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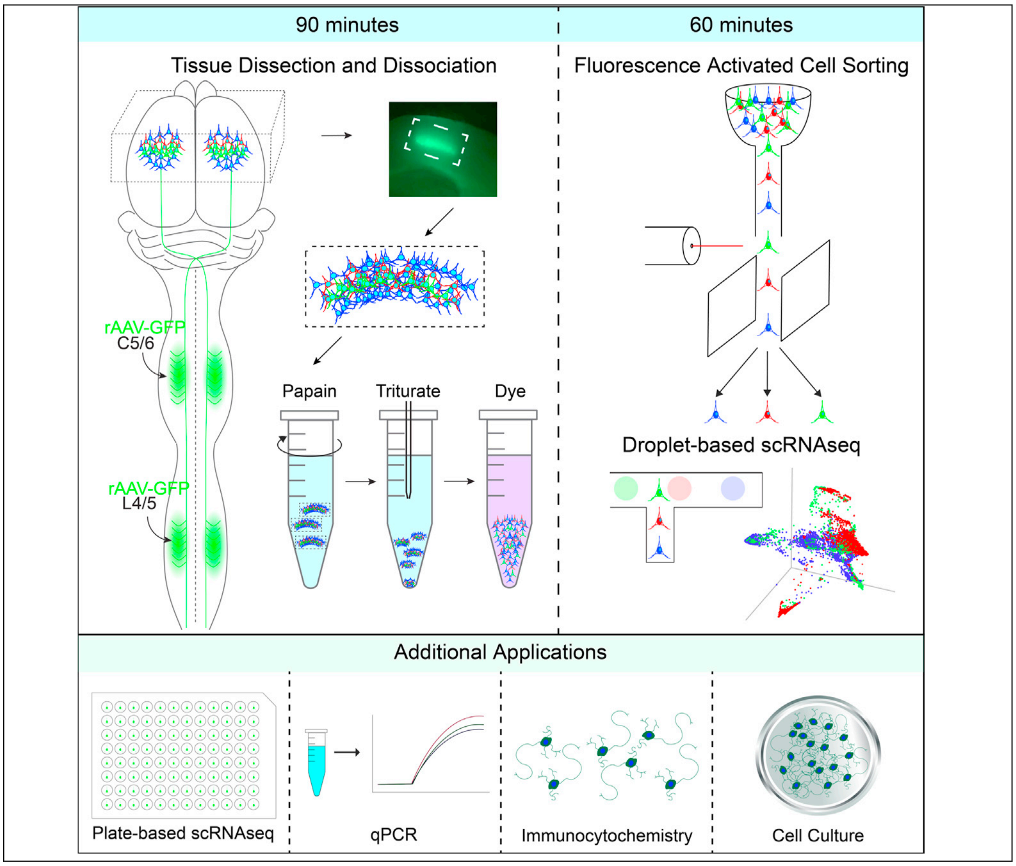
Protocol

Dissociation of intact adult mouse cortical projection neurons for single-cell RNA-seq



This protocol provides an improved pipeline for dissociating intact projection neurons from adult mouse cortex for applications including droplet and plate-based single-cell RNA sequencing, qPCR, immunocytochemistry, and long-term *in vitro* cell culture. This protocol provides a robust and reproducible dissociation pipeline that uses exclusively off-the-shelf reagents, not requiring the use of expensive dissociation kits. The unique incubation steps, in combination with the FACS gating strategy, results in unparalleled enrichment for intact cortical neurons from the adult brain.

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Highlights

Protocol to dissociate neonatal and adult mouse cortical projection neurons for scRNA-seq

Gentle dissociation ensures reliable plasma membrane and cytoplasmic integrity

Neuronal subtype selection via fluorescent viral labeling and FACS

Uses exclusively off-the-shelf reagents, rather than expensive dissociation kits

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Protocol

Dissociation of intact adult mouse cortical projection neurons for single-cell RNA-seq

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SUMMARY

This protocol provides an improved pipeline for dissociating intact projection neurons from adult mouse cortex for applications including droplet and plate-based single-cell RNA sequencing, qPCR, immunocytochemistry, and long-term *in vitro* cell culture. This protocol provides a robust and reproducible dissociation pipeline that uses exclusively off-the-shelf reagents, not requiring the use of expensive dissociation kits. The unique incubation steps, in combination with the FACS gating strategy, results in unparalleled enrichment for intact cortical neurons from the adult brain.

For complete details on the use and execution of this protocol, please refer to Golan et al. (2021).

BEFORE YOU BEGIN

Viral tracing methods are powerful tools in the functional dissection of neuronal circuits. Precision labeling of anatomically defined subsets of neuronal pathways allows for exploring the molecular mechanism that build, refine, and define these circuits under homeostatic and pathological conditions. While cortical projection neurons are well labeled via retrograde AAV uptake, maintaining an intact soma during dissociation has proved difficult due to the large amount of membrane devoted to their axons, which are lost during the dissociation procedure. Here we provide step-by-step instructions to dissociate adult neurons from primary motor cortex that were traced from the spinal cord using retrograde AAVs (Wang et al., 2018; Saunders et al., 2018; Tasic et al., 2018; Tervo et al., 2016) that maintains cytosplasmic intregrity of these neurons for comprehensive mRNA sequencing analysis. Additionally, we have also used this protocol to isolate cortical neurons from adult transgenic mice that express a floxed stop codon 5′ to TdTomato to isolate intrinsically fluorescent and nonfluorescent neurons.

We recommend preparing all reagents the night before the experiment to allow solutions to be at the correct temperature for the following procedures.

Preparation of pasteur pipette tips

Timing: 30 min

1. Fire polish salinized pipettes tips using an oil burner flame to a final internal diameter of 600 μm, 300 μm and 150 μm (Figure 1).

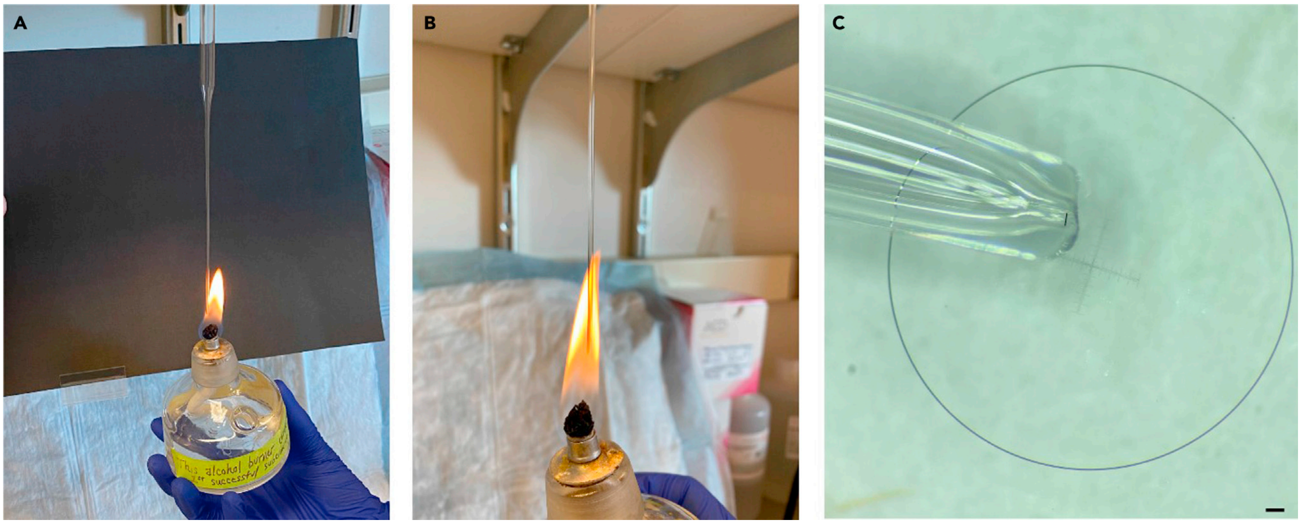

Figure 1. Preparation of pasteur pipettes
(A) Suspend Pasteur pipette under an oil burner.
(B) Allow flame to heat the pipette tip.
(C) Measure the internal diameter using a calibration slide under a dissection microscope.
Scale bar = 200 μm.

a. Measure tip diameters using a calibration slide under a dissection microscope at 20X magnification.

Note: Measure the internal diameter of flamed tips every 10 s of heat exposure to achieve the desired diameters. These times may depend on the temperature of the flame and will require optimization to avoid sealing the tip entirely.

Note: Make fresh tips for each dissociation experiment.

Preparation of artificial cerebrospinal fluid (aCSF)

Timing: 3 h

2. Prepare the aCSF
   a. Prepare the 1M stock solutions (following the recipe in "materials and equipment").
   b. Add reagents to 500 mL of water to make aCSF. Do not add the HCl.
3. Carbogenate the aCSF for 10 min.
4. pH the carbogenated aCSF and add HCl until the pH=7.3
5. Chill in the fridge until the aCSF is at 4°C, ~3 h.

⚠ CRITICAL: Carbogenate the aCSF before adding the HCl. The carbogen changes the pH of the solution.

Preparation of enzyme solution for tissue dissociation

Timing: 30 min

6. Warm dissociation buffer (3 mL per sample; the recipe is in "materials and equipment") to 34°C.
7. Activation of papain

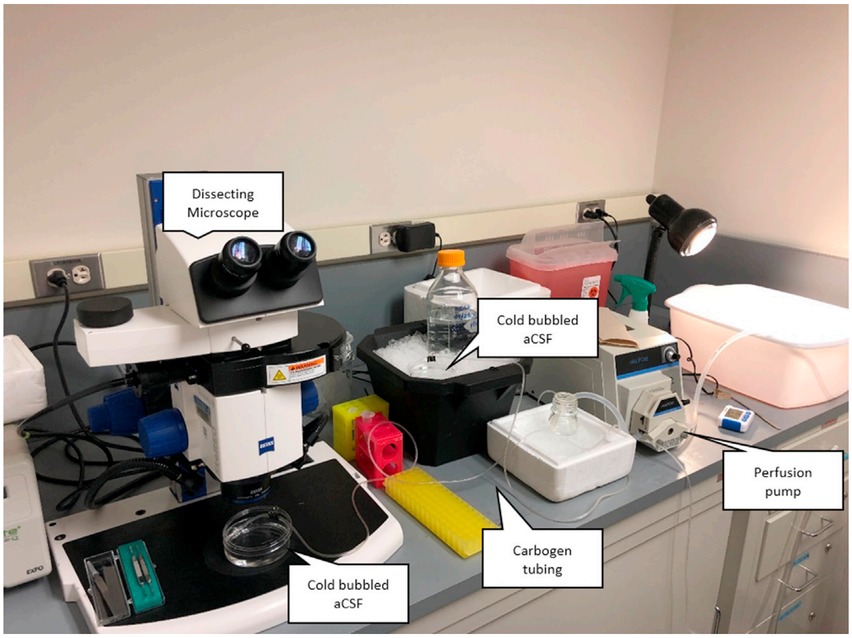

Figure 2. Dissociation set-up
Proximity of all equipment is critical to ensure brevity of protocol to maintain health of dissociated neurons. All surfaces should be wiped with 70% ethanol.

a. Add 5 mL of warmed dissociation buffer into a vial of papain to a concentration of 26 units/mL and allow to activate at 34°C for 30 min.
b. When ready to dissociate, dilute papain solution 1:2 in dissociation buffer. Each sample should digest in 3 mL of total solution in a 5 mL microfuge tube.

Setting up for the dissociation

Timing: 10 min

8. Prior to beginning the experiment, set up two petri dishes filled with aCSF. One of them on ice, one of them under a fluorescent dissection microscope.
9. Using ethanol-wiped binder clips, clip the tubing to each culture dish, ensuring that the aCSF gets continuously carbogenated throughout the entire experiment.
10. Set up the perfusion pump with clean tubing and run cold aCSF through the tubing in preparation for the perfusion.

⚠ CRITICAL: Ensure that the tubing for the perfusion pump has never been used to perfuse paraformaldehyde or any other fixative.

⚠ CRITICAL: See Figure 2 for an example set-up that ensures all solutions remain cold and carbogenated.

Pre-cooling the centrifuge

Timing: 10 min

11. Prior to beginning the experiment, set centrifuge to cool to 4°C and ensure 15 mL tube inserts are installed.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
rAAV-CAG-GFP	Addgene	37825-AAVrg
rAAV-CAG-tdTomato	Addgene	59462-AAVrg
Chemicals, peptides, and recombinant proteins		
Water	Milli-Q	N/A
$CaCl_2$	J.T. Baker	1332–01
NaH_2PO_4	J.T. Baker	3818–01
KCl	Sigma-Aldrich	746436
sodium L-ascorbate	ChemCruz	sc215877A
Taurine	Alfa Aesar	A12403
Thiourea	Alfa Aesar	A12828
Glucose	Sigma-Aldrich	G5767
HEPES	AmericanBio	AB00892
$MgSO_4$	AmericanBio	AB09013
myo-inositol	Sigma	15125
N-acetylcysteine	Acros	160280250
N-Methyl-D-Glucamine (NMDG)	mpbio	191506
$NaHCO_3$	J.T. Baker	3506–01
Sodium Pyruvate	Gibco	11360070
Trehalose	Alfa Aesar	A19434
HCL	J.T. Baker	953500
Na_2SO_4	Honeywell	239313
K_2SO_4	Sigma Aldrich	P0772
MgC_{l2}	AmericanBio	AB09006-00100
Ovomucoid Protease Inhibitor	Worthington Biochemical Corporation	LK003182
Papain	Worthington Biochemical Corporation	LK003176
Bovine Serum Albumin	AmericanBio	AB01088
Phosphate Buffered Saline	Sigma	P4417-50TAB
Cytoplasmic Dye	Invitrogen	L34974
Laminin	Sigma-Aldrich	L2020
Poly-D-Lysine	Sigma-Aldrich	P7405
Experimental models: Organisms/strains		
C57BL/6, Male and Female mice, P56	The Jackson Laboratory	000664
Ai14, Male and Female mice, P56	The Jackson Laboratory	007908
Rbp4 Cre, Male and Female mice, P56	Gifted from Dr. David Berson, Brown University	N/A
Other		
Carbogen gas (95% O2 and 5% CO2)	Airgas	n/a
Petri Dish 100 X 20mm	Corning	353003
Isoflurane	Henry Schein NDC	11695-6776-2
Adult Mouse Brain Matrix, 0.5mm	Braintree Scientific, Inc.	BS-SS 505C
Tygon S3 E-3603 Bio-based Tubing for the carbogen	Saint-Gobain	ACF00001
Razor Blades	Personna	94-120-71
Cell Sorter	Sony	SH800
130 μm Microfluidic sorting Chip	Sony	LE-C3213
Flow Cytometry polystyrene particle standard, 8μm–12.9μm	Sphereotech	PPS-5
Flow Cytometry polystyrene particle standard, 13μm–17.9μm	Sphereotech	PPs-6
Perfusion Pump	Merck Millipore	XX80EL004
High-Performance Precision Pump Tubing	Masterflex	96410–15
9 Inch Silanized Glass Pasteur Pipette	BrainBits	FPP

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Calibration slide	Motic	1101002300142
Eppendorf Tubes (5 mL)	Eppendorf	0030119460
Stovall Belly Dancer Shaker	Stovall Life Science, Inc.	n/a
Centrifuge	Eppendorf	5810 R
Epifluorescent Dissecting Microscope	ZEISS	495015-0008-000
5 mL polystyrene round-bottom tube with cells-strainer cap	Falcon	352235
Amnis ImageStream	Luminex	Mk II

MATERIALS AND EQUIPMENT

Artificial Cerebrospinal Fluid (aCSF) stock solutions

Reagent	Final concentration	Amount
$CaCl_2$	1M	1.47 g in 10 mL of water
NaH_2PO_4	1M	1.19 g in 10 mL of water
KCl	1M	1 g in 13.43 mL of water
sodium L-ascorbate	1M	1.98 g in 10 mL of water
Taurine	1M	1.25 g in 10 mL of water **Heat up to get in solution
thiourea	1M	1 g in 13.14 mL of water

Make stock solutions fresh for each preparation. Do not store more than 24 h. Store at room temperature (20°C–22°C).

Artificial Cerebrospinal Fluid (aCSF)

Reagent	Final concentration	Amount
Water	n/a	Up to 500mL
$CaCl_2$ (1M)	0.5mM	250 μL of 1M solution
Glucose	25mM	2.254g
HEPES	20mM	2.383g
$MgSO_4$ (1M)	10mM	5 mL
NaH_2PO_4 (1M)	1.25mM	625μL of 1M solution
myo-inositol	3mM	0.27g
N-acetylcysteine	12mM	0.97g
NMDG	96mM	9.3g
KCl (1M)	2.5mM	1.25 mL of 1M solution
$NaHCO_3$	25mM	1.05g
sodium L-ascorbate (1M)	5mM	2.5 mL of 1M solution
sodium pyruvate (100 mM)	3mM	15 mL
taurine (1 M)	0.01 mM	5μL of 1M solution
thiourea (1M)	2mM	1 mL of 1M solution
Trehalose (13.2 mM)	13.2mM	2.5g
HCl (96 mM)[a]		Add until pH = 7.3
Total	**n/a**	**500mL**

Make aCSF fresh for each preparation. Do not store more than 24 h. Store at 4°C.
[a] Carbogenate for 10 min before adding HCl as the carbogen will change the pH of the solution

Dissociation buffer

Reagent	Final concentration	Amount
Water	n/a	Up to 100mL
$Na_2\,SO_4$ (82mM)	82mM	1.17g
$K_2\,SO_4$ (30mM)	30mM	0.52g

(*Continued on next page*)

Continued		
Reagent	Final concentration	Amount
HEPES (10 mM)	10 mM	0.24g
Glucose (10mM)	10mM	0.18g
$MgCl_2$ (1M)	5mM	0.5 mL of 1M solution
Total	n/a	100mL

Make this solution fresh for each preparation. Do not store for more than 24 h. Store at 4°C. Warm to 34°C prior to adding papain.

Stop solution		
Reagent	Final concentration	Amount
Dissociation Buffer	n/a	5mL
Ovomucoid Protease Inhibitor	n/a	5mg
Bovine Serum Albumin	n/a	10mg
Total	n/a	5mL

Make this solution fresh for each preparation. Do not store for more than 24 h. Store at 4°C.

STEP-BY-STEP METHOD DETAILS

Perfusion and region of interest (ROI) dissection

Timing: 10 min

Note: Adult mice that have been injected with retrograde AAVs to label cortical projection neurons in the region of interest (e.g., primary motor cortex) or adult transgenic mice with fluorescence-labeled cortical neurons have been used in this protocol.

1. Perfusion
   a. Euthanize mice (postnatal day 28 and older, male and female) with isoflurane (open drop method), if using neonatal mice, euthanize on ice.
   b. Transcardially perfuse with cold, carbogenated aCSF for 3 min at a rate of 3.75 mL/min.
   c. Dissect out the entire brain and place in one of the petri dishes filled with aCSF and continuously bubbled with carbogen on ice for 3 min.
2. Dissection
   a. Place the cold brain in the adult brain matrix and cut 500 μm thick sections through primary motor cortex using a fresh razor blade.
      i. Immediately place sections in cold carbogenated aCSF under the epifluorescent dissecting microscope.
   b. Under an epifluorescent dissecting microscope, macro dissect the regions of interest based on the location of fluorescent cell bodies as shown in Figure 3.

CRITICAL: This step must be completed quickly to avoid neuronal death.

Note: Dissociate an extra piece of tissue that does not contain your region of interest but is from a comparable location in cortex. This will serve as a negative fluorescent control for FACS. For our experiment, we dissected out primary motor cortex from a wildtype mouse that did not receive any viral labeling.

Tissue digestion and manual dissociation

Timing: Variable depending on age of mouse, up to 70 min

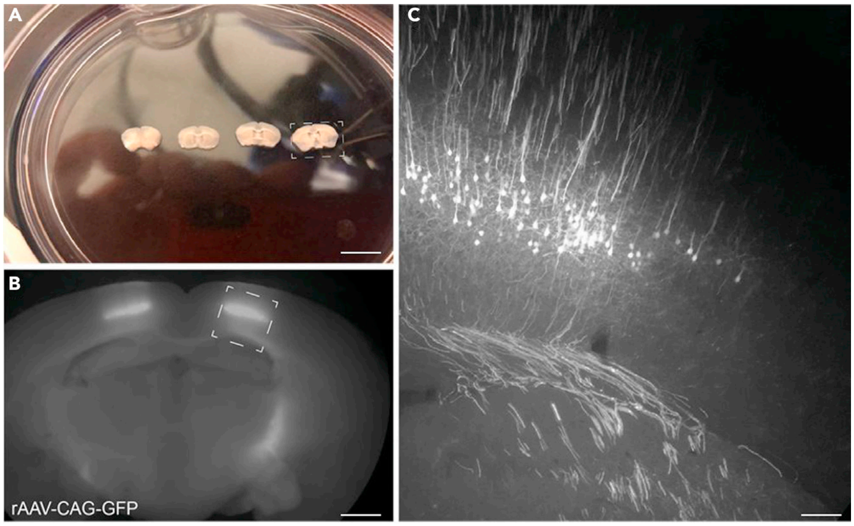

Figure 3. Tissue macro dissection

(A) 500 μm thick sections of cortex were cut using the adult brain matrix and placed in a petri dish with carbogenated aCSF.

(B) One section of cortex was visualized under epifluorescence. The area in the white box was dissected.

(C). High magnification of the area in the box from (B), showing individual cell bodies and axonal projections of corticospinal neurons.

Scale bar = 2 mm, 500 μm, 50 μm.

3. Transfer dissected tissue to 5 mL microfuge tubes containing 3 mL enzyme solution (dissociation buffer + Papain).

   Note: This is the only solution to contain Papain.

4. Place 5 mL tubes on a tube rack and secure the rack onto an orbital shaker in an incubation oven set to 34°C.
   a. To calculate incubation time, add 15 min to the animal's age in days to a max of 70 min). E.g., postnatal day 28 incubate for 43 min, postnatal day 56 incubate for 70 min.
5. Remove papain solution with a P1000 pipette, allowing the tissue chunks to remain at the bottom of the tube. Add 2 mL of Stop solution and place on ice and incubate for 5 min. Do not shake or mix the solution at this step.
6. Remove stop solution with a P1000 pipette and replace with 800μL of cold dissociation buffer.

   ⚠ CRITICAL: for this step, ensure that the dissociation buffer is cold and does not contain any papain.

7. Triturate sample with fire-polished pipettes (Methods video S1).
   a. Start with pipette with a diameter of 600 μm. Triturate 10 times.
   b. Triturate 10 times with the pipette of 300 μm diameter.
   c. Triturate 6 times with the pipette of 150 μm diameter.

   ⚠ CRITICAL: Triturate very carefully to avoid air bubbles.

   ⚠ CRITICAL: Tips should be thoroughly flushed with water and then dissociation buffer between samples.

8. Place samples on ice until ready for FACS.

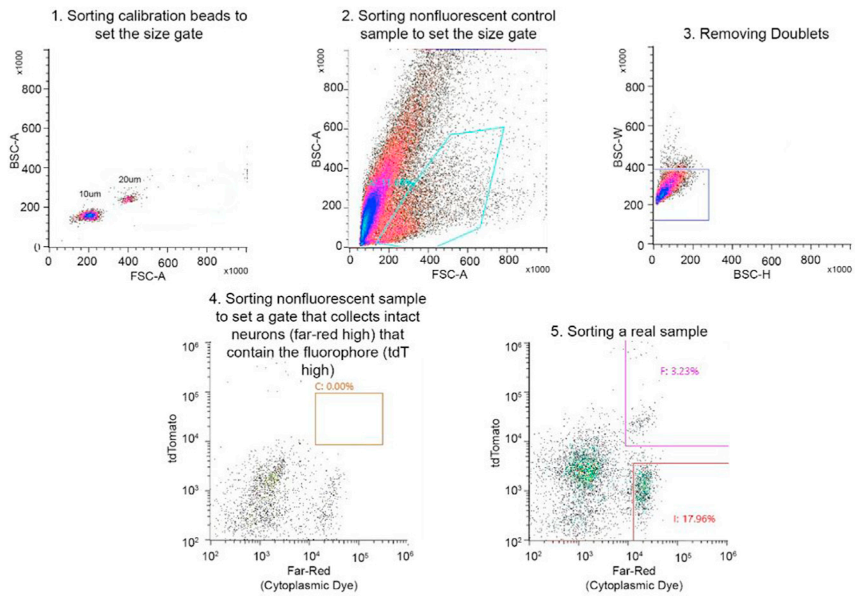

Figure 4. Example FACS gating strategy for collecting *Rbp4*+ neurons from *Rbp4* cre mice crossed with tdTomato reporter mice (ai14)

1) Polystyrene beads allow for gating on known size. 2) Based on beads size, size gate of sample is set. 3) Backscatter height and width are used to remove cellular doublets or intact cells plus debris. 4) Non-fluorescent control sample is used to set fluorescence gate (tdTomato from reporter mouse). 5) Collection shows intact cells expressing tdTomato and enriched in cytoplasmic dye.

Preparation of sony cell sorter for FACS

Based on morphological analyses, pyramidal neurons have a soma diameter of 10–20 μm (Oswald et al., 2013), while astrocytes, oligodendrocytes, and microglia have an average diameter of 6–8μms (Chai et al., 2017; Karasek et al., 2004; Davis et al., 2017). One of the key methods for neuronal enrichment is to set the initial size gate such that anything smaller than 10 μm is gated out.

Timing: 30 min

9. Turn on Sony cell sorter and insert a 130 μm Microfluidic sorting Chip.
10. Add a drop of 8μm–12.9 μm polystyrene particle standard beads and 13μm–17.9 μm polystyrene particle standard beads into 1× PBS.
11. Sort the bead solution with forward scatter area and back scatter area to establish the first gate (Figure 4).

Note: Cortical neurons have a soma size of approximately 10μm–20μm. Anything <10μm is likely debris.

Note: This can be done while waiting for the enzymatic dissociation.

Sample preparation for FACS

Incubation in cytoplasmic dye will allow for selection of intact neurons based on visualization of the cytoplasm using FACS. As this protocol dissociates long-distance projection neurons, the dissociation itself results in removal of the primary axon of the neuron. This leads to inevitable membrane disruption/ permeability which allows for uptake of the cytoplasmic dye and binding to intracellular amines primarily in neurons (as other cell-types remain intact). Our experiments culturing these dissociated neurons after FACS confirm that while they do take up the dye, they remain viable during and after dissociation.

Timing: 15 min

12. Add cytoplasmic dye directly to the sample at a concentration of 1:1000.
    a. Gently flick tube to mix into solution.

Note: The cytoplasmic labeling dye used here is marketed as a live/dead stain as it is used to differentiate between cells that have an intact or disrupted plasma membrane. It doesn't identify any cellular biochemical processes that suggest the cell is overtly dying *per se*. Removal of the primary axon from projection neurons during our dissociation protocol will transiently disrupt the plasma membrane allowing entry of the dye and thus identification of intact neuronal somata as evidenced from our robust single cell sequencing data.

13. Incubate on ice for 5 min.

Note: such dyes can exhibit toxicity to cells, and therefore incubation should not exceed 20 min.

14. Spin cells at 300 *g* for 10 min at 4°C.
15. Carefully remove supernatant with a P1000 pipette, being careful to not disturb the loose pellet.
16. Resuspend in 500μL dissociation buffer and triturate very carefully with a P1000 pipette to resuspend the cells.
17. Run the final cell solution through a 35 μm nylon mesh cap of a round bottom polystyrene test tube to ensure no large debris is present in the sample.

Sorting the sample

Timing: 45 min

18. Load the nonfluorescent sample that has been incubated in cytoplasmic dye into the sorter to set the remaining gates (Figure 4).
    a. Backscatter width and backscatter height are set to exclude doublets.
    b. Sorting based on far-red (the spectrum of the cytoplasmic dye) and tdTomato (fluorophore expressed by the neurons of interest) will allow for drawing of the gate to select for intact fluorescent neurons: tdTomato high, far-red (cytoplasmic dye) high.

Note: For our experiments, we did not need to perform compensation using this sorter. This may differ if using different fluorophores or an alternate sorter.

Note: When we sorted our retrogradely traced cells, the cells of interest were quite rare (0.88% of cells sorted) and the division between fluorescent and non-fluorescent cells could only be set by first sorting a negative control.

19. Using the gates drawn with the negative control, sort the samples.
    a. If doing 10X sequencing or other droplet-based sequencing methods, sort the samples directly into a microfuge tube containing 500 μL of cold dissociation buffer. Immediately proceed to sequencing step.

Note: Use the cell count obtained through sorting for estimation of the number of cells for 10X sequencing rather than using a cell counter- especially if collecting a rare or fragile population of neurons.

Note: For our experiments, we collected between 2,000 and 10,000 cells and collected them into 500μl of cold dissociation buffer. As the chromium chip allows for a maximum loading

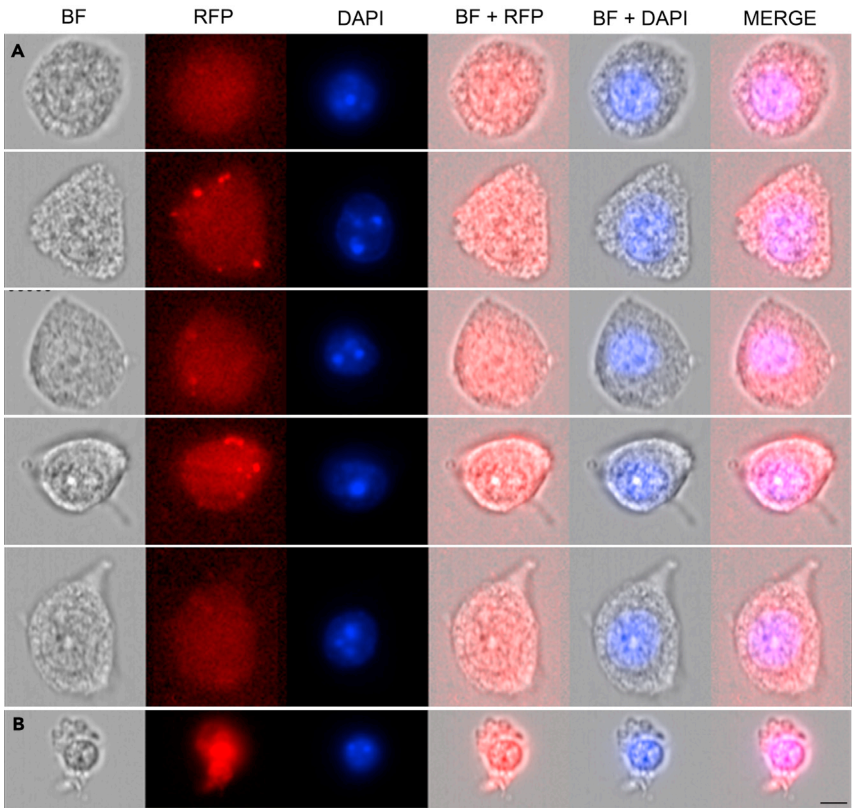

Figure 5. Neurons following dissociation

(A) Corticospinal neurons retrogradely labeled with rAAV-CAG-tdTomato were dissociated according to the protocol above. Robust detection of tdTomato confirms an intact plasma membrane and retained cytoplasm.

(B) Enzymatic digestion with 1 mg/mL pronase solution at room temperature without mixing results in a loss of cell integrity and adhesion of non-specific fluorescent debris to nuclei. Neurons were imaged prior to FACS using an Amnis Imagestream.

Scale bar = 5 μm.

volume of 34μl, we first spun down the collected cells at 500g for 5 min, and very carefully removed much of the supernatant. We then load all of the cells in 34μl of cold dissociation buffer into the chromium chip.

b. If using plate-based sequencing, sort directly into the plate.
c. If using cells for qPCR, sort up to 5×10^5 cells into 350μL of lysis buffer.
d. If using cells for immunocytochemistry, sort directly into a 96-well plate into fix.
e. If using cells for long-term tissue culture, sort into 20μL of warm media per 10,000 cells sorted. Add 100μL of warm media along with the sorted cells into each well of a 0.01% poly-D-lysine- and 10 μg/mL laminin-coated 24-well plate. For best results, plate 30,000 cells per well. Allow to adhere for 30 min. Remove media to remove debris and replace with 500μL of warm media. Maintain with 50% media replacement every other day.

EXPECTED OUTCOMES

This protocol will enrich for intact neurons from adult mouse cortex (Figure 5A). scRNAseq of cells collected using this protocol shows that 98% of cells sequenced are neurons and 2% of cells sequenced are endothelial cells. Of the neurons, all of them express *Ywhaz* (Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Zeta), a whole-neuron specific marker (Yao et al., 2020).

If tracing from the spinal cord, expect to collect approximately 500 neurons per mouse. If collecting all layer V cortical neurons from motor cortex, expect to collect 10,000+ neurons per mouse.

LIMITATIONS

This protocol dissects out primary motor cortex from adult mice after retrograde AAV labeling from the spinal cord. We found that viral production of a fluorophore (either GFP or tdTomato) makes cortical neurons more fragile and thus harder to keep intact during dissociation (Suriano et al., 2021). For our experiments, we dissected neurons 7–10 days after viral injection. Pilot experiments demonstrated robust expression of retrograde AVV-mediated fluorophore expression by this time point when tracing from either the cervical or lumbar spinal cord. We recommend using the protocol as early after fluorophore expression is confirmed. Additionally, neurons that were transduced with multiple fluorescent viruses simultaneously appeared too fragile to be dissociated using this protocol.

We also found that neuron fragility was associated with axon length. Cortical neurons with axons terminating in the lumbar spinal cord were more challenging to dissociate than cortical neurons with axons terminating in the cervical spinal cord.

TROUBLESHOOTING

Problem 1

Neurons are not intact following dissociation, before FACS (Figure 5B; step 4 and step 7).

Potential solution

This protocol is designed to ensure optimal cell integrity. If neurons do not have a cytoplasm after going through the protocol, prior to FACS, the most likely reasons are the enzymatic and manual dissociation steps. For the enzymatic dissociation (step 4), ensure that the size of the tissue pieces is small (no more than 2 mm in diameter). Additionally, ensure that solutions are well carbogenated prior to adding tissue. Once the tissue has been added, proper shaking of the solution in the incubator at 34°C is vital, as it allows the enzyme to property access all the tissue.

For manual trituration (step 7), it is vital to avoid air bubbles. Be sure to triturate slowly to avoid causing neuronal damage during this step.

Problem 2

Neurons are intact prior to FACS, but do not maintain their integrity during the sort (step 18).

Potential solution

In our experience, we were only able to achieve high cell viability using a chip-based sorter (such as the Sony Sh800) with a 130 μm chip. We failed to successfully maintain cell viability with a non-chip based FACS machine including the BD Aria, even with the 130 μm nozzle.

Additionally, ensuring that the FACS machine is pre-set to 4°C was critical.

Problem 3

Contamination of non-neuronal cells (step 12).

Potential solution

Incubation in cytoplasmic dye is key for neuronal enrichment (step 12). This protocol is specifically designed to dissociate projection neurons and therefore, even when being very gentle, the primary axon gets removed from the cell body. This results in slight permeability of the membrane only in neurons, as microglia and oligodendrocytes remain intact and do not take up the dye. Our sequencing supports this as less than 2% of sequencing cells were non-neuronal. If experiencing contamination, incubate with dye for an additional 5 min prior to spin.

Additionally, the FACS size gate is designed to exclude cells with a soma diameter of less than 10 μm, which based on morphological analysis, will only include pyramidal neurons.

Problem 4

Cell yield is lower than expected (step 15).

Potential solution

If cell yield is lower than expected, one possibility is that after the spin step (step 15), the pellet is removed along with the supernatant. The pellet is likely quite small and very loosely packed. To avoid, aspirate the supernatant very slowly and very carefully. If that is not sufficient, skip spin step altogether and sort the cells directly. This is not recommended as the cytoplasmic dye can be toxic; however, if the sort is quick (less than 20 min), the cells remain viable.

Problem 5

Multiple mice need to be pooled together for an experiment (step 1).

Potential solution

If multiple mice need to be pooled together, perfuse each mouse and proceed until step 2a. Once all of the brains have been cut into 500 μm thick sections, macrodissect the ROIs (step 2b) and leave the macrodissected chunks in cold bubbled aCSF. Only once all of the sections have been macrodissected, they should be transferred to the papain solution (step 3) and processed together moving forward. In our experiments, we routinely pooled 6–8 mice per prep.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, William Cafferty, william.cafferty@yale.edu.

Materials availability

This study did not generate new unique reagents.

Data and code availability

The published article includes all datasets generated or analyzed during this study.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2021.100941.

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AUTHOR CONTRIBUTIONS

Conceptualization, N.G. and W.B.C.; methodology, N.G. and W.B.C.; formal analysis, N.G. and W.B.C.; investigation, N.G., and W.B.C.; writing – original draft, N.G. and W.B.C.; supervision, W.B.C.; funding acquisition, W.B.C.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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