Wide-field imaging of cortical neuronal activity with red-shifted functional indicators during motor task execution

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Abstract

Genetically encoded calcium indicators (GECIs) can be used to monitor the intracellular concentration of free calcium in populations of neurons. GECIs with long emission wavelengths are particularly attractive for deep tissue microscopy in vivo, and have the additional advantage of avoiding spectral overlap with commonly used neuronal actuators such as channelrhodopsin.

The aim of this work is to evaluate the performances of four red-shifted GECIs (jRCaMP1a, jRCaMP1b, jRGECO1a, jRGECO1b) using both ex vivo and in vivo experimental techniques.

Cortical neurons were infected with adeno-associated virus (AAV) expressing one of the red GECI variants. First we characterized the transfection ex vivo in terms of extension and intensity of the indicator. Next, we monitored the neuronal activity over the right cortical hemisphere of a jRCaMP1a-transfected mouse during a goal-directed motor task. To this aim, we combined wide-field fluorescence microscopy with a robotic device for simultaneous recording of cortical neuronal activity, force applied and forelimb position during task execution.

Our results show that jRCaMP1a has sufficient sensitivity to monitor in vivo neuronal activity simultaneously over multiple functional areas, and can be successfully used to perform longitudinal imaging sessions in awake mice.

Keywords: wide-field microscopy, GECI, calcium imaging, fluorescence, in vivo

(Some figures may appear in colour only in the online journal)
1. Introduction

Calcium concentration is widely used in neuroscience as a competent indirect reporter of neural activity. One of the main advantages is that concentration fluctuations of this ion in its free form during neuronal activity are among the highest [1, 2].

Genetically encoded calcium indicators (GECIs) have partially replaced both organic sensors and electrophysiological methods since they present considerable advantages, such as the possibility of addressing their expression into specific cellular populations and their stable transfection over time that allows long-term studies of neuronal activity in vivo [3, 4].

GCaMP is the most used GECI, since this green emitting sensor presents a fast kinetics of calcium binding, high brightness and great sensitivity [5]. Nevertheless, the GCaMP indicator has several limitations due to its excitation and emission spectra. Indeed, the blue excitation light used in standard fluorescence microscopy can cause photodamage and it is highly scattered in tissue. Furthermore, the green emission of GCaMP is absorbed by hemoglobin [6], which reduces the penetration depth of the imaging in vivo. Finally, the GCaMP excitation spectrum overlapped with that of commonly-used light-sensitive ion channels, such as channelrhodopsin-2 (ChR2), which limits the simultaneous use of green GECIs and optogenetic techniques [7, 8].

These limitations led to an increasing interest in developing a red variant of GECIs, namely R-GECIs, which have a structure similar to that of the GCaMP but possess a longer emission wavelength. R-GECIs are composed by a circular permutated thermostable red fluorescent protein (RFP), calmodulin (CaM) and M13, a peptide sequence from myosin light chain kinase [9, 10]. Among the most commonly R-GECIs used, there are two variants based on two different RFPs: mRuby (like in jRCaMP1a and jRCaMP1b) and mApple (jRGECO1a and jRECO1b) which have similar spectral profiles, with excitation peaks centered at 558 nm, and 568 nm, and emission peaks at 605 nm and 592 nm, respectively [8].

R-GECIs present multiple advantages for in vivo imaging: (i) a longer excitation wavelength, which allows deeper penetration into tissue due to a reduced scattering by endogenous fluorophores; (ii) red fluorescence is less absorbed than green fluorescence by both endogenous fluorophores and hemoglobin in mammalian tissue [8–11].

In recent years, the biophysical properties of red-GECIs have been analysed using two-photon fluorescence microscopy (TPFM). Several features of the calcium response were investigated in single cells and small networks in vitro and in vivo, showing their capacity of discriminating single action potentials in a variety of organisms and cell populations [8–12]. In addition, these works discriminated between photostability and photobleaching of VSD [14], or (ii) the combination of GCaMP6s and red-shifted C1V1, which was limited by C1V1 photoswitching when illuminated by blue light [15]. On the other hand, R-GECIs are more easily compatible with ChR2 activation for the purpose of simultaneous neural circuit activity monitoring and manipulation over time [13].

Currently, the most flexible approach for inducing the expression of GECI is viral transfection that can target specific cellular populations in selected functional areas. Then, the fluorescence emission of GECI can be recorded in vivo using imaging techniques like wide-field fluorescence microscopy (WFFM). This technique allows the recording of neuronal activity on distant functional cortical areas over both cortical hemispheres with millisecond resolution [16]. Compared to two-photon fluorescence microscopy (TPFM), WFFM does not provide information at the single cell level nor from single cell layers. In addition, the visible light used in WFFM for fluorescence excitation produces more photodamage than the infrared light used in TPFM [17]. However, WFFM uses a simpler light source as compared to TPFM which requires an ultrafast laser. Furthermore, WFFM allows performing correlations of functional connectivity at brain level over one or both cortical hemispheres in anesthetized and awake mice [18, 19]. These large-scale recordings could be correlated with functional measures on humans, like EEG and fMRI. Finally, the combination of WFFM with GECIs was recently demonstrated to be a new tool to improve our understanding of how learning changes the macroscopic dynamics in the cortex [20].

In order to choose the best red-shifted GECI for long-term in vivo studies using WFFM, here we examined four different commercially available R-GECIs in terms of both intensity and extension of the transfection. Further, we tested if WFFM in jRCaMP1a transfected mice could allow monitoring neuronal activity over one hemisphere of the dorsal cortex in resting state and during the execution of a motor task. Finally, we tested the applicability of this combination of techniques for the evaluation of the functional connectivity in different behavioral states.

2. Methods

2.1. Animals

Adult male and females C57BL mice (age >1 years) used in this study were housed in a temperature- and humidity-controlled room with food and water ad libitum. Animal handling and experimental procedures were performed in accordance with the guidelines of the Italian Minister of Health.

2.2. Viral injection and optical window

All surgeries were conducted under isoflurane anesthesia (1.5%–2%); local anesthetic, lidocaine 2%, was applied during the incision of the skin and on the exposed bone.

For the viral injection, the animals were placed into a stereotaxic apparatus and both the skin over the skull and periosteum were removed. A small hole (Ø 0.4 mm) was then drilled...
into the skull over the right hemisphere. For each indicator ($n_{\text{mice}} = 4$ on each group), we injected a volume of 250 nl of AAV at two depths (0.4 mm and 0.8 mm from the dura), into the somatomotor cortex (+1.5 mm mediolateral and −1.5 anteroposterior from bregma). The injection was performed by means of glass capillary (ϕ of the tip: 50 μm) connected to a Picospritzer (Picospritzer III—Science Products™) with a delivery speed of 10 μl min⁻¹.

The viral vectors used were:

- jRCaMP1a: pGP-AAV-syn-NES-jRCaMP1a-WPRE.211.1488
- jRCaMP1b: pGP-AAV-syn-NES-jRCaMP1b-WPRE.211.1519
- jRGECO1a: pGP-AAV-syn-NES-jRGECO1a-WPRE.111.1670
- jRGECO1b: pGP-AAV-syn-NES-jRGECO1b-WPRE.111.1721.

The AAV9 serotype was selected to allow indicator expression at the central nervous system level. Moreover, the neuro-specific promoter (synapsin) targeted both excitatory and inhibitory neurons [21] while the NES motif limited expression to the cytoplasm.

In order to study the cortical activity during the motor task execution, we injected 500 nl of the jRCaMP1a construct into the primary motor cortex (+1.75 mm mediolateral and +0.5 mm anteroposterior from bregma) of three mice at a depth of 0.5 mm.

All mice were implanted with a cover glass attached to the bone via transparent dental cement (Super Bond C&B—Sun Medical) to allow free optical access to the cortex. A custom-made aluminum head-bar was attached to the skull on the interparietal bone to allow the fixation of the head during awake imaging.

2.3. Wide-field fluorescence microscope

A custom-made widefield imaging setup was used to image the fluorescence signal of the GECIs. This setup was equipped with an excitation source for imaging RGECIs fluorescence (595 nm LED light, M595L3 Thorlabs, New Jersey, United States), and a bandpass filter (578/21 nm, Semrock, Rochester, New York, USA) to select the excitation band. A dichroic filter (606 nm, Semrock, Rochester, New York USA) located on the objective (EC Plan Neofluar, NA 0.085) reflected the excitation light beam towards the sample. Finally, a 3D motorized platform (M-229 for xy plane, M-126 for z-axis movement; Physik Instrumente, Karlsruhe, Germany) allowed animal positioning under the objective.

The fluorescence signal was collected through a bandpass filter (630/69, Semrock, Rochester, New York, USA) with an excitation source for imaging RGECIs fluorescence peak. The acquired images at a resolution of 512 by 512 px² covering 4.4 × 4.4 mm² of cortex.

2.4. Wide-field calcium imaging

Calcium imaging sessions were performed from 2 to 4 weeks after surgery in a resting state condition (i.e. the animals were awake, but they were not subjected to stimuli). Repeated in vivo acquisitions were performed daily or weekly on the same mouse. At the beginning of each recording session, the head position was adjusted so that the imaging field of view matched the acquisition performed on the first day for each mouse. Each session consisted in 40 s of recordings (sampling rate: 25 Hz).

During the last week, one experimental group (AAV-jRCaMP1a injected in motor cortex, $n_{\text{mice}} = 3$) was subjected to behavioral training for five consecutive days (see section 2.4.6 below).

2.4.1. Data analysis. The analysis has been performed using ImageJ, OriginPro and Mesoscale Brain Explorer (MBE) software. The fluorescence traces were analysed both on the whole field of view and on five regions of interest (ROIs, area 0.24 mm²), located on specific functional areas: primary and secondary motor cortex (M1 and M2), primary sensory cortex of barrel field and forelimb region (S1BL and S1FL) and retrosplenial cortex (RS) (figure 4(B)).

For each recording session changes in the fluorescence signal were expressed as $\Delta F/F$, according to the following formula:

$$\Delta [Ca^{2+}] \propto \frac{\Delta F}{F} = \frac{[F_t - F_0]}{F_0} \%,$$

where $F_0$ was the average of baseline fluorescence intensity and $F_t$ was the fluorescence issued at a given time. We only considered fluorescence peaks with intensity 20% higher than the baseline. The threshold value was calculated for every week as the average of the intensity in a ROI (0.24 mm²) far from the site of injection.

2.4.2. Full width half-maximum. For each indicator, we chose three frames corresponding to the maximum fluorescence peak reached during the recording session on the fourth week (exposure time 40 ms, LED power 23 mW, 32 mW cm⁻²). On the average of these frames, we calculated the full width at half maximum (FWHM) of fluorescence profile over two orthogonal lines passing through the injection site (rostro-caudal and medium-lateral plan). Moreover, on the same profiles we measured the peak amplitude (figure 2).

2.4.3. Ex vivo imaging. Four weeks after injection, mice were perfused with 20–30 ml of 0.01 M PBS (pH 7.6) and 150 ml of 4% paraformaldehyde (PFA). After the perfusion, we obtained brain coronal slices (100 μm thick) using a vibratome (Vibratome Series 1500—Tissue Sectioning System). On each slice, we studied the rostro-caudal transfection extension by WFFM (figure 3(A), exposure time 12 ms, LED power 23 mW).
In addition, on the three brightest slices, we have evaluated the signal to noise ratio (SNR, figure 3(C)) calculated as follows:

$$\text{SNR} = \frac{S}{\sqrt{R}}$$

where S was the fluorescence intensity averaged on a 0.24 mm ROI (red rectangle in figure 3(A)) centered on the brightest area and mediated on the three brightest slices, while R was the mean intensity of noise obtained in the same slices but in an area furthest from the transfection site (~2 mm).

2.4.4. Correlation matrix. The correlation matrixes were realized using data from a single mouse and analyzed by mesoscale brain explorer (MBE) software [22]. We calculated the correlation index (r) of cortical activity (ΔF/F) between the following five functional areas: (1) primary motor cortex (M1, brain area highly involved in motor control [23]); (2) somatosensory representation of forelimb region (S1FL, highly connected to M1; sensory feedbacks are known to play an important role in driven learned movements [24, 25]); (3) secondary motor cortex (M2, has a predictive role to drive goal-directed movement, thus playing a fundamental role into the neuronal circuit mechanism of voluntary actions [20–26]; it receives several sensory afferences and has reciprocal connection with retrosplenial cortex); (4) retrosplenial cortex (RS, supports spatial working memory, but its projections to M2 suggest a role in motor control and sensorimotor integration too [20–28]); (5) sensory cortex in barrel-field region (S1BF, principally involved in whisker-dependent behaviours; functional activity in this region should not be primarily involved in lever pulling movement [29]).

The correlation index depicts how the activity in one area correlates with other areas in the matrix, and accounts for both (i) synchronicity of fluctuations in fluorescence intensity and (ii) time delays with which the same peaks occur in two different areas.

The ‘motor task’ correlation matrix (figure 4(F)) was calculated on 12 of 15 consecutive retraction trials. We selected imaging subsets that were satisfying a specific condition, i.e. when the peak force necessary to pull the slide in an attempt managed to produce displacement of the slide (peaks 2 and 3 in figure 4(C)) and when it was associated with a reward (rewarded pull, peak 4 in figure 4(C)). We discarded the trials where the paw slipped away from the slide.
On the same mouse, the correlation matrix during resting state condition was obtained on a collated sequence of 12 randomly-selected baseline imaging datasets.

2.4.5. Scatter distribution. We selected the peaks of maximum fluorescence in M1, associated with force peaks exerted \( n_{\text{peak}} = 19 \). The threshold was obtained averaging the intensity of maximum peaks during resting state condition in the same mouse \( n_{\text{peak}} = 19 \) (figure 4(G)).

2.4.6. Robotic platform. Mice expressing jRCaMP1a in motor cortex performed five consecutive days of training in the robotic platform device [30]. The single daily session consisted in 15 cycles or ‘trials’ of active retraction of left forelimb associated with a sweetened condensed milk reward (10 \( \mu l \)) upon reaching of the target (fully-retracted) position. Each day, before the training, we recorded the ‘baseline’, which was the spontaneous neuronal activity in resting state condition (40 s of acquisitions, 40 ms of exposure time).

The robotic device (M-platform) is based on the one described in Spalletti et al [30]. It is composed of a linear actuator, a 6-axis load cell (Nano 17, ATI Industrial Automation, USA), a precision linear slide with an adjustable friction system and a custom-designed handle where the left wrist of the mouse was allocated, which allowed a transfer of the force applied by animals to the sensor.

In each trial, first a linear motor pushed the slide and extended the mouse left forelimb by 10 mm (passive phase). Then, the motor was quickly decoupled from the slide and the mouse was free to voluntarily pull the handle back (active phase). Two acoustic signals informed the mouse of the end of passive phase (0.5 s) and the reaching of a target position (1 s), which was associated with reward.

During the exercise, the robotic device integrated in the wide-field microscope allows simultaneous recording of: (i) cortical activity over the right hemisphere, measured as a change in fluorescence signal, (ii) the force applied by left forelimb and (iii) the limb position, estimated by the movement of the slide using an IR position sensor located on the slide and recorded by an IR camera (EXIS WEBCAM #17003, Trust) (figure 4(A)).

3. Results

3.1. Experimental timeline

We characterized four different red-GECIs (jRCaMP1a, jRCaMP1b, jRGECO1a, jRGECO1b) locally expressed at somatomotor cortex level by using \textit{in vivo} and \textit{ex vivo} wide-field fluorescence imaging.

To this aim, we performed intraparenchimal injections of adeno-associated viruses expressing one of the red GECI

![Figure 2](https://example.com/figure2.png)
variants under the human synapsin1 (neuron-specific) promoter (AAV-SYN1-red GECI variant). In order to evaluate both the stability of transfection over the weeks and the in vivo indicator distribution on the cortex, an imaging session was performed at two, three and four weeks after surgery. To quantify more precisely the extension of the transfection, we performed ex vivo imaging on brain slices four weeks after the injection (figure 1(A)).

3.2. In vivo wide-field calcium imaging over 4 weeks

During the imaging session in vivo we recorded the spontaneous cortical activity to evaluate the sensitivity of the indicators during a resting state condition (figure 1(B)).

Next, we performed a comparison of the average cortical activity between each week. Our results showed that only jRCaMP1b indicator had a very low expression level, which was comparable to the background noise. Conversely, among the other three indicators, jRGECO1a had the best average values (figure 1(B)).

We also showed that the indicator intensity was stable over time for all the indicators (figure 1(B)). This condition allows long-term studies of neuronal activity.

3.3. In vivo fluorescence distribution in the space of four R-GECIs

Four weeks after injection, for the brightest frames, we quantified in vivo spatial extension of the fluorescence in both the rostro-caudal and the medio-lateral plane (figure 2(A)). To this aim, we evaluated the full width at half maximum (FWHM) and the peak amplitude of fluorescence profiles for each indicator.

We first evaluated the FWHM (figure 2(B)), which is an index of indicator distribution in space. We found that the indicator expression was isotropic from the injection site along both planes for all the indicators (figure 2(B)). In addition, jRCaMP1a and jRGECO1b exhibited the largest in vivo fluorescence extension along both planes.

We further analyzed the peak amplitude, which gives an indirect estimation of indicator sensitivity and concentration in situ. Our results showed that jRCaMP1a was the brightest at the injection site (figure 2(C)).

Since jRGECO1b exhibited lower expression level than jRCaMP1a (figure 2(C)), we identified the RCaMP1a indicator as the best in terms of in vivo distribution in space, sensitivity and concentration level.
Figure 4. Cortical neuronal activity in resting state and during execution of motor task. (A) Schematic view of the M-platform used for training integrated with the wide-field microscope, which allows simultaneous recording of three different data: the force applied by forelimb (green), the position of left forelimb (blue) and the contralateral cortical activity as a change in the indicator fluorescence intensity (red). (B) Schematic of field of view of the microscope, highlighted by the black dotted square, superimposed to the cortical hemispheres. The five cortical functional areas used for correlation matrix are reported (inset). The ROIs are primary and secondary motor cortex (M1 and M2, green and black respectively), primary sensory cortex in both barrel field and forelimb region (S1BF and S1FL, red and yellow respectively) and retrosplenial cortex (RS, blue). Bregma is reported as red spot. (C) Top: example of force trace obtained by the load cell on the M-platform during a single trial in the forelimb active retraction phase. The forelimb position is in orange, the force applied by the mouse in blue. The force peaks trace shows the attempts to move the slide (red spot), the force peaks associated with movement of the slide (purple spot) and the pulling associated with reward (black star). Bottom: calcium response recorded in the R-M1 simultaneously with the force trace shown on top. (D) Example of neuronal activity simultaneously recorded on the five cortical areas during the single trial shown in (C). (E) Representative image sequence of cortical activation during a rewarded pull. The white dot indicates bregma. Scale bar: 1 mm (F) Linear average correlation matrices between five selected ROIs, obtained both during resting state condition (left, \( n_{\text{trials}} = 12 \)) and the last day of motor task execution (right, \( n_{\text{trials}} = 12 \)) in the same mouse. (G) Scatter distribution of the relationship between the maximum fluorescence activity of M1 and the corresponding force peak during the last day of training (\( n_{\text{mouse}} = 1, n_{\text{peak}} = 19 \)). The black line shows the best fit (intercept = 2.1 ± 0.5, slope = 5 ± 1). Orange dashed line reports the threshold measured as the average of M1 maximum activity in resting state condition in the same mouse (\( n_{\text{peak}} = 19; \Delta F/F = 2.06 \)). Values reported as average ± SEM.
3.4. Ex vivo characterization of R-GECIs transfection

To finely quantify the expression profile of the indicators throughout the entire right hemisphere, we further assessed both the rostro-caudal extension and the expression level of transfection ex vivo.

Our results confirmed that three out of four indicators were successfully transfected (figure 3(A)), while the expression level of jRCaMP1b indicator was not detectable in our analysis. Compared to the other indicators, jRCaMP1a construct showed both the widest transfection at cortical level (figure 3(B)) and the best signal to noise ratio (figure 3(C)), which can be associated with an higher in situ expression level.

Taken together, these results allowed us to identify the best red-shifted calcium indicator for wide-field imaging: jRCaMP1a, which was characterized by both wide in vivo fluorescence distribution and high sensitivity.

3.5. Evaluation of jRCaMP1a sensitivity to neuronal activity during motor task execution

We further tested the performance of jRCaMP1a on in vivo awake imaging of dorsal cortex during the execution of a motor task. In these experiments, we transfected jRCaMP1a at motor cortex level and monitored neuronal activity four weeks after the injection on five different functional areas during motor task execution on a robotic device (M-platform [19]).

The robotic platform allowed us to record simultaneously force and position of left forelimb while monitoring the contralateral cortical activity (figure 4(A)).

The motor task consisted of 15 consecutive active retraction of left forelimb; after reaching the target (fully-retracted) position, the mouse received a milk reward. This training session was performed daily for 5 d.

The correlation matrices were computed for both the resting state and the task execution conditions (figure 4(E)). In the last case, we selected only the timeframes of the imaging dataset corresponding to force peaks associated with forelimb movement (peak 4 in figures 4(C) and (D)).

Our results showed a high and widespread correlation of neuronal activity during the motor task compared to resting state. The higher variation of correlation index took place between two ROIs pair: R-M2/R-S1FL and R-RS/R-S1FL (figure 4(F)).

Moreover, we analyzed the potential of our paradigm in detecting the neuronal activation in motor cortex associated with a broad range of forces applied. We found that when the force applied during the active retraction by the forelimb has small intensity, the M1 activity associated is detectable and slightly higher than the average value measured in resting state condition. As the applied force increases, the maximum activity of M1 increases linearly (figure 4(G)).

These results allowed us to conclude that the extension of the transfection was sufficient to simultaneously record the activation of several cortical areas distant from injection site, and that the jRCaMP1a is sufficiently sensitive to report the variation of neuronal activity associated with the modulation of applied forces.

4. Discussion and conclusion

In this study, we performed in vivo and ex vivo characterizations of the expression patterns of four different red-shifted GECIs transfected at cortical level. We confirmed that the indicator expression is stable over time for all sensors except for the jRCaMP1b indicator, which showed transfection level comparable to noise in each analysis performed. On the other indicators, we show that there is a slight reduction (statistically non-significant) of average ΔF/F over the weeks.

Although the mice were acclimated to the environment for a few minutes before each imaging session, this decrease could still be due to changes in the emotional state of the animals over the days and the weeks.

jRGECO1a indicator (mApple based) showed the highest response amplitude over the weeks (figure 1(B)), in agreement with previous studies demonstrating that it is the most sensitive indicator with fastest rise kinetics [8]. Nevertheless, it has been shown that both mApple based-indicators (jRGECO1a and jRGECO1b) are affected by photoswitching effect when illuminated by blue light [8–12], thus limiting their use for optogenetic studies where blue activated opsins are used.

The in vivo and ex vivo quantification of indicator distribution over the dorsal cortex of the right hemisphere showed jRCaMP1a as the best sensor in terms of sensitivity and signal to noise ratio (figures 2(B) and (C)). Our results are in agreement with previous studies using two-photon where jRCaMP1a is identified as the brightest indicator in the calcium-bond state [8]. In addition, we found that jRCaMP1a construct is associated with the widest rostro-caudal extension of transfection at four weeks after injection. jRCaMP1a expression involved several well-defined functional areas along the right hemisphere, allowing in combination with wide-field microscopy the simultaneous study of the functionality of five cortical areas.

In conclusion, although jRGECO1a has been previously identified as the most sensitive and with faster rise kinetics compared to the other indicators [8], our results indicated jRCaMP1a construct as the more suitable for in vivo wide-field imaging studies.

We therefore chose jRCaMP1a for an exploratory study using WFFM of the functional activation of cortical neurons over one hemisphere during a goal-directed motor task.

We demonstrated that our approach allowed investigating the interplay between the selected functional areas during an active behavior involving retraction of the limb. In agreement with previous studies [25–31], we showed the involvement of retrosplenial and sensorimotor-areas in movement control. The barrel-field region showed (i) the lowest correlation degree with the other functional areas during the exercise and (ii) the lowest variation of correlation index with R-M1 between resting state and motor task execution. On the other hand, we showed an extensive increase in correlation of neuronal activity during the task compared to resting state for each ROIs pair.

A limitation of WFFM is that the fluorescence signal recorded with this technique derives from the summation of the firing activity from several neurons. Indeed, the temporal
resolution of WFFM does not allow the detection of single APs. In addition, the viral transfection labels neurons throughout the entire cortex (figure 1(A)) therefore the contribution from the different layers is hardly quantifiable. Nevertheless, the highest contribution is likely to come from superficial layers of the cortex, since tissue scattering may prevent photons originating from deep layers to reach the detector.

In addition, the combination of WFFM with a robotic device for motor training [30] allowed performing a preliminary correlation of the neuronal activity in M1 and the force applied during the retraction task. Our preliminary result (figure 4(G)) is in agreement with previous studies where the activity of corticomotoneuronal cells increased linearly with the modulation of force levels [32].

Our preliminary analysis showed the potential of using a combination of AAV-transfected RGECs and WFFM to analyze functional connectivity changes in different behavioral states (resting versus task-evoked). We anticipate that we plan to extend the analysis to verify longitudinal alteration in whole-cortex functional connectivity induced by learning of the motor task.

In conclusion, by using wide-field imaging we demonstrated that jRCaMP1a transfected in one cortical hemisphere allows simultaneous recordings of the activity in several functional areas. Furthermore, this combination of tools is capable of detecting the variation of neuronal activity at motor cortex level associated with a broad range of applied forces (figure 4(G)).

Finally, we anticipate that the well-defined excitation and emission spectra of ChR2 and jRCaMP1a will enable simultaneous all-optical manipulation and wide-field recording of neuronal activity in awake animals. Our approach will be targeted to the investigation of optogenetically induced neuronal activation associated with complex movements. The clear calcium response evoked by ChR2 stimulation will allow to better understand neural processes underlying specific behaviors.

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