Characterization of gait analysis, memory, and neuromuscular junction integrity in a mouse model of ALS-Parkinsonism Complex

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INTRODUCTION
Amyotrophic lateral sclerosis (ALS) is a fatal paralytic disease that targets motor neurons, leading to widespread denervation atrophy of muscle. Transgenic mice that carry mutations in the gene encoding Cu, Zn superoxide dismutase (SOD) are by far the most common model of ALS. Such models have their limitations since 5 – 10% of ALS cases are familial and of these, only 20 – 25% are due to mutations in the gene encoding SOD. Previously, a mouse model of ALS-parkinsonism dementia (ALS-PDC) was generated by in vivo feeding of washed cycad flour. These mice show behavioural and neuropathological deficits that mimic human pathology. ALS-PDC is a neurological condition where motor neuron disease resulting from the ALS component may occur in conjunction with parkinsonism features and cognitive deficits. This syndrome has been described as the ‘Guam’ form of ALS, with linkage to the seeds of cycad as a traditional food in Guam and also a source of ingested toxin. In the present study, we assessed previously unexplored aspects of the behavioural and neuropathological pattern of cycad-fed mice, namely detailed gait analysis, social and object memory, and neuromuscular junction integrity.

1. RESULTS SUMMARY
Cycad-fed animals show a transient, but significant, deficit in their gait pattern, as revealed by ventral plane videography. In addition, subtle deficits in tasks of social recognition were also detected, whereas object memory seemed to be preserved. Quantitative analysis in spinal cord showed a significant increase in microglial activation at each time point as determined by two-way ANOVA, whereas no differences in astrocyte proliferation were apparent. At 33 weeks, motor neuron loss was greater in cycad-fed animals whereas there was no evidence of significant end-plate denervation in either group.

3. GAIT ANALYSIS

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<thead>
<tr>
<th>Treatment</th>
<th>F1,1538 = 15.6 (p &lt; 0.001)</th>
<th>F2,1538 = 12.0 (p &lt; 0.005)</th>
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4. MEMORY

Social Memory

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<tr>
<th>Variables measured</th>
<th>Group: F1,18 = 0.0571 (n.s.)</th>
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Object Memory

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<tr>
<th>Variables measured</th>
<th>Group: F1,18 = 0.0571 (n.s.)</th>
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5. CNS PATHOLOGY

Motor neuron loss and microglial activation in cycad-fed mice occurs by the earliest time point in the ventral horn of lumbar cord. Immunostaining for motor neurons with ChAT reveals lower counts in cycad-fed mice (E) as compared to control mice (D) at 12 weeks. 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).

6. CONCLUSIONS

Microglial activation around motor neurons in the absence of distal axon degeneration suggests 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 degeneration suggests survival of surviving motor neurons. These differences in disease progression provide the most plausible evidence of what may be different forms of ALS. The spontaneous recovery in the paw angle deficit suggests the action of compensatory functional or morphological mechanisms. The presence of social recognition deficits might be related to impairment in olfactory or limbic functions.

REFERENCES


METHODS

Male 6.5-8.5 weeks old weanling male C57Bl/6J mice were fed washed cycad flour or regular white flour as part of their daily diet (0.5g cycad or regular flour/day) and subjected weekly to a detailed gait analysis using ventral plane videography (DigigaitTM, Mouse Specifics Inc.). Social interaction was evaluated as the ratio of time spent in the vicinity of a cage with and without a foreign mouse. Social memory was evaluated as a function of decreased social interaction during a second visit, one day later, relative to the foreign mouse. Object recognition and memory was evaluated as a function of decreased exploration of a familiar object during a second visit to an arena containing a familiar and an unfamiliar object. 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 Iba1 or GFAP to visualise microglia or astrocyte proliferation, respectively. Motor neurons were stained with FITC-Choline acetyltransferase (ChAT). Gastrocnemius muscles were immunostained for α-dystroglycan (neurolintiment/SV2), and endplates acetylcholine receptors were stained with Alexa Fluor 594-α-bungarotoxin. This work was supported by grants from the NIMH, ALSA, and the Scottish Rites Charitable Foundation of Canada.