| | Average Velocity (μm/s) | | % of Time in Motion | | Stop Frequency | Turn Back Frequency | Length (μm) |
|---|---|---|---|---|---|---|---|
| | Anterograde | Retrograde | Anterograde | Retrograde | | | |
| RFP-mito, SYP-YFP | 0.12 ±0.01 (n=32) | 0.12 ±0.01 (n=40) | 14 ±2.6 (n=152) | 15 ±2.4 (n=152) | 0.13 ±0.03 (n=152) | 0.02 ±0.01 (n=152) | 1.6 ±0.1 (n=111) |
| RFP-mito, Miro, SYP-YFP | 0.10 ±0.01 (n=23) | 0.12 ±0.01 (n=26) | 12 ±2.4 (n=145) | 14 ±2.6 (n=145) | 0.08 ±0.02 (n=145) | 0.00 ±0.00 (n=145) | 1.6 ±0.1 (n=118) |
| RFP-mito, Miro$^{KK}$, SYP-YFP | 0.13 ±0.01 (n=30) | 0.13 ±0.01 (n=26) | 17 ±2.8 (n=156) | 13 ±2.6 (n=156) | 0.06 ±0.02 (n=156) | 0.00 ±0.00 (n=156) | 1.7 ±0.1 (n=122) |
| RFP-mito, Miro, KHC, milton | 0.12 ±0.01 (n=21) | 0.12 ±0.02 (n=17) | 11 ±2.5 (n=144) | 9 ±2.3 (n=144) | 0.05 ±0.02 (n=144) | 0.00 ±0.00 (n=144) | 1.4 ±0.1 (n=121) |
| RFP-mito, Miro$^{KK}$, KHC, milton | 0.11 ±0.01 (n=28) | 0.12 ±0.01 (n=24) | 15 ±2.7 (n=147) | 14 ±2.7 (n=147) | 0.07 ±0.02 (n=147) | 0.01 ±0.01 (n=147) | 1.5 ±0.1 (n=122) |
| One-Way Anova | 0.843 | 0.858 | 0.604 | 0.518 | 0.087 | 0.228 | 0.064 |

**Table S1.1 Movement Parameters of Mitochondria before Calcimycin Treatment**

Average Velocity is defined as the average velocity of all instantaneous velocities that are NOT zero for a mitochondrion; % of time in Motion is calculated by the numbers of instantaneous velocities that are not zero divided by the numbers of total instantaneous velocities (including both zero and non-zero) for a mitochondrion; Stop Frequency is defined as the frequency per mitochondrion that the instantaneous velocity changes to zero; Turn Back Frequency is defined as the frequency per mitochondrion that the direction of instantaneous velocity reverses. The definitions are for all tables. Data is from 10 axons from 4 transfections.

| | Average Velocity (µm/s) | | % of Time in Motion | | Stop Frequency | Turn Back Frequency | Length (µm) |
|---|---|---|---|---|---|---|---|
| | Anterograde | Retrograde | Anterograde | Retrograde | | | |
| RFP-mito, SYP-YFP | 0.17 ±0.04 (n=8) | 0.16 ±0.03 (n=16) | 3 ±1.2 (n=147) | 5 ±1.4 (n=147) | 0.11 ±0.03 (n=147) | 0.00 ±0.00 (n=147) | 1.6 ±0.1 (n=112) |
| RFP-mito, Miro, SYP-YFP | 0.12 ±0.03 (n=4) | 0.10 ±0.02 (n=6) | 1 ±0.6 (n=136) | 3 ±1.3 (n=136) | 0.03 ±0.01 (n=136) | 0.00 ±0.00 (n=136) | 1.6 ±0.1 (n=116) |
| RFP-mito, Miro$^{KK}$, SYP-YFP | 0.13 ±0.02 (n=27) | 0.14 ±0.03 (n=27) | 11 ±2.3 (n=154) | 12 ±2.3 (n=154) | 0.07 ±0.02 (n=154) | 0.00 ±0.00 (n=154) | 1.7 ±0.1 (n=121) |
| RFP-mito, Miro, KHC, milton | 0.15 ±0.06 (n=5) | 0.11 ±0.02 (n=5) | 3 ±1.3 (n=135) | 3 ±1.4 (n=135) | 0.01 ±0.01 (n=135) | 0.00 ±0.00 (n=135) | 1.5 ±0.1 (n=119) |
| RFP-mito, Miro$^{KK}$, KHC, milton | 0.14 ±0.02 (n=29) | 0.10 ±0.01 (n=28) | 18 ±3.1 (n=140) | 16 ±2.9 (n=140) | 0.07 ±0.02 (n=140) | 0.04 ±0.03 (n=140) | 1.5 ±0.1 (n=121) |
| One-Way Anova | 0.870 | 0.259 | <0.001 | <0.001 | 0.013 | 0.096 | 0.212 |

**Table S1.2 Movement Parameters of Mitochondria after Calcimycin Treatment**

Data is from 10 axons from 4 transfections.

| RFP-mito, SYP-YFP | Average Velocity (μm/s) | | % of Time in Motion | | Stop Frequency | Turn Back Frequency |
|---|---|---|---|---|---|---|
| | Anterograde | Retrograde | Anterograde | Retrograde | | |
| Before | 0.10 ±0.01 (n=12) | 0.10 ±0.02 (n=9) | 26 ±6.2 (n=44) | 8 ±3.6 (n=44) | 0.11 ±0.05 (n=44) | 0.02 ±0.02 (n=44) |
| After | 0.10 ±0.01 (n=11) | 0.13 ±0.01 (n=8) | 22 ±5.8 (n=43) | 14 ±4.9 (n=43) | 0.19 ±0.06 (n=43) | 0.00 ±0.00 (n=43) |
| Mann-Whitney *U* | 0.833 | 0.252 | 0.599 | 0.207 | 0.346 | 0.303 |

**Table S2 Movement Parameters of Synaptophysin-YFP before and after Calcimycin Treatment**

Data is from 4 axons from 3 transfections.

| | Average Velocity (μm/s) | | % of Time in Motion | | Stop Frequency | Turn Back Frequency |
|---|---|---|---|---|---|---|
| | Anterograde | Retrograde | Anterograde | Retrograde | | |
| RFP-mito, SYP-YFP | 0.11 ±0.01 (n=12) | 0.12 ±0.01 (n=17) | 15 ±4.3 (n=57) | 16 ±4.1 (n=57) | 0.18 ±0.05 (n=57) | 0.00 ±0.00 (n=57) |
| RFP-mito, KIF5C1-335-YFP | 0.13 ±0.02 (n=14) | 0.12 ±0.02 (n=10) | 18 ±4.7 (n=54) | 14 ±4.5 (n=54) | 0.07 ±0.04 (n=54) | 0.02 ±0.02 (n=54) |
| Mann-Whitney *U* | 0.782 | 0.749 | 0.567 | 0.269 | 0.109 | 0.304 |

**Table S3.1 Movement Parameters of Mitochondria before Calcimycin Treatment**

| | Average Velocity (μm/s) | | % of Time in Motion | | Stop Frequency | Turn Back Frequency |
|---|---|---|---|---|---|---|
| | Anterograde | Retrograde | Anterograde | Retrograde | | |
| RFP-mito, SYP-YFP | 0.14 ±0.04 (n=2) | 0.14 ±0.06 (n=3) | 2 ±1.5 (n=55) | 2 ±1.5 (n=55) | 0.11 ±0.04 (n=55) | 0.00 ±0.00 (n=55) |
| RFP-mito, KIF5C1-335-YFP | 0.17 ±0.03 (n=10) | 0.13 ±0.01 (n=9) | 21 ±5.7 (n=48) | 19 ±5.6 (n=48) | 0.03 ±0.00 (n=48) | 0.00 ±0.00 (n=48) |
| Mann-Whitney *U* | 0.909 | 0.727 | 0.003 | 0.023 | 0.077 | 1 |

**Table S3.2 Movement Parameters of Mitochondria after Calcimycin Treatment**

Data is from 6 axons from 3 transfections.

| | Average Velocity (μm/s) | | % of Time in Motion | | Stop Frequency | Turn Back Frequency |
|---|---|---|---|---|---|---|
| | Anterograde | Retrograde | Anterograde | Retrograde | | |
| RFP-mito, Miro, PSD95-YFP | 0.11 ±0.01 (n=13) | 0.14 ±0.01 (n=15) | 18 ±4.7 (n=58) | 23 ±5.4 (n=58) | 0.09 ±0.04 (n=58) | 0.02 ±0.02 (n=58) |
| RFP-mito, Miro$^{KK}$, PSD95-YFP | 0.14 ±0.02 (n=15) | 0.13 ±0.02 (n=15) | 21 ±5.2 (n=61) | 22 ±5.2 (n=61) | 0.33 ±0.02 (n=61) | 0.00 ±0.00 (n=61) |
| Mann-Whitney *U* | 0.217 | 0.775 | 0.968 | 0.872 | 0.218 | 1 |

**Table S4.1 Movement Parameters of Mitochondria before Glutamate Treatment**

| | Average Velocity (μm/s) | | % of Time in Motion | | Stop Frequency | Turn Back Frequency |
|---|---|---|---|---|---|---|
| | Anterograde | Retrograde | Anterograde | Retrograde | | |
| RFP-mito, Miro, PSD95-YFP | N.A. | 0.08 ±0.01 (n=2) | 0 ±0.0 (n=56) | 3 ±1.9 (n=56) | 0.00 ±0.00 (n=56) | 0.00 ±0.00 (n=56) |
| RFP-mito, Miro$^{KK}$, PSD95-YFP | 0.15 ±0.02 (n=14) | 0.10 ±0.01 (n=15) | 19 ±5.0 (n=53) | 21 ±5.0 (n=53) | 0.11 ±0.04 (n=53) | 0.00 ±0.00 (n=53) |
| Mann-Whitney *U* | N.A. | 0.618 | <0.001 | <0.001 | 0.01 | 1 |

**Table S4.2 Movement Parameters of Mitochondria after Glutamate Treatment**

Data is from 6 axons from 3 transfections. N.A., not available.