

Supplemental Material

Materials and Methods

Accelerating rotarod and static rod. Motor coordination was assessed using both the accelerating rotarod and static rod tests at P42 and P70. For the rotarod test, mice were placed on the beam of a rotarod (Ugo Basile) revolving at the default 5 rpm, facing in the opposite orientation to rotation. After 30 s, the speed was gradually accelerated to a maximum of 30 rpm over a 6 min test session. The latency before falling was measured up to a maximum total time on the rod of 6 min for 3 trials at 1 h intervals on 3 consecutive days. For the static rod test, mice were placed on the protruding end of wooden rod 60 cm in length and 28 mm in diameter, facing the supported end. The time taken to fall from the rod while attempting to reach the supported end was recorded.

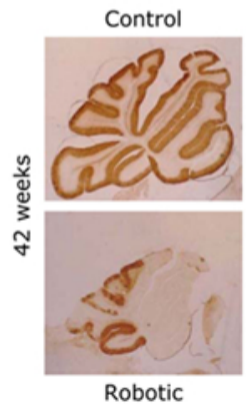
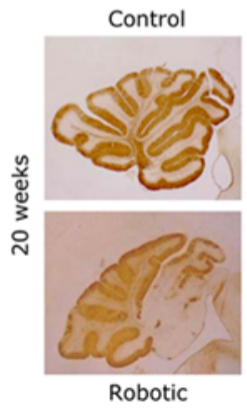
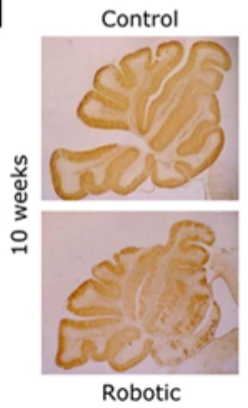
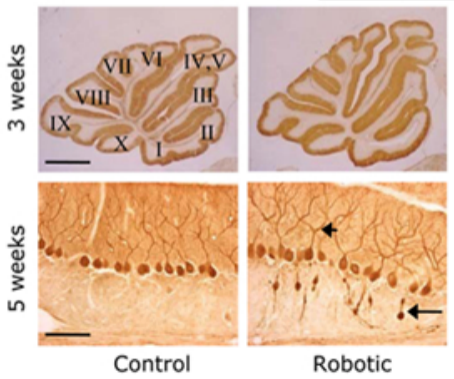
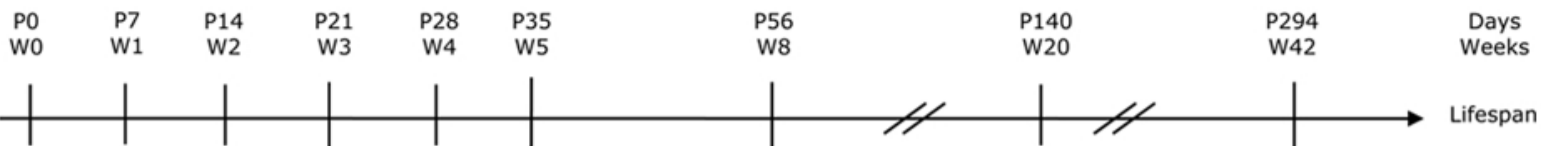
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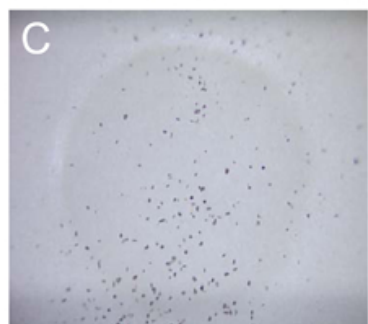
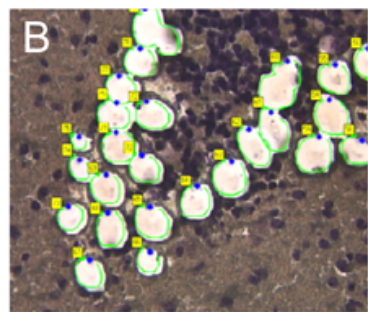
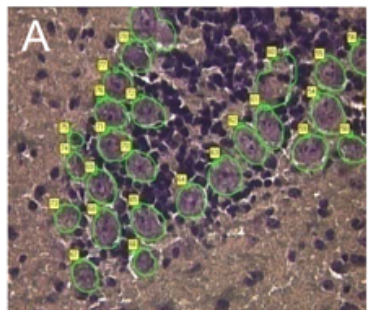
Supplemental Figure S1. Progressive adult-onset region-specific PC loss in the robotic cerebellum. Calbindin-stained vermal parasagittal sections. PC degeneration is first noted in the cerebellum around 21 days postnatally (P21) and is particularly evident at 5 weeks (W5) of age with swelling of cell bodies, dendrites (arrowhead) and axonal torpedoes (arrow); ataxia follows shortly after. From 8 weeks, a progressive region-specific PC death process takes place that affects most severely the anterior lobes (I to V) whilst lobe X is spared throughout. By 42 weeks, only 30% of the total PC population is still alive: virtually no PCs have survived in the anterior region and posterior lobes have undergone massive PC loss, resulting in a decrease in size of the molecular layer and shrinking of the cerebellum; robotic mice die shortly after of respiratory

arrest. Scale bars, 1mm and 100 μ m at 3, 10, 20 and 42 weeks, and 5 weeks, respectively. Figure modified from Isaacs et al. (2003) by copyright permission from the Journal of Neuroscience, and reproduced from Bitoun and Davies (2009) with kind permission of Springer Science and Business Media.

Supplemental Figure S2. LCM and microarray analysis of robotic PCs. **A-C**, LCM of PCs from cresyl violet-stained parasagittal cerebellum sections. Boundaries of PCs are drawn using the Carl Zeiss PALM RoboSoftware (**A**) and laser-microdissected cells (**B**) are catapulted into the cap of a tube placed above the sections (**C**). Pictures were taken with 40x (**A-B**) and 5x (**C**) objectives. **D**, Gene expression changes in robotic PCs. Heat map shows PC expression profiles in wild-type (WT) and robotic (ROB) mice. Each column represents one of four RNA samples and each row represents one gene. Expression levels are depicted according to the colour scale at the bottom with increased expression ranging from blue to red.

Supplemental Figure S3. Treatment of robotic mice with recombinant IGF-1 does not prevent ataxia. Analysis of motor coordination of robotic mice following administration of IGF-1 (n= 8) or vehicle (n= 9) at P70 using the accelerating rotarod and static rod did not show any significant difference in the mean latency to fall.





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