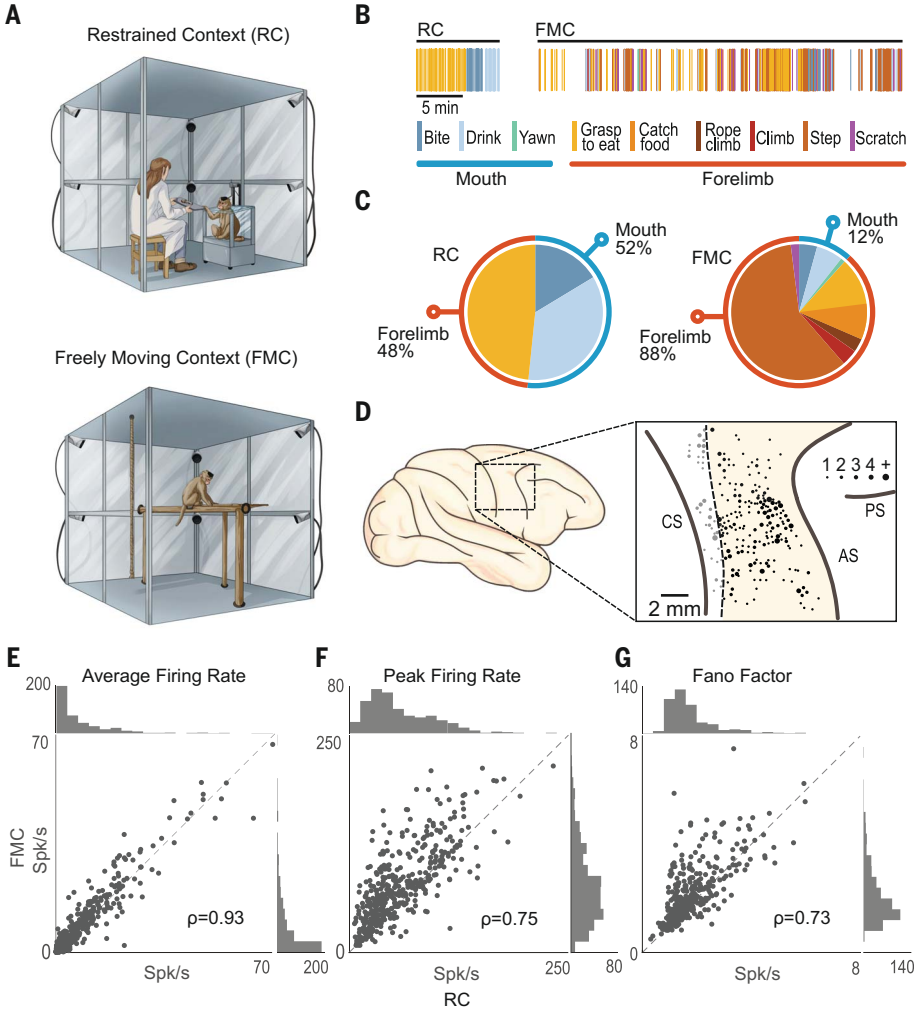

Fig. 1. Stable multielectrode recording of premotor neurons activity in RCs and FMCs. (**A**) Experimental setup within the NER for neurophysiological recordings in head-fixed RCs and in FMCs (fig. S1). (**B**) Raster plot of actions according to the used ethogram (fig. S2) in RCs and FMCs in an example session of Mk1. (**C**) Frequency distribution of the occurrences of all actions ($n = 703$ in RC and 2732 in FMC) performed by both monkeys in all ($n = 10$) sessions. Color codes are as in (B). (**D**) Reconstruction of the anatomical location and frequency distribution of all the recorded neurons ($n = 424$) in a template brain obtained by warping the recorded regions in the right and left hemispheres of Mk1 and Mk2 (fig. S3). The gray and black dots correspond to neurons recorded caudally ($n = 65$) and rostrally ($n = 359$) to the putative border (dashed line) between the primary motor cortex and the premotor cortex (inset, colored sector), respectively. Superimposed landmarks are AS, arcuate sulci; CS, central sulcus; and PS, principal sulcus. (**E**) Average firing rate of individual neurons in RC and FMC (Wilcoxon signed-rank test, $Z = 5.64$, $P = 1.7 \times 10^{-8}$). Spk, spike. (**F**) Absolute peak of firing rate of individual neurons in RCs and FMCs ($Z = 11.83$, $P = 2.6 \times 10^{-32}$). (**G**) Firing variability (Fano factor) of individual neurons in RCs and FMCs ($Z = 10.14$, $P = 3.5 \times 10^{-24}$).

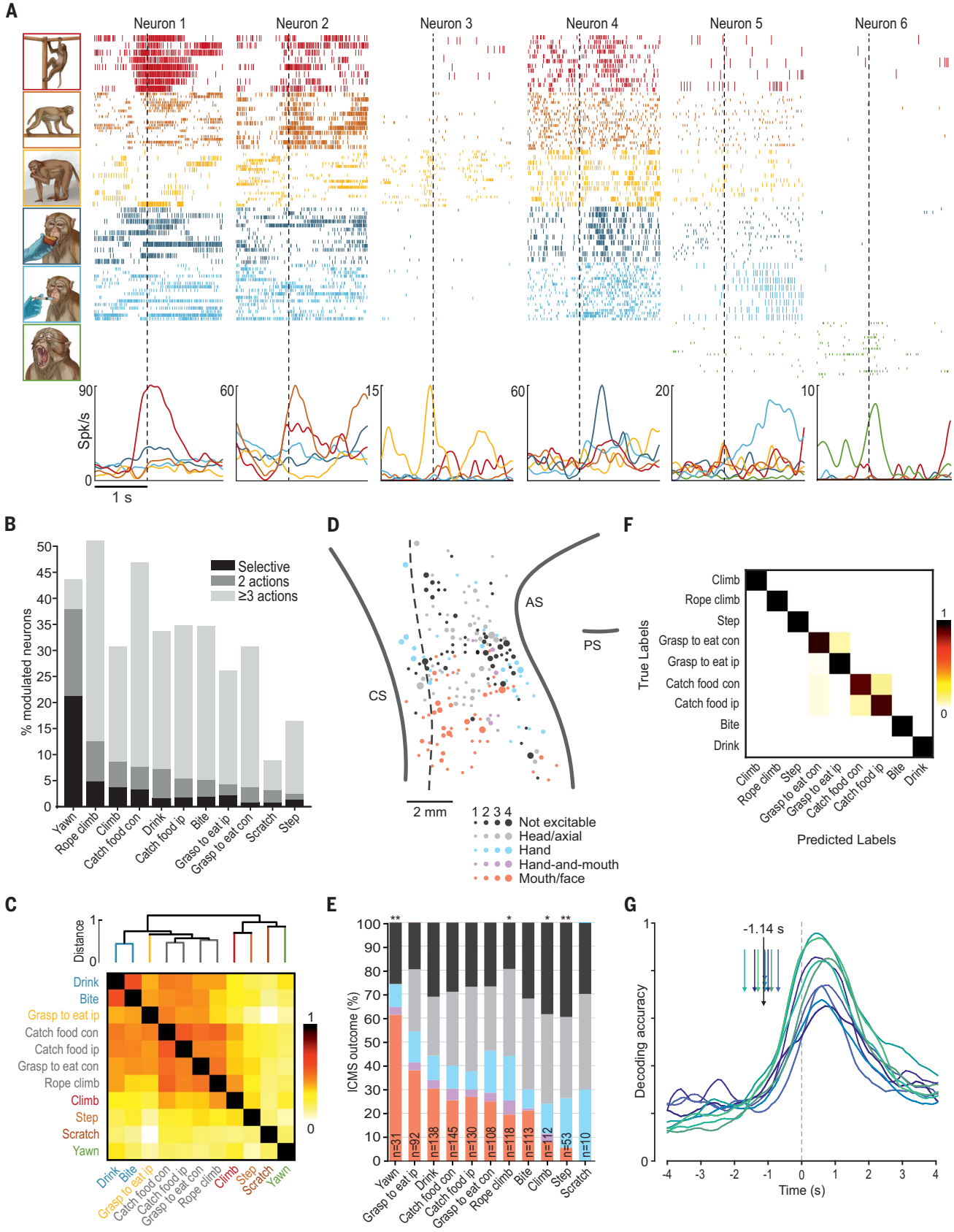

Fig. 2. Single-neuron activity during natural actions. (**A**) Neurons show selective tuning for one or two actions in FMCs. Shown from top to bottom are climb, step, grasp to eat, bite, drink, and yawn. (**B**) Percentage of neurons modulated during one, two, or multiple (≥3) actions in FMCs. con, contralateral; ip, ipsilateral. (**C**) Sørensen-Dice coefficient (SDC) matrix illustrating the degree of overlap between neuronal tuning for each pair of actions. Dendrogram shows the distances

(d = 1 − SDC) between actions grouped according to Ward's criterion (*20*). (**D**) Anatomical distribution of the stimulated cortical sites in terms of type of ICMS-evoked movements (color code) and number of modulated neurons recorded in each site (size of the dot) (conventions are as in Fig. 1D). (**E**) Fraction of all the recorded neurons responsive to each of the tested actions recorded from nonexcitable sites or sites in which ICMS triggered head/axial, hand, hand-and-mouth, or mouth/face movements [color code as in (D)]. *P < 0.05; **P < 0.001. (**F**) Decoding accuracy of an example session of FMC in Mk2 (figs. S7 and S8). (**G**) Classification accuracy as a function of the time lag between the neural activity windows used to train the decoder and behavioral events (movie S3), averaged across actions in each session. Color code of lines and arrows indicate the distinct recording sessions. Colored arrows indicate for each session the time point when the classification accuracy starts to increase according to the elbow method (black arrow indicates the average) (*20*).

cortical motor synergies that underlie the control of forelimb actions (*26*). Our findings suggest the possible extension of this model to include synergistic control of head and axial components in addition to distal components.

Despite this complexity, we could accurately decode distinct natural actions from the readout of single-neuron activity during FMCs by applying a support vector machine (SVM) classifier to the time-varying (500-ms bin) neuronal response profile (*20*) within a 2-s window centered on the alignment point of each action (Fig. 2F and fig. S7). Enriching single-neuron with multiunit activity yielded even higher decoding accuracies in most sessions (fig. S8), regardless of the parameters used (fig. S9). By measuring the decoding accuracy obtained at different time points relative to the target action (*20*), we found that the highest accuracy is obtained in correspondence and slightly after the alignment point of the target action, which likely corresponds to the peak of somatosensory and proprioceptive feedback that characterizes all actions involving contact between a part of the body and an external object (or another body part). The decoding accuracy starts to increase more than 1 s before the reference event (Fig. 2G and movie S3). Thus, the activity of premotor neurons in freely moving monkeys encodes a variety of natural actions and generates anticipatory signals that may be exploited to predict upcoming spontaneous behaviors.

Single-neuron and population dynamics largely differ in restrained and FMCs

On the basis of the current knowledge on the organization of actions in the motor system, the findings from head-fixed recordings should generalize to freely moving subjects. We directly tested this prediction by focusing on those actions that were performed in both contexts—that is, drinking, biting, and grasping food with the contralateral or ipsilateral hand.

For each of the tested actions (Fig. 3A), we found that a small fraction of the neurons modulated during its unfolding exhibited context-invariant discharge (Fig. 3B, example neuron 1), which ranged from 15.9% for drinking to 21.7% for grasping with the contralateral hand, with no difference in this proportion across the studied actions (Fisher's exact test, $P \geq 0.05$ for all pairwise comparisons). Most of the tested cells (Fig. 3A) exhibited radically different activity patterns between the two contexts in terms of temporal profile of discharge, discharge intensity, or both (Fig. 3C, example neuron 2), which indicates that context-specific features influence neuronal responses. One may argue that context-invariant coding is a hallmark feature of premotor (*8*) but not of the primary motor cortex (*27*); by contrast, we could directly verify that the frequency of context-invariant neurons (fig. S10) was the same in the primary motor (14.3%) and premotor (15.0%) regions (χ^2 = 0.06, P = 0.8). Furthermore, one might expect that context-invariant neurons selectively encode a specific action or body posture; yet we found that during FMCs, context-invariant neurons were modulated by a significantly higher number of actions (5.01 ± 2.25) compared with that for context-dependent neurons (2.70 ± 1.83; Mann-Whitney U test, P < 0.001). These findings indicate that single-neuron encoding of actions is strongly context dependent, which suggests that premotor mechanisms of action organization are more flexible than previously hypothesized on the basis of the results of constrained experiments.

Next, we wanted to explore whether and to what extent individual actions during RCs and FMCs retained similar dynamics at the population level. To this purpose, we identified the neural subspace optimal for each action in each context (RC and FMC) and then projected on this subspace the trajectory of the population activity associated with all the other tested actions and contexts (fig. S11). Next, we expressed the similarity in the neural population trajectories as an alignment index (*20*) so that the results of all possible pairwise comparisons could be expressed in a similarity matrix (Fig. 3, D and E). Context-invariant neurons encoded the same actions similarly across context and segregated actions that were performed with the mouth and the contralateral and the ipsilateral hand (Fig. 3D). By contrast, context-dependent neurons encoded more similarly different actions that involved the same effector in the same context (Fig. 3E, branches of the same color) than the same action across the two contexts.

Together, these findings support the idea that premotor representation of actions relies on flexible motor synergies at variable degrees of complexity, which can hardly be captured in head-fixed settings and thereby challenges the possibility of generalizing the findings from RCs to FMCs.

Cross-context neural readout of natural actions

Next, we leveraged our dataset to directly test whether and to what extent the joint contribution of single- and multiunit dynamics can allow us to decode the four actions occurring in both RCs and FMCs by using a SVM classifier (*20*). We found a high decoding accuracy both within RCs and FMCs, as well as in cross-context decoding (Fig. 4A), which indicates the presence of a certain amount of information that is shared across contexts, at least for this set of actions. Cross-context decoding yielded an overall lower classification accuracy compared with the one obtained in both RCs and FMCs. Nonetheless, it remained largely above chance, and in all the recording sessions, the decoding performance was better when the classifier was trained on FMC data and then tested on RC data as compared with the reverse (Fig. 4B).

By leveraging a dimensionality reduction technique, t-distributed stochastic neighbor embedding (*20*), for visualizing neural data in the two contexts, we found that the four actions were generally more dispersed and intertwined in FMCs than in RCs, in which they appeared much more distinct and clustered in smaller areas (movie S4). Therefore, if the SVM hyperplanes are learned in the narrower interclass spaces of FMCs (fig. S12), decoding the same actions in the highly clustered RC space becomes easier, provided that each action occupies a similar region across contexts. Conversely, decoding actions in FMCs by using a model trained in RCs, in which a wider range of hyperplanes would easily allow optimal performances, leads to higher decoding error rates.

These findings indicate that neural activity recorded during FMCs is richer, rather than just noisier, and more generalizable than that recorded in RCs, which supports the relevance and value of investigating the neuronal mechanisms of action planning and control in more naturalistic contexts.

Discussion

We used a stepwise approach to track the same single neurons' activity in both a classical head-restrained laboratory context (RC) and during spontaneous behavior in a FMC. We found notable differences in neurons' firing pattern and response dynamics between the two contexts and showed that a FMC provides a richer and more complex neural characterization of spontaneous actions than a RC.

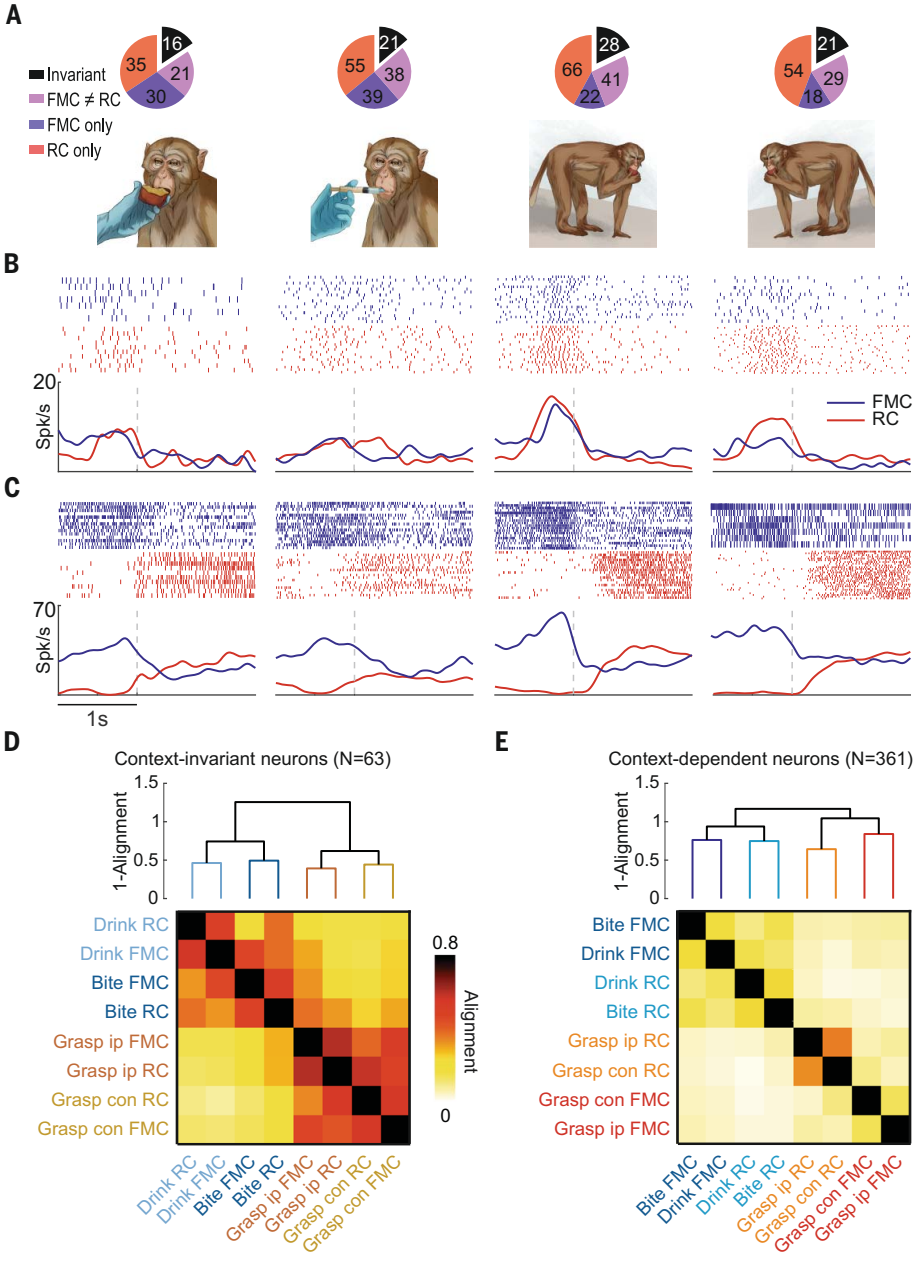

Fig. 3. Single-neuron and population activity during action execution in restrained and FMCs. (**A**) For each action, pie charts show the number of neurons modulated in either a similar or different manner in RC and FMC during each of the four tested actions: biting, drinking, and grasping with the ipsilateral and contralateral hands (with the grasp-to-eat and catch food categories in FMC merged). (**B**) Example neuron exhibiting a similar modulation in the two contexts during the four tested actions. (**C**) Example neuron exhibiting a different modulation in the two contexts during the four tested actions. (**D**) Matrix of alignment indexes between each pair of actions within and across contexts, defined as the fraction of residual variance after projecting the activity of context-invariant neurons associated with one action into the neural subspace of another. Ward's hierarchical clustering method was applied to the symmetrized distance matrix, defined as 1 − alignment, to generate the agglomerative dendrogram and sort the matrix accordingly (*20*). By applying a bootstrap procedure resampling neurons 1000 times, we found that the alignment between the same mouth action across the two contexts is higher than that between different mouth actions within the same context ($P = 0.037$), and a similar trend emerges for ipsilateral and contralateral grasping actions ($P = 0.098$); alignment between actions involving the same effector (such as the hand or the mouth) is significantly higher than that between actions involving different effectors ($P < 1 \times 10^{-15}$), independently from the context. (**E**) Matrix of alignment indexes computed as in (D) for context-dependent neurons. Alignment between different mouth actions in the same context is significantly higher than that between the same mouth action across the two contexts ($P < 1 \times 10^{-15}$), and the same effect emerges for ipsilateral and contralateral grasping actions ($P < 1 \times 10^{-15}$); alignment between actions involving the same effector is significantly higher than that between actions involving different effectors ($P < 1 \times 10^{-15}$), independently from the context. Random resampling of smaller ($n = 63$) sets of context-dependent neurons consistently leads to the same clustering result (>95%), which indicates that differences in clustering results between (D) and (E) could not be accounted for by different numerosity of the neuronal populations.

The prevailing view emphasizes the relevance of dense coding in the frontal cortex, with neuronal population dynamics shared by multiple behaviors (*28*). By contrast, in FMCs, we identified neurons with selectivity for individual natural actions, which suggests the existence of a certain degree of sparse coding of motor plans in the premotor cortex, likewise previously shown for input signals in sensory areas (*18*, *29*). Apparently, this finding confirms and extends to FMCs the classical concept of "vocabulary of motor acts" (*9*) deemed to be the premotor representational format of actions in terms of their immediate goal (*8*, *10*). However, according to this notion, single-neuron tuning for a given action should be context-invariant as much as its goal, whereas we found that only a minority of the tested neurons exhibited consistent modulations across the two contexts. Furthermore, context-invariant neurons encoded a larger variety of spontaneous actions when recorded in FMCs relative to context-dependent cells, which suggests that they may encode motor synergies shared by a variety of spontaneous actions rather than a specific motor goal. In line with this hypothesis, context-dependent neuronal populations consistently showed greater similarity in their dynamics for different actions performed with the same effector in the same context (RC or FMC) rather than for the same action performed in the two contexts. In addition, representational similarity measures applied to FMC data show that natural actions relying on the same effector, such as the forelimb or the mouth, are associated with more similar neural codes, which supports the idea that partially mixed selectivity (*30*) could represent a strategy to facilitate a flexible organization of natural actions. Together, these findings suggest that an interpretation in terms of cortical motor synergies constitutes a more parsimonious and useful framework than the action goal hypothesis to unravel the premotor underpinnings of spontaneous actions.

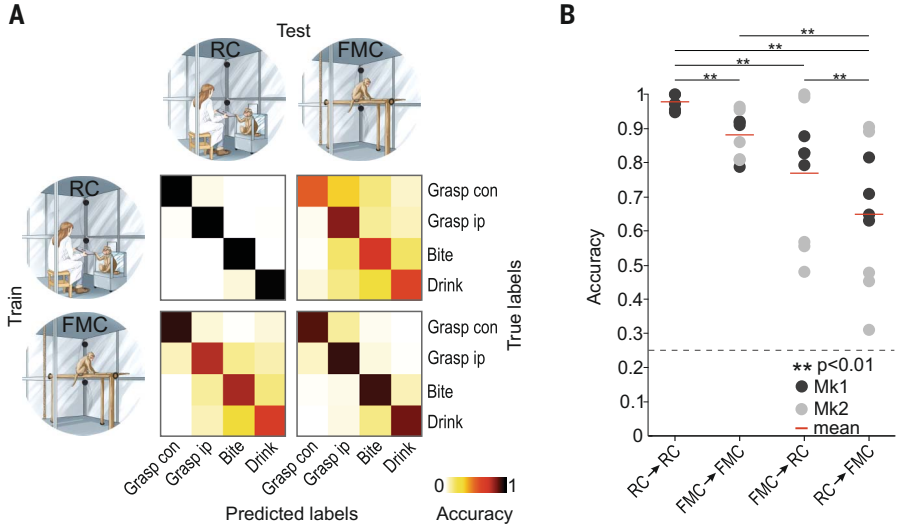

Fig. 4. Cross-context decoding. (**A**) Confusion matrices averaged over sessions and that result from four training-validation set combinations. The first row shows the decoding performances when RC data are used for training the SVM model subsequently tested with RC and FMC data, respectively, and the second row shows the decoding performance when the model is trained with FMC data and then tested with RC and FMC data, respectively. (**B**) Individual decoding accuracies for each session and directionality of cross-context decoding, as indicated with the arrows (from train to test dataset). The horizontal dashed line corresponds to chance, and significance was determined by using the Wilcoxon signed-rank test.

An alternative and view on premotor organization of action derived from constrained ICMS experiments is based on the concept of final body posture coding. By performing ICMS while the monkey was sitting on the primate chair but with its head free, we found a widespread presence of head and axial movements, such as neck extension and flexion or head rotation, either alone or combined with other movements of the mouth, face, or forelimb. This finding suggests that previous reports of trunk movements evoked by ICMS in the premotor cortex (*22*, *31*) might be misattributions owing to head-fixed conditions, which emphasizes the relevance of a combined control of distal body parts and axio-proximal components related to the neck and the head. All neural representations of actions, except for yawning, extensively co-localize with ICMS-evoked head and neck movements, which are shared among a variety of natural actions that require the coordination of head movements with those of other effectors. A model based on static final postures that form an "action map" hardly accounts for the variability of motor and premotor neurons that fire during spontaneous movement (*32*, *33*). Thus, we propose that a model based on a "vocabulary of motor synergies" at various levels of complexity, which encompasses primary motor and premotor cortices, can reconcile the "vocabulary of motor acts" (*34*) and "ethologically relevant action map" (*35*) models to provide a more flexible and useful framework to explain the neural control of goal-directed actions as well as the cortical modulation of motor synergies primarily controlled by subcortical mechanisms, such as those required for yawning or walking.

Despite the variety of actions and their execution modes being considerably greater in FMCs than in RCs, we could accurately decode various actions in both contexts. In this respect, the RC can be seen as an overfitting model in which the limited variability ensured by the behavioral constraints makes it possible to achieve the highest decoding performance, but at the expense of flexibility and generalizability to real-world contexts. Cross-context decoding performs better when generalizing from FMCs to RCs than in the opposite direction, which suggests that freedom of movement adds richness to the neural codes without compromising their reproducibility. By revealing neural strategies for the control of natural actions, our findings could inform the development of more effective neuromodulation and robot-assisted neurorehabilitation approaches (*36*) for restoring sensory-motor functions after neurological disorders.

Together with recent technological and methodological progresses in the study of navigation (*37*, *38*), decision-making (*39*), and social behavior (*40*, *41*) in monkeys, our study contributes to paving the way for the transition from primate neurophysiology to primate neuroethology and advances our understanding of the voluntary control of natural actions in real-world situations and its possible use for translational research.

REFERENCES AND NOTES

1. E. A. Buffalo, J. A. Movshon, R. H. Wurtz, *Proc. Natl. Acad. Sci. U.S.A.* **116**, 26167–26172 (2019).
2. R. P. Dum, P. L. Strick, *Physiol. Behav.* **77**, 677–682 (2002).
3. G. Rizzolatti, G. Luppino, *Neuron* **31**, 889–901 (2001).
4. P. Cisek, J. F. Kalaska, *Annu. Rev. Neurosci.* **33**, 269–298 (2010).
5. M. Graziano, *Annu. Rev. Neurosci.* **29**, 105–134 (2006).
6. J. H. Kaas, I. Stepniewska, *J. Comp. Neurol.* **524**, 595–608 (2016).
7. S. Kakei, D. S. Hoffman, P. L. Strick, *Nat. Neurosci.* **4**, 1020–1025 (2001).
8. M. A. Umiltà *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 2209–2213 (2008).
9. G. Rizzolatti *et al.*, *Exp. Brain Res.* **67**, 220–224 (1987).
10. L. Bonini *et al.*, *J. Neurosci.* **31**, 5876–5886 (2011).
11. G. Rizzolatti, J. F. Kalaska, *Princ. Neur. Sci.* **5**, 865–893 (2013).
12. V. Jovanovic, A. R. Fishbein, L. de la Mothe, K.-F. Lee, C. T. Miller, *Neuron* **110**, 1318–1326.e4 (2022).
13. M. M. Yartsev, N. Ulanovsky, *Science* **340**, 367–372 (2013).
14. J. O'Keefe, J. Dostrovsky, *Brain Res.* **34**, 171–175 (1971).
15. B. Mimica, B. A. Dunn, T. Tombaz, V. P. T. N. C. S. Bojja, J. R. Whitlock, *Science* **362**, 584–589 (2018).
16. T. C. Foster, C. A. Castro, B. L. McNaughton, *Science* **244**, 1580–1582 (1989).
17. C. T. Miller *et al.*, *Curr. Biol.* **32**, R482–R493 (2022).
18. B. A. Olshausen, D. J. Field, *Curr. Opin. Neurobiol.* **14**, 481–487 (2004).
19. P. Cisek, A. M. Green, *Curr. Opin. Neurobiol.* **86**, 102859 (2024).
20. Materials and methods are available as supplementary materials.
21. O. Friard, M. Gamba, *Methods Ecol. Evol.* **7**, 1325–1330 (2016).
22. M. Maranesi *et al.*, *Eur. J. Neurosci.* **36**, 3376–3387 (2012).
23. J. E. Chung *et al.*, *Neuron* **95**, 1381–1394.e6 (2017).
24. M. S. A. Graziano, C. S. R. Taylor, T. Moore, *Neuron* **34**, 841–851 (2002).
25. O. A. Gharbawie, I. Stepniewska, J. H. Kaas, *Cereb. Cortex* **21**, 1981–2002 (2011).
26. S. A. Overduin, A. d'Avella, J. Roh, J. M. Carmena, E. Bizzi, *J. Neurosci.* **35**, 12615–12624 (2015).
27. J. A. Gallego *et al.*, *Nat. Commun.* **9**, 4233 (2018).
28. M. M. Churchland, K. V. Shenoy, *Nat. Rev. Neurosci.* **25**, 213–236 (2024).
29. M. Beyeler, E. L. Rounds, K. D. Carlson, N. Dutt, J. L. Krichmar, *PLOS Comput. Biol.* **15**, e1006908 (2019).
30. C. Y. Zhang *et al.*, *Neuron* **95**, 697–708.e4 (2017).
31. V. Raos, G. Franchi, V. Gallese, L. Fogassi, *J. Neurophysiol.* **89**, 1503–1518 (2003).
32. T. N. Aflalo, M. S. A. Graziano, *J. Neurosci.* **27**, 2760–2780 (2007).
33. T. N. Aflalo, M. S. A. Graziano, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 2909–2914 (2006).
34. G. Rizzolatti *et al.*, *Exp. Brain Res.* **71**, 491–507 (1988).
35. M. S. A. Graziano, C. S. R. Taylor, T. Moore, D. F. Cooke, *Neuron* **36**, 349–362 (2002).
36. M. Coscia *et al.*, *Brain* **142**, 2182–2197 (2019).
37. A. Nourizonoz *et al.*, *Nat. Methods* **17**, 1052–1059 (2020).
38. D. Mao *et al.*, *Neuron* **109**, 3521–3534.e6 (2021).
39. B. Voloh *et al.*, *Cell Rep.* **42**, 113091 (2023).
40. M. Franch *et al.*, *Nature* **627**, 174–181 (2024).
41. C. Testard *et al.*, *Nature* **628**, 381–390 (2024).

ACKNOWLEDGMENTS

We thank F. Mastrandrea for drawings; A. Guida for animal care and technical assistance; F. Leonardi for veterinary assistance and anesthesia; M. Arconte, J. Arcuri, F. Cimmelli, M. Delgrosso, G. Rigamonti, and R. Sini for help in animal handling and data acquisition; and F. Caruana for comments on the manuscript. **Funding:** This work was supported by European Research Council (ERC) StG-2015 grant WIRELESS 678307 to L.B.; ERC CoG-2020 grant EMACTIVE 101002704 to L.B.; ERC PoC-2020 grant FUTURE-NHP 957538 to L.B.); NEXTGENERATIONEU project MNESYS (PE0000006–DN 1553 11.10.2022 to L.B. and S.M.); NEXTGENERATIONEU project BRIEF (IR0000036–Biorobotics Research and Innovation Engineering Facilities DN 103 17.06.2022 to S.M., A.M., and L.B.); NEXTGENERATIONEU project THE (IECS00000017–Tuscany Health Ecosystem DN 1553 11.10.2022 to S.M. and A.M.); the Italian Ministry of University and Research (PRIN 2022 22SP5K99 to M.M.); and the University of Parma (FIL_2023_PROGETTI_B_ALBERTINI to D.A.). **Author contributions:** Conceptualization: L.B., M.M., F.L., M.L., and D.A. Animal preparation and data acquisition: F.L., M.M., M.L., E.F., and L.B. Data analysis and visualization: D.A., F.L., E.H.R.,

M.M., E.F., and L.B. Supervision: L.B., A.M., and S.M. Funding acquisition: L.B., S.M., A.M., M.M., and D.A. Project administration: L.B. Writing – original draft: L.B., F.L., M.M., E.H.R., and D.A. Writing – review and editing: L.B., F.L., M.M., E.H.R., D.A., E.F., M.L., A.M., and S.M. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** The data and code that support the conclusions of this study are deposited in Open Science Framework (https://osf.io/82jfr/). **License information:** Copyright © 2025 the authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original US government works. https://www.science.org/about/science-licenses-journal-article-reuse

SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.adq6510
Materials and Methods
Supplementary Text
Figs S1 to S12
Table S1
References (*42*–*45*)
Movies S1 to S4

Submitted 24 May 2024; resubmitted 22 September 2024
Accepted 8 November 2024
10.1126/science.adq6510