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Analyzing Mitochondrial Transport and Morphology in Human Induced Pluripotent Stem Cell-Derived Neurons in Hereditary Spastic Paraplegia

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Abstract

Neurons have intense demands for high energy in order to support their functions. Impaired mitochondrial transport along axons has been observed in human neurons, which may contribute to neurodegeneration in various disease states. Although it is challenging to examine mitochondrial dynamics in live human nerves, such paradigms are critical for studying the role of mitochondria in neurodegeneration. Described here is a protocol for analyzing mitochondrial transport and mitochondrial morphology in forebrain neuron axons derived from human induced pluripotent stem cells (iPSCs). The iPSCs are differentiated into telencephalic glutamatergic neurons using well-established methods. Mitochondria of the neurons are stained with MitoTracker CMXRos, and mitochondrial movement within the axons are captured using a live-cell imaging microscope equipped with an incubator for cell culture. Time-lapse images are analyzed using software with “MultiKymograph”, “Bioformat importer”, and “Macros” plugins. Kymographs of mitochondrial transport are generated, and average mitochondrial velocity in the anterograde and retrograde directions is read from the kymograph. Regarding mitochondrial morphology analysis, mitochondrial length, area, and aspect ratio are obtained using the ImageJ. In summary, this protocol allows characterization of mitochondrial trafficking along axons and analysis of their morphology to facilitate studies of neurodegenerative diseases.

SUMMARY

Impaired mitochondrial transport and morphology are involved in various neurodegenerative diseases. The presented protocol uses induced pluripotent stem cell-derived forebrain neurons to assess mitochondrial transport and morphology in hereditary spastic paraplegia. This protocol allows characterization of mitochondrial trafficking along axons and analysis of their morphology, which will facilitate the study of neurodegenerative disease.

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DISCLOSURES

The authors declare no competing financial interests.

Keywords

mitochondrial transport; mitochondrial morphology; forebrain neurons; induced pluripotent stem cells; axonal degeneration; hereditary spastic paraplegia

INTRODUCTION

Mitochondrial motility and distribution play a vital role in fulfilling variable and specialized energetic demands in polarized neurons. Neurons can extend extremely long axons to connect with targets through the formation of synapses, which demand high levels of energy for Ca^{2+} buffering and ion currents. Transport of mitochondria from soma to axon is critical for supporting axonal and synaptic function of neurons. Spatially and temporally dynamic mitochondrial movement is conducted by fast axonal transport at rates of several micrometers per second[1].

Specifically, motor or adaptor proteins, such as kinesin and dynein, participate in the fast organelle transport along microtubules to control the movement of mitochondria[2,3]. Normal neuronal activity requires proper transport of newly assembled mitochondria from neuronal soma to distal axon (anterograde axonal transport) and reverse transport of mitochondria from the distal axon back to the cell body (retrograde transport). Recent studies have indicated that improper mitochondrial allocation is strongly associated with neuronal defects and motor neuron degenerative diseases[4,5]. Therefore, to dissect the role of mitochondria in neurodegeneration, it is important to establish methods for examining mitochondrial movement along axons in live cultures.

There are two main challenges in examining and analyzing the tracking of mitochondria: (1) identifying mitochondria from the background in every frame, and (2) analyzing and generating the connections between every frame. In resolving the first challenge, a fluorescence labeling approach is used widely to distinguish mitochondria from the background, such as MitoTracker dye or transfection of fluorescence-fused mitochondrial targeting protein (e.g., mito-GFP)[6–8]. To analyze the association between frames, several algorithms and software tools have been described in previous studies[9]. In a recent paper, researchers compared four different automated tools (e.g., Volocity, Imaris, wrMTrck, and Difference Tracker) to quantify mitochondrial transport. The results showed that despite discrepancies in track length, mitochondrial displacement, movement duration, and velocity, these automated tools are suitable for evaluating transport difference after treatment[10]. In addition to these tools, an integrated plugin “Macros” for ImageJ (written by Rietdorf and Seitz) has been widely used for analyzing mitochondrial transport[11]. This method generates kymographs that can be used to analyze mitochondrial movement, including velocity in both anterograde and retrograde directions.

Mitochondria are highly dynamic organelles that constantly change in number and morphology in response to both physiological and pathological conditions. Mitochondrial fission and fusion tightly regulate mitochondrial morphology and homeostasis. The imbalance between mitochondrial fission and fusion can induce extremely short or long mitochondrial networks, which can impair mitochondrial function and result in abnormal

neuronal activities and neurodegeneration. Impaired mitochondrial transport and morphology are involved in various neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and hereditary spastic paraplegia (HSP)[12–15]. HSP is a heterogeneous group of inherited neurological disorders characterized by the degeneration of the corticospinal tract and subsequent failure to control lower limb muscles[16,17]. In this study, iPSC-derived forebrain neurons are used to assess mitochondrial transport and morphology in HSP. This method provides a unique paradigm for examining mitochondrial dynamics of neuronal axons in live cultures.

PROTOCOL

1. Generation of telencephalic glutamatergic neurons from iPSCs

NOTE: The detailed protocol for maintaining iPSCs and their differentiation into telencephalic glutamatergic neurons are similar to those described previously[18]. Here, the critical process during the differentiation of human pluripotent stem cells is introduced and highlighted.

1.1. Culture iPSCs on mouse embryonic fibroblast (MEF) feeders in human embryonic stem cell (hESC) medium supplemented with fibroblast growth factor (bFGF, 4 ng/mL).

1.2. After incubation with dispase (1 mg/mL) for 3 min, dissociate the iPSCs into small clumps. Then, culture the iPSC aggregates in suspension in the hESC medium for 4 days. Refer to this timepoint, when the iPSCs are starting as the suspension culture, as day 1 (D1). Change the medium daily for 4 days.

1.3. At D5, collect the iPSC aggregates after centrifugation at 200 *x g* for 2 min and culture in neural induction media (NIM) for 3 days. Culture the cell aggregates in suspension for 3 days by changing half of the media every 2 days. Add DMH-1 (2 μM) and SB431542 (2 μM) to NIM medium to increase neural induction efficiency.

1.4. At D8, collect the iPSC aggregates after centrifugation at 200 *x g* for 2 min and let them adhere to 6 well plates (around 20–30 aggregates per well of the plate) by culturing in NIM with 10% FBS (or with laminin-coated plates) overnight. On the following day, remove the old medium and change to NIM every 2 days until D17.

NOTE: The generation of neuroepithelial cells, which is indicated by the formation of columnar cells with rosette structures, is observed in this period.

1.5. At D17, mechanically isolate the neuroepithelial cells in the center of colonies (lifting off directly by applying a small pressure to the center of the colonies) or enzymatically (treated with dispase). Transfer the isolated neuroepithelial cells to non-treated tissue culture T25 flask containing 8 mL of NIM with addition of B27 (1x), cAMP (1 μM), and IGF (10 ng/mL).

NOTE: The suspension culture allows cells to form neurospheres that are enriched with cortical projection neural progenitors.

1.6. After D35, plate the neurospheres on the poly-ornithine and LDEV-free reduced growth factor basement membrane matrix (Table of Materials)-coated 35 mm glass bottom dishes to generate telencephalic glutamatergic neurons (cortical projection neurons). Before plating, dissociate the neurospheres to small clusters by incubating with 1 mg/mL cell detachment solution for 2 min at 37 °C.

1.7. Remove the cell detachment solution by centrifugation and resuspend in 1 mL of neural differentiation medium (NDM). Plate cells on the glass bottom dish (around five clusters in 100 μL of medium per dish) to let them attach. Then, add NDM (1 mL per dish) and culture the cells in NDM containing B27 (1x), cAMP (1 μM), IGF (10 ng/mL), hBDNF (10 ng/mL), and GDNF (10 ng/mL).

NOTE: Small clusters are plated because they survive well and give rise to long projection neurons. Alternatively, cells can be dissociated and plated at a density around 20,000 cells per dish for better separation.

1.8. Characterize the telencephalic glutamatergic neurons by immunostaining the Tbr1 and βIII-tubulin markers.

2. Examination of mitochondrial transport along axons of telencephalic glutamatergic neurons

2.1. Warm up the NDM and turn on the incubator for the fluorescence microscope set at 37 °C and 5% CO_2.

2.2. To visualize mitochondria along the axons of forebrain neurons, incubate the neurons with 50 nM red fluorescent dye to stain mitochondria in live cells (e.g., MitoTracker CMXRos) in NDM for 3 min at 37 °C. Next, wash cells 2x with warmed NDM. The neurons are incubated in NDM.

2.3. In order to record the mitochondrial transport along the axons, take time-lapse images of mitochondrial movement using the 40x objective with a fluorescence microscope.

NOTE: To stabilize the culture, perform the live cell imaging when the cells are incubated in the incubator for 20 min following mitochondrial staining.

2.4. Under phase field, identify the axons based on morphological characteristics (directly emerge from neuronal cell body, constant thin long neurites with no branching). Mitochondria move in anterograde (from cell body to distal axon) and retrograde directions (from axonal terminal to cell body). To distinguish the direction of mitochondrial movement along axons, clearly identify the cell bodies of neurons. In this step, focus axons under phase field to reduce photobleaching.

2.5. After distinguishing the cell body and axon, adjust the exposure time and focus of mitochondria in axons. Then, capture the transport of mitochondria within axons every 5 s for a total duration of 5 min, yielding 60 frames. Randomly capture at least five locations for each dish and repeat 3x for each group.

3. Data analysis of mitochondrial transport and morphology in cortical neurons

NOTE: Analyze the collected data on mitochondrial transport using an image analysis software (e.g., ImageJ or MetaMorph[19]). Since ImageJ is readily available, perform the analysis of mitochondrial transport and morphology using ImageJ with the MultiKymograph, Macros, and Analyze particles plugins.

3.1. Analyzing mitochondrial transport using ImageJ

3.1.1. After capturing the time-lapse images of mitochondrial motility, analyze the velocity and movement of mitochondria using the MultiKymograph plugin. The images are saved as .tiff format files, which are analyzed by Fiji software following a previously reported method[20].

3.1.2. Download the Fiji software. Download plugins that will be needed for analyzing mitochondrial moving velocity from kymographs. Download the Bio-formats Package and Kymograph Plugin along with these four .class plugins, including: MultipleKymograph.class, MultipleOverlay.class, StackDifference.class, and WalkingAverage.class (Table of Materials). Move these files to the Fiji plugins folder. Download “tsp050706.txt” plugins to the plugins folder. Restart the Fiji software when the files are moved to the plugins folder.

3.1.3. Open Fiji software. Choose plugins, then import images of the .tiff series through Bio-Formats Importer under Bio-Formats. Make sure to choose Standard ImageJ in Stack viewing, choose Open all series, check Autoscale, then click OK. Take note of the frame number and size of images in pixels, which are displayed on the top of the image.

NOTE: Take 60 frames per image.

3.1.4. Consider making the images clearer by adjusting brightness/contrast under the Image menu for all 60 frames.

3.1.5. Right-click the line tool to choose segmented line and draw a segmented line starting from the cell body and ending at the terminal axon. Generate the kymograph by choosing MultipleKymograph under Plugins. The selection of line width is prompted after choosing the MultipleKymograph. Ensure that this is an odd number. Choose 1 for the line width. A kymograph is generated after this step.

NOTE: Several important pieces of information can be read from the kymograph. The y-axis of kymograph is the time of duration (5 min) for the 60 frames. The x-axis represents the position of the selected axons.

3.1.6. Use a diagonal line in the kymograph to determine the movement direction of mitochondria (anterograde, retrograde, or stable). For example, a line going down to the right along the y-axis indicates anterograde movement, and a line going down to the left along the y-axis indicates retrograde movement. A vertical line indicates that there was no movement in the mitochondrion.

3.1.7. Measure the distance, time values, and velocity for the moving mitochondria using the Macros plugin in Fiji software. Go to Plugins | Macros | Install | tsp050607.txt. Draw a segmented line over the trace of mitochondrial movement on the kymograph, and always draw the line from the superior to inferior region (y-axis).

3.1.8. After drawing the line, go to Plugins | Macros | read velocities from tsp. Since a segmented line along the trace is drawn, the plugin reads segmented velocities corresponding to the line.

NOTE: The unit for all data is pixels. dy sum is the time consumed from the starting point, and dx sum indicates the distance of the mitochondrion moving in the x-axis. dy now and dx now shows the period time and movement distance for every segment, respectively. actual speed shows the speed for every segment (dx now/dy now). average speed indicates the average speed for the mitochondrial moving (dx sum/dy sum).

3.1.9. Change the unit of dx now from pixel to μm as the ratio of pixel in μm by measuring the scale bar. Convert the unit for distance from pixel to μm by measuring scale bars in images. Use the line tool to draw a line along the scale bar and measure the length of the line by choose Measure' under "Analyze. Change the time from pixel to seconds, as 1 pixel=5 seconds in this experiment.

3.1.10. In the spreadsheet file, calculate the average velocity and correspondingly label retrograde or anterograde movement. Average the anterograde or retrograde movement velocity.

3.1.11. Determine the percentage of stationary and motile mitochondria from the kymograph. The anterograde or retrograde moving mitochondria are defined as moving 5 μm forward or backward from the origin during the entire period[21]. Mitochondria are considered stationary if they did not move more than 5 μm during the 5 min.

3.2. Analysis of mitochondrial morphology using ImageJ

NOTE: Determine mitochondrial morphology by measuring mitochondrial length, area, and aspect ratio using ImageJ. To do so, follow the steps below.

3.2.1. In order to analyze the mitochondrial length and area within axons, download the Straighten_.jar plugin from ImageJ website (see Table of Materials) and move it to the folder of plugins. Restart ImageJ software.

3.2.2. Open the picture through the open function under File and convert the 32 bit image to 8 bit using Type under Image.

3.2.3. Draw a segmented line along the axons. Choose Straighten under Plugins and set 50 pixels for Width of Filament/Wide Line. This will generate a straightened axon.

3.2.4. Adjust the threshold under Image and set the measurement under Analyze by choosing "Area | Perimeter | Fit ellipse | Shape descriptors.

3.2.5. Measure the scale bar using the line function and set the scale under Analyze by filling the distance in pixel, know distance, and the unit of length. Then, choose Global to set this scale setting.

3.2.6. Use Analyze Particles under Analyze to determine the area. The prompt parameters are size (pixel^2) = 0.20–infinity, circularity = 0.00–1.00, and show = ellipses. Choose Display results.

NOTE: The measurement will yield the results of multiple mitochondrial morphology parameters including area, perimeters, length (major), width (minor), and aspect ratio (AR). The corresponding mitochondrial number is also listed.

3.2.7. Calculate the mitochondrial number per axon (μm) using the mitochondrial number divided by axonal length.

REPRESENTATIVE RESULTS

Here, human iPSCs were differentiated into telencephalic glutamatergic neurons, which were characterized by immunostaining with Tbr1 and βIII tubulin markers (Figure 1A). To examine the axonal transport of mitochondria, these cells were stained with red fluorescent dye, and time-lapse imaging was performed. Since ImageJ is readily available and easier to obtain, mitochondrial transport was further analyzed with the “MultiKymograph” and “Macros” ImageJ plugins, shown in Figure 1.

There are three respective states of mitochondrial motion, including static, anterograde, and retrograde movement (Figure 1B,C). A single mitochondrion can remain static or move in anterograde or retrograde direction within an axon, and here, a segmented line was drawn along the track of mitochondrial movement to determine velocity (Figure 1D). The velocity of mitochondrial movement along the axon is shown in Figure 1E and corresponds to the segmented lines in Figure 1D after reading by “Macros” in ImageJ. Similar to the MetaMorph software, ImageJ can be used to determine the mitochondrial velocity and percentage of motile mitochondria based on the kymograph generated by ImageJ (Figure 1F). Using commercially available analysis software (e.g., MetaMorph), previous data showed that the percentage of motile mitochondria was significantly reduced in SPG3A neurons compared to normal neurons, while velocity was not altered[19]. To evaluate the ImageJ software, the percentage of motile mitochondria was examined, and a similar reduction in the percentage of motile mitochondria in SPG3A neurons compared to control wild-type (WT) neurons was observed (Figure 1G).

Regarding analysis of mitochondrial morphology, the mitochondrial area, length, and AR were analyzed using the “analyze particles” function in ImageJ. Axons were straightened using the “straighten” plugin (Figure 2A,B). Mitochondria were chosen clearly by adjusting the threshold (Figure 2C,D). Finally, the mitochondrial area, length (major), width (minor), aspect ratio, and perimeter were obtained from the straightened axon using the “Analyze Particles” plugin (Figure 2E,F). Abnormal mitochondrial morphology was (and has previously been) observed in HSP iPSC-derived telencephalic glutamatergic neurons, including SPG15 cells (Figure 2G,H,I)[13,22]. Both the mitochondrial length and aspect ratio

were significantly reduced in SPG15 neuron axons compared to control WT neuron axons (Figure 2G,H,I).

DISCUSSION

This article describes a method to analyze mitochondrial transport and morphology in neuronal axons using red fluorescent dye and ImageJ software, both of which provide a unique platform to study axonal degeneration and mitochondrial morphology in neurodegenerative disease. There are several critical steps in the protocol, including staining of mitochondria, live cell imaging, and analyzing the images. In this method, a fluorescent dye was used to stain mitochondria. Since human iPSC-derived neurons are easily detached from the dish, it is important to leave some solution in the dish and gently add neurobasal medium. The washing can be performed three or four times to remove the dye. In addition, mitochondria can be labeled with other reporters to measure their transport along axons, such as fluorescent protein fused mitochondria targeting proteins[6]. In long-term tracking, several probes (i.e., NPA-TPP, 2,1,3-benzothiadiazole [BTD] fluorescent derivatives, and a specific Raman probe) showed great potential in long-term mitochondrial tracking and mitochondrial dynamics analysis[23–25]. The advantages and disadvantages of various probes can be found in the Table 1.

After mitochondrial staining, live cell imaging is performed using a microscope equipped with an incubator. To effectively focus the neurons during imaging, neural samples should be kept in the 37 °C incubator with 5% CO_2 and in a humid environment for at least 15 min. To minimize the out-of-focus effects, images at different Z-positions can be taken to make a Z-stack, or the auto-focus function can be utilized. Importantly, to identify the direction of mitochondrial transport (anterograde or retrograde), phase images are taken to distinguish the neuronal cell body and axons. Another critical issue is photobleaching of fluorescent samples, which must be prevented to obtain efficient mitochondrial transport time-lapse images. An effective method to minimize photobleaching is to focus samples through the eyepiece and set all imaging parameters under the phase channel, except for exposure time. Moreover, the automatic scaling pattern can decrease photobleaching of fluorescence.

Mitochondria are highly dynamic organelles and can move in both anterograde and retrograde directions. In neurons, a few mitochondria within axons stay stationary during the recording. Among the moving mitochondria, motion status can vary over time. This phenomenon raises the important question of which mitochondria type is considered stationary or moving. This can be resolved by setting the threshold during mitochondrial axonal transport analysis. To distinguish the static mitochondria, Neumann et al. used the mitochondrial track center, which is defined as the mean of its position coordinates over time, then set the threshold to 350 nm/s so that the maximum deviation distance of the mitochondrion from its track center is in the first frame[26].

In another study, the authors set 50 nm/s as the threshold to distinguish stationary status from the moving status[27]. A 300 nm/s threshold was used here to distinguish the microtubule-based transport as done in previous reports[28,29]. Although the threshold for stationary and moving mitochondria is different, setting the threshold can provide important

relative information on mitochondrial movement within axons between wild-type and degenerative neurons.

Most protocols involving mitochondrial transport analysis have used kymograms, which are two-dimensional representation of positions versus time. Multiple automated tools have been developed for the analysis of particle tracking[26,30–34]. These can accurately separate each frame. In addition to the velocity and motile percentage that ImageJ can measure, this method can measure motile events accurately. However, these are not free to use. Here, the analysis of mitochondrial transport was performed using ImageJ with the “Multikymograph” and “Macros” plugins. These plugins can effectively measure mitochondrial movement. The advantage of these plugins is their ease of use and ability to indicate alterations in mitochondrial axonal transport in the form of kymographs and velocity over time.

Motile mitochondria were analyzed in SPG3A and control neurons. A similar reduction in the percentage of motile mitochondria was observed using two different analysis methods, confirming the usefulness of ImageJ to analyze axonal transport. In addition, mitochondrial morphology can be analyzed using the same set of images, which provides important readouts for studying mitochondrial dysfunction in various neurological diseases. Since axonal degeneration and mitochondrial dysfunction usually occur during earlier stages, before neurons die, this method can be used to examine early pathological changes to help identify molecular pathways and screen therapeutics to rescue neurodegeneration.

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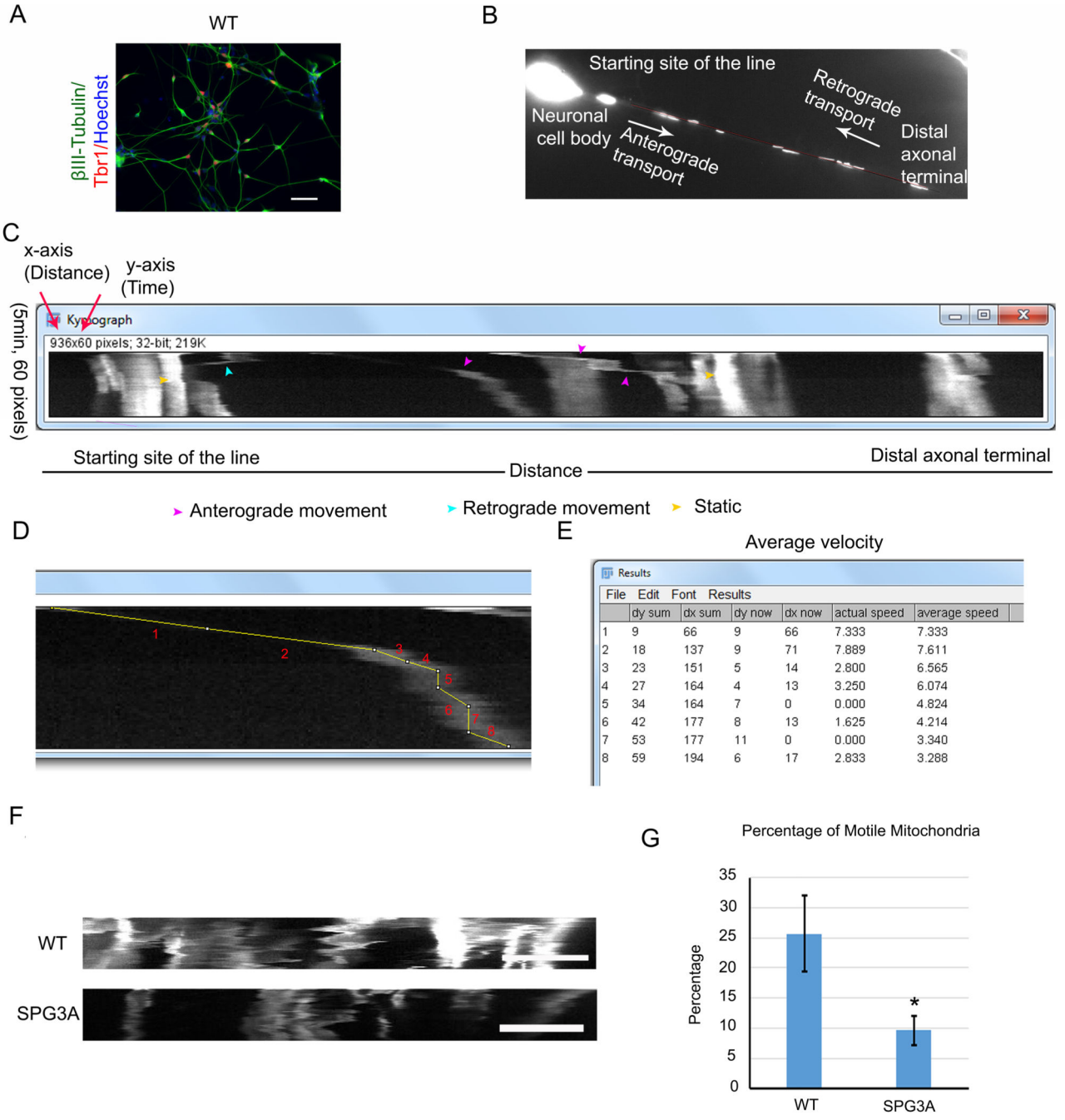

Figure 1: Analysis of mitochondrial transport using ImageJ.

(**A**) Immunostaining showing the generation of telencephalic glutamatergic neurons. Tbr1 (red), βIII tubulin (green), and Hoechst (blue). Scale bars = 50 μm. This figure has been modified from a previous study[19]. (**B**) Mitochondrial transport within axons of neurons. Anterograde mitochondrial movement is defined as the mitochondria moving from the cell body to distal axon, and retrograde mitochondrial movement originates from the distal axon and extends to the neuronal cell body. The segment line is drawn along axons from neuronal cell body to distal axonal terminal in order to generate kymographs. (**C**) Kymograph is

generated using ImageJ; x-axis is the axonal position and (B) y-axis is time. One continuous white line is the mitochondrion moving in anterograde direction (pink arrowhead), retrograde direction (blue arrowhead), or static state (yellow arrowhead). (**D**) The segmented line is drawn to read the velocity using the Macros plugin. Numbers 1–8 describe the segment of the line. Anterograde movement (1–4, 6, 8) and static state (5, 7) can be distinguished from this magnified kymograph. (**E**) Time and moving distance for each segment, actual and average velocity (in pixels). (**F**) Kymograph of mitochondrial transport for WT and SPG3A cortical PNs using ImageJ. Scale bar = 10 μm. (G) Motile mitochondrial ratio in WT and SPG3A cortical PNs using ImageJ (*$p < 0.05$).

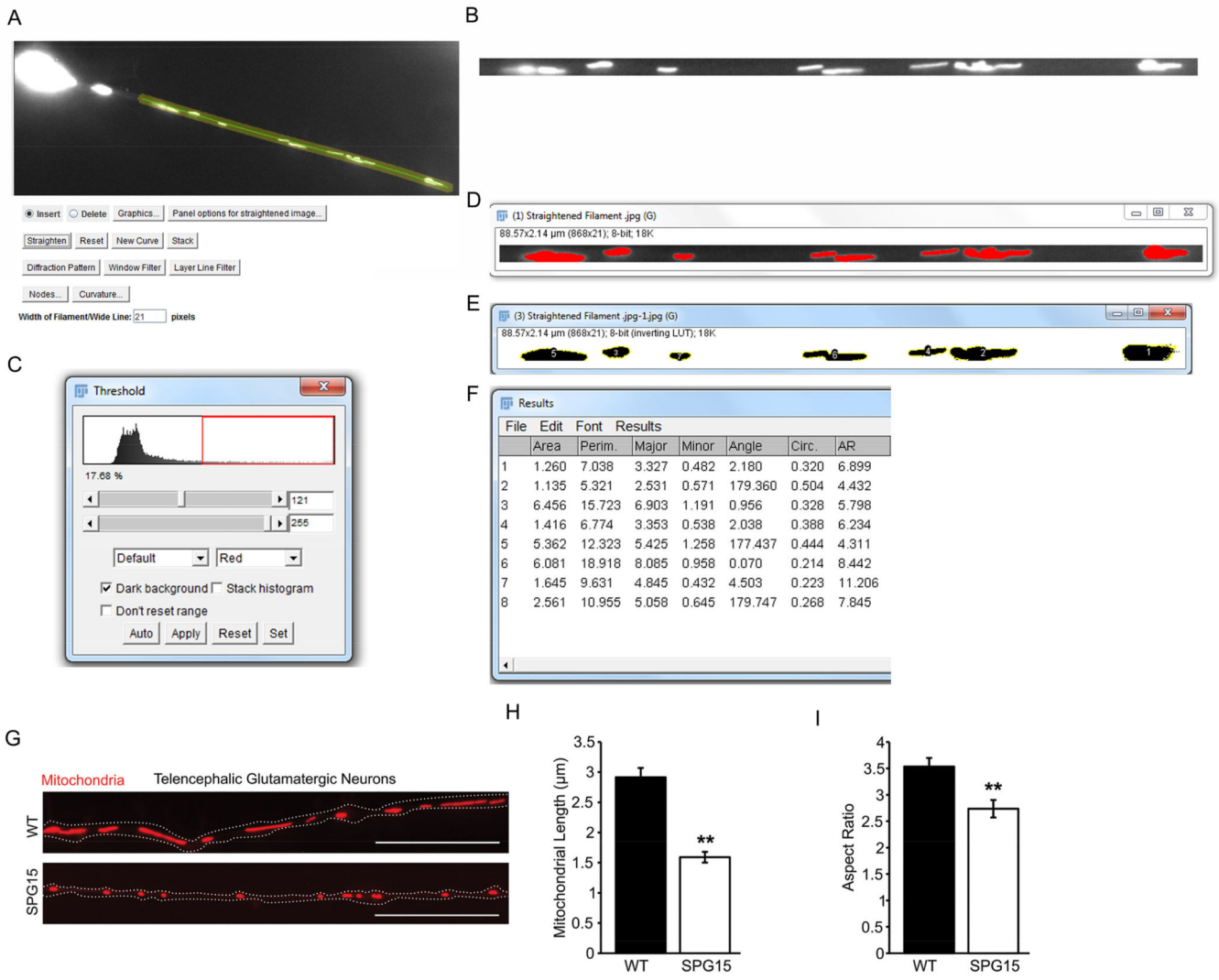

Figure 2: Analyzing mitochondrial morphology using ImageJ.

(**A**) Parameters for straightening the axon. (**B**) The representative straightened axon. (**C**,**D**) Adjustment of threshold to choose mitochondria. (**E**,**F)** The measured mitochondrial area, perimeter, length (major), width (minor), and aspect ratio (AR) corresponding to the selected mitochondria in (E). (**G**) Representative pictures of mitochondria in WT and SPG15 telencephalic glutamatergic neurons. Scale bars = 20 µm. (**H**) Mitochondrial length in WT and SPG15 neurons. (**I**) Aspect ratio of mitochondria in WT and SPG15 neurons (**p < 0.01) *vs.* WT. (G), (H), and (I) are modified from a previous study[13].

Table 1:

Advantages and disadvantages of some fluorescent tools for mitochondrial labeling

Fluorescent Tools	Advantages	Disadvantages	References
MitoTracker	MitoTracker is mitochondrial potential-independent and can be used to analyze the colocalization of mitochondria with autophagosome or lysosome.	MitoTracker is not photostable enough for long-term research.	35
NPA-TPP	This dye is a novel photostable mitochondrial labeling reagent.	The synthesis and purification process of this dye is time-consuming and costly.	23
MitoBADY	This dye can be used for mitochondrial visualization using Raman microscopy with high sensitivity and specificity.	Using this dye requires Raman microscope.	25
TMRE	This dye is a non-toxic, specific mitochondrial staining dye with low concentration and no quenching effect.	TMRE labeling mitochondria depends on the mitochondrial membrane potential.	36, 37
Mitochondria-targeted fluorescent proteins	Mitochondria-targeted fluorescent proteins are more specific and stable.	This method needs transfection and transfection efficiency is different for different cell types.	38, 39

Name of Material/ Equipment	Company	Catalog Number
Accutase Cell Detachment Solution	Innovative Cell Technologies	AT104
Biosafety hood	Thermo Scientific	1300 SERIES A2
Bovine serum albumin (BSA)	Sigma	A-7906
Brain derived neurotrophic factor (BDNF)	Peprotech	450-02
Centrifuge	Thermo Scientific	Sorvall Legend X1R/ 75004261
Coverslips	Chemiglass Life Sciences	1760-012
Cyclic AMP (cAMP)	Sigma-Aldrich	D0627
Dispase	Gibco	17105-041
Dorsomorphin	Selleckchem	S7146
Dulbecco's modified eagle medium with F12 nutrient mixture (DMEM/F12)	Corning	10-092-CV
FBS	Gibco	16141-002
Fibroblast growth factor 2 (FGF2, bFGF)	Peprotech	100-18B
Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix	Gibco	A1413201
Gem21 NeuroPlex Serum-Free Supplement	Gemini	400-160
Glass Bottom Dishes	MatTek	P35G-0.170-14-C
9" glass pipetes	VWR	14673-043
Glial derived neurotrophic factor (BDNF)	Sigma-Aldrich	D0627
GlutaMAX-I	Gibco	35050-061
Heparin	Sigma	H3149
Insulin growth factor 1 (IGF1)	Invitrogen	M7512
Knockout Serum Replacer	Gibco	A31815
Laminin	Sigma	L-6274
2-Mercaptoethanol	Sigma	M3148-100ML
MitoTracker CMXRos	Invitrogen	M7512
Neurobasal medium	Gibco	21103-049
Non Essential Amino Acids	Gibco	11140-050
N2 NeuroPle Serum-Free Supplement	Gemini	400-163
Olympus microscope IX83	Olympus	IX83-ZDC2
PBS	Corning	21-031-CV
Phase contrast microscope	Olympus	CKX41/ IX2-SLP
6 well plates	Corning	353046
24 well plates	Corning	353047
Poly-L-ornithine hydrobromide (polyornithine))	Sigma-Aldrich	P3655
SB431542	Stemgent	04-0010
Sterile 50ml Disposable Vacuum Filtration System 0.22 μm Millipore Express® Plus Membrane	Millipore	SCGP00525
Stericup 500/1000 ml Durapore 0.22 μM PVDF	Millipore	SCGVU10RE

Name of Material/ Equipment	Company	Catalog Number
Tbr1 antibody (1:2000)	Chemicon	AB9616
Trypsin inhibitor	Gibco	17075029
50 ml tubes	Phenix	SS-PH50R
15 ml tubes	Phenix	SS-PH15R
T25 flasks (untreated)	VWR	10861-572
Plugins for softwares		
Bio-formats Package	http://downloads.openmicroscopy.org/bio-formats/5.1.0/	
Fiji software	https://fiji.sc/	
Kymograph Plugin	https://www.embl.de/eamnet/html/body_kymograph.html	
MultipleKymograph.class	https://www.embl.de/eamnet/html/body_kymograph.html	
MultipleOverlay.class	https://www.embl.de/eamnet/html/body_kymograph.html	
WalkingAverage.class	https://www.embl.de/eamnet/html/body_kymograph.html	
StackDifference.class	https://www.embl.de/eamnet/html/body_kymograph.html	
Straighten_.jar	https://imagej.nih.gov/ij/plugins/straighten.html	
tsp050706.txt	https://www.embl.de/eamnet/html/body_kymograph.html	