**Title: Axolemmal nanoruptures arising from paranodal membrane injury induce secondary axon degeneration in murine Guillain-Barre syndrome**

Study Aim: This study used established *ex* *vivo* acute and subacute mouse models ofl Guillain-Barre syndrome (GBS) to study the mechanisms of secondary axon degeneration.

For immunostaining, images were captured using Zeiss LSM or Zeiss AxioImager Z1 microscopes and observed using Zeiss Zen or ImageJ (FIJI) software. Quantification was performed using the same. Statistical analysis was performed using GraphPad software. Reposited data are Graphpad files of collated data.

Figure 1a

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| --- | --- | --- | --- | --- |
| File name | Mice | Tissue | Purpose | Staining and output |
| GFP intensity | B6CGTGN n=3 | Ex vivo TS  (half TS con, half injured) | Assess S100 GFP following acute injury with anti-sulfatide Ab and complement *ex vivo* | S100 GFP  Myelin basic protein (MBP)  Bungarotoxin (BTx)  Membrane attack complex (MAC) or C3c  AxioImager .czi files |

Figure 1.b,c

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| File name | Mice | Tissue | Purpose | Staining and output |
| glialxhCAST vs AK295 ex vivo 4h inj AnkB pNFasc stats | glialxhCAST n=3  glial n=3 | Ex vivo TS  (half TS con, half injured)  (half injured half injured + AK295) | Assess nodal protein integrity following acute injury with AgAb and complement *ex vivo* and various calpain inhibition paradigms | AnkyrinB or pan-neurofascin  Myelin basic protein (MBP)  Bungarotoxin (BTx)  Membrane attack complex (MAC) or C3c  AxioImager .czi files |

Figure 2c

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| File name | Mice | Tissue | Purpose | Staining and output |
| NFH loss - combined | glialxhCAST n=3  PLP n=3  SARM1ko vs WT n=3/genotype  glial n=3  WT n=3  YCG063  WT n=6 | Ex vivo TS  (half TS con, half injured) | Assess axonal integrity following sub-acute injury with AgAb and complement *ex vivo*  And various protection strategies | NFH or CFP  Myelin basic protein (MBP)  Bungarotoxin (BTx)  Membrane attack complex (MAC) or C3c  AxioImager .czi files |

Figure 2d

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| File name | Mice | Tissue | Purpose | Staining and output |
| CFP loss | B6CGTGN n=12 total | Ex vivo TS  (half TS con, half injured) | Assess S100 GFP following acute injury with anti-sulfatide Ab and complement *ex vivo* | S100 GFP  Myelin basic protein (MBP)  Bungarotoxin (BTx)  Membrane attack complex (MAC) or C3c  AxioImager .czi files |

Figure 3b,c,d,e

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| File name | Mice | Tissue | Purpose | Staining and output |
| Calcium imaging | TNXXL n=8 total | Ex vivo TS | Live Calcium imaging and morphology analysis | FRET Calcium images (CFP and YFP) |

Figure 3f

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| File name | Mice | Tissue | Purpose | Staining and output |
| Dextran dyes | B6CGTGN n=9 total | Ex vivo TS  (half TS con, half injured) | Assessing presence of different sized dyes in axons | Axonal CFP  Bungarotoxin (BTx)  Dextran 3 kDa or 70 kDa dye |