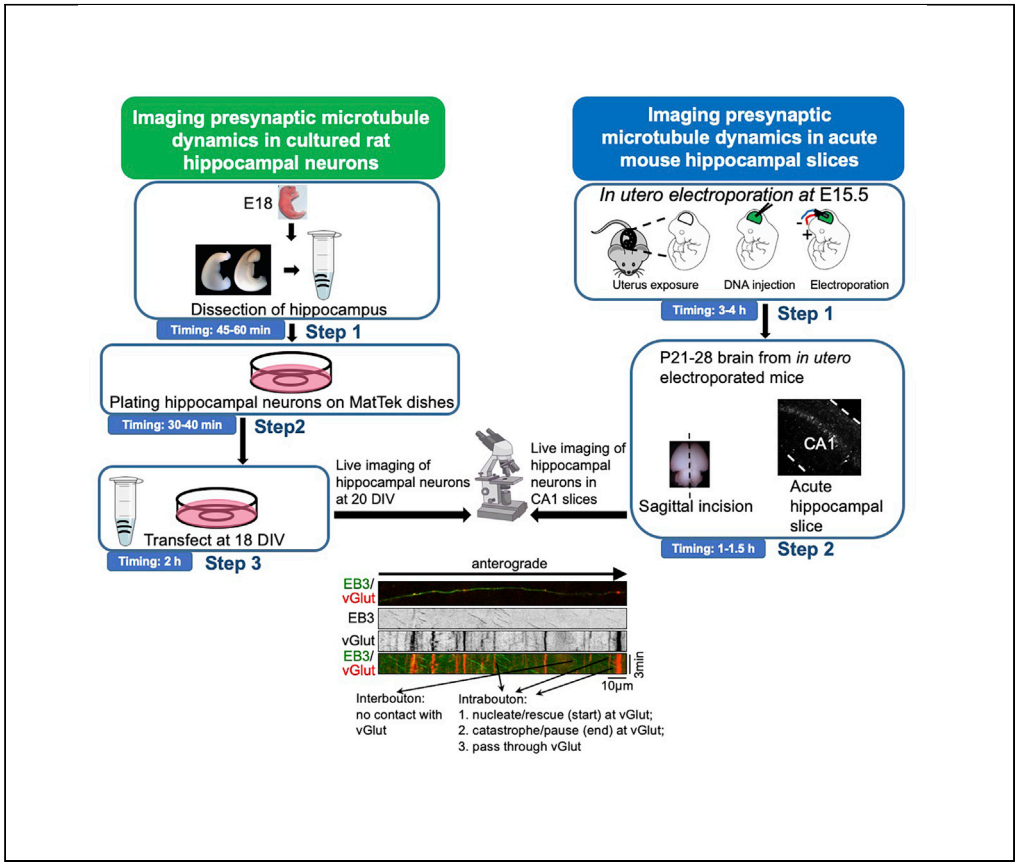
STAR Protocols

CellPress
OPEN ACCESS

Protocol

Live imaging of microtubule dynamics at excitatory presynaptic boutons in primary hippocampal neurons and acute hippocampal slices



Analyses of microtubule (MT) plus end dynamics at glutamatergic *en passant* boutons can be carried out in cultured primary neurons isolated from mouse or rat embryos or *ex vivo* in acute slices isolated from mice that had been electroporated *in utero*. Here, we describe a protocol for setting up and analyzing live image recordings of primary neurons and acute hippocampal slices expressing tagged versions of the MT plus end binding protein EB3 and the presynaptic vesicle markers vGlut1 or VAMP2.

Xiaoyi Qu, Atul Kumar, Francesca Bartolini

fb2131@columbia.edu

HIGHLIGHTS

Analysis of MT plus end dynamics at excitatory boutons in cultured neurons

Analysis of MT plus end dynamics at excitatory boutons in acute hippocampal slices

Pharmacological stimulation of MT nucleation at boutons in cultured neurons

Qu et al., STAR Protocols 2, 100342
March 19, 2021
https://doi.org/10.1016/j.xpro.2021.100342

STAR Protocols

CellPress
OPEN ACCESS

Protocol

Live imaging of microtubule dynamics at excitatory presynaptic boutons in primary hippocampal neurons and acute hippocampal slices

Xiaoyi Qu,[1,2,3] Atul Kumar,[1,3] and Francesca Bartolini[1,4,*]

[1]Department of Pathology & Cell Biology, Columbia University Medical Center, New York, NY 10032, USA

[2]Present address: Genentech, Inc., South San Francisco, CA 94080, USA

[3]Technical contact

[4]Lead contact

*Correspondence: fb2131@columbia.edu
https://doi.org/10.1016/j.xpro.2021.100342

SUMMARY

Analyses of microtubule (MT) plus end dynamics at glutamatergic *en passant* boutons can be carried out in cultured primary neurons isolated from mouse or rat embryos or *ex vivo* in acute slices isolated from mice that had been electroporated *in utero*. Here, we describe a protocol for setting up and analyzing live image recordings of primary neurons and acute hippocampal slices expressing tagged versions of the MT plus end binding protein EB3 and the presynaptic vesicle markers vGlut1 or VAMP2.

For complete information on the use and execution of this protocol, please refer to Qu et al. (2019).

BEFORE YOU BEGIN

Experimental considerations

There are two potential starting points: primary neurons or acute slices.

Primary neurons have the following advantages: (1) less laborious; (2) the success rate does not depend on animal survival; (3) faster image acquisition (fewer z-stacks required) to capture all MT nucleation/rescue events; (4) easy for tracking both the proximal and distal portion of the axon; (5) lower chance of interfering with baseline MT dynamics by shorter EB3-EGFP expression. However, the disadvantages are: (1) the circuit is not intact; (2) embryonic neurons; (3) healthy cultures need to be maintained for several weeks *in vitro*; (4) no correlation with behavioral readouts.

> ***Note:*** To follow the protocol using primary neurons, follow steps 1–9 in Before you begin and then proceed to 1–7 in Step-by-step method details.

Acute slices have several advantages: (1) spatial compartmentalization is maintained; (2) genetically engineered animals can be used; (3) acute slices are isolated from P21-P28 developed brains in contrast to cultured neurons that are derived from embryos; (4) no high-resolution live-imaging approach is available to perform optical studies in the hippocampus of live animals so acute slices offer a valuable alternative; (5) can be coupled with electrophysiological recordings of specific circuits. The disadvantages are: (1) more laborious and relies on the overexpression of wild-type or mutant genes that are hard to transfect or infect using viral particles *ex vivo*; (2) the success rate depends on mouse survival from electroporation *in utero*; (3) not easily adapted for high throughput studies or drug screenings; (4) no correlation with behavioral readouts.

Note: To follow the protocol using acute slices, follow steps 10–28 in Before you begin and then proceed to 8-13 in Step-by-step method details.

Primary rat hippocampal neuronal cultures

Day 1

Timing: 10 min

1. Coat MatTek live-imaging dishes (35 mm Petri dish, 14 mm microwell) with 0.1 mg/mL poly-D-lysine (PDL) in 1× sterile borate buffer for 24–48 h at 37°C. The microwell holds ~300 µL volume. PDL only needs to cover the microwell.

Day 2

Timing: 2–3 h

2. Wash microwells 3 times with ddH_2O.

Pause point: If not seeding cells immediately, cover the microwells with culturing medium. Do not let them dry.

Culture primary hippocampal neurons from E18 rats. Seed 50,000–70,000 cells into microwells of coated MatTek dishes. Spread the cells evenly in the center. After 2 h, add 2 mL of culturing medium.

Days 3–18

Timing: 30 min/medium change

3. To maintain the cultures, change medium every 3–4 days. If starting volume is ~2 mL, take 300–400 µL out and add 500–600 µL of conditioned medium.

⚠ CRITICAL: Using conditioned medium collected from dense cultures (100,000 neurons/cm^2) is critical during the first 2 weeks of culturing. Conditioned medium is composed of growth medium from dense cultures at the same DIV and fresh growth medium at 1:1 volume ratio.

⚠ CRITICAL: When adding or removing volumes from the dish, pipette from the side. Do not pipette directly into the microwell.

4. Healthy neurons will be evenly spread in the imaging chamber with multiple extended and branched dendrites and high spine density (0.5–0.8 spine/µm). This is critical for transfection efficiency and expected EB3 puncta motility (Figure 1).

⚠ CRITICAL: If the neuronal culture does not appear homogenously healthy prior to transfection, trash the cells and do not proceed to the next step. Unhealthy neurons will have short or very few dendritic branches, aggregated neurites, or lift off from the bottom of the well.

Transfection of primary rat hippocampal neurons

Using this protocol, the transfection efficiency of cultured neurons at 18 DIV is typically no higher than 1% regardless of density of plating, offering the advantage to readily identify single axons even in high density cultures.

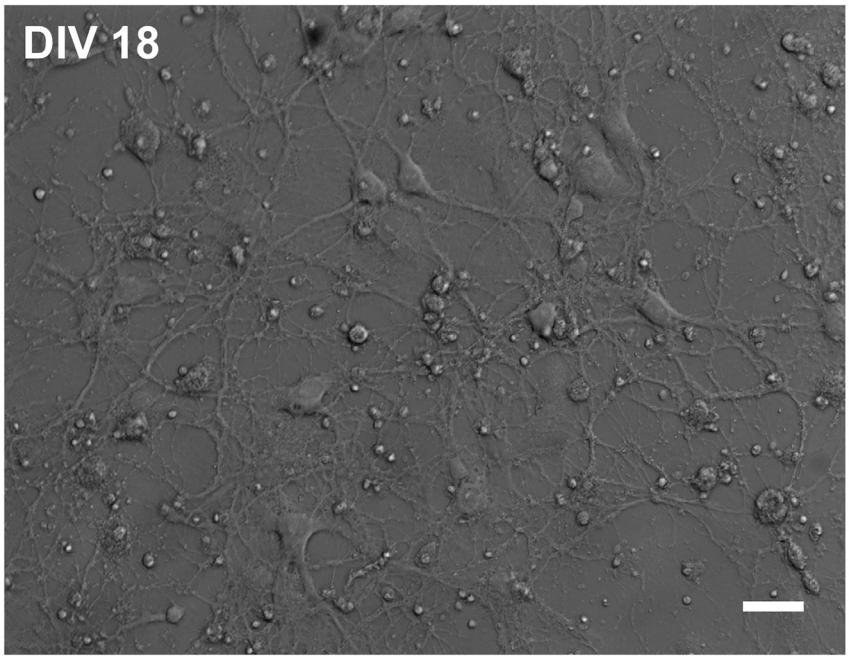


Figure 1. Neuronal morphology and density of an healthy culture at 18 DIV

Representative bright field image (20×) of hippocampal neurons isolated from E18 rat embryos and grown in culture for 18 days to illustrate appropriate cellular density and neurite arborization at this step of the protocol. Scale bar, 100 μm.

Day 19

Timing: 2.5–3 h

5. Hippocampal neurons are transfected at 18 DIV with Lipofectamine 2000 reagent. For one dish, mix 0.5–2.5 μg of plasmid DNA with 300 μL of incomplete neurobasal medium (no B27, no glutaMAX, no antibiotics → critical for transfection efficiency).
   For co-transfection, mix the 2 plasmid DNAs together into incomplete neurobasal medium at this step.

   Note: plasmid DNAs were made from low endotoxin maxiprep kit for example PureLink HiPure Plasmid Filter Maxiprep Kit and were titrated for each batch to make sure cells are expressing the proper level of proteins. Usually for EB3-EGFP (gift from Franck Polleux), use 1.2–1.5 μg /dish; VAMP2-mCherry (gift from Clarissa Waites) or vGlut1-mCherry (gift from Clarissa Waites), 0.5–0.8 μg/dish. EB3 expression should be bright enough to detect comets during live imaging, while having low cytosolic background. Similarly, for VAMP2 or vGlut1 expression, boutons should appear as bright puncta without interfering with the comet signals in the other channel.

6. For one dish, add 3 μL of Lipofectamine 2000 to the diluted plasmid DNA-media mix followed by vortexing. Incubate Lipofectamine-DNA mix at 20°C–25°C for 30 min.
7. Pipette original conditioned medium out from the dishes and save in conical tubes. Add lipofectamine-plasmid DNA mix dropwise to the microwells and make sure the microwells are well covered. Put dishes back into the incubator and the conditioned medium in conical tube in a 37°C water bath.
8. Incubate for 2 h.

9. Take dishes out of the incubator and aspirate out transfecting reagents. Tilt the dish so that the liquid is aspirated out completely and the pipette tip does not touch the cells. Add back 2 mL of conditioned medium immediately. Do not let the cells dry.

Brain *in utero* electroporation followed by acute hippocampal slicing

Timing: 3–4 h

Note: This protocol is adapted from Stoppini et al. (1991).

10. Monitor breeding females daily to assess for vaginal plugging and timed pregnant mouse at E15.5.

⚠ CRITICAL: The success of the procedure can be highly strain dependent, i.e., B6 mice are not good at caring for the pups after birth. We cross SV129 with B6 and use the F1s for most of our procedures but CD1 also work well.

11. Prepare plasmid DNA (1–2 μg/μL) in 0.5% Fast green dye (Sigma-Aldrich).
12. Prepare a pulled glass pipette (6 inch; OD/ID: 1/0.58) using dual stage glass micropipette puller (PC-10; Narishige's).
13. Weigh the mouse and give the mouse drug injections:
    a. Subcutaneous Buprenex – 0.1 mg/kg
    b. Subcutaneous Rimadyl – 5 mg/kg

Note: The use of drugs depends on institution animal rules.

14. Inject a mix of endotoxin-free plasmid preparation and Fast Green into one lateral hemisphere of E15.5 embryos using a Picospritzer III (Parker).
15. Electroporation (ECM 830, BTX) is performed to target hippocampal progenitors in E15.5 embryos by placing the anode (positively charged electrode) on the side of DNA injection and the cathode on the other side of the head.
16. Five pulses of 45 V for 50 ms at 500 ms intervals are used for electroporation.
17. After electroporation, it is best not to touch the mouse/change the cage following the injections until a few days after birth (usually around 5–7 days after birth).

Acute hippocampal slices preparation

Timing: 1–2 h

Note: This protocol is adapted from Stoppini et al. (1991) and Yasumatsu et al. (2008).

18. Anesthetize 21–28 days old electroporated mice with 5% isoflurane.
19. Check for the absence of reflex (tail or paw pinching) before decapitation.
20. Remove the brain from the animal: Slice the scalp down the midline with a scalpel, and then carefully cut the skull along the midline with a fine pair of scissors, taking care to not cut the underlying brain tissue.
21. Transfer the brain in ice cold cHBSS buffer.
22. In the case of the hippocampus: place the brain ventral side down, locate the superior colliculi, make a transverse cut, and discard the caudal part.
23. Flip the brain ventral side up and make a transverse cut.
24. Spread glue on the cutting plate of vibratome and gently lift the brain with spatula and place it over glue with brain rostral side up and ventral side facing you.
25. Transfer slicing chamber in vibratome, lock it, and pour cHBSS to submerge the brain.

26. Prepare the vibratome by placing a mix of ice and water in the tray surrounding the slicing chamber. Cut a razor blade in half and place one half in the blade holder of the vibratome.
27. 200 μm thick slices (speed of the vibratome 0.08–0.1 mm/s) are prepared with a vibratome (Leica Biosystem VT 1000S) and collected in 6 well plates with complete cHBSS media.
28. Transfer acute slices into 35 mm MatTek dishes for live imaging.

⚠ CRITICAL: Live cell imaging of EB3 comets in acute hippocampal slices is performed immediately after preparation of slices

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Recombinant DNA		
EB3-EGFP	Gift from Franck Polleux	N/A
vGlut1-mCherry	Gift from Clarissa Waites	N/A
VAMP2-mCherry	Gift from Clarissa Waites	N/A
Chemicals, peptides, and recombinant proteins		
Lipofectamine 2000	Thermo Fisher	11668019
D-Glucose	Sigma-Aldrich	G8270
$CaCl_2$	Sigma-Aldrich	C5670
$MgSO_4$	Sigma-Aldrich	M2643
$NaHCO_3$	Sigma-Aldrich	144-55-8
Neurobasal media	Thermo Fisher	1103049
B-27 supplement (50×)	Thermo Fisher	7504044
GlutaMAX supplement	Thermo Fisher	35050061
Penicillin-streptomycin	Thermo Fisher	15140163
Trypsin 0.05% EDTA	Thermo Fisher	25300054
10× HBSS	Thermo Fisher	14065056
1 M HEPES (pH7.4)	Thermo Fisher	15630080
Poly-D-lysine	Sigma-Aldrich	P1149
D-AP5	Sigma-Aldrich	A8054
Bicuculline	Sigma-Aldrich	14343
Ammonium chloride	Sigma-Aldrich	254134
BDNF	R&D Systems	248-BDB
Fast green	Sigma-Aldrich	F7252
Isoflurane	Primal Healthcare	66794-017-25
Critical commercial assays		
PureLink HiPure Plasmid Maxiprep Kit	Thermo Fisher	K210007
Experimental models: organisms/strains		
DH5α	New England BioLabs	C2987H
Mouse: C57BL/6J	Charles River Laboratories	RRID:IMSR_CRL:027
Rat: Sprague-Dawley	Charles River Laboratories	RRID:RGD_734476
Software and algorithms		
ImageJ (Fiji)	NIH	RRID: SCR_002285
MetaMorph microscopy automation and image analysis software	Molecular Devices	RRID: SCR_002368
Andor iQ3	Oxford Instruments	RRID:SCR_014461
GraphPad Prism	GraphPad	RRID: SCR_002798
Nikon Elements software	Nikon Instrument	RRID:SCR_014329
Other		
35 mm MatTek dishes	Mattek corporation	P35G-1.5-14-C
Millicell membrane	Millipore	HAWP02500

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Vibratome	Leica Biosystem	1000S
Electroporator	BTX	ECM 830
Micropipette puller	TriTechResearch	PC-10
Picospritzer III	Parker	052-0500-900
Electrodes	Nepagene	CUY650P5
Microcapillaries	WPI	1BF00F-6

MATERIALS AND EQUIPMENT

Neuronal culture medium			
Component	Stock concentration	Final concentration	Volume
Neurobasal medium	1×	1×	483.75 mL
B-27 supplement	50×	1×	10 mL
Pen/strep	100×	1×	5 mL
GlutaMAX	200 mM	0.5 mM	1.25 mL
Total			500 mL

Aliquot in 50 mL conical tubes and store at 4°C. Use within 1 week.

Complete HBSS (cHBSS)			
Component	Stock concentration	Final concentration	Volume
HBSS	10×	1×	50 mL
HEPES (pH7.4)	1 M	2.5 mM	1.25 mL
D-Glucose	1 M	30 mM	15 mL
$CaCl_2$	100 mM	1 mM	5 mL
$MgSO_4$	100 mM	1 mM	5 mL
$NaHCO_3$	1 M	4 mM	2 mL
ddH_2O			421.75 mL
Total			500 mL
Sterile filter with a 0.2 μm filter. Store at 4°C.			

Microscope setup

Live imaging in cultured hippocampal neurons is performed on IX83 Andor Revolution XD Spinning Disk Confocal System. The microscope is equipped with a 100×/1.49 oil UApo objective, a multi-axis stage controller (ASI MS-2000), and a controlled temperature and CO_2 incubator. Movies are acquired with an Andor iXon Ultra EMCCD camera and Andor iQ 3.6.2 live cell imaging software at 0.5–2 s/frame for 3 min.

Live imaging in acute hippocampal slices is performed with a Nikon A1R GaAsP multi detector unit on an inverted Nikon Ti-E microscope (60× objective NA1.4) with Nikon Elements Software at 1frame/3 s. In all, 488 and 561 nm lasers shuttered by Acousto-Optic Tunable Filters (AOTF) were used for the light source, and a custom quad-band excitation/dichroic/emission cube (based off Chroma, 89400) were applied for excitation and emission.

STEP-BY-STEP METHOD DETAILS

Live cell imaging in primary hippocampal neurons

Timing: 6–12 h pretreatment + 2–6 h imaging time depending on number of replicates

Here we describe how to perform live imaging of MT dynamics at presynaptic boutons before and after pharmacological induction of neuronal activity.

1. Live cell imaging of EB3 comets in hippocampal neuronal culture is performed 24–72 h after transfection.
2. Prepare complete HBSS as recording medium and warm it up in a 37°C water bath.
3. For presynaptic MT dynamics we use an IX83 Andor Revolution XD Spinning Disk Confocal System with 37°C incubator and 5% CO_2. Stabilize the temperature and humidity of incubator before imaging. The microscope is equipped with a 100×/1.49 oil UApo objective, a multi-axis stage controller (ASI MS-2000), and a controlled temperature and CO_2 incubator.

   Note: Axons are selected based on morphology (smaller diameter than dendrites and no spine structures along the neurite) and uniformly anterograde movement (toward the distal tip of the neurite) of EB3 comets. Proximal axonal segments (within 100 μm from the cell body according to our definition) are determined by first locating the cell body and then tracing the distance from cell body based on the real distance in μm in a still image taken with the camera.

4. Movies are acquired with an Andor iXon Ultra EMCCD camera and Andor iQ 3.6.2 live cell imaging software at 1 frame/3–4 s, 3 z-stacks at 0.4 μm step size for 3 min for dual channel acquisition.
5. For pharmacological induction of neuronal activity, neurons are pretreated with 50 μM D-AP5 for 6–12 h prior to live imaging in complete neurobasal medium, then changed to complete HBSS medium supplemented with 50 μM D-AP5. To induce neuronal activity, neurons are washed 3× with complete HBSS medium prior to addition of complete HBSS medium supplemented with 20 μM bicuculline or DMSO vehicle control. For BDNF induction of neuronal activity, 50 ng/mL BDNF is directly added to complete HBSS medium during live imaging.
6. Always acquire movies starting 1 min after induction of neuronal activity to ensure consistency among different experimental groups and to allow temperature and CO_2 flow to stabilize after addition of inducing medium.
7. Maximum intensity projections of movies are performed by Image Math within Andor software, exported as Tiff files, and analyzed in ImageJ.

Live cell imaging in acute hippocampal slices

Timing: 2–3 h

Here we describe how to perform live imaging of MT dynamics at presynaptic boutons in acute hippocampal slices. This is based on the protocol from (Yasumatsu et al., 2008)

8. Acute slices are inspected under fluorescence illumination to identify the expressing regions of interest (CA1 and CA3) in the hippocampus.
9. Acute slices are transferred onto millicell membrane upside down in MatTek dishes with 1 mL of warm fresh sterile medium.
10. The MatTek dish is transferred in a temperature-controlled chamber attached to the microscope. Both temperature (37°C) and CO_2 flow (5%) are controlled and maintained to promote cell viability and optical stability during live imaging.
11. Electroporated hippocampal neurons from the CA1 region are selected and imaged using an inverted Nikon Ti-E microscope operated by Nikon Elements Software.
12. 488 and 561 nm lasers are used as light sources and shuttered by Acousto-Optic Tunable Filters (AOTF).
13. Live-imaging acquisition of EB3 comets relative to vGlut1 positive stable puncta within 50–100 μm of cell bodies is performed for 300 s using a 60× oil-immersion objective (NA1.4) at 1 frame/3 s.

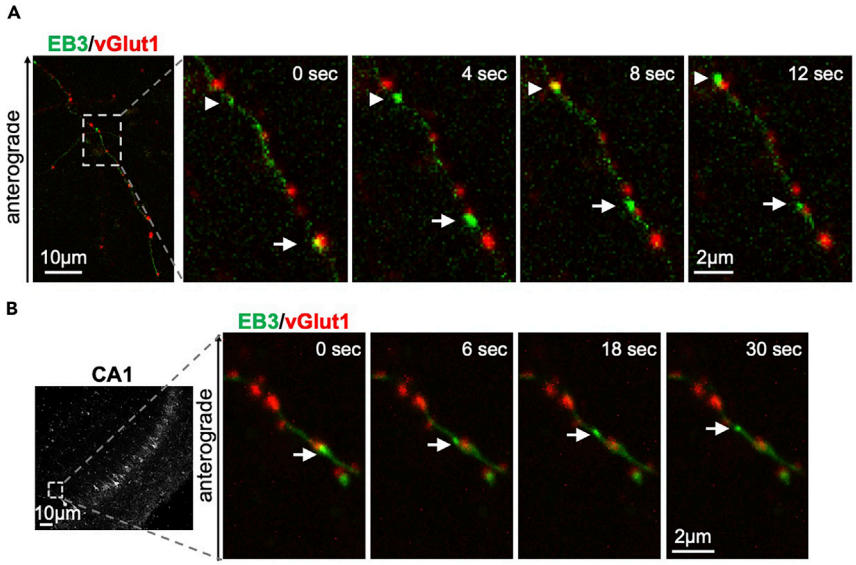


Figure 2. EB3 comet motion relative to vGlut$^+$ *en passant* boutons in hippocampal neurons in culture and in acute slices

(A) Maximum intensity projection of a time-lapse showing a proximal axon of a cultured hippocampal neuron (20 DIV) that was transfected with EB3-EGFP and vGlut1-mCherry plasmid DNAs 48 h prior to imaging. The arrow shows a comet starting at a vGlut1$^+$ bouton while the arrowhead shows a comet passing through a vGlut1$^+$ bouton.

(B) Maximum intensity projection of a time-lapse showing a proximal axon residing in the CA1 region of a mouse acute hippocampal slice 28 days after *in utero* electroporation with EB3-EGFP and vGlut1-mCherry plasmid DNAs. The arrow shows a comet starting at a vGlut1$^+$ bouton.

EXPECTED OUTCOMES

EB3 comets and vGlut1/VAMP2 puncta can be observed in axons of primary hippocampal neurons in culture and acute hippocampal slices (Qu et al., 2019). In proximal axons (up to 100 μm from the cell body) of cultured hippocampal neurons EB3 comets move distally from the cell body while 72.3% of vGlut1 or 68.6% of VAMP2 puncta are stationary. An EB3 comet can appear in between (arrowhead) or right at (arrow) vGlut1/VAMP2 puncta and move along the axon until it falls off the MT plus end (Figure 2 and Methods Video S1 and S2).

QUANTIFICATION AND STATISTICAL ANALYSIS

1. Tiff files can be opened by ImageJ software. To better visualize the comets and reduce background, the average intensity projection image of a time-lapse can be subtracted from each frame. Click Image → stacks → z projects, then choose average intensity. The average intensity projection image of the movie will show up. Click process → image calculator, then subtract the average intensity projection image from every frame of the movie. Save as a tiff file.
2. Kymographs are generated by drawing a region on the axon from the cell body to the most distal visible end of the neurite. Axons are identified based on morphology and uniform anterograde movement of EB3-labeled MT plus ends. Open the tiff file movie in MetaMorph software to generate kymographs. Draw a line along the neurite from the cell body to the most distal visible end of the axon. Click stack → kymograph. Click measure → calibrate distance to set pixel to μm conversion. Measure only comet tracks moving within 100 μm from the cell body. If doing the analysis in ImageJ, install KymoResliceWide plugin. Click Analyze → set scale to convert px to μm. Draw the line along axons from cell the body to the end of the neurite. Normally axons need 10–15 px wide lines. Click plugin → KymoResliceWide to generate a kymograph. Length of growth can be measured directly on the comet track as X-axis length. Comet lifetime is derived by the measured Y-axis length (Y μm) in the ratio of total kymograph width (D μm), which

represents the total length of movie time. For example, in a 180 s movie, comet lifetime (seconds) = 180 × Y/D. Then the growth rate can be calculated by growth length/comet lifetime.

3. Generate kymographs and distinguish between two groups of MTs, intrabouton and interbouton MTs as illustrated in the Graphical Abstract and in (Qu et al., 2019). Presynaptic MTs are classified based on their plus end contacts with stable vGlut1 or VAMP2 labeled boutons. Intrabouton MTs consist of MTs that nucleate/rescue at, catastrophe at, or pass through stable vGlut1 or VAMP2 labeled boutons. Interbouton MTs are those with no contact with vGlut1 or VAMP2 labeled boutons during the recording. In our measurements of EB3 tracks starting or ending at boutons, we also include those that start or end at a bouton and pass through the next distal or proximal boutons. Kymographs from both channels are overlaid to observe relative location of the comet tracks to stable boutons.
4. All MT dynamics parameters are defined as in (Qu et al., 2017; Qu et al., 2019; Stepanova et al., 2010) and calculated separately for intrabouton MTs and interbouton MTs.

Parameters describing MT dynamics are defined as follows:

MT dynamics parameter	Definition
Rescue/nucleation frequency	number of rescue or nucleation events per μm^2 per min
Catastrophe frequency	number of full tracks/total duration of growth
Comet density	number of comets per μm^2 per min
Growth length	comet movement length in μm
Comet lifetime	duration of growth in seconds
Growth rate	growth length/comet lifetime

5. Import data to GraphPad Prism and plot. If there are less than 15 axons per group or the dataset does not pass normality test, non-parametric tests will be used. Parametric/non-parametric T tests or two-way ANOVA are applied based on number of axons for all parameters. Averages of growth length, comet lifetime, and growth rate are calculated for each axon and statistical analysis is performed on those averages.

LIMITATIONS

The limitations of using primary neuronal cultures are predominantly associated with the challenge of maintaining healthy cultures for several weeks *in vitro* prior to transfection and the restrictions imposed by the embryonic nature of the cultures and loss of an intact circuit upon dissociation. The acute hippocampal slice approach is more laborious, and hence not well suited for drug screening or high throughput studies, and its success rate greatly depends on mouse survival from *in utero* electroporation.

TROUBLESHOOTING

Problem 1

Very few EB3 comets per axon or EB3 puncta do not appear to move in transfected neurons (steps 1–3).

Potential solution

This problem is typically caused by poor neuronal health after transfection or during live imaging. Be careful when changing to conditioned media and avoid creating shear stress on the neurons while pipetting. Plasmid DNA should be prepared using endotoxin-free maxi prep kits. Live imaging should always be performed in controlled and steady atmospheric conditions at 37°C with 5% CO_2, and over 90% humidity.

Problem 2

It is hard to visualize EB3 comets in the *in utero* electroporated acute brain slice if not used immediately after slicing (steps 5–7).

Potential solution

The duration between preparing the brain slices and live imaging should be kept at minimum. After cutting the slices, they should be immediately transferred into steady atmospheric conditions at 37°C with 5% CO_2. These conditions must be maintained steady during the entire live-imaging session.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Francesca Bartolini (fb2131@columbia.edu)

Materials availability

This study did not generate unique materials or reagents.

Data and code availability

This study did not generate unique datasets or codes.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.xpro.2021.100342.

ACKNOWLEDGMENTS

The authors would like to thank all Bartolini lab members, past and present, for contributing to this work at various stages. Special gratitude goes to Heike Blockus for assisting us with the *in utero* electroporation. We are grateful to Franck Polleux, David Sulzer, and Clarissa Waites for stimulating discussions and help with the experimental design. This work was supported by NIH grants RO1AG050658 (NIA), RO3AG060025 (NIA), and R21NS120076 (NINDS) to F.B.

AUTHOR CONTRIBUTIONS

Conceptualization, X.Q. and F.B.; investigation, X.Q. and A.K.; writing – original draft, X.Q. and A.K.; writing – review & editing, X.Q., A.K., and F.B.; funding acquisition and supervision, F.B.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Qu, X., Yuan, F.N., Corona, C., Pasini, S., Pero, M.E., Gundersen, G.G., Shelanski, M.L., and Bartolini, F. (2017). Stabilization of dynamic microtubules by mDia1 drives Tau-dependent Aβ1-42 synaptotoxicity. J. Cell Biol. *216*, 3161–3178.

Stoppini, L., Buchs, P., and Muller, D. (1991). A simple method for organotypic cultures of nervous tissue. J. Neurosci. Methods *37*, 173–182.

Yasumatsu, N., Matsuzaki, M., Miyazaki, T., Noguchi, J., and Kasai, H. (2008). Principles of long-term dynamics of dendritic spines. J. Neurosci. *28*, 13592–13608.

Qu, X., Kumar, A., Blockus, H., Waites, C., and Bartolini, F. (2019). Activity-dependent nucleation of dynamic microtubules at presynaptic boutons controls neurotransmission. Curr. Biol. *29*, 4231–4240.e5.

Stepanova, T., Smal, I., van Haren, J., Akinci, U., Liu, Z., Miedema, M., Limpens, R., van Ham, M., van der Reijden, M., Poot, R., et al. (2010). History-dependent catastrophes regulate axonal microtubule behavior. Curr. Biol. *20*, 1023–1028.