

Rewired glycosyltransferase activity promotes scarless regeneration and functional recovery in spiny mice after complete spinal cord transection



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Introduction

During development, CNS neurons possess an active molecular machinery that drives axon elongation. However, upon maturation, axon growth capacity is repressed to allow for synaptic wiring and, in general, is not re-engaged after injury. Hence, regeneration of the adult mammalian CNS is abortive, which remains an obstacle to treat spinal cord injuries (SCI). Here we reveal that the spiny mouse (*Acomys cahirinus*), an unique mammal with extensive regenerative capacity in non-CNS tissues, can spontaneously regenerate after SCI, overturning the established dogma.

Acomys spontaneously recovers function following complete SCI

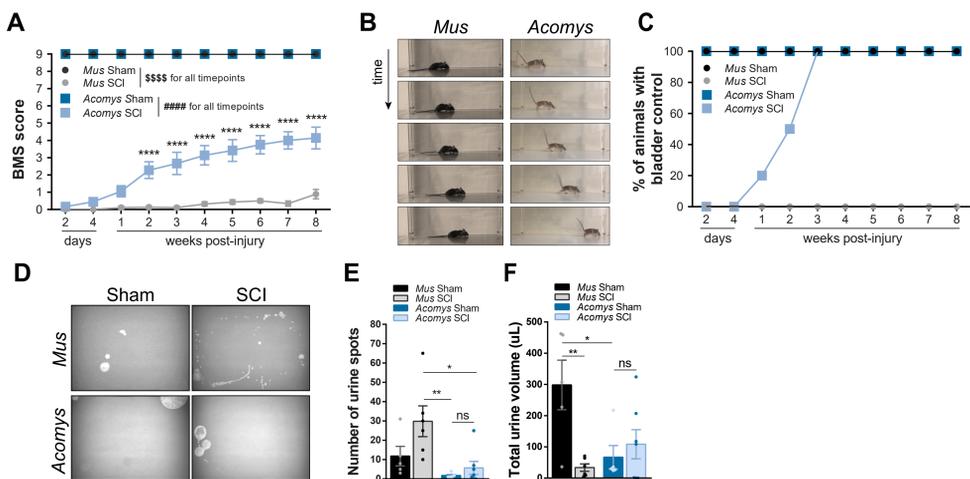


Fig. 1 - (A) Assessment of motor function of injured *Mus* and *Acomys* using the BMS score shows locomotion recovery of *Acomys*; **(B)** Representative sequential images of locomotion improvement of injured *Mus* and *Acomys*; **(C)** Percentage of animals recovering bladder control in *Mus* and *Acomys*; **(D)** Representative images of the urine spots of *Mus* (upper) and *Acomys* (lower); **(E)** Quantification of number and **(F)** volume of voided urine spots indicate *Acomys* faster recovery from the bladder areflexia period typically present after SCI.

Injured *Acomys* exhibits axon regeneration, synapse formation and signal propagation

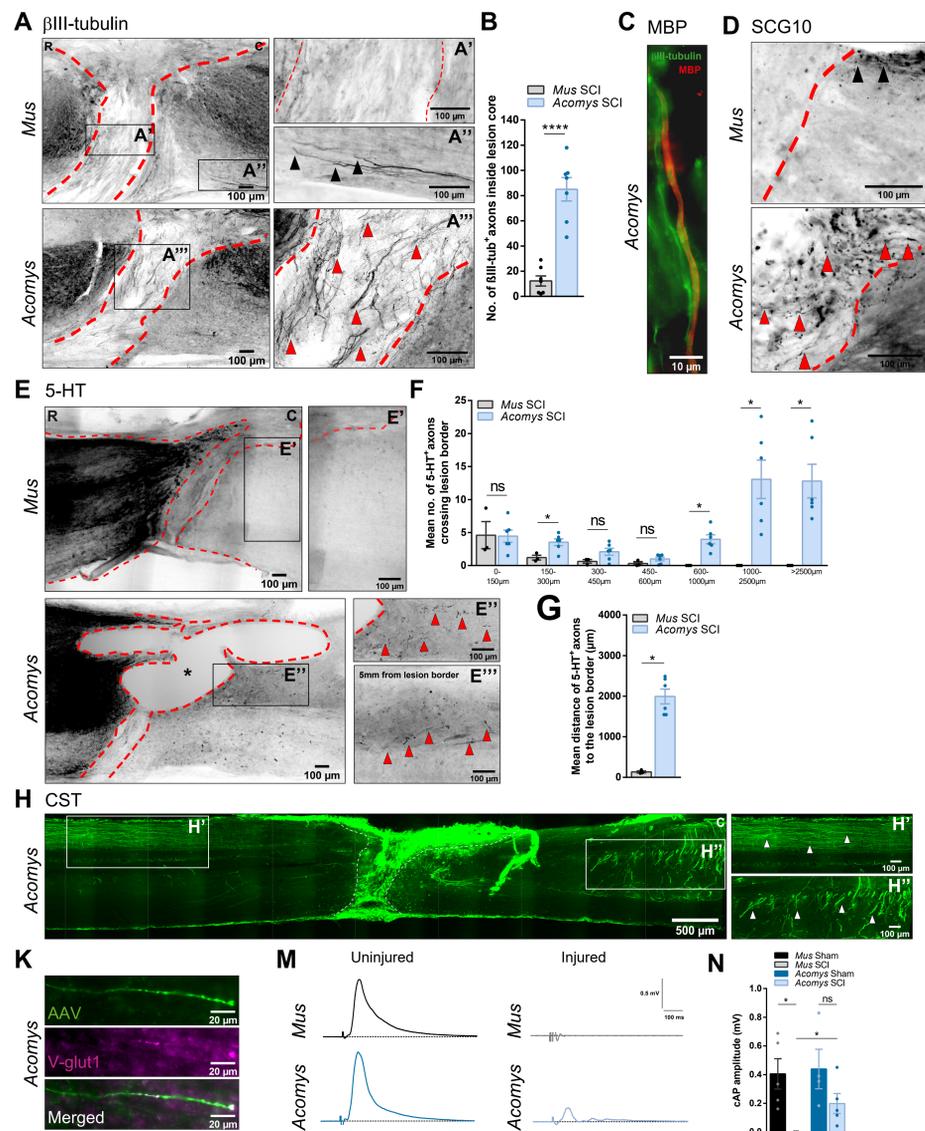


Fig. 2 - (A) Sagittal spinal cord sections of *Acomys* stained with βIII-tubulin confirmed the existence of regenerating axons penetrating and crossing the bridging tissue (A'). In *Mus*, no axons are seen in the injury site (A) and are only found retracted in relation to the injury area (A'); **(B)** Quantification related to A; **(C)** *Acomys* spinal cord lesion core stained with βIII-tubulin (green) and myelin-basic protein (MBP) (red); **(D)** Representative sagittal spinal cord sections of *Mus* and *Acomys* stained with SCG10 (ascending sensory axons). In *Mus*, SCG10+ axons do not cross the injury border whereas in *Acomys* SCG10+ axons cross the injury site; **(E)** Representative sagittal spinal cord sections of *Mus* and *Acomys* stained with 5-HT (descending motor axons) show that *Acomys* axons are able to regenerate within the injured tissue (E') whereas in *Mus* no regenerating axons are found (E''); **(F,G)** Quantification related to E; **(H)** Cortical AAV-GFP injections in injured *Acomys* reveal motor corticospinal tract axons traced rostrally to injury site (H') and robust axon regeneration caudally (H''); **(I)** Presynaptic VGLUT1 marker staining of regenerating axons in injured *Acomys*; **(M)** Nerve conduction of descending motor tracts show compound action potentials being conducted in the injured *Acomys* spinal cord. In contrast, no signal conduction across the lesion was detected in *Mus*, as expected by the absence of functional recovery. **(N)** Quantification related to M.

Acknowledgments

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Acomys sustains growth under inhibitory conditions and express blue RAGs

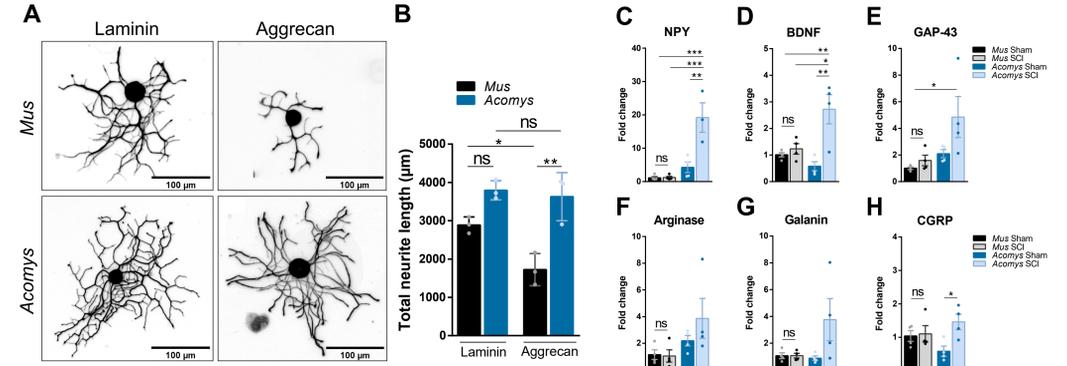


Fig. 3 - (A) βIII-tubulin staining of DRG neurons from *Mus* and *Acomys* grown in permissive (laminin) or inhibitory conditions (aggrecan) indicates that *Acomys* DRG neurons sustain growth under inhibitory conditions, overshooting repulsive cues. **(B)** Total neurite length related to A; **(C-H)** qPCR of DRGs from uninjured and injured *Mus* and *Acomys* reveal that SCI induced a generalized increased expression of the major RAGs in injured *Acomys*, suggesting that this species are able to mount a pro-regenerative neuron intrinsic program after CNS injury.

Scarless regeneration and rewired glycosylation are signatures of *Acomys* pro-regenerative tissue

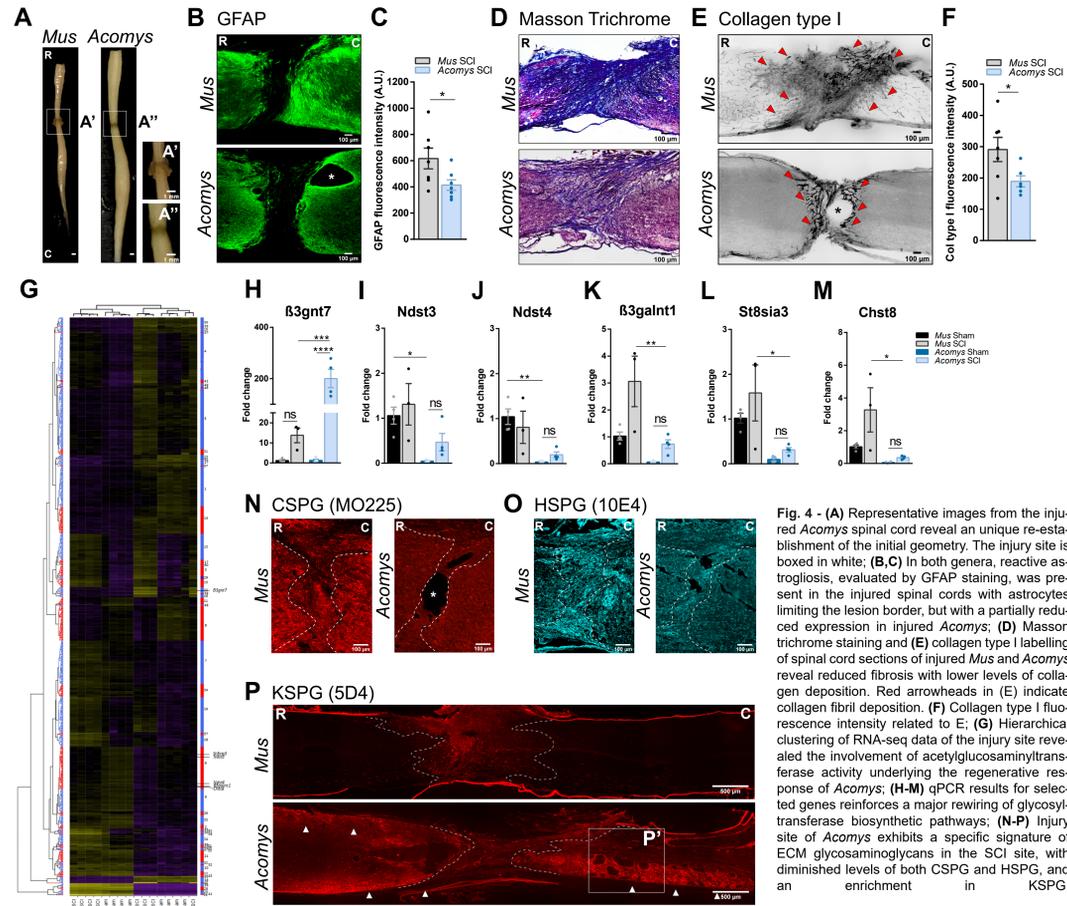


Fig. 4 - (A) Representative images from the injured *Acomys* spinal cord reveal a unique re-establishment of the initial geometry. The injury site is boxed in white; **(B,C)** In both genera, reactive astroglia, evaluated by GFAP staining, was present in the injured spinal cords with astrocytes limiting the lesion border, but with a partially reduced expression in injured *Acomys*; **(D)** Masson trichrome staining and **(E)** collagen type I labelling of spinal cord sections of injured *Mus* and *Acomys* reveal reduced fibrosis with lower levels of collagen deposition. Red arrowheads in **(E)** indicate collagen fibril deposition. **(F)** Collagen type I fluorescence intensity related to E; **(G)** Hierarchical clustering of RNA-seq data of the injury site revealed the involvement of acetylglucosaminyltransferase activity underlying the regenerative response of *Acomys*; **(H-M)** qPCR results for selected genes reinforces a major rewiring of glycosyltransferase biosynthetic pathways; **(N-P)** Injury site of *Acomys* exhibits a specific signature of ECM glycosaminoglycans in the SCI site, with diminished levels of both CSPG and HSPG, and an enrichment in KSPG.

Conclusions

- After complete SCI, *Acomys* spontaneously recovers sensorimotor and urinary functions.
- The scarless *Acomys* SCI site shows abundant regenerating axons from multiple tracts, new synapse formation and signal propagation.
- *In vitro*, *Acomys* neurons sustain growth on inhibitory substrates, and *in vivo* SCI triggers increased expression of RAGs.
- *Acomys* assembles a pro-regenerative environment with reduced astroglia and fibrosis and rewired glycosyltransferase activity.

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Understanding the mechanisms that underlie *Acomys* CNS regeneration will enable the design of interventions to induce axon regrowth in non-regenerative mammals, including therapeutic options to human SCI patients.

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