1 Manuscript title

2 A distributed neocortical action map
3 associated with reach-to-grasp

4 Abbreviated title

5 Neocortical dynamics during reach-to-grasp

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34 **Author contributions**

35 EQ conceived the study, developed the experimental setup, performed the
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 37 formed the analysis, produced the figures and wrote the manuscript. AS
 38 wrote the analysis algorithms, performed the analysis; JL participated in the
 39 experiments, participated in the analysis; LS developed the setup; ALAM
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 41 analysis; FSP conceived the study. All authors discussed and iterated on the
 42 analysis and its results, and all authors revised the final manuscript.

43 ABSTRACT

44 Reach-to-Grasp (RtG) is known to be dependent upon neocortical circuits
 45 and extensive research has provided insights into how selected neocortical
 46 areas contribute to control dexterous movements. Surprisingly, little infor-
 47 mation is available on the global neocortical computations underlying RtG
 48 in the mouse. Here, we characterized, employing fluorescence wide-field cal-
 49 cium imaging, the neocortex-wide dynamics from mice engaging in a RtG
 50 task. We demonstrate that, beyond canonical motor regions, several areas,
 51 such as the visual and the retrosplenial cortices, also increase their activ-
 52 ity levels during successful RtGs. Intriguingly, homologous regions across
 53 the ipsilateral hemisphere are also involved. Functional connectivity among
 54 areas increases transiently from rest to planning, and decreases during move-
 55 ment. Two anti-correlated neocortical networks emerged during movement.
 56 At variance, neural activity levels scale linearly with kinematics measures of
 57 successful RtGs in secondary motor areas. Our findings establish the coex-
 58 istence of distributed and localized neocortical dynamics for efficient control
 59 of complex movements.

60 SIGNIFICANCE STATEMENT

61 In mammals, including humans, the cerebral cortex is known to be critical for
 62 the correct execution of dexterous movements. Despite the importance of the
 63 mouse for elucidating the neural circuitry for motor control, its neocortex-
 64 wide dynamics during RtG are largely unexplored. We used in-vivo fluores-
 65 cence microscopy to characterize the neural activity across the neocortex as
 66 mice performed a reach-to-grasp task. We show that for such complex move-
 67 ments, a large network of neocortical areas gets involved, while movement
 68 kinematics correlates with neural activity in secondary motor areas. These
 69 findings indicate the coexistence, at the mesoscale level, of distributed and
 70 localized neocortical dynamics for the execution of fine movements. This
 71 study offers a novel view on the neocortical correlates of motor control, with
 72 potential implications for neural repair.

73 INTRODUCTION

74 Deciphering how the CNS produces goal-directed movements, such as the
 75 reach-to-grasp (RtG), is crucial for expanding our understanding on the neu-
 76 ral computations underlying motor ability and more generally, for animal
 77 cognition (Bayne et al., 2019). RtG is known to be dependent upon neocor-
 78 tical circuits and intensive research has provided detailed insight into how
 79 neural activity from motor areas of the neocortex contribute to the control
 80 of movements, (Fritsch and Hitzig, 1870; Evarts, 1968; Georgopoulos et al.,
 81 1986; Graziano et al., 2002; Churchland et al., 2012). In the mouse, optical
 82 methods, which provide mean for minimal invasive neural recordings, are
 83 gradually replacing electrophysiological approaches to investigate the neural
 84 bases of motor control, however most investigations to date have focused
 85 on segregated neocortical areas (Guo et al., 2015; Estebanez et al., 2017).
 86 Nevertheless, how neural systems give rise to behavior cannot be fully com-
 87 prehended from segregated analysis of its components. Indeed, parallel to the
 88 patterns of neural activity at the local level, information processing occur-
 89 ring at the cortico-cortical level is necessary for complex behavior (Peters et
 90 al., 2014; Allen et al., 2017, Battaglia-Mayer and Caminiti, 2019). However,
 91 despite the growing adoption of this organism for elucidating the neural un-
 92 derpinnings of motor control (Ölveczky, 2011), the neocortex-wide dynamics
 93 during RtG remain largely unexplored. Here, we inquired about the topo-
 94 graphic organization of neural activity during movement, that is, if during
 95 RtG specific, large-scale communication schemes emerge across the neocorti-

cal mantle. Movement kinematics are associated with neural activity in the contralateral motor areas, and information about such activity can also be employed to predict several features of the executed movement (Prsa et al., 2017, Li et al., 2019). While growing knowledge reported ipsilateral activity during motor behavior, information on the relationship between RtG kinematics and neural activity across the neocortex is still scanty. Filling this gap could provide an important step forward for the understanding of neural mechanics underlying RtG behavior. To tackle these questions, we developed a novel setup and performed a combined analysis of RtG and mesoscale cortical activity from head-fixed mice. The present characterization of the neural activity patterns provide, to our best knowledge, the first evidences indicating that neocortex-wide activity is a prominent neural signature of RtG movements.

MATERIALS AND METHODS

Transgenic mice

All experimental procedures were authorized by the Italian Ministry of Health (Authorization Nr. 127-2018-PR). A total of 6 transgenic mice (C57BL/6J-Tg(Thy1-GCaMP6f)GP5.17Dkim/J, stock nr. 025393, herein referred to as GCaMP6f, Chen et al., 2013) aged between 3-9 months were used in this study. Animals were housed on a 12-hr light/dark cycle with ad libitum water. Mice undergoing behavioral training were food restricted to 80-90% of original body weight by limiting food intake to 2-3 g/day. Animals were

118 monitored and weighted daily and food ratios was increased if necessary.
119 Veterinary staff monitored animals twice a week.

120 *Surgery*

121 The day before surgery, mice were give a subcutaneous injection of en-
122 rofloxacin (10 mg/kg) and each day thereafter for 3 days, to prevent in-
123 fections. The day of surgery, animals were anesthetized with isofluorane
124 (3% induction, 1.5% maintenance) and placed on a stereotaxic apparatus
125 (Kopf Instruments; Tujunga, CA, USA). Absence of tail reflex and toe-pinch
126 reflex was tested to confirm that the mouse was adequately anesthetized.
127 Throughout the surgery, temperature was kept constant at 37 C by means
128 of an electric heating pad controlled by a rectal temperature sensor (Stoelt-
129 ing; Wood Dale, IL, USA). To prevent cornea dehydration ophthalmic gel
130 was applied over the eyes. The scalp was first scrubbed with ethanol and
131 betadine, then a depilatory cream was applied to remove hairs, then a few
132 drops of topic agent (Lidocaine Hydrochloride 2%) were applied as analgesic
133 measure. A circular piece of scalp was removed and the anatomical points
134 of reference (bregma and lambda) were stained with a permanent marker.
135 Two semicircular coverslip glasses, one per hemisphere, were glued on the
136 skull above the cortex using a transparent dental cement. A custom-build
137 head post was placed ca. 0.5 mm posterior to lambda. At the end of the
138 surgery, subcutaneous injections of analgesic and anti-inflammatory (carpro-
139 fen, 5mg/kg) drugs were given to facilitate recovery. To this end, 200 ml
140 lactated ringer of the 0.9% saline was given to each mouse at the end of

141 surgery. Mice were allowed to recover at least one week following surgery.
142 During this period animals were given ad libitum food and water.

143 *Behavioral apparatus*

144 The experimental apparatus (Fig. 1A) comprised a stage (size 300 x 300 x 20
145 mm, Thorlabs, Inc.) and walls to form an enclosure and secured above a lab
146 jack (MLJ050/M, Thorlabs, Inc.) and was inspired by the work of Guo and
147 collaborators (Guo et al., 2015). The enclosure is equipped with a base for
148 the animal, a perch, a speaker to deliver the auditory cue, and a turn-table
149 attached to a servo-motor equipped with a contactless magnetic rotary en-
150 coder to ensure consistent pellet positioning (MX-28AT) and controlled by a
151 controller board (ArbotiX-M Robocontroller, Troussier Robotics). The servo
152 is controlled via an ad-hoc program. Chocolate pellets (10 mg, 5TUL Puri-
153 fied 10mg pellets catalog id. 1811529, TestDiet) were employed to induce the
154 reach-to-grasp movements, serving thus as stimulus. Interstimulus interval
155 (ISI) was 6 to 20 seconds. The enclosure was placed within a custom-made
156 a sensory-isolated enclosed imaging recording chamber that is kept dark and
157 has been insulated with acoustic foam to further reduce ambient sounds (RS
158 components and woods). The behavioral data were collected at 200 frames/s
159 via a high-speed camera (CM3-U3-13YC-CS, Chameleon3, FLIR) with 2.8-
160 8mm varifocal objective lenses (LENS-30F2-V80CS, Fujinon), placed to the
161 left of the head-fixed animal. A 470 nm LED illuminating the stage was used
162 as light source to create a high-contrast image for processing and quantifica-
163 tion. Movies were recorded using the FlyCapture software (FLIR).

164 *Wide-field microscopy*

165 Imaging was performed through the intact skull using a custom-made mi-
 166 croscope. The microscope consisted of back-to-back 50 mm f/1.2 camera
 167 lenses (Nikon), separated by a FF495-Di03-50.8-D dichroic mirror (Semrock),
 168 mounted in a 60 mm cube (Thorlabs). To excite the GCaMP6f indicator, a
 169 470 nm light source (LED, M470L3 Thorlabs, NJ, USA) was deflected by a
 170 dichroic filter (DC FF 495-DI02 Semrock, NY, USA) on the objective (TL2X-
 171 SAP 2X Super Apochromatic Microscope Objective, 0.1 NA, 56.3 mm WD).
 172 The fluorescence signal was selected by a band pass filter (525/50 Semrock,
 173 NY, USA) and collected by a CMOS camera (ORCA-Flash 4.0 V2, Hama-
 174 matsu). Images were acquired at 25 Hz, with a resolution of 512×512 pixels
 175 with the field of view about 18×18 mm (depth 16-bit) via the HCLImage
 176 Live software (Hamamatsu). The microscope thus allows a FOV embracing
 177 the entire dorsal prospect of the mouse neocortex. To reduce unwanted light
 178 scattering on the mouse eyes (which could serve as visual stimuli, inducing
 179 unwanted neural activity), an iris (ID15/M - Mounted Standard Iris, 15 mm
 180 Max Aperture, TR75/M Post, Thorlabs Inc.) was placed 1-2 mm above the
 181 mouse skull. Imaging sessions were performed for each behavioral training
 182 session. The synchronization of the components of the experimental setup
 183 occurred via a common hardware trigger signal at the start of each block (see
 184 Experimental design for the definition).

185 *Experimental design*

186 Following recovery from surgery for at least one week, mice were habituated
187 to the experimental setup for 4 or more days (30 min a day/mouse). Then,
188 mice were gently placed under the microscope objective and fixed (Fig. 1B).
189 Each session contained at least 5 blocks, and each block lasted between 65
190 seconds and 6 minutes, 50 seconds . Here, we analyzed the first 5 days
191 starting from when the mouse performed the first successful RtG. To en-
192 sure consistency, only trajectories from successful RtG, defined as forelimb
193 movements that ended with the pellet eaten, were analyzed.

194 *Image processing and data analysis*

195 For the movement data, image sequences were loaded onto FIJI (ImageJ)
196 and the metacarpus position was tracked using the manual tracking plugin,
197 in order to obtain the xy coordinates of the successful RtGs. Considering
198 the central aim of the goal-directed task, our investigation was centered on
199 successful RTGs, i.e., those forelimb movements that ended with the pellet
200 being eaten by the mouse. The timing of the movement onset (MoveON)
201 was defined as the frame for which the operator detected a variation in the
202 metacarpus position. The metacarpi coordinates were then employed to re-
203 construct the movement trajectories and compute the movement kinematics.
204 Here we present the results on movement distance (i.e., arc length), move-
205 ment duration (in seconds) and the number of movement speed peaks, the
206 latter used as a measure of movement smoothness (Lai et al., 2015).

207 For the neural data, image stacks for each animal collected from different
208 sessions were registered manually using custom made software, by taking

into account the bregma and lambda position. To dissect the contribution of each cortical area for RtG, the processed stacks we also registered the cortex to the surface of the Allen Institute Mouse Brain Atlas (www.brain-map.org) projected to our plane of imaging. We further applied a mask to exclude the medial-most areas because they lay over the superior sagittal sinus and therefore the neuronal signal in this region could be more easily affected by hemodynamic artifacts. Areas laying on the most lateral parts of the mouse cortex were also excluded. Also, areas smaller than 100px² were also excluded. This parcellation of the neocortex created 17 areas for each hemisphere, plus three formed by the left, the right and both hemispheres, for a total of 37 areas, whose abbreviations and extended names are: CTX, neocortex; MOs, Secondary motor area; RFA, Rostral Forelimb Area; MOp, Primary motor area; CFA, Caudal Forelimb Area; SSp-bfd, Primary somatosensory area- barrel field; SSp-ll, Primary somatosensory area- lower limb; SSp-n, Primary somatosensory area-nose; SSp-tr, Primary somatosensory area- trunk; SSp-ul, Primary somatosensory area- upper limb; SSp-un, Primary somatosensory area- unassigned; RSPagl, Retrosplenial area- lateral agranular part; RSPd, Retrosplenial area- dorsal part; VISrl, Rostrolateral visual area; VISa, Anterior area; VISam, Anteromedial visual area; VISp, Primary visual area; VISpm, posteromedial visual area. Throughout the text and figures, we added the suffix L and R to term cortical areas of the left (ipsilateral with respect to the paw executing the movement) or right (contralateral with respect to the paw executing the movement), respectively

(e.g., MOs_L, MOs_R).

For each block, the image stacks were processed to obtain the estimates of $\Delta F/F_0$. Briefly, the image stacks were processed considering the equation $\Delta F/F_0 = (F - F_0)/F_0$, where F defines the value of the fluorescence signal in a given moment, F_0 defines mean fluorescence over each block. Cortical areas were considered associated with movement if their activity during any moment of the time window crossed a threshold defined as the mean+1.5 times the standard deviation of the neural signal occurring during the baseline (-2 to -1 sec with respect to MoveON). The highest value reached in any moment of the 4 second time window was defined as the PeakMax (Fig. 2B). Also, in order to statistically determine if the transaction between the behavioral phases is associated to different neural activity levels, we employed a two-way ANOVA followed, if appropriate, by Tukeys HSD Post-Hoc tests. To evaluate the functional connectivity (FC) between the areas during RtGs, cross-correlations between pairs of areas for each behavioral phase were computed. The fluorescent time series were chunked according to the behavioral phase of interest and are presented as cross-correlation matrices (CCM). We investigated the network membership of these regions by using a hierarchical clustering algorithm (HCA) that was based on the Wards linkage method (Ward, 1963), which minimizes the variant between the clusters. The proximity was then interpreted as an indirect measure of FC and represented graphically by means of dendrograms. All data are reported as *mean* $\pm$ *SD* if not stated otherwise. Sample size and appropriate statistical analyses are

255 specified in each figure legend. Statistical significance was defined as $\alpha < 0.05$
 256 if not stated otherwise. Multiple comparisons were corrected for by Tukey
 257 HSD or Bonferroni correction. No statistical methods were employed to
 258 pre-determine sample size. All statistical analyses and related figures were
 259 created using either Python or R, and were assembled in Inkscape.

260 RESULTS

261 *Wide-field calcium imaging during RtG*

262 We conceived that for a mouse to perform complex, goal-directed movements
 263 such as RtG a specific neural network within the neocortex could be recruited.
 264 To gain information about such dynamics, our setup integrates a large field
 265 of view microscope to simultaneously image the entirety of the neocortical
 266 surface and a behavioral apparatus for inducing, delivering and videotaping
 267 the RtGs (Fig. 1A). Mice were trained to perform the task while head-
 268 fixed under the microscope (Fig. 1B). Once trained, animals executed RtG
 269 movements to eat chocolate pellets that were placed on a rotating table placed
 270 in front of them (Fig. 1C, left). The time-aligned image stacks obtained from
 271 the camera and from the microscope allowed the monitoring of neural activity
 272 across functional areas of the neocortex, throughout the behavioral task (Fig.
 273 1C and D).

Figure 1: Mesoscale imaging of neocortical dynamics during RtGs

A. Experimental setup. Schematics of the experimental setup, which comprises a custom-made widefield microscope to acquire images, 512×512 pixels, from the intact skull of GCaMP6f transgenic mice with a sampling rate of 25 Hz. Behavioral data were obtained via a high-speed (200 fps) camera placed on the left side of the head-fixed animal. Chocolate pellets were employed to induce voluntary RtG movements. A turn-table attached to a servo-motor was used for pellet delivery. All components of the experimental setup were synchronized by using a common TTL trigger. **B.** Experimental timeline. Mice underwent a surgery to expose the skull and head-bar implantation. Following recovery from surgery (1 week), mice were food-restricted to 80-90% of their baseline body weight and habituated to the experimental setup for at least 4 days (30 min a day/mouse). After habituation, mice were gently placed under the microscope and head-fixed below the objective. Training consisted of daily sessions (30-45 min/session), and lasted at least 3-4 weeks (5 sessions/week). Each session consisted of several (at least 3) blocks, during which tones (square wave at 440 Hz) preceded by 200ms the rotation of the turntable. Interstimulus interval (ISI) was between 6 to 20 seconds.

[continues in the next page]

Figure 1: caption, continued from previous page

C. Mesoscale cortical activity during a single RtG movement. Left, image sequence of a mouse performing a successful RtG. Right, image sequence of cortical activity during the same peri-movement epoch (between -0.2 sec and +0.56 sec relative to the movement onset, sampling time 40 msec). Color bar (viridis) represents the range of $\Delta F/F_0$ (%). In this case, the movement lasts ca. 560 ms. Black dot = bregma. Activity is higher in the medial part of the cortex during early phases of movement. Following grasping phase of the movement, the activity is more localized on the contralateral motor areas. D. Neural activity in right primary motor cortex is associated to repeated RtGs. Left, image showing the typical field of view obtained during recordings. The Allen Mouse Brain Atlas mask was applied on the fluorescence image stacks to parcellate the signals according to the cortical areas of interest. Right, graph depicting the fluorescent and movement signals. F/F_0 (%) extracted from the right primary motor cortex (gold-colored trace). Onsets of RtGs movements are illustrated by the blue rasterplot. Increase activity in the selected cortical area is observed for each RtG attempt. The asterisks denote successful RtGs.

274 *Neural activity increases among several areas spanning both*
 275 *hemispheres during reach-to-grasp behavior*

276 Single-pixel analysis of cortex-wide activity indicate that on average, for all
 277 movements and for all subjects, the neural activity increases throughout the
 278 whole cortical mantle between -2 to -1.5 and between -0.5 to -0.04 seconds
 279 with respect to MoveON (Fig. 2A). According to this observation, we an-
 280 alyzed the different contributions of cortical activation during three tempo-
 281 ral lapses, namely rest (2 to 1.5 sec before MoveON), plan (0.5 to 0.04 sec
 282 before MoveON), and move (0 to 0.5 sec after MoveON, Fig. 2B-C), col-
 283 lectively termed behavioral states or behavioral phases (Scherberger et al.,
 284 2005; Hwang and Andersen, 2009; Schieman et al., 2015). During movement
 285 execution, bilateral activation in more rostral areas becomes apparent, while
 286 during the latter phase of movement execution a more pronounced increase
 287 in neural activity levels emerges in the the contralateral hemisphere (Fig.
 288 2A).

Figure 2: Single-pixel analysis of neocortex-wide activity during RtGs

A. Perievent (-2+2 seconds with respect to MoveON) image sequence. Images display the average signal obtained from aligned stacks of all subjects, for all peri-movement epochs (n=113 movements, 6 mice). On average, activity increases before movement onset in medial regions of the cortex and spreads medio-laterally thereafter. This increase involves areas of both hemispheres. During a latter phase of movement execution we observed a partial bias toward the contralateral hemisphere, possibly associated with the grasping subcomponent of the movement. Black dot = bregma. Scale bar = 2 mm. Color bar (viridis) represents the range of $\Delta F/F_0$ (%). B. Top, Averaged activity across the behavioral phases rest (-2,-1.5s relative to MoveON), plan (-0.5,-0.04s), and move (0,0.5s). Scale bar = 2 mm. Bottom, cartoon of calcium transient. The vertical lines in the graph depict the division of the investigated behavioral phases. Red dot = PeakMax

289 To characterize the contribution of functional areas across the neocortex dur-
 290 ing RtG, we parceled the acquired image stacks according to the Allen In-
 291 stitute Mouse Brain Atlas (Fig. 3A, left). The resulting parcellation allowed
 292 to extract the calcium signal for each neocortical area. The increase in the
 293 levels of neural activity encompassing whole neocortical mantle is observed
 294 also on each hemisphere separately (Fig. 3B). Several areas displayed a vigor-
 295 ous increase in neural activity levels with movement execution, including the
 296 motor areas and the retrosplenial cortices of both hemispheres, while other
 297 areas, such as the barrel field (SSp-bfd), displayed a more modest increase in
 298 activity (Fig. 3C-D). Overall, the activity levels across homologous areas of

299 both hemispheres, including motor, somatosensory, retrosplenial, and visual
300 areas appeared comparable.

Figure 3: Widespread increase of neural activity during RtGs

A. Illustration of the areas from which the traces displayed in panel B were obtained, which include also the bilateral Rostral Forelimb Area and the Caudal Forelimb Area. Areas and subsequent traces are color-coded accordingly to the left (blue), and right (red) hemisphere. Neural activity traces refer to the peri-movements epochs ($n=113$ movements, 6 mice). B. $\Delta F/F_0$ (%) activity across blocks (shaded lines) and averaged value (thicker, black line) for the whole cortex, the left and the right hemisphere, for a time window of -2,+2 sec around the movement onset (MoveON). C. Single and mean (thicker, black lines) traces of neural activity for a selection of 8 areas across the two neocortical hemispheres. The MOS, RFA, MOp, CFA and RSPd, and to a lesser extent the SSp-bfd and the VISp, display increased activity levels during RtGs.

301 *Calcium signals from neocortical areas*

302 *increases before movement onset*

303 To quantify our observations, we compared the temporal profile of the parcel-
304 lated fluorescent calcium signal across the perievent time window. All areas
305 emerged as associated to movement, with the rostral-most areas displaying
306 the highest PeakMax and other areas spread across both hemispheres, form-
307 ing a distributed cortical network associated with reach-to-grasp movements
308 (Fig. 4A). To determine the temporal involvement of cortical areas during
309 the task we computed the latency necessary to reach the 50% and the 100% of
310 the PeakMax (latency to T-halfMax and latency to PeakMax, respectively)
311 in each of the responsive areas (namely the ones that showed activity levels
312 higher than threshold, as described in the materials and methods, 37 cortical
313 areas in total).

314 We observed that, across the responsive areas, the T-halfMax was reached be-
315 fore movement onset, which indicates that the increases in the amplitude lev-
316 els of the fluorescent signal anticipate the movement onset (Fig. 4B, left), at
317 variance with the latency to reach PeakMax, occurring mostly after the move-
318 ment onset (Fig. 4C, right). While the T-halfMax was comparable across
319 areas, the latency to reach PeakMax indicated a higher variability, with so-
320 matosensory areas and the contralateral CFA displaying a delay to reach the
321 PeakMax compared to other areas. ANOVA revealed that the levels of neural
322 activity were significantly affected by the cortical area [$F(33,11424)=19.214$,
323 $p\leq 0.0001$], by the behavioral [$F(2,11424)=2920.965$, $p\leq 0.0001$], and by the

324 interaction of these two factors [$F(66,11424)=6.382, p \leq 0.0001$].

325 Tukey's HSD Post-Hoc results indicate statistically significant increases from

326 phase rest to movement for several distributed movement-associated areas,

327 including the MOs, the RFA, the MOp, the CFA, the RSPd, the somatosen-

328 sory primary cortex upper limb (SSp-ul), and the visual areas. Mean activity

329 levels were found to be already different when comparing the rest vs the plan-

330 ning phase, e.g., for the MOs, the RFA, the MOp, the CFA, the RSPd and

331 the SSp-ul, but were not found to be different for the comparisons involving

332 the primary visual areas (for all pairwise comparisons, see supplementary

333 table 1). These findings suggest that a global network of areas is activated

334 during movement execution, with the majority of them increasing their levels

335 of activity before movement onset.

336 *Motor planning is associated with a global increase in FC*

337 Considering the increase in neural activity levels across the areas from rest to

338 movement, we next asked whether such variation was associated with modu-

339 lation of functional connectivity (FC) parameters across areas, and whether

340 FC was modulated throughout behavioural phases. The HCA revealed that,

341 in the context of the planning phase, all functional areas tend to form a single

342 functional aggregate highlighted by the fact that they meet lower on the den-

343 drogram compared to the rest phase (Fig. 5A-B, top row). This tendency is

344 illustrated by the CCM (Fig. 5B, bottom row), indicating an increase in the

345 correlation coefficients in the transition from the rest phase to the plan phase

346 (0.5-0.04s with respect to MoveON, Fig. 5B, left and center, respectively).

Figure 4: Bilateral and state-associated neural dynamics emerge during RtG

A. Color plots showing the mean activity for all neocortical areas, separated for each hemisphere (ipsi = left, ipsilateral hemisphere, on the left; contra = right, contralateral hemisphere, on the right side). Several homologous areas across the hemispheres exhibit increased activity levels. Colorbar reflects the levels of F/F_0 (%) activity. B. Color raster plots showing the mean, normalized activity for the movement-associated areas. Left, the areas in the plot are ranked by latency to reach T-half max%, in ascending order (areas requiring less time to reach the T-half max% are placed higher in the plot). The black squares indicate the latency to T-half max% for each area. Right, the areas in the plot are ranked by latency to reach the PeakMax, in ascending order (areas requiring less time to reach their PeakMax are placed higher in the plot). The black squares indicate the latency to T-half max% for each area. D. Emergence of a state-associated increase of activity levels across cortical areas. Notched boxplots (upper and lower whiskers refer to maximal and minimal value, respectively, excluding outliers) indicate the activity levels for all movement-responsive areas, ranked based on PeakMax, in a descending order from left to right. Left, movement-responsive areas within ipsilateral hemisphere. Bottom, movement-responsive areas within contralateral hemisphere.

347 Upon movement execution, the FC transitions to the opposite direction, i.e.,
348 there is a decrease in the correlation coefficients (Fig. 5C, right).

349 ***Two anticorrelated networks emerge during movement execution***

350 We next sought to determine whether such scheme of communication is main-
351 tained in a context of single-pixel analysis. To this end, the contralateral MOs
352 was used as seed and the cross-correlation of every pixel with each individual
353 area was computed (Fig. 5A-C, bottom row). The transition from resting to
354 planning was associated with a robust increase in the cross-correlation (Fig.
355 5A-B, bottom row). Upon movement execution, two anticorrelated networks
356 emerged for all seeds (Fig. 5A-B, bottom row). The first network contained
357 areas associated with higher-level transformation, including all subdivisions
358 of the retrosplenial cortex, the secondary motor areas of both hemispheres
359 (MOs and RFA) as well as the visual areas (which could be regarded as the
360 sensorimotor transformation network). The second network includes almost
361 exclusively subdivisions of the primary sensory cortex, the primary motor
362 cortices of both hemispheres (and could viewed as the executive network).
363 Our approach provides evidence for the existence of segregated functional
364 networks emerging during motor execution. Overall, these FC results suggest
365 that the cortico-cortical communication scheme during goal-directed forelimb
366 movements can be decomposed into two parts: the earlier phase, with high
367 increase in cross-correlation, and a latter, where two anti-correlated networks
368 emerge.

Figure 5: Motor planning is associated with transient increases in FC

The functional connectivity (FC) was analyzed by means of cross-correlation matrices (CCM), for each of the three behavioral phases and are shown in columns from left to right. Cross-correlation coefficient in the matrices are shown from +1 (in blue) to -1 (in red). The order of appearance of each cortical area in the CCM is based upon area position along the dendrogram. A, B. Strong increase of FC during planning with respect to rest. This increase is transient, as during the movement phase there is an abrupt shift towards rearrangement of the functional connectivity, shown by high anti-correlations between selected areas. C. During the RtG execution, some areas maintain highly positive cross-correlation values. A marked anticorrelation arises between the associative and the executive clusters during movement. In panels A-C Seed-pixel analysis (SCA) across each behavioral phase is . The ipsilateral MOs (B), MOp (C), and RSPd (D) were used as seeds, respectively. SCA indicate that from rest to plan vs the activity is highly correlated across the neocortex. This analysis also highlights that during movement execution (move) two anticorrelated networks emerge across both hemispheres. The anticorrelated network overlaps, at least partially, with the canonical coordinates of CFA. Correlation coefficients (CC, -1 denote anticorrelation, +1 denote correlation) is color-coded (jet colormap). Black dots = bregma scalebar = 1mm.

Neural activity levels in the MOs and RFA scale linearly with movement kinematics

Considering the increase in activity observed across the neocortical areas, we sought to determine the extend of association between neural activity and movement parameters. To this end we first reconstructed the spatial trajectories for the successful RtGs and computed the movement metrics (Fig. 6A-C). The movements distance was $52.8 \pm 18.0\text{mm}$ (Fig. 6D), the movements lasted 0.7 ± 0.2 seconds (Fig. 6E), while the number of speed peaks was 5.4 ± 2.7 (Fig. 6F).

Figure 6: Kinematic trajectories of successful RTGs

A. Example of RtG movement tracked for reconstruction and kinematic analysis.

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Figure 6: caption, continued from previous page

A. In this stereotypical reach-to-grasp sequence, the yellow line in each image represent the path covered by the metacarpus. B. Reconstructed reach-to-grasp trajectories of left-paw movements for each animal. Trajectories of successful movements are shown, separated for each animal. Scalebar = 10 mm for both directions. C. Speed profile reconstruction from a successful RtG movement. Speed peaks exceeding the mean speed (horizontal blue line) are used to compute the movement smoothness (red triangles). C. Distribution of the movement distance. Histogram (bin size = 10) showing the absolute frequency distribution for the distances (arc length) covered by the paw to reach the chocolate pellet and eat it. The vertical line illustrate the median value (47.47 mm), the shaded curve indicate the density distribution, lines of the rug plots (bottom) illustrate the raw distribution for each movement. D. Distribution of the movement duration. Histogram (bin size = 10) showing the absolute frequency distribution for the movement durations. The vertical line illustrate the median value (0.61 s), the shaded curve indicate the density distribution, lines of the rug plots (bottom) illustrate the raw distribution for each movement. D. Distribution of the number of speed peaks. Histogram (bin size = 10) showing the absolute frequency distribution for the speed peaks. The vertical line illustrate the median value (5), the shaded curve indicate the density distribution, lines of the rug plots (bottom) illustrate the raw distribution for each movement (note: the speed peaks being computed as natural numbers, overlap frequently in the rug plot).

378 Since during movement execution we observed the emergence of differentiated
379 activation among areas, we asked to what extent each area contributed to
380 specif kinematic parameter of RtGs movement.

381 To this end, we performed correlation analysis between PeakMax in selected
382 areas of the neocortex and the extracted parameters of movement kinematics:
383 movement distance, movement durations and movement smoothness (Fig.
384 7). A moderate association between the distance covered and the Peak Max
385 in the MOs of both hemispheres (ipsi: $r=0.42$; $p=4.5e-06$, contra: $r=0.42$;
386 $p=4.2e-06$) as well as the RFA (ipsi: $r=0.44$; $p=1.2e-06$, contra: $r=0.37$;
387 $p=4.8e-05$) was found. Interestingly, in the MOp or the CFA the results in-
388 dicate that statistical significance was not reached, not for MOp (ipsi: $r=0.24$;
389 $p=0.011$, contra: $r=0.13$; $p=0.16$) nor for CFA (ipsi: $r=0.23$; $p=0.013$, contra:
390 $r=0.15$; $p=0.12$), suggesting that in these areas the PeakMax may not be as-
391 sociated with movement distance. No evidence for linear relationship among
392 the same parameter was found in the RSPd and in the VISpm (Fig. 7A).
393 The same pattern was observed when considering the relationship between

394 PeakMax and movement duration. Indeed, the MOs (ipsi: $r=0.4$; $p=1.5e-06$,
395 contra: $r=0.48$; $p=7.8e-08$) and the RFA (ipsi: $r=0.45$; $p=4.8e-07$, contra:
396 $r=0.4$; $p=1.2e-05$) were the only areas whose activity was robustly associated
397 with this movement metric (Fig. 7B). We next investigated the relationship
398 between the neural activity levels and the movement smoothness (e.g., Lai
399 et al., 2015). Very high statistical significance for moderate positive associ-
400 ation between PeakMax and movement smoothness was found in the MOs
401 (ipsi: $r=0.43$; $p=2.4e-06$, contra: $r=0.41$; $p=7.8e-06$) and in the RFA (ipsi:
402 $r=0.41$; $p=6.9e-06$, contra: $r=0.43$; $p=1.9e-06$). No evidence for linear rela-
403 tionship was found in the other areas, including the RSPd and the VISPM
404 (Fig. 7C). Therefore, while activity level increase neocortex-wide, secondary
405 motor areas contained the most information related to kinematic parameters
406 of RtGs.

Figure 7: Neural activity levels in both hemispheres scale linearly with movement kinematics

For all panels, the data from the ipsilateral cortical areas are shown as blue diamonds, and the data from the contralateral cortical areas are illustrated as red circles. The PeakMax occurring for each movement within a temporal window of -0.2 ± 0.8 seconds from MoveON was extracted, for each area across both hemispheres and correlate to movement distance (A), movement duration (B), and movement smoothness (C). For the correlation analysis of each pair of parameters, we set a Bonferroni-adjusted significance level of $\alpha < 5 \times 10^{-5}$. Panels with star denote statistical significance, panels with cross denote statistically non-significant trends.

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Figure 7: caption, continued from previous page

The scatterplots show the relationship between PeakMax and the distance covered, across selected homologous areas of the ipsi- and contralateral hemisphere. PeakMax observed in the RFA and MOs of the ipsi- and contralateral hemisphere were found to be positively correlated with the distance covered during the RtGs. This relationship was not observed in the MOp, in the CFA nor in the RSPd nor in the VISp. B. The scatterplots show the relationship between PeakMax and the movement duration, across selected homologous areas of the ipsi- and contralateral hemisphere. PeakMax observed in the bilateral RFA and MOs were found to be positively correlated with the duration of the RtGs. This relationship was not observed in the MOp, in the CFA nor in the RSPd nor in the VISp. C. The scatterplots show the relationship between PeakMax and the speed peaks, across selected homologous areas of the ipsi- and contralateral hemisphere. PeakMax observed in the bilateral RFA and MOs were found to be positively correlated with the speed peaks of the RtGs. This relationship was not observed in the MOp, in the CFA nor in the RSPd nor in the VISp.

DISCUSSION

In the present work, we report results from experiments in which we employed a wide-field fluorescent imaging approach to provide an unprecedented view of the mesoscale level neocortical dynamics from mice performing a voluntary reach-and-grasp task. To the best of our knowledge, this is the first reported step towards an all-optical characterization of neocortex-wide neural dynamics in rodents performing voluntary RtG movements. We found that during RtG, a distributed network of cortical areas, beyond the regions classically associated with the production of skilled movements, displays increased activity levels. Also, the increased activity is observed in both hemispheres, a finding quite unexpected from a unilateral motor task. The FC among those movement-associated areas undergo a brief (<1 sec), transition, i.e., towards an increased connectivity from rest to the planning phase, and towards a decreased connectivity from planning to movement execution. Our analyses indicate the emergence of functional clusters during the movement execution. At variance with the global communication scheme during successful RtG, the internal representation of movement parameters appears localized. Indeed,

a robust association between the levels of neural activity and selected RtGs kinematics was found primarily in the secondary motor areas (MOs and RFA of the contra- and ipsilateral hemispheres).

In rodents, it was shown that areas such as the contralateral RFA are both necessary and sufficient to elicit goal-directed and it was also demonstrated that different areas subserve discrete steps of such behaviors (Guo et al., 2015; Morandell and Huber 2017; Wang et al., 2017; Galiñanes et al., 2018). However, these studies were focusing on sensorimotor areas and may have missed important information on the dynamics taking place in other cortical areas during RtG. Our work expands upon this line of research, by providing supporting additional evidence for an involvement of sensorimotor areas during RtG and, more importantly, by directly observing all areas of the neocortex, hence providing an integrative framework not reported previously. The global communication scheme found in the present work is consistent with recent reports on other types of behavioral tasks (Goard et al., 2016; Kyriakatos et al., 2016; Wekselblatt et al., 2016; Allen et al., 2017; Makino et al., 2017; Salkoff et al., 2019; Stringer et al., 2019, Musall et al., 2019). However to our best knowledge, similar approaches were not yet applied to investigate neural dynamics underlying the control of unilateral forelimb movements requiring to locate, reach and grasp for an object. Also, a direct correlative analysis between neocortex-wide neural activity and RtG kinematics was still largely unexplored. Our work provides thus the first direct evidence indicating that while neural activity increases globally during RtG, neuro-kinematic

447 correlations are significant only for the secondary motor areas, suggesting
 448 that movement planning and execution may involve large neural networks
 449 across the neocortex while encoding of movement parameters occurs in seg-
 450 regated areas. Our work also suggests that during successful reach-to-grasp
 451 movements the information processing occurring in the neocortex for multi-
 452 joint movements is distributed, at least when contextualized to our imaging
 453 approach, the temporal windows analyzed and the cortical parcellation em-
 454 ployed. Our results point the attention also to areas beyond the MOs, the
 455 MOp and their subdivisions (RFA and CFA) towards other territories such as
 456 the RSC, the SSp and the visual areas, which were previously not recorded
 457 and studied simultaneously within the context of the RtG. For the RSC,
 458 while its participation to RtG in the mouse is demonstrated here for the
 459 first time, these findings are supported by anatomical evidence, indicating
 460 monosynaptic communication between the RSC and the MOs (Yamawaki et
 461 al., 2016). At the functional level, the contribution of RSC may be explained
 462 by its known involvement in the processing of visual information necessary to
 463 locate the target relevant for the behavioral tasks (Czajkowski et al., 2014).
 464 There are some aspects that needs to be considered when interpreting the
 465 results. The one-photon, wide-field calcium imaging approach is sensitive to
 466 light scattering potentially leading to artifacts in the signals extracted from
 467 the cortical areas, which could increase the risk of erroneously consider as
 468 an area as movement-associated (Yang and Yuste, 2017). Such possibility
 469 is reduced by the fact that areas even in close proximity (for example the

470 barrel fields vs the upper limb portion of the somatosensory cortex) do not
 471 display the same levels of activity (Fig. 5 A). The analyzed neural activity
 472 is inferred from the fluctuations of fluorescence intensity throughout time, a
 473 signal composed also by non-neuronal sources, notoriously of hemodynamic
 474 origin, which represents another artifact that needs to be taken into account
 475 (Ma et al., 2016). However, the hemodynamic component of the signal has
 476 a much slower rise and decay time with respect to the neuronal signal. This
 477 temporal feature of the hemodynamic component of the GCaMP6f signal
 478 reduces its contribution relative to the results reported here (Scott et al.,
 479 2018). Even considering such limitations, our findings remains unexpected
 480 and provide experimental evidence to put into questions some dogmas of
 481 the classical literature in motor control (Omrani et al., 2017), at least when
 482 investigating the *modus operandi* of rodent cortex, adding experimental ev-
 483 idence to the relatively new idea of ipsilateral representation of movements
 484 parameters, which is also a computational feature of the human neocortex
 485 and is increasingly getting attention in the motor control community (Bundy
 486 et al., 2018; Bundy and Leuthardt, 2019). In rodents, a study restricted to
 487 the posterior parietal cortex recently reported evidence for ipsilateral con-
 488 trol of limb movement (Soma et al., 2019). We concede that the number of
 489 neocortical clusters emerging during movement could be influenced by the
 490 Allen Institute Mouse Brain Atlas parcellation of the cortex, which may in-
 491 troduce spurious segregations beyond those that are physiologically present.
 492 Importantly, the overlap between the results obtained from the SCA and the

493 CCM, supports the existence of two neocortical networks during voluntary
494 movement motor control.

495 Overall, these associations suggest the secondary motor areas could encode
496 for the movement duration, movement distance, and movement smoothness,
497 encouraging future investigations toward decoding of movement kinematics
498 from neural data. Decoding approaches have been long and very well es-
499 tablished for electrophysiological studies of motor control, and more recently
500 applied to fluorescent imaging data of neural activity (Prsa et al., 2017; Li
501 et al., 2019). Our findings extend these studies by providing evidence for
502 correlation between large scale, area-level activity, and specific metrics of
503 movement. Within the context of motor control, our findings also encour-
504 age to study the role of areas found to be active during movement (such as
505 the visual areas) commonly disregarded in the association to forelimb move-
506 ment, at the cellular-level (Galiñanes et al., 2018, Karandell and Huber,
507 2017, Ebina et al., 2018). In the future, it would also be relevant to assess
508 the neocortical dynamics during RtG in a freely moving condition or during
509 performance refinement, considering that cortical areas undergo functional
510 reorganization during learning (Makino et al., 2017; Whishaw et al., 2017;
511 Bolu et al., 2019; Hwang et al., 2019). In sum, our investigation identified
512 a global network of neocortical area associated to successful RtG execution.
513 Since we observed that several areas across both hemispheres of the cerebral
514 cortex display movement associated activity, we asked whether this activ-
515 ity could be associated with metrics of RtGs movements. We found that

among the responsive areas, the secondary motor areas of both hemispheres displayed the most robust association between the neural and the movement kinematics. Previous work showed that these areas is encodes performance rate in a licking task (Salkoff et al., 2019). Our current results suggest that the secondary motor areas could encode for the forelimb movement duration, movement distance, and movement smoothness, encouraging future investigations toward decoding of movement kinematics from neural data. Decoding approaches have been long and very well established for electrophysiological studies of motor control, and more recently applied to fluorescent imaging data of neural activity (Prsa et al., 2017; Li et al., 2019; Salkoff et al., 2019). Our findings extend these studies by providing evidence for correlation between large scale, area-level activity, and specific metrics of RtG movements. Within the context of motor control, our findings also encourage to study the role of areas found to be active during movement (such as the visual areas) commonly disregarded in the association to forelimb movement, at the cellular-level (Galianes et al., 2018, Karandell and Huber, 2017, Ebina et al., 2018). In the future, it would also be relevant to assess the neocortical dynamics during RtG in a freely moving condition or during performance refinement (Makino et al., 2017; Whishaw et al., 2017; Bollu et al., 2019). In sum, our investigation identified a global network of neocortical areas beyond those previously known to be associated with goal-directed arm movements and suggest that the operating mode of the mouse neocortex underlying unilateral goal-directed movements involves distant communication across both

539 hemispheres, while encoding of movement parameters occurs locally, and, in
540 the context of our investigations, mostly in the secondary motor areas.

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