



Jul 12, 2019

SPARC Retrograde Neuroanatomical Tracing of Phrenic Motor Neurons Using Intrapleural Injections of Cholera Toxin B Fragment

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ABSTRACT

This protocol describes the procedure for retrogradely labeling bilateral phrenic motor neuron pools in the cervical spinal cord using intrapleural injections of cholera toxin B fragment

- 1 Anesthesia is induced in a closed chamber (3.5% isoflurane in 100% O₂) then transferred to a heated surgical station and anesthesia is maintained via nose cone at 2-2.5%
- 2 Sterilized 25 µL Hamilton syringes with 9.52-mm sterile needles are prepared to administer CtB intrapleurally in rats.
- 3 Cholera toxin B fragment (CtB) is prepared as follows: 0.2% weight/volume; dissolved in sterile H₂O; Calbiochem, Billerica, MA.
- 4 25 µL of CtB is loaded into a 25-µL Hamilton syringe and 12.5 µL is injected per side (25 µL total per animal).
- 5 Prep injection site for injection using aseptic methods:
 - 5.1 shave and scrub injection sites per Institutionally approved animal protocol
- 6 Rats were turned sideways to inject CtB at the 5th intercostal space at a depth of ~6 mm.
- 7 Following injections, discontinue isoflurane and allow rat to recover fully while monitoring for signs of respiratory compromise or distress



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