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SELECTIVE FUNCTIONAL ASSESSMENT OF SENSORY FIBRES IN THE RAT SCIATIC NERVE USING OPTOGENETICS

Background. Peripheral nerve injuries resulting from accidents, military operations, domestic or iatrogenic injuries, are one of the leading causes of long-term loss of limb function. The development of highly selective *in vivo* testing methods for comprehensive assessment of functional regeneration is crucial for understanding the dynamics of tissue recovery. This work aimed to develop a protocol for optogenetic stimulation of somatosensory fibres by refining a technique for subepineural injection of adeno-associated viral (AAV) vectors expressing the light-sensitive cation channel, channelrhodopsin-2 (ChR2), into the plasma membrane of rat sciatic nerve axons. **Methods.** AAV1-CAG-hChR(H134R)-mCherry.WPRE.SV40 (Addgene, 100054-AAV1) construct was injected subepineurally to the sciatic nerve of Wistar rats (2 m.o.; n = 5). The safety of the surgical procedure was evaluated over an 8-week observation period using Hargreaves and von Frey behaviour testing. Pain-like responses to photostimulations of different regions of the rat plantar area were assessed using a blue LED at 450 nm. **Results.** The developed protocol for subepineural administration of viral vectors proved safe with no evidence of iatrogenic neuropathic pain or motor dysfunction. Optical stimulation reliably evoked a nociceptive behavioural responses in the ipsilateral limb, indicating successful functional expression of ChR2. Behavioural responses peaked at 4 weeks post-injection, reflecting the temporal dynamics of the construct's functional expression. The highest response rate was observed during optical stimulation of the lateral and central regions of the limb, corresponding to the innervation territory of the sciatic nerve, while the response rate in the medial region, mainly innervated by the saphenous nerve, was the lowest. The specificity of the method was confirmed by minimal responses in the contralateral limb and the absence of false-positive responses when using a non-specific-wavelength LED (520 nm). **Conclusions.** The developed technique is well tolerated in animals, does not induce neuropathic pain, and enables selective functional assessment of sensory fibres within the distinct innervation territories of the targeted nerve. **Funding.** V.U. was supported by NASU grant 0126U002342. V.U., T.P. & P.B. were supported by NASU grants 0124U001556 and 0124U001557.

Keywords: sciatic nerve, optogenetics, channelrhodopsin-2, adeno-associated viral vectors, subepineural injection, behavioural responses.

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