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Intraarticular injection of fast blue

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for injection of fast blue into the knee joint cavity for retrograde labelling of intraarticular afferents.

Materials

Equipment/drugs:

Heating blanket

Ketamine (20mg/ml)

Medetomidine (0.2mg/ml)

Atipamezole (0.5mg/ml)

Fast blue (2% in sterile water; Polysciences, cat.#: 105190-94-1)

Electric shaver

70% Ethanol swab

10ul Hamilton syringe

30g Hamilton syringe removable needle

1ml Syringes

26 G needle

Warm recovery chamber

0.9% saline

Preparing animal and induction of anaesthesia

- 1 Weigh mouse.
- 2 Prepare heat mat to keep animal warm during anaesthesia.
- 3 Using 1ml syringe with 26g needle attached, perform intraperitoneal (i.p.) injection of ketamine (75mg/kg) and medetomidine (1mg/kg).
- 4 Once mouse has lost consciousness, use a shaver to remove the fur from around the knee joint you intend to inject.
- 5 Wipe skin around knee joint with 70% ethanol swab.

Intraarticular injection of Fast blue

- 6 Place the mouse belly up and hold the leg at the ankle with the knee in a slightly bent position.
- 7 Run the side of the needle up and down the front of the leg until it rests in the 'groove', running from the lateral to medial meniscus. Press with the side of the needle to indent the skin slightly, so that you can visualise the location of the ridge.
- 8 Using a 30g removable needle attached to a 10ul Hamilton syringe, insert the needle from the front (with the needle perpendicular to the front of the knee) about 2-2.5mm depth. You will know that the needle has entered the articular space as you will feel a slight 'give' as the needle passes into the space rather than hitting bone.
- 9 Inject 2ul of Fast blue into the knee joint. Note that this is reported to be the maximum volume that can be injected without it leaking out of the knee joint¹.
- 10 Take care not to move the mouse around too much to ensure that the knee is not flexed/extended. Leave mouse on heat pad for 1-1.5 hours - the idea behind this waiting period is that it gives the Fast blue more time to be absorbed, and there is reduced chance that it leaks out. However, we have never directly tested whether shorter periods would lead to more leakage.



Animal recovery

- 11 Ensure mouse is hydrated during recovery by administering 0.2ml 0.9% sterile saline subcutaneously.
- 12 Using a 1ml syringe with 26g needle attached, reverse medetomidine anaesthesia by giving an i.p. injection of atipamezole (1mg/kg).
- 13 Place mouse in warm heated chamber to recover for ~1.5-2 hours.
- 14 Once mice have fully recovered from the ketamine anaesthesia, place back into home cage.

Protocol references

- 1 Xu Yang, V. C., Alberto Carli, Matthew B. Shirley, & Olufunmilayo Adebayo, M. C. H. v. d. M., F. Patrick Ross, Mathias P.G. Bostrom. 2016 Annual Meeting of the Orthopaedic Research Society Orlando, FL March 5-8, 2016 - Refining Intra-articular and Intramedullary Injection Techniques to Establish Joint, Bone and Periprosthetic Joint Infection in Mice. *J Orthop Res* **34 Suppl 1**, S1, doi:10.1002/jor.23247 (2016).