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AAV Craniotomy

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ASAP Collaborative Rese… | Kaplitt Protocols

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We use this protocol and it's working

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Abstract

This protocol is for injection of AAV into a mouse brain using a stereotactic frame and Hamilton syringe.

Guidelines

Note that sterile technique must be followed during this procedure to avoid health complications.

Anesthesia

1 Weigh mice.

2 Administer intraperitoneal mixture of ketamine (ButlerAnimal Health Supply) and xylazine (Lloyd Laboratories) at concentrations of 110 and 10 mg/kg of body weight, respectively.

3 Confirm induction of anesthesia.

Injection

4 Place mouse into stereotactic frame (David Kopf Instruments) and place eye lubricant.

5 Remove fur from skull area using either electric shaver or depilatory cream.

6 Sterilize skull area and then cut open with scalpel.

7 Adjust brain so that it is level. Locate bregma and zero X and Y axes on stereotaxic frame.

8 Move to injection coordinates and drill small hole into the skull.

9 Zero the Z axis on the stereotaxic frame.

10 Prepare AAV solution by diluting AAV in PBS with 500mM Mg2/Cl to a final dilution of total of $2x10^{11}$ genomic particles/mL.

11 Using a 10 µL stereotactic syringe with a 33 G needle attached to a micro-infusion pump (World Precision Instruments), withdraw the AAV solution.

12 With the loaded syringe, navigate the needle to the desired X, Y, Z coordinates.

13 Infuse the brain with AAV at a flow rate of 0.1 - 0.4 µL/min.

14 To prevent reflux, after each infusion, leave the injection needle in place for 5 min, withdraw a short distance (0.3 - 0.5 mm), and then leave in the new position for an additional 2 min before complete removal of the needle.

15 Close skin with non-absorbable sutures.

AAV Preparation

16 Inject a total of $2x10^{11}$ genomic particles/mL of vector is injected into the appropriate coordinates.

Post-procedure

17 Give meloxicam 2mg/kg subcutaneously.

18 Perform health checks every day for the first 72 hrs post-injection.

19 Remove sutures 15 days post-injection.