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Optogenetic Stimulation of superior mesenteric ganglion in a model of septic shock

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ABSTRACT

Protocol details how to use optogenetics to activate neurons in the superior mesenteric ganglion of a mouse.

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MATERIALS TEXT

Materials:

Solutions:
LPS (Invivogen cat#TLRL-PB5LPS)
1x PBS

Animals:
TH.Cre YFP-CHR2 Mice

Optogenetics:
Optogenetic controller
Radiant software
PlexonBright LED *465nm blue*

Anesthesia:
Heating Pad
Isoflurane + nose

Retroorbital injection:
1/2ml Tuberculin Needle
26g Needle

Blood collection:
1 ml syringe

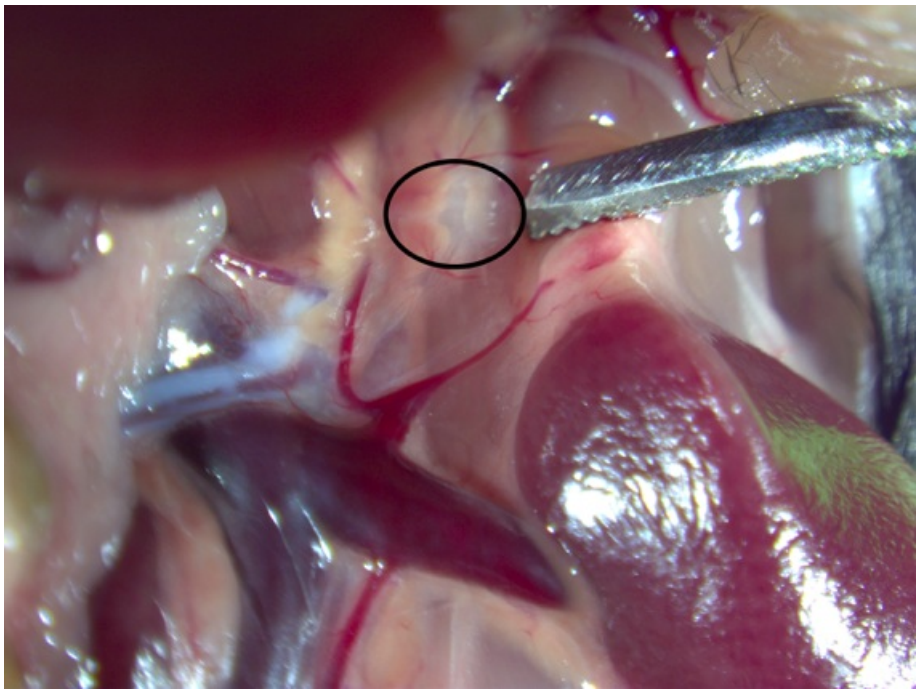
TNFα Measurements:
ELISA TNFα kit (ThermoFisher)

Optogenetic Stimulation

1 Place anesthetized mouse supine on heating pad under stereo dissecting scope

2 Make a 1-inch incision on the right side of the mouse, directly under the rib cage

3 Locate the SMG



superior mesenteric ganglion

4 Begin optogenetc stimulation (250mA, 10Hz, 2ms) of superior mesenteric ganglion (SMG) by placing the fiber 1-2mm over SMG

5 Stimulate for 10 minutes

6 Retro-orbital IV injection of 4mg/kg LPS

7 Continue optogenetic stimulation of SMG for another 10 minutes

8 Keep tissues moist with continual application of sterile 1X PBS and cover wound with sterile gauze

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9 Allow mouse to rest (under anesthesia) for 50 minutes

Blood Collection

10 Perform a cardiac puncture to draw blood serum and collect in serum separator tube

11 Euthanize mouse

12 Centrifuge blood at 15000rpm for 5 mins, 4C

13 Remove top layer and store at -80C

ELISA Protocol

14 Coat Corning Costar 9018 ELISA plate with 100ul/Well of capture antibody buffer
- Add 48ul capture antibody to 12ml 1x Coating Buffer
- Seal plate, incubate **overnight** 2-8C

15 Aspirate wells and wash 3x with >250ul/well washing buffer
- wash buffer: 1x PBS, 0.05% Tween 20
- Allow ~1 minute soak between washing
- Blot on absorbent paper

16 Dilute 5x ELISA/ELISAPOT diluent with 4 parts DI water and block wells with 200ul/well of 1x ELISA/ELISAPOT diluent
- 10ml of 5x ELISA/ELISAPOT to 40ml DI water
- Incubate RT for **1 hour**

17 Optional: aspirate and wash with wash buffer

18 Reconstitute lypohilized standards, sit for 15 minutes with gentle agitation prior to diluting further
- Add 0.8ml DI water to vial

19 Dilute reconstituted standard with 1x ELISA/ELISAPOT diluent
- Add 100ul of standard to 1400ul of 1x ELISA/ELISAPOT diluent
- Add 100ul/well of top standard concentration to appropriate wells
- Perform 2-fold serial dilutions of the top standards to make a standard curve of a total of 8 points: Add 100ul/well
- Include at least 2 wells with 100ul/well of 1x ELISA/ELISAPOT diluent as blanks
- Seal plate and incubate at RT for **2 hours** (or overnight 2-8C)

20 Aspirate and wash for 3-5 washes

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21 Add 100ul/well of detection antibody diluted in 1x ELISA/ELISAPOT diluent
- 48ul detection antibody to 12ml of 1x ELISA/ELISAPOT diluent
- seal plate and incubate **1 hour** at RT

22 Aspirate and wash for 3-5 washes

23 Add 100ul/well of Avidin-HRP diluted in ELISA/ELISAPOT diluent
- 48ul enzyme to 12ml 1x ELISA/ELISAPOT diluent
- seal and incubate 30 minutes at RT

24 Aspirate and wash, soak wells in wash buffer 1-2 minutes prior to aspiration
- repeat 5-7 washes

25 Add 100ul/well of 1x TMB solution to each well
- Incubate at RT 15 minutes, or until fully developed

26 Add 50ul of stop solution to each well

27 Read plate at 450nm- if λ subtraction is available, subtract the values of 570nm from those of 450nm and analyze

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