Microglia phagocytose myelin sheaths to modify developmental myelination

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLES

Supplementary Table 1. Summary of Ca^{2+} events detected by AQuA in microglia at 4 dpf

Parameter	Area (μm²)	Max dF/F	Duration (s) (50% to 50%)	Event distance from soma (μm)	Event frequency (events / s)
Mean +/- SD n = 645 events (31 microglia/31 fish)	8.81 +/- 15.98	0.079 +/- 0.03	76.87 +/- 74.57	18.70 +/- 16.99	0.053 +/- 0.034

Supplementary Table 2. Fiji macros to detect sheath disappearance

Purpose	Use with	Language	
Select first and last frames; last frame subtract first frame	A single channel time-series image to detect overall changes between first and last frames (anything present in first, but gone by last, will appear void)	Python (Jython)	from ij import IJ, ImagePlus from ij.plugin import Duplicator from ij.plugin import ImageCalculator imp = IJ.getImage(); nframes=imp.getNFrames() imp2 = Duplicator().run(imp, 1, 1) imp3 = Duplicator().run(imp, nframes, nframes) imp2.setTitle("first") imp3.setTitle("last") imp2.show() imp3.show() imp4 = ImageCalculator().run("subtract create 32 bit", imp3, imp2) imp4.setTitle("lastSUBfirst") imp4.show()

Duplicate myelin channel (2); bleach correct (prepare for XOR scheme)	A sum-projected 2-channel time-series (myelin channel is 2 in our acquisitions; change as necessary)	ImageJ Macro	run("Correct 3D drift", "channel=2 multi_time_scale sub_pixel only=0 lowest=1 highest=1");
			run("Duplicate...", "duplicate channels=2");
			run("16-bit");
			run("Bleach Correction", "correction=[Exponential Fit]")

Perform consecutive XOR operation to detect change over time	The output of previous step (bleach corrected myelin time-series)	ImageJ Macro	setPasteMode("xor");
			run("Set Slice...", "slice="+nSlices);
			run("Select All");
			for(i=1; i<nSlices; i++) {
			run("Previous Slice [<]");
			run("Copy");
			run("Next Slice [>]");
			run("Paste");
			run("Previous Slice [<]");
			}

Supplementary Table 3. AQuA parameters used for event detection in microglia.

AQuA steps	Values
Signal: intensity threshold	2.5
Signal: smoothing	0.5
Signal: minimum size	8 px
Voxel: temporal cut	2.5
Voxel: growing z	1.5
Event: rising time uncertainty	3
Event: slowest delay in propagation	2
Event: propagation smoothness	1.0
Clean: Z-score threshold	6
Merging, reconstruction, delay Tau	Ignore all

SUPPLEMENTARY VIDEOS

Supplementary Videos 1, 2. Microglia exhibit Ca^{2+} transients at sheath- and non-sheath-contacting processes. Microglia in *Tg(mpeg1.1:GCaMP6s-CAAX; sox10:mRFP)* 4 dpf larvae. Ca^{2+} events (cyan) are visible at process tips, along branches, and in the soma. Note moving engulfed mRFP+ material inside microglia and at points of microglia contact with sheaths. Videos are representative of microglial Ca^{2+} events (31/31 microglia imaged exhibited transients during 10 min). Scale bar, 10 µm.

Supplementary Videos 3, 4. Microglia Ca^{2+} transients prior to phagocytosis. Microglia in *Tg(mpeg1.1:GCaMP6s-CAAX; sox10:mRFP)* 4 dpf larvae. (3) A Ca^{2+} event at the distal process is followed by beading, engulfment, and intracellular movement of mRFP+ phagocytosed material. (4) A Ca^{2+} event in the first frame (arrowhead) is followed by breakdown of the associated sheath (rectangle); a later event (second arrowhead) is followed by sheath beading but not loss within the acquisition time. Scale bars, 10 µm.

Supplementary Video 5. Engulfment of myelin sheaths by microglia. Top, timelapse imaging (~5 min/frame, timestamped in upper left) of a microglia (yellow) interacting with an oligodendrocyte (magenta) in a *Tg(mpeg1.1:mVenus-CAAX; sox10:mRFP)* larva. Two myelin sheaths (magenta rectangles) are intact and visibly surrounded by microglial processes at first but become engulfed by the microglia soon after. Bottom panel, same video but only the RFP channel is shown to highlight the nascent sheaths that are engulfed. Scale bar, 10 µm.

Supplementary Video 6. 3D rotation of a microglia containing myelin inclusions. A microglia (yellow) in a *Tg(mpeg1.1:mVenus-CAAX; mbpa:mCherry-CAAX)* larva containing phagocytosed myelin (magenta

inclusions). Note intact myelin sheaths next to the microglia (magenta tubes). Movie generated with 3D Viewer plugin in Fiji.

Supplementary Video 7. 3D rotation of microglia, myelin, and neurons in the dorsal optic tectum. A 3D rendering of a ~40 µm z-stack of dorsal optic tectum in a 6 dpf *Tg(mpeg1.1:mVenus-CAAX; mbpa:mCherry-CAAX)* larva mosaically expressing neuroD:mTagBFP-CAAX to sparsely label neurons. Microglia (yellow) are situated between the tectal commissure (magenta) and tectal neurons (cyan). Movie generated with 3D Viewer plugin in Fiji.

Supplementary Video 8. Microglial response to glutamate uncaging in tectal cell body layer. Timelapse imaging (1 min/frame) of a microglia in a *Tg(mpeg1.1:mVenus-CAAX)* larva with MNI-glutamate focally uncaged in the tectal cell body layer. Closed circle after the third minute represents the time and area of 405 nm laser application to uncage glutamate; open circle for remaining frames indicates the area that glutamate was uncaged. Scale bar, 20 µm.