Characterisation of axonopathy in a mouse model of ALS-parkinsonism dementia complex
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OBJECTIVES
We have carried out a preliminary investigation of the morphology of innervation at neuromuscular junctions in a mouse model of ALS-PDC in relation to the timing of motor neuron loss, in order to establish the directionality of axonopathy. Results from a recent communication suggest that the mSOD model of ALS shows initial damage at motor endplates, and that motor neuron loss reflected a dying back of the denervated axons.

1. INTRODUCTION
Amyotrophic lateral sclerosis – parkinsonism dementia complex (ALS-PDC) is a unique progressive neurological disorder first characterized among the indigenous Chamorro people of Guam. The clinical phenotype of ALS-PDC is one of either classical ALS/motor neuron disease, or an Alzheimer’s dementia with parkinsonism features, or a combination of both. In a lengthy epidemiologic surveillance of the disease, evidence revealed that the strongest epidemiologic link was the correlation to consumption of the seeds from Cycas micronesica, a species of cycad. We have previously generated a mouse model of ALS-PDC by in vivo feeding of washed cycad flour. Treated mice show phenotypes that mimic or correspond to human pathology in all aspects, especially the expression of an ALS phenotype: mice show inappropriate clasping reflexes, tremor, and deterioration of motor coordination and/or muscle strength and a loss of motor neurons in lumbar cord and motor cortex. In the present study, immunohistochemistry together with fluorescence microscopy was used to compare the morphological features of axons and their endplates of cycad-fed and control mice.

2. MORPHOLOGY
Wild type control.

Motor endplate innervation at neuromuscular junction.

Immunostaining for axons with neurofilament/SV2 (green) together with AChR staining with α-bungarotoxin (red) reveals morphology of innervation at neuromuscular junctions. Endplates were depicted as fully innervated (A) when there is complete overlap of the axon terminal with the endplate, where yellow indicates overlap (inset). Neuromuscular junctions associated with a preterminal axon only, or showing partial overlap of the axon terminal with the endplate were depicted as having intermediate innervation (B). Complete denervation is evident when the endplate is not associated with an axon (arrow in C). All pictures taken at the final, 33 week time point. (Scale bar 50μm).

Quantification of motor endplate innervation status at 33 weeks shows no difference of endplate denervation between groups. Data are shown as percent of total endplates classified as innervated, intermediate, or denervated per group. Numbers in parentheses indicate the total number of endplates quantified per group. At this time point, a majority of endplates are completely innervated in both groups: 79.38 ± 5.98% for control animals and 84.22 ± 14.46% for cycad-fed animals. Denervated endplates made up 15.41 ± 5.72% for control animals and 13.45 ± 13.03% for cycad-fed animals.

3. BEHAVIOUR
Body weight measurements and wire mesh hang tests measuring muscle strength were performed weekly during the duration of their diet. Body weight measurements show no differences between groups, although all animals exhibit a steady weight gain with ageing (D). Animals fed control pellets on average performed better than animals receiving cycad pellets as part of their daily diet (E). For both animal groups, there is a trend towards decreased performance.

4. CNS HISTOLOGY
Motor neuron loss is significant in cycad-fed mice and neurones by the earliest time point.

Immunostaining for motor neurons with ChAT reveals lower counts in cycad-fed mice (G) as compared to control mice (F) at 12 weeks. Quantification of motor neurons in lumbar cord reveals lower counts in cycad-fed mice at all time points (graph). Mean counts were compared by ANOVA (Scale bar 20μm).

Microglial activation is evident around motor neurons in the ventral horn of lumbar cord.

Immunostaining for microglia with Iba-1 shows significantly greater microglial activation in cycad-fed animals (I) than control animals (H) at 12 weeks. Quantification of microglia in lumbar cord reveals lower counts in control mice at all time points (graph). Microglial activation around motor neurons was identified in the absence of distal axon degeneration from motor endplates, suggesting that motor neuron pathology may begin at the motor neuron soma and proceed distally. Mean counts were compared by ANOVA (Scale bar 20μm).

Out-bred CD1 wild-type mice were fed cycad flour or regular white flour as part of their daily diet (0.5g cycad or regular flour) and subjected weekly to motor function tests. (The daily cycad diet commenced 5 weeks after the start of behaviour tests.) Cohorts of these mice were killed at three time points, and spinal cord and gastrocnemius muscles were dissected after perfusion with paraformaldehyde. Tissues were processed to be frozen for cryosectioning. Lumbar cord sections were either immunostained with Iba-1 or GFAP to visualise microglia or astrocyte proliferation, respectively. Motor neurons were stained with FITC-Online acetylcholinesterase (ChAT). Gastrocnemius muscles were immunostained for astrocyte GFAP and endplates acetylcholine receptors were stained with Alexa Fluor 647-conjugated. This work was supported by grants from the US Army Medical Research and Materiel Command and CHIR.

5. CONCLUSION
Microglial activation around motor neurons was identified in the absence of distal axon degeneration, suggesting that motor neuron pathology may begin at the motor neuron soma and proceed distally. This is different from the more widely studied mSOD mouse model of ALS, where axonopathy was shown to begin distally at the motor end-plates and proceed proximally. The loss of motor neurons in the absence of endplate denervation suggests sprouting of surviving motor neurons.

These differences in disease progression also provide the most plausible evidence of what may be different forms of ALS.

REFERENCES

METHODS
Out-bred CD1 wild-type mice were fed cycad flour or regular white flour as part of their daily diet (0.5g cycad or regular flour) and subjected weekly to motor function tests. (The daily cycad diet commenced 5 weeks after the start of behaviour tests.) Cohorts of these mice were killed at three time points, and spinal cord and gastrocnemius muscles were dissected after perfusion with paraformaldehyde. Tissues were processed to be frozen for cryosectioning. Lumbar cord sections were either immunostained with Iba-1 or GFAP to visualise microglia or astrocyte proliferation, respectively. Motor neurons were stained with FITC-Online acetylcholinesterase (ChAT). Gastrocnemius muscles were immunostained for astrocyte GFAP and endplates acetylcholine receptors were stained with Alexa Fluor 647-conjugated. This work was supported by grants from the US Army Medical Research and Materiel Command and CHIR.