Supplemental methods

Mouse model of severe sciatic nerve crush injury

Sciatic nerve crush injury was performed as previously described by our lab (Tseng et al., 2016; Clark et al., 2019; Hsu et al., 2020). Briefly, after deep anesthesia with intraperitoneal (IP) injection of ketamine (100 mg/kg)/xylazine (10 mg/kg) mixture (supplied by animal care facility), the right hindlimb was shaved and prepared with alcohol swabs and McKesson 10% povidone-iodine solution (Cat# 854301PA, McKesson Corporation, NY, USA). Under a precision stereo zoom binocular microscope (Model PZMIII, World Precision Instruments, Sarasota, FL, USA), a lateral skin incision (~2.5 cm) was made along the length of the femur and the sciatic nerve (SN) was bluntly exposed through the iliotibial band. Crush injury was performed ~3 mm proximal to the SN trifurcation using a needle driver (3 mm tip width; RH2560, V. Mueller; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for 30 seconds. The skin was closed by surgical staples and post-operative slow release buprenorphine (0.05 mg/kg) was given subcutaneously to all animals as an analgesic. The experimental animals (9/group) were randomly assigned to intraperitoneal saline and different doses of 4-AP (10, 40, and 80 µg per 20 g mouse). Functional recovery as sciatic function index (SFI) was assessed by walking track analysis before crush injury (baseline) and on post-injury days 3, 7, 14 and 21.

References

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