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TITLE:

In Vivo Wireless Optogenetic Control of Skilled Motor Behavior

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SUMMARY:

The present protocol describes how to use wireless optogenetics combined with high-speed videography in a single pellet reach-to-grasp task to characterize the neural circuits involved in the performance of skilled motor behavior in freely moving mice.

ABSTRACT:

Fine motor skills are essential in everyday life and can be compromised in several nervous system disorders. The acquisition and performance of these tasks require sensory-motor integration and involve precise control of bilateral brain circuits. Implementing unimanual behavioral paradigms in animal models will improve the understanding of the contribution of brain structures, like the striatum, to complex motor behavior as it allows manipulation and recording of neural activity of specific nuclei in control conditions and disease during the performance of the task.

Since its creation, optogenetics has been a dominant tool for interrogating the brain by enabling selective and targeted activation or inhibition of neuronal populations. The combination of optogenetics with behavioral assays sheds light on the underlying mechanisms of specific brain functions. Wireless head-mounted systems with miniaturized light-emitting diodes (LEDs) allow remote optogenetic control in an entirely free-moving animal. This avoids the limitations of a wired system being less restrictive for animals' behavior without compromising light emission efficiency. The current protocol combines a wireless optogenetics approach with high-speed

videography in a unimanual dexterity task to dissect the contribution of specific neuronal populations to fine motor behavior.

INTRODUCTION:

Motor skilled behavior is present during most movements performed by us, and it is known to be affected in several brain disorders[1-6]. Implementing tasks that allow studying the development, learning, and performance of skilled movements is crucial to understanding the motor function's neurobiological underpinnings, especially in models of brain injury, neurodegenerative and neurodevelopmental disorders[2,7-13]. Reaching far and retrieving objects is done routinely in everyday life actions, and it is one of the first motor skills acquired during early development and then refined through the years[5,6]. It comprises a complex behavior that requires sensory-motor processes such as the perception of the object's features, movement planning, action selection, movement execution, body coordination, and speed modulation[7,14-16]. Thus, unimanual high dexterity tasks require the participation of many brain structures of both hemispheres[16-22]. In mice, the single pellet reach-to-grasp task is characterized for several phases that can be controlled and analyzed separately[7,13,23]. This feature allows to study the contribution of specific neuronal subpopulations at different stages of acquisition and behavior performance and provides a platform for detailed studies of motor systems[13,23,24]. The movement occurs in a couple of seconds; thus, high-speed videography should be used for kinematic analysis in distinct stages of the skilled motor trajectory[7,25]. Several parameters can be extracted from the videos, including body posture, trajectory, velocity, and type of errors[25]. Kinematic analysis can be used to detect subtle changes during wireless optogenetic manipulation[7,23].

Using miniaturized light-emitting diodes (LEDs) to deliver light *via* a wireless head-mounted system makes it possible to have remote optogenetic control while the animal performs the task. The wireless optogenetic controller accepts single-pulse or continuous trigger commands from a stimulator and sends infrared (IR) signals to a receiver connected to the miniaturized LED[23,26]. The current protocol combines this wireless optogenetics approach with high-speed videography of a dexterity task to dissect the role of specific neuronal populations during the performance of fine motor behavior[23]. Since it is a unimanual task, it allows for assessing the participation of structures in both hemispheres. Traditionally, the brain controls the body movement in a highly asymmetric manner; however, high dexterity tasks require careful coordination and control from many brain structures, including ipsilateral nuclei and differential contribution of neuronal subpopulations within nuclei[10,20-23]. This protocol shows that subcortical structures from both hemispheres control the trajectory of the forelimb[23]. This paradigm can be suitable to study other brain regions and models of brain disease.

PROTOCOL:

The procedures involving animal use were conducted following local and national guidelines and approved by the corresponding Institutional Animal Care and Use Committee (Institute of Cellular Physiology IACUC protocol VLH151-19). Drd1-Cre transgenic male mice[27], 35-40 days postnatal with C57BL/6 background were used in the current protocol. Mice were kept under the following conditions: temperature 22±1 °C; humidity 55%; light schedule 12/12 h with lights off at 7 p.m. and were weaned at postnatal day 21. Weaned pups were housed in same-sex groups of 2–5.

Standard rodent pellets and water were provided *ad libitum*, except when noted.

1. Surgical procedures

1.1. Prepare an LED cannula at the desired length according to the dorsoventral coordinates of the structure of interest (ideally 0.5 mm longer to account for the thickness of the skull, for the dorsolateral striatum 3.5 mm) **(Figure 1)**.

1.1.1. Cut the glass fiber to a length longer than the final desired size, grind the fiber tip to the target length with rough sandpaper, and finally, polish the fiber tip with fine sandpaper.

NOTE: LED cannula is a glass optical fiber of 250 µm diameter attached to an infrared receiver (see **Table of Materials**).

1.2. Pull glass pipettes (1.14 mm outer diameter, 0.53 mm inner diameter, and 3.5 in length) for the nano-injector with a horizontal puller (see **Table of Materials**) and store them for later. Program the puller in one loop to get a 15–20 µm tip diameter with a long gradual slope taper (4-5 mm).

1.3. Prepare the surgery area by thoroughly disinfecting the stereotaxic apparatus, hood, micro-injector (see **Table of Materials**), and surrounding surfaces with 70% ethanol.

NOTE: A mouse stereotaxic apparatus is essential to inject Adeno Associated Virus (AAVs) precisely and place the LED cannula in the region of interest.

1.4. Wear the appropriate personal protective equipment for the procedure, including a clean lab coat or disposable surgical gown, sterile gloves, face mask, and disposable head cap.

1.5. Place the necessary equipment close to the surgery area, such as sterile surgical tools, cotton tips, solutions, micropipette, pipette tips, capillaries, micro-fill with mineral oil, and marker.

1.6. Fill a pipette for microinjections with mineral oil and place it in the micro-injector. Make sure that the micro-injector is working correctly by ejecting some mineral oil.

1.7. Anesthetize animals with gaseous isoflurane 4-5% to induce anesthesia and 1.2% throughout the surgery with 0.5-1 L/min pure oxygen. The surgery begins only after the animal has reached a point of deep anesthesia, assessed by the absence of paw withdrawal after a slight pinch.

1.7.1. Continuously monitor the breathing rate and temperature of the animal. Maintain the body temperature by a heating pad set at 34 °C.

1.8. Apply an ophthalmic ointment. Remove hair from the scalp with a trimer and hair removal

cream. Wipe the scalp with cotton swabs having 8% povidone-iodine (see **Table of Materials**) and 70% ethanol alternated three times each.

1.9. Place the mouse in the stereotaxic apparatus and secure the head, ensuring that the skull is leveled in the mediolateral and anterior-posterior axes.

1.10. Make a 1 cm incision with a scalpel through the scalp at the level of the eyes along the sagittal axis. Retract the skin to expose the skull and clean the periosteum with cotton swabs.

1.11. Clean the cranium surface with saline solution and cotton swabs. Resolve any bleeding at the surface using sterile absorbent eye spears (see **Table of Materials**) or similar sterile absorbent material.

1.12. Apply a drop of 2.5% hydrogen peroxide with a cotton swab and let it act for a few seconds to make the skull sutures visible and have a better reference. After a few seconds, clean thoroughly with a clean cotton swab.

1.13. With the glass pipette (15 µm final tip diameter), locate bregma and lambda to check that the skull is leveled in the anterior-posterior axis.

NOTE: It is recommended to have a stereoscopic microscope or USB microscope to see the tip of the glass pipette. In case it is needed, adjust the height of the mouth holder to level the skull.

1.14. Move the capillary toward the selected anterior-posterior (AP) and medial-lateral (ML) coordinates (dorsolateral striatum AP 1.2 mm, ML 2.28 mm). Paint a reference point in the scalp above the selected coordinates with a marker.

1.15. In the reference point, perform an ~1 mm diameter craniotomy applying gentle pressure to the skull with a rotary tool or dental drill at a low to medium speed with a small round dental drill bit (see **Table of Materials**).

1.16. Load the capillary with 300-400 nL of Cre-dependent adeno-associated virus (AAV) such as AAV1-dflox-hChR-2-mCherry to express Channelrhodopsin or an AAV to express only the reporter protein (e.g., mCherry) as a control in the region of interest (see **Table of Materials**). Check that the tip is not clogged, then introduce the glass pipette in the brain at the desired dorso-ventral (DV) coordinates (dorsolateral striatum DV -3.35 mm).

1.16.1. Inject 200 nL using an automatic injector at a rate of 23 nL/s. Wait for 10 min after finishing the injection, withdraw the glass pipette slowly to avoid spillage.

NOTE: It is possible to use a 30 G needle to inject with the appropriate micro-injector.

1.17. Clean and dry any residues with cotton swabs.

1.18. Attach the glass LED cannula to the stereotaxic arm and calibrate the coordinates using bregma as a reference. Insert the cannula very slowly to avoid tissue damage and place it 100 µm above the injection site.

1.19. Once the LED cannula is in place, add a drop (100 µL) of tissue adhesive at the edge of the craniotomy.

1.20. Prepare dental cement mixture (see **Table of Materials**) following the manufacturer's instructions to fix the fiber to the skull.

NOTE: Briefly, use a chilled porcelain dish to have more working time before cement sets. Add 2 scoops of resin clear powder to the porcelain dish, add 4 drops of quick base and 1 drop of catalyst, then mix well. The powder/liquid ratio can be adjusted if a thinner or thicker viscosity is needed.

1.21. Using a sterile brush, apply the dental cement mixture around the cannula connector little by little, building layers until the skull is covered and the connector is securely attached to the skull, leaving the pins completely free. Avoid getting dental cement on the skin of the mouse.

1.22. Allow drying completely.

1.23. Close the skin around the implant using tissue adhesive (see **Table of Materials**).

1.24. Place the mouse in a recovery cage over a heating pad at 33° C. Observe animals for signs of discomfort or pain.

NOTE: Keep the mouse individually caged during all the procedures to avoid implant detaching. In case of detachment of the cannula, perform euthanasia by injecting 150 mg/kg of sodium pentobarbital followed by decapitation after deep anesthesia is reached.

1.25. Inject subcutaneously (SC) meloxicam 1 mg/kg once daily for three days post-surgery to provide analgesia.

1.26. Wait at least 7 days for complete recovery and 14 days for opsin expression before further procedures.

2. Reach-to-grasp training

2.1. On day 7 post-surgery, start the food deprivation protocol[28]. Weigh mice for three consecutive days to determine their average ad libitum body weight. Then, schedule food restrictions so that the animals receive enough nutrients to maintain approximately 90% and not less than 85% of body weight.

NOTE: This is achieved by providing 2.5-3 g of food daily. Monitor animals' weight daily and score

for overall well-being observing animals' behavior and appearance, for example, coat and eyes appearance. Use the body condition scoring system from Reference[29].

2.2. During the pre-training, training, and testing periods, provide each mouse with 20 pellets (20 mg of dustless chocolate-flavored pellets) daily (see **Table of Materials**) (eaten during the task or after) besides the standard food pellets.

2.3. Three days before habituation, scatter 0.4 g/animal/day 20 mg of dustless chocolate-flavored pellets in their home cages, so mice get acquainted with the pellets that serve as a reward during the reach-to-grasp task.

2.4. Habituate mice by placing them 10 min in the testing chamber one day before pre-training with pellets scattered on the chamber floor (**Figure 1A**).

2.5. Allow food daily after training and testing. Keep a similar schedule every day.

2.6. On the first day of pre-training, place the mice in the reach-to-grasp chamber and observe from the front. Place the pellets in front of the chamber close to the opening so that they start consuming the pellets. At this stage, mice are allowed to grab the pellets in any form.

2.7. On day two of pre-training, place the pellets further and further from the opening until getting them to the indentation (1 cm from the opening) so mice can shape their reach-to-grasp movement (**Figure 1C**).

2.8. Train mice to run to the rear of the cage and return to the cage opening to receive the next food pellet as a strategy to individualize trials.

NOTE: This can be achieved by waiting until the mouse is in the rear of the cage before placing a pellet in the indentation for each trial.

2.9. Place pellets to be grasped by either their right or left paw.

NOTE: Mice start using preferentially one paw to grasp, which will be used the following days of training and testing.

2.10. Train animals for 6 days in daily sessions lasting 20 trials or until a maximum of 10 min elapse. From day 2 of training, put the mock receiver (dimensions 12 x 18 x 7 mm, 1 g, see **Table of Materials**), so mice get habituated to the weight while performing the task (**Figure 1B**). Each day score the number of hits and missed trials.

2.11. Record behavior with a regular camera and capture 30-60 frames/s from the front of the chamber. Additionally, one can place a mirror under the training chamber at a 45° angle to monitor the animals' posture (**Figure 1D,E**).

2.12. For *post-hoc* kinematic analysis (**Figure 2**), mount a high-speed camera (see **Table of Materials**) at an angle of 45° to record from the side of the cage. If a 3D analysis is required, place a second high-speed camera to record at a 35° angle from the front of the chamber; both cameras should be placed in the right or left side of the cage depending on animals' sidedness and should capture at the same frame rate and be synchronized[7] (**Figure 3D,E**).

2.13. Set the high-speed cameras to 100 frames/s with a resolution of 376 x 252 pixels or more if possible. Place white Styrofoam walls behind the sides and back of the chamber to reduce background and increase contrast (**Figure 1E**).

2.14. On test day, replace the mock unit with an infrared receiver for wireless optogenetic stimulation (**Figure 1B,C**).

2.15. When mice start reaching, turn the LED cannula manually with the remote controller to have a continuous stimulation for the time the behavior is performed and for no longer than 2 s. Programming an automatic stimulation paradigm is preferable. The stimulation device triggers an LED of 470 nm (blue light) with intensity at the tip of 1.0 mW/mm^2.

2.16. Collect the videos for further examination, including scoring and kinematic analysis.

3. Post-hoc histological confirmation

3.1. Upon completion of an experiment, confirm viral expression and LED cannula placement. Anesthetize the animal with a cocktail of ketamine 100 mg/kg and xylazine 10 mg/kg. Once the mouse presents signs of deep anesthesia (step 1.7), perfuse with ice-cold phosphate-buffered saline (PBS) followed by 4% PFA.

3.2. Remove implanted cannula carefully by firmly grasping the connector with forceps and pulling up gently.

3.3. Extract and post-fix the brain for 24 h in 4% PFA[23].

3.4. Perform 3–10 min washes with PBS.

3.5. Cut the brain in 50 µm sections using a microtome (see **Table of Materials**).

3.6. Mount the sections in slides with hard-set mounting media with DAPI to stain nuclei and cover slides.

3.7. After drying, observe the sections under the confocal microscope and verify the implanted cannula location and expression of Ch2R fused with any fluorescent protein.

REPRESENTATIVE RESULTS:

The reach-to-grasp task is a paradigm widely used to study shaping, learning, performance, and

kinematics of fine skill movement under different experimental manipulations. Mice learn to execute the task in a couple of days and achieve more than 55% accuracy reaching a plateau after 5 days of training (**Figure 2A,B**). Similar to what has been previously reported, a percentage of animals do not perform the task appropriately (29.62%), and those should be excluded from further analysis[30]. These include a subset of non-learner mice (6/54 mice, 11.1%) that from the beginning of training miss the target aiming too far from the pellet or perform the grasping movement before they are in the correct position over the pellet. During the first training days, another group performed the task with high accuracy but started performing poorly by aiming too far from the pellet by day 3-4 (10/54 mice, 18.51%). Within this group, some mice start training using a preferred paw but change their preference after a few days; this has been previously discussed by Chen et al., 2014[30].

The reach-to-grasp movement is highly stereotypical from trial to trial and within animals (**Figure 2**). The use of high-speed videography allows tracking the trajectory of movements making it possible to analyze kinematics at different phases in the control condition and during optogenetic stimulation (**Figure 1E** and **Figure 2C**). This approximation results in a quantifiable assessment of parameters such as distance traveled, velocity, acceleration, end-point, and trajectory (**Figure 2 C-E**). It is possible to analyze both multi-reach trials, where the mouse reaches multiple times before retrieving the pellet, and single-trial events, where the mouse retrieves the pellet in a single reaching movement. The trial is finished when animals push the pellet away or reinitiate the trial by going to the rear of the cage. A quantitative comparison of trajectories under different experimental conditions is achieved with principal component analysis (PCA) followed by k-means clustering (**Figure 3J-K**)[23,25].

During most training sessions, mice sometimes fail to grasp the pellet (missed trials). Some manipulations change the number of missed trials and hence the accuracy of the task. Then it is essential to analyze differences between hit and missed trials. In our hands, missed trials result from changes in three distinct phases of the movement: (1) the paw modifies its trajectory before it crosses the chamber opening (initial error), (2) the paw modifies its trajectory after the paw crosses the opening (final error), and, (3) failure to collect the pellet (grasp error) (**Figure 2 I,J**)[13]. A general characteristic of missed trials is that mice start the grasping movement further away from the pellet (end-point) compared to hit trials (**Figure 2G**). Additionally, misses associated with the mouse posture are measured as significant differences in body angle between hit and missed trials (**Figure 2H**).

Depending on the structure or neuronal population targeted with optogenetics, one can expect differential effects over behavior[7,19,23,31-33]. The current protocol describes the impact of activating spiny projection neurons (SPNs) in the striatum in contralateral or ipsilateral hemispheres about the preferred paw used by the mouse during the reaching movement (**Figure 3**). Contralateral activation of D1 dopamine expressing SPNs, which give origin to the basal ganglia direct pathway, reduced grasping success to 64.9±8.8% compared to control conditions (**Figure 3B**). Kinematic analysis reveals that during optogenetic stimulation, the paw trajectory described an oscillatory pattern, shown by an increase in the traveled distance to 218.4±19.2% of control, leading to the incapability to target the pellet and an increase in initial error type I

(**Figure 3F)**. PCA analysis shows that all trials' trajectories during contralateral D1 SPNs activation separated in a cluster with almost no overlap with a control cluster, indicating a low similarity (**Figure 3J-K**).

On the other hand, activation of dSPNs in the ipsilateral side lead to an increase in trajectory dispersion shown by PCA analysis (**Figure 3K**) without affecting reaching success (120.7±23.6%, n = 4), total distance traveled (136.3±35.5%), or maximum velocity during the reaching phase (117.3±10.3%) (**Figure 3C**), indicating that ipsilateral D1 SPNs activation modified in some degree the reaching trajectory without changing the behavioral outcome (**Figure 3G-I**). Kinematic analysis indicates subtle changes in movement control by ipsilateral manipulation. Finally, body posture analysis shows a shift in body angle during contralateral D1SPNs activation (**Figure 3L**). It is highlighted that even this simple task has many components that permit proper movement execution to attain a goal.

FIGURE LEGENDS:

Figure 1: **Experimental setup.** (**A**) Schematics of the behavioral chamber. A chamber made with acrylic sheet having the following dimensions in cm: 18.5 (h) x 8.5 (w) x 20 (d) with a front window 1 (w) x 5 (h) and a small shelf 8.5 (w) x 4 (d). (**B**) Photograph of LED cannula (left) and wireless receiver (right). (**C**) Side view of a mouse with the implanted LED cannula connected to the receiver while performing the reach-to-grasp task (the white arrowhead shows the receiver, the asterisk shows the dental cement holding the cannula, and the empty arrowhead shows the pellet). (**D**) Sketch of the experimental setup. Two high-speed cameras record the reach in two dimensions, while a third collected a panoramic view of the task, including the mouse's location from the mirror under the chamber. Animals were free to choose their preferred paw, and stimulation sides always refer to the side of the preferred paw. (**E**) The exact position of the cameras and representative image of each camera during a trial. This figure has been adapted from Reference[23].

Figure 2: **Kinematic analysis of reaching behavior.** (**A**) Timeline of the experiment from day-0 (d0) to day-25 (d25). (**B**) Performance during the reach-to-grasp task over time measured as pellet retrieval accuracy (total number of successful grasps/total number of trials x 100). (**C**) Example of trajectory tracking from a high-speed video. (**D**) Individual trajectories of the paw during the hit and missed trials. (**E**) Total distance traveled by the paw during the hit and missed trials. (**F**) Acceleration of the paw through the trajectory in the hit and missed trials plotted as distance *vs*. velocity. (**G**) Summary of end-points distance in hits = 3.16 mm, misses 6.08 mm (Mann-Whitney-Wilcoxon test$_{\text{missed vs. hit}}$ $U = 4184$, $p<0.0001$, $n = 28$ mice). (**H**) Differences in body angle in the two kinds of trials, misses = 8.4±5.3°, hits 6.7±4° (Mann-Whitney-Wilcoxon test $U = 6437$, $P = 0.0243$, $n = 28$ mice). (**I**) Schematics of the three types of errors. (**J**) Proportions of the three kinds of errors made by the mice in control conditions. This figure has been adapted from Reference[23].

Figure 3: Optogenetic activation of contralateral and ipsilateral D1 SPNs during reach-to-grasp behavior. (**A**) Schematics of the stimulation paradigm. (**B**) Success rate compared to non-

stimulation trials. (**C**) Change in traveled distance compared to control conditions. (**D**), (**G**) Two-dimensional plots of paths made by the paw with and without optogenetic stimulation. (**E**)**,** (**H**) Total distance traveled by the paw during reaching movements. (**F**)**,** (**I**) Summary of the distributions of the different kinds of errors. D1 contralateral: Initial error or type I (control = 18.2±11.6 %, stimulation = 79.9±8.2 % Fisher's exact test, $p<0.0001$). (**J**) Example of PCA analysis of the trajectories in control conditions compared to contralateral activation of D1SPNs. The shaded area represents the cluster of each condition, and the star is the cluster centroid. (**K**) Summary of the overlap between clusters of the different experimental conditions. (**L**) Changes in body angle. This figure has been modified from Reference[23].

DISCUSSION:

The use of optogenetic manipulation of neuronal populations in well-defined behavioral paradigms is advancing our knowledge about the mechanisms underlying motor control[7,23]. Wireless methods are especially suitable for tasks that require tests on multiple animals or free movement[34,35]. Nevertheless, as techniques and devices are refined, it should be the go-to option for any behavioral task combined with optogenetics[34,36].

The current method has many advantages because miniaturized LEDs provide a reliable light source with high intensity, and implants can be used in studies requiring stimulation over several days. Nevertheless, insertion of an optic fiber for opsin stimulation may mechanically damage brain tissue, cause infections and occasionally inflammation at the location of the canula[37]. Long-lasting high-frequency optogenetic stimulation has been demonstrated to produce heat and could cause phototoxity[37]. It is possible to reduce phototoxicity by using red-shifted effector opsins that are activated with red or even near-infrared light, which reduces the generation of heat[38].

Also, since the pins to connect the receiver remain outside of the skull, sometimes mice can cause displacement or detachment of the cannula if dental cement is not applied correctly; this often leads to damage of brain tissue and decreases the number of subjects to be taken into account for further analysis. Recent developments have introduced fiberless optogenetics, which uses particles that can emit visible light through up-conversion luminescence in response to near-infrared light penetrating deep in the brain tissue[36]. Fiberless devices bring the opportunity to stimulate optogenetically over longer time frames in freely behaving animals with unnoticeable implants[35]. This allows for unrestrained motion even in water mazes, to have multiple animals housed together (to avoid the impact of social isolation), and to study animals in more naturalistic settings[35,36].

Even with all the advantages that fiberless optogenetics offers, it still faces biocompatibility and heat generation challenges. The efficiency of photon conversion also limits it. Finally, further improvement is required for high emission efficiency[34,36].

The combination of this paradigm with high-speed videography allows for kinematic analysis under different experimental conditions. This offers sensitive detection of even subtle effects over distinct components of behavior and motor control. As more analytic tools develop, it is

possible to have online kinematic analysis and an in-depth characterization of motor behavior in different contexts. A thorough quantification of mouse reaching movement kinematics has been recently published by Becker et al.[25].

The possibility of selectively manipulating neuronal populations in freely moving animals with minimally invasive techniques allows one to dissect the contribution of specific neuronal types in precise behavioral tasks[23]. The reach-to-grasp task is a translatable paradigm for motor behavior[13,19]. It is known that conserved brain structures participate in the different phases of acquisition, learning, and performance of the task[7,12,23]. Revealing the neural circuits that underlie this behavior will increase the understanding of motor control. Several studies highlight the importance of bi-hemispheric control over unimanual tasks, especially when high dexterity is needed[20-22]. The kinematic analysis combined with optogenetic manipulations allows for the investigation of the different mechanisms of this complex behavior. It could help to analyze the contribution of sensory-motor feedback in normal conditions and disease models.

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The authors declare no disclosures.

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Figure 1

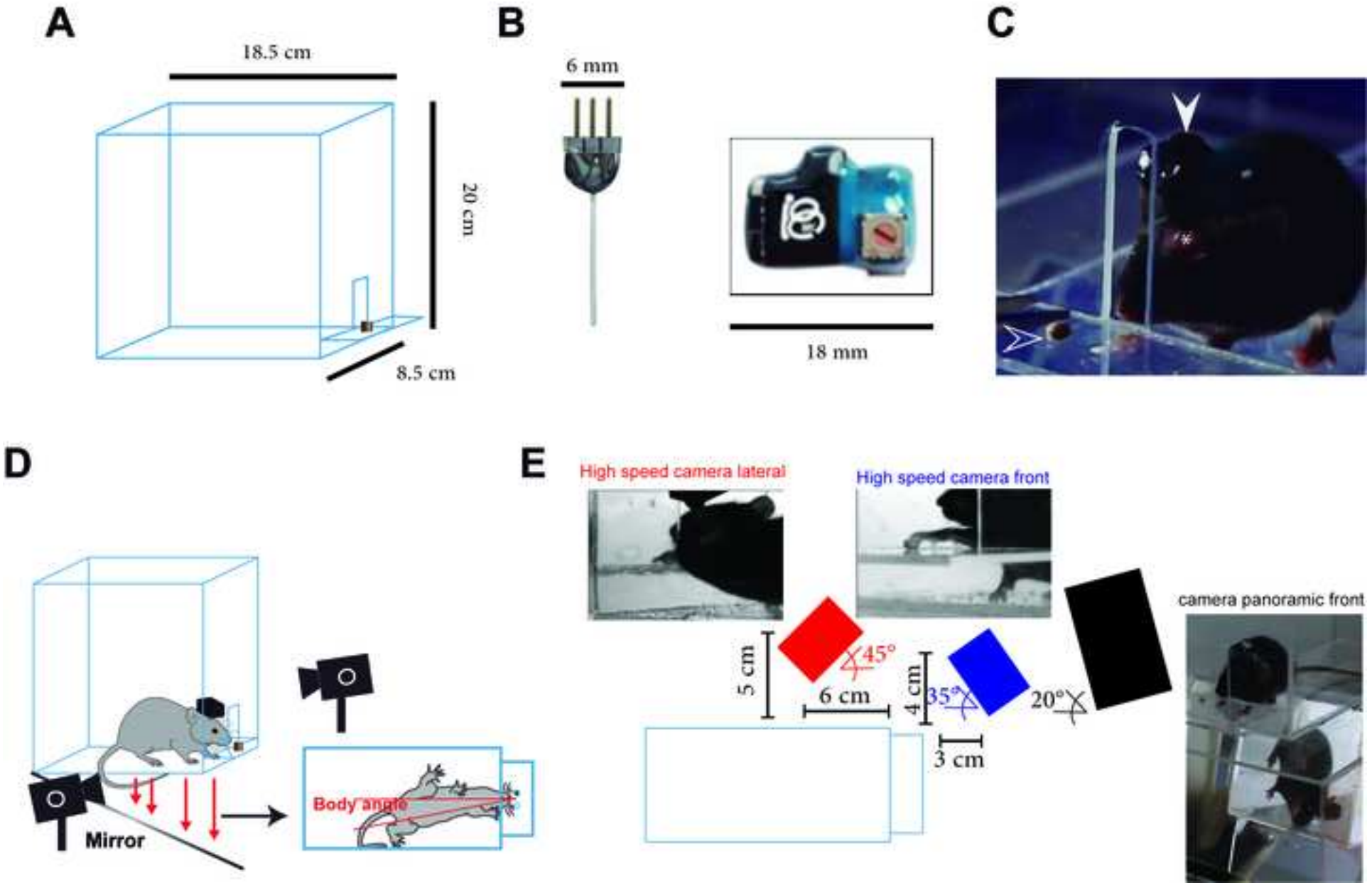
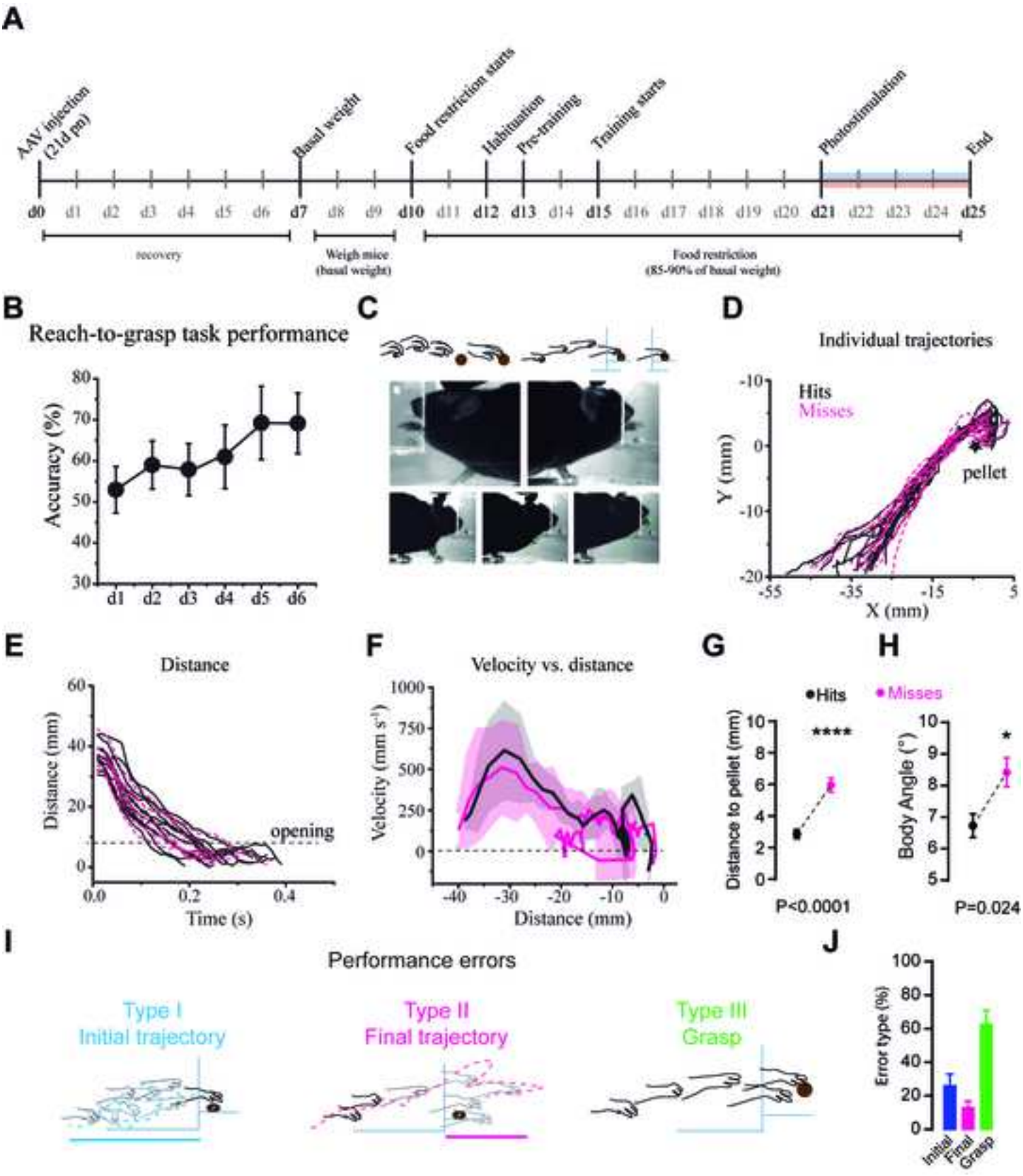
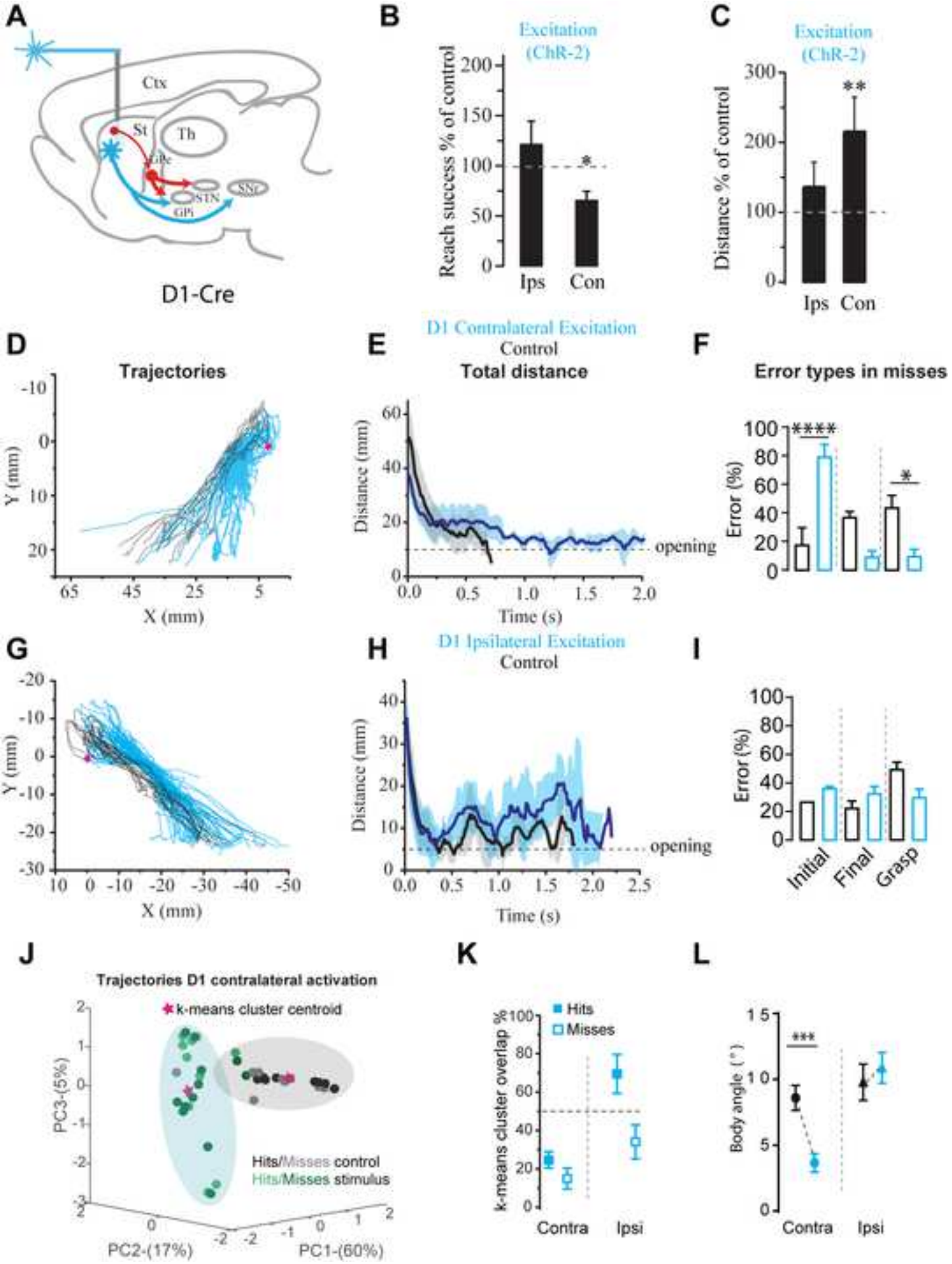


Figure 2





UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
Instituto de Fisiología Celular
Neurodevelopment and physiology department

Instituto de Fisiología Celular

October 6th, 2021

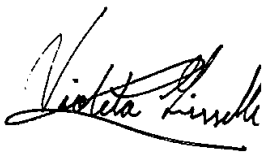
Dear Editor,

Please receive the revised manuscript entitled 'In vivo wireless optogenetic control of skilled motor behavior' by Diana L. Rodriguez-Munoz, Omar Jaidar, Marcela Palomero-Rivero, Mario A. Arias-Garcia, Gordon W. Arbuthnott and Violeta G. Lopez-Huerta.

We appreciate very much your work on the manuscript to improve the writing and attach to the journal guidelines. We have revised the manuscript and done the pertinent changes. We also reply to the reviewer concerns and added a few lines in the manuscript to address the comments.

We thank for your and reviewers' time and comments that have substantially improved the paper and we hope that after the revision the manuscript is now suitable for publication.

Sincerely



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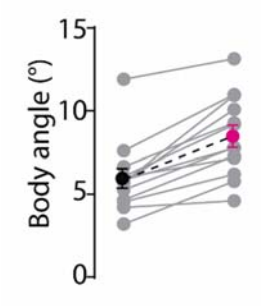
Minor Concerns:
The data in figure 2G&H (formerly 2D&F) is comparing differences in successful execution of complex behavior and should be between animals. A paired comparison of data from the 28 individual mice is suitable, but comparison of individual reaches pooled across all mice is not appropriate.

Thank you for your accurate observation. We have done the analysis as paired comparisons with a subset of animals chosen randomly and we get a very similar result and figure (shown below).



The definition of trials and reaching success need to be defined in the protocol, not just in the response to reviewers.

We have included this information in lines 325-328.

Are cameras position on the same side regardless of which paw is used for reaching, or are cameras repositioned based on paw preference? If so, this should be indicated in the protocol.

Thank you for noting this point. Cameras are positioned left or right of the chamber depending on sidedness of the animal. We have included this information in lines 267 and 268 (step 2.12).

In the optogenetics experiments shown, was the stimulation started as the mouse approached the front of the chamber, or as the reach was initiated? The movement is so quick that it seems that manual triggering of the stimulus must be done prior to reach onset and may therefore affect the stance and movements prior to reach initiation. Do you have any quantification of results that would inform the reader of inter-user reliability with manual triggering?

The stimulation was triggered when the mouse took the stance to initiate the movement. Only one experimenter performed all the experiments. As the reviewer points out we also thought the posture of the animal could change with our manipulations and we performed different analysis of the stance as step width and length and body angle. We found differences in body angle but not in the other parameters. Additionally, kinematic analysis shows that not only the posture is affected but also the trajectory of the movement changes with optogenetic stimulation. We are aware of the inconvenience and downsides of manual stimulation and we are currently working to automatize the system. At the

moment, we do not have any data to inform the reader of inter-user reliability. We have added a recommendation in line 280.

Is the difference in distance between ipsi and contra stimulation (Fig 3B) indicative of a larger effect of contralateral stimulation? In E2, the duration and distance in control mice appears significantly different from controls in D2 and the sample traces in Fig 2E.

Thank you, we do observe a larger effect with contralateral manipulations. We also observe some differences between the control condition of animals implanted in contralateral and ipsilateral hemispheres in relation to the paw but the overall performance is not significantly different among controls. For example, we noticed that the distance in control of ipsi and contralateral conditions is different but the performance of the animals is not significantly different and the proportion of errors is also similar in both controls. It is possible that the cannula is damaging some involved regions and that is the reason we observe differences in the controls.

What is the distance from the inside of the front chamber wall to the pellet? Is this distance similar across trials?

The distance from the inside of the front chamber wall to the pellet is 10 mm, additionally the mice took the stance to perform the reaching movement at around 15-20mm from the inside of the chamber. The distances are very similar across trials and individuals.

UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
Instituto de Fisiología Celular
Neurodevelopment and physiology department

Instituto de Fisiología Celular

August 23th 2021

Dear Editor,

Please receive the revised manuscript entitled 'In vivo wireless optogenetic control of skilled motor behavior' by Diana L. Rodriguez-Munoz, Omar Jaidar, Marcela Palomero-Rivero, Mario A. Arias-Garcia, Gordon W. Arbuthnott and Violeta G. Lopez-Huerta.

In response to the accurate and appropriate comments from the reviewers, we have detailed several steps of the protocol and revised the figures for clarity. Also, we have edited the manuscript to address their concerns.

We really appreciate the reviewers' comments that encouraged us to improve the paper and we hope that after the revision the manuscript is now suitable for publication. In the following pages you can find a point by point response to each comment.

Sincerely

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Comments: We thank the editor and reviewers for their constructive suggestions and insightful comments that we believe have improved the clarity of our protocol. Corrections and changes have been highlighted in the text (blue text).

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. We have read the manuscript several times to spot and correct spelling issues and use grammar to the best of our knowledge.
2. Please revise the following lines to avoid previously published work: 185-199, 201-203, 204-206, 245-247. We have rephrased the text to avoid wording of previously published work. Please note that the line numbers have changed.
3. Please rephrase the Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “The present protocol describes. …”. Here the word limit is exceeding. We have simplified our summary, lines 15-18.
4. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.). We have revised our manuscript to avoid the use of personal pronouns.
5. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials (including reagents, instruments, software, etc.). Please sort the Materials Table alphabetically by the name of the material. We have removed any commercial names and added more details in our Materials table.
6. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes. We have adjusted the numbering in our protocol steps.
7. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. We have rewrite some of the steps in the protocol to offer more detailed information on how it is done.
8. Please add more details to your protocol steps:

Line 82: Please mention in detail what is meant by clean the surgery area? Please mention specifically what tasks are to be done. Done step 1.3 Line 96.
Line 95: Please write detail about the stereotaxic apparatus. Also, mention its relevance with the current study. Done in step 1.4 Line 99.
Line 104: Please specify the needle size. Done step 1.2 and 1.14 Line 92 and Line 134.
Line 109: Please mention in detail how the step is performed. Done step 1.16 Line 143.
Line 120: Please mention the composition of the mixture to be applied. Done step 1.21.
Line 125: Please mention the suture size. Modified step 1.24 Line177.
Line 173: Please mention how the euthanization was carried out. Modified steps 3.1 Line

259.
Line 177: Please mention how the processes will be carried out. In case you don't need to film this step, citations to published References should suffice. Modified steps 3.3-3.7 Lines 267-275.
9. Please include one-line space between each protocol step and then highlight in yellow up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader. Done.
10. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next and is in line with the Title of the manuscript. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in the imperative tense.
11. Please note that the Figure legends are overlapping with the previously published works. Reword them or provide Reprint permission if they are being extracted from any previously published work. We will provide reprint permission from previously published work.
12. Please spell out the journal titles in the References. Done, we apologize for these errors it seems the citation software had some problems.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

In, "In vivo wireless optogenetic control of skilled motor behavior," Rodriguez-Munoz et al. describe the use of miniature, wireless, head-mounted optogenetic control of skilled forelimb behavior. The single pellet reach to grasp task is a key tool for assessing skilled forelimb behavior in rodents and has been used to probe the roles of multiple motor circuits in skilled motor control. Forelimb movement during the task has been previously segmented into individual components by Ian Whishaw and the use of high-speed imaging would allow for a sensitive evaluation of deficits induced by injury or optogenetic control. Unfortunately, the extent of the analysis feasible with high-speed imaging is not touched upon in the manuscript. Movement errors are broken down into deficits in initiating reach, targeting the pellet, and grasping the pellet, while earlier work has segmented the reach into more discrete components. The most robust effect on success presented in the manuscript is the stance and initial positioning of the mouse, which does not depend on high-speed imaging.

We thank the reviewer for the insightful comments, indeed an important part of the current protocol is the high-speed imaging that we employed to perform kinematic analysis of the reaching movement. We apologize for not emphasizing its importance from the beginning but the analytical part is extensive, and we preferred not to focus the protocol on analysis. We observed that high-speed imaging allows to study even subtle effects over behavior that wouldn't be reflected by other forms of scoring. We added panel F in figure 3 to show how we used principal component analysis (PCA) to compare trajectories obtained with high-speed videography in different experimental conditions. Interestingly ipsilateral activation of D1SPNs does not change any performance parameters but it affects the

trajectory of the paw in hit and missed trials. We think that kinematic analysis can be useful in disease models where fine motor control is altered. We added a citation of the work by Becker et al, 2020 where they do a thorough analysis of the reaching movement kinematic.

Major Concerns:

What percentage of mice "never perform the task appropriately," (line 183) and what does that entail? Are these mice that continue to reach for the pellet with their tongues, or are they the over-shaped or nonlearner mice described by Chen, Gilmore, and Zuo (JoVE 2014)?

We apologize for not being clear before. We have included a paragraph (lines 232- 240) explaining in more detail the behavior of animals that “do not perform the task appropriately”. We have observed that all the animals are able to do a reaching movement but not all can successfully grasp the pellet either from the beginning of the training sessions or throughout them (those that have a ‘regression’ in performance), now we mention the percentages of non-learners 11.1% (6/54 mice) and over-shaped mice 18.51% (10/54 animals). As you correctly noted this has been discussed by Chen et al, 2014 and we have included this citation in the manuscript.

The camera position is confusing. I am assuming that the high-speed camera is meant to capture the forepaw movement; however, the statement that the high-speed camera is to "record from the rear of the cage," along with the illustration in Fig 1E don't indicate that the forepaw would be in the field of view. As the front camera is set at 30-60 fps, I am assuming that it is only for evaluating the position and stance. Is 100 fps for the high-speed camera sufficient to characterize reach when the entire movement is over in less than half of a second? Sathyamurthy et al recorded at 240 fps and Azim et al at 500 fps. Additionally, the figure legend indicates that two high speed cameras were used. The figure needs to be revised for clarity.

Thank you for noting this important point. We are sorry for the confusion and not explaining correctly, the camera is not placed at the rear of the cage but in the lateral side of the cage to have a rear view of the behavior. We have revised Figure 1 and added panel E to clarify this point with the exact measurements and position of the cameras. In our experience, you can track the trajectory with one high speed camera in 2 dimensions but if 3 dimensional analysis is needed a second high-speed camera should be added as in Azim et al, 2014. In our hands, 100-120 frames per second is enough to track the trajectory of the movement, we get around 60-70 points per reach in control conditions and usually that is increased with optogenetic manipulations. In a recent paper by Becker et al, 2020 they also employ 120 and obtain enough data for kinematic analysis.

Experimental details are sparse throughout. What is the strain and age of the mice used in these experiments? Strain plays a critical role in behavior and may affect outcomes on this task.

We have added lines 77-81 to give more details about our experimental model.

Please provide details on the optogenetics experiments. How was expression restricted to spiny projection neurons?

We now mentioned in line 76 that we used Drd1-Cre transgenic mice and we mention in line 147 that a cre-dependent AAV is used to restrict expression to spiny neurons.

How was the stimulus triggered? What were the stimulation parameters (mW, duration, frequency of stimuli, etc.)? What is the control? Is it stimulation with control vector or no light stimulus, and therefore, no additional heat?

The stimulus is triggered manually by the researcher with a remote controller, the stimulus was a continuous pulse 1.5-2 seconds and the intensity at the tip was 1 mW, we added details of the stimulation in lines 209-211. We have some control animals where we did not express the opsin (n=5). In these mice the light stimulation did not affect behavior.

It is unclear whether the analysis of initial distance and body angle (Fig 2D,F) represents effects of trials between animals or across trials regardless of animal.

In this analysis we pooled data from hit and missed trials of 28 mice.

Are trials defined as any reach beyond the opening of the box? Occasionally mice will reach out of the box in rapid succession; does this count as multiple trials? If the mice fail to make contact with the pellet, but rather mis-target, does that count as a trial?

We analyzed both multi-reach trials, where the mouse reached multiple times before retrieving the pellet, and single-trial events, where the mouse retrieved the pellet in a single reaching movement. The trial was finished when animals pushed the pellet away or reinitiate the trial by going to the rear of the cage.

As the higher error rates appear to be related to stance (distance to pellet, body angle), do these parameters change with the optogenetic stimulation?
We observe that the body angle changes with contralateral activation of dSPNs but it does not change when the ipsilateral hemisphere is activated. The end-point distance to the pellet also increases with some manipulations that affect the target accuracy, we did not show in the current manuscript but activation of the striatal indirect pathway neurons changes significantly this parameter.

Minor Concerns:
In describing the analysis of separate phases of single pellet reach behavior (lines 54-55), the authors should include mention of work by Ian Q. Whishaw (eg. Whishaw and Pellis 1990). Thank you for noting this important reference we were missing.

Are 20 trials sufficient to account for variability? Chen et al (JoVE 2014) performed 30 training reaches, Azim et al (Nature 2014) 20 successful reaches, and Sathyamurthy et al (Cell Reports 2020) 40 trials.
In our hands 20 trials are enough but we are aware that it depends on the goal of the study and animal model used.

The authors would be best served by employing the same scales for reaching trajectories throughout.

The trajectories come from a left view (right handed animal) and right view (left handed animal) and that's the reason why they are not the same.

References 6, 25, 27-29 are missing journal information.

We have corrected this important issue.

What is the information on the high-speed camera used?

We included this information in the Materials table.

What is a Mototool?

It is the name we have in Mexico for the rotary tool (dental drill). We have changed the name in text to avoid confusion.

Reviewer #2:
Manuscript Summary:
Wireless optogenetics is an important technique. One great chanllenge in studying circuits involved in motor behaviors is to perturb the circuit activity with high spatial and temporal precision, but least interrupt the motor actions. Using the wireless technology to control optogenetic stimulation can be one of the solutions. Therefore, I appreciate authors' effort of putting together this protocol. But I think authors need to add lots of details. As one important goal of this paper, readers should be able to replicate the procedure based on this protocol. But I doubt this current version provides enough guidance.

We really appreciate the reviewer's accurate observations to make possible to replicate the protocol.

Major Concerns:
The quality of surgery matters to the success of experiments. To be able to perform the surgery in the right way, details are needed. However, I don't think the paper provided enough.
(1) In surgery procedures step 1, what is actually done to prepare the surgery area, whate PPE should be worn?

We added this information in steps 1.3 and 1.5 lines 96 and 102.

(2) 5% of isoflurane is pretty high. Do you keep the concentration at high throughout the procedure? Or only use high concentration to induce anesethia? In other words, what is the isoflurane concentration to induce anesthesia, what is the concentration to maintain anesthesia?

The reviewer is right, we apologize for the missing information. We have specified the concentration for induction and maintenance in step 1.8 line 111.
(3) In surgery procedures step 9, how many body axes need to be adjusted? only pitch?

We have added details in step 1.10 line 120.

(4) In surgery procedures step 12, what kind of pipette is used? glass pipette? how to prepare the pipette? What is the diameter and shape of its tip? What is the speed of viral injection? How much volume?

Thank you for pointing out these important missing information, we have included all the details in steps 1.2 line 92, 1.17 line 147 and 151.

(5) What is the purpose of meloxicam? Analgesic?

Yes, we have added it in step 1.26 line 184.

(6) In reach-to-grasp training, I don't fully understand how the food restriction is done. For example, appoximately how much food is provided a day, how long does the food restriction last? Every day throughout the training period? What signs indicate health issues resulting from food restriction? Is water also restricted or not?

We have modified our figure 2 panel A to clarify the time line of the experiment, there we specify the length of the food restriction period. We have added information about the amount of food during food restriction in step 2.1 line 194. We have included details about health evaluation of the mouse in lines 195-197.

(7) During habituation and pretraining (reach-to-grasp training steps 3-6), how much of pellets is given?

Since habituation until the last day of testing mice are given 20 chocolate pellets equivalent to 0.4 grams and 2.1-2.6 grams of standard pellets a day. We included this information in steps 2.2 line 199.

(8) In reach-to-grasp training step 7, how to train mice to run to the rear of the cage?

This can be achieved by waiting until the mouse is in the rear of the cage before placing a pellet in the indentation for each trial. We have added this information in lines 222-224.

(9) In reach-to-grasp training step 11, how the LED is turned on? Manually or controlled automatically by the video feedback?

Thank you for noting this information. The stimulus is triggered manually by the researcher with a remote controller, the stimulus was a continuous pulse 1.5-2 seconds and the intensity at the tip was 1 mW, we added details of the stimulation in lines 251-254. Although it would be ideal to have automatic video feedback we did not have the tools to develop such stimulation paradigm at the time.

(10) In reach-to-grasp training step 12, are those three cameras different types with different frame rate? Why is that? which one(s) are used to derive movement trajectory?

Thank you for noting this important point. We are sorry for the confusion and not explaining correctly. We have 2 high-speed cameras and one “normal” camera. In our experience, you can track the trajectory in 2 dimensions with one high speed camera but if 3 dimensional analysis is needed a second high-speed camera with the same characteristics of the first one should be added as in Azim et al, 2014. In our case we extracted trajectory data from only one camera (the high-speed camera in the side of the cage with a rear view of the behavior). The two high-speed cameras have a small field of view that does not allow to monitor the complete behavior. Then, we had a third camera (normal frame rate) to have a panoramic view of the behavior and a view of the mirror to collect body posture data. We have revised Figure 1 and added panel E to clarify this point with the exact measurements and position of the cameras.

(11) Is the cannula reusable? If yes, how to clean it, especially considering it is contaminated by PFA?

We have not re-used the LED cannula. But we would like to do in the future so we will start exploring ways to achieve this.

(12) What software or method is used to analyze movies to derive movement trajectory?
Minor Concerns:
Some part numbers and order information are needed.
(1) diameter and type of optical fiber glass optical fiber of 250 µm diameter line 85
(2) part number of eye spears We added the information in the Materials table
(3) size and part number of drill bit We added the information in the Materials table

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General guidelines

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- Figures should be submitted as separate image files rather than be embedded in the manuscript; please do not send figure panels individually
- For initial submission, we prefer TIFF or PDF files, but we will also accept JPEG or EPS files; PDF file size should be less than 3 MB
- For final production, we prefer high-resolution TIFF, PDF, or CDX (ChemDraw) files; each file should be no more than 20 MB
    - NOTE: The journal *Cell* prefers source files (AI, PSD, or PPT) for Leading Edge figures

    - NOTE: If possible, please create and save ChemDraw files on a PC rather than a Mac because Mac-created files may occasionally lose certain details (e.g., symbols) when processed on a PC, which we use in production
- For color figures, the resolution should be 300 dpi at the desired print size
- For black and white figures, the resolution should be 500 dpi at the desired print size
- For line-art figures, the resolution should be 1,000 dpi at the desired print size
- Make sure that any raster artwork within the source document is at the appropriate minimum resolution
- If used, color should be encoded as RGB, and to accommodate all viewers, red and green should not be used together
- Always embed fonts and use only Arial fonts
- Different panels should be labeled with capital letters
- Text should be about 6–8 pt at the desired print size
- Limit vertical space between parts of an illustration to only what is necessary for visual clarity
- Line weights or stroke widths should be in the 0.5–1.5 pt range
- Gray fills should be kept at least 20% different from other fills and no lighter than 10% or darker than 80%
- When using layers, reduce to one layer (flatten Artwork) before saving your image (except for the journal *Cell*)
- A scale bar, rather than magnification, must be provided for any micrographs

NOTE: Please review the data processing policy at the bottom of this page and confirm that your figures and manuscript adhere to this policy.

Journal-specific guidelines

Please note the following journal-specific figure sizes:

AJHG, Cell, Cancer Cell, Cell Chemical Biology, Cell Genomics, Cell Host & Microbe, Cell Metabolism, Cell Reports, Cell Reports Medicine, Cell Stem Cell, Cell Systems, Current Biology, Developmental Cell, HGG Advances, Immunity, Molecular Cell, Molecular Therapy and sister journals, Neuron, One Earth, Patterns, Stem Cell Reports, Structure, and *The Innovation*:

- For 2-column formats (such as research articles and reviews), the sizes are 85 mm (1 column), 114 mm (1.5 columns), and 174 mm (full width of the page)
- For 3-column formats (such as previews and commentaries), the sizes are 55 mm (1 column), 114 mm (2 columns), and 174 mm (full width of the page)

Cell Reports Physical Science, Chem, Chem Catalysis, Joule, Matter, and *Med*:

- For 1-column formats (such as research articles and reviews), the sizes are 112 mm (1 column) and 172 mm (full width of the page)

- For 3-column formats (such as previews and commentaries), the sizes are 53 mm (1 column), 112 mm (2 columns), and 172 mm (the full width of the page)

iScience and *STAR Protocols*:

- For all formats, the sizes are 134.6 mm (1 column) and 172 mm (full width of the page)

File types

TIFF, PDF, and CDX (ChemDraw) files are the preferred types for final production files.

TIFF

Virtually all common artwork-creation software is capable of saving files in TIFF format. TIFF is the recommended file format for bitmap, grayscale, and color images. TIFF supports several good compression schemes, ensuring that file sizes are kept to a minimum to aid easy file transfer. The LZW compression option should always be used.

CDX

CDX files are preferred for chemical structures. ChemDraw settings should be as follows:

- Chain bond angle = 120°
- Fixed bond angle = 15°
- Bond length = 0.45 cm
- Bond width = 0.015 cm
- Bold bond width = 0.05 cm
- Double bond space = 20% of bond length
- Stereo bond width = 0.056 cm
- Hash spacing = 0.065 cm
- Captions and atom labels* = Arial, 7 pt
- Atom labels "Show labels on Terminal Carbons" and "Hide Implicit Hydrogens" should be unchecked.

For initial submission, ChemDraw files must either be embedded in the text or supplied separately as TIFF or PDF files. For final submission, please use our one-column or full-page template.

NOTE: If possible, please create and save ChemDraw files on a PC rather than a Mac because Mac-created files may occasionally lose certain details (e.g., symbols) when processed on a PC, which we use in production.

EPS

Virtually all common artwork-creation software is capable of saving files in EPS format.

MS Office (Word, Excel, PPT figures)

Please convert your MS Office files to PDF. Make sure that any artwork placed into MS Office applications is at the appropriate minimum resolution. Do not reduce or enlarge any images after placement in an MS Office application, as this can lead to loss of image quality.

AI (Illustrator)

The AI file type should only be used for Leading Edge figures in the journal *Cell*. Please embed all fonts when saving your AI files. Please embed all images when saving your AI files (to embed external images, please refer to the "links" menu in Illustrator). If fonts or images are not properly embedded, they may drop out during reproduction or printing.

File sizes for articles under review

For newly submitted articles or revisions still under review, please keep figure files as small as possible (1–2 MB) so that editors and reviewers can more easily work with the files. If your article is accepted for publication, we will request higher-quality production figure files if necessary.

Solutions to common figure problems

File types not accepted by editorial manager

Editorial Manager accepts TIFF, PDF, CDX, JPEG, and EPS files. Other formats should be converted to one of these accepted file types. Virtually all common artwork-creation software, such as Adobe Photoshop, Adobe Illustrator, Canvas, ChemDraw, CorelDraw, SigmaPlot, Origin Lab, and others, is capable of saving files in PDF format. This option can normally be found under the "Save As" or "Export" commands in the "File" menu.

Files are too large

We place an upper limit of 20 MB on individual figure files. To reduce file size, you may try flattening layers or downsampling the file if the resolution exceeds our guidelines. If you are working with TIFF files, please use LZW compression. If you are working with PDF files, you may try selecting the "Reduced Size PDF" option from the File > Save As menu. If you are unable to reduce the file size below 20 MB without a loss of quality, please contact the journal's editorial operations associate for help.

Figure360

Authors are also encouraged to submit Figure360 videos to accompany their static figures. Each F360 video focuses on one figure from the article and is published alongside that figure. There is no limit to the number of figures that can be accompanied by a F360 video; however, each video must focus on only one figure. Videos are generally 2–3 minutes long and give readers a fun way to quickly and effectively grasp the major points of that figure. For additional information, examples, and instructions on how to create a Figure360 video, please visit our Figure360 page for authors.

Graphical abstracts

Please refer to the Cell Press graphical abstracts guidelines(opens in a new window) page for examples of and comprehensive instructions for graphical abstracts.

The graphical abstract is one single-panel, square image that is designed to give readers an immediate understanding of the take-home message of the paper. Its intent is to encourage browsing, promote interdisciplinary scholarship, and help readers quickly identify which papers are most relevant to their research interests.

Technical requirements are as follows:

- Size: 1,200 pixels ***square*** at 300 dpi
- Font: Arial, 12–16 points; smaller fonts will not be legible online
- Accepted file types: TIFF, PDF, JPEG
- Content: the graphical abstract should consist of one single panel

Please note that not all Cell Press journals publish graphical abstracts. If you are unsure of whether the journal that you are publishing in accepts graphical abstracts, please consult the journal's information for authors page.

Data processing policy

Authors should make every attempt to reduce the amount of post-acquisition processing of data. Some degree of processing may be unavoidable in certain instances and is permitted provided that the final data accurately reflect that of the original. In the case of image processing, alterations must be applied to the entire image (e.g., brightness, contrast, color balance). In rare instances for which this is not possible (e.g., alterations to a single color channel on a microscopy image), any alterations must be clearly stated in both the figure legend and the methods section. Groupings and consolidation of data (e.g., cropping of images or removal of lanes from gels and blots) must be made apparent and should be explicitly indicated in the appropriate figure legends. Data comparisons should only be made from comparative experiments, and individual data should not be utilized across multiple figures. In cases in which data are used multiple times (e.g., multiple experiments were performed simultaneously with a single control experiment), this must be clearly stated in both the figure legend and the methods section. In the event that it is deemed necessary for proper evaluation of the manuscript, authors will be required to make the original unprocessed data available to the editors of the journal. All accepted manuscripts will be taken through a data presentation image screening process before publication.

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Research Journals

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- Cancer Cell
- Cell Chemical Biology
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- Cell Host & Microbe
- Cell Metabolism
- Cell Reports
- Cell Reports Medicine
- Cell Reports Methods
- Cell Reports Physical Science
- Cell Stem Cell
- Cell Systems
- Chem
- Chem Catalysis
- Current Biology
- Developmental Cell
- Heliyon
- Immunity
- iScience
- Joule
- Matter
- Med
- Molecular Cell
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- One Earth
- Patterns

- STAR Protocols
- Structure

Trends Reviews Journals

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- Genetics
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- Neurosciences
- Parasitology
- Pharmacological Sciences
- Plant Science

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