STEREOTACTIC INJECTIONS IN MOUSE AND RAT

Rat Cocktail (60 mg/kg Ketamine; 0,4 mg/kg medetomidine)

Anesthesia

0,60 ml Nimatek + 0,40 mL Domitor + 1,00 ml Saline = 2 ml anesthetic cocktail Use 0,15 ml / 100g of body weight

Administration route IP

If the injection is given properly, the rat will sleep in 2 minutes

Reversal of anesthesia

1,0 ml Antisedan + 4,0 ml Saline = 5 ml antidote Use 0,2 ml / 100g of body weight Administration route IP

Mouse Cocktail (75 mg/kg ketamine; 1 mg/kg medetomidine)

Anesthesia

0,15 ml Nimatek + 0,20 ml Domitor + 1,65 ml Saline = 2 ml anesthetic cocktail Use 0,1 ml / 10g of body weight Administration route IP

If the injection is given properly, the mouse will sleep in 2 minutes

Reversal of anesthesia

0,1 ml Antisedan + 9,9 ml Saline = 10 ml antidote Use 0,1 ml / 10g of body weight Administration route IP

Analgesia

Post - operative analgesia

Dilute Vetergesic 10x

Rats: 150μl / 100g of body weight Mice: 30μl / 10g of body weight

Analgesic effect will last 8-12 hours

Put liquid Xylocaïne drops upon the skull if you notice that the animal is suffering pain.

<u>Material</u>

- ✓ Big scissors (for the hair), small scissors, curved forceps, needleholder, spatula, scalpelholder
- ✓ Xylocaïne 2%, joodalcohol, Vidisic
- ✓ Chip(holder)
- ✓ Magnifier, blade nr 10, wire 3-0 (Rat) / 4-0 (Mouse)
- √ 10ml syringe + pink needle, small tissues (sterile)
- ✓ Small pots to rinse the Hamilton syringe (RBS, ETOH, PBS, PBS, AD)
- √ 4x 1ml syringe+needle (anesthesia, reversed, painkiller, +1)
- ✓ Hamilton syringe + needle
- ✓ Pipet + tips + eppendorfs

Procedure

- Remove hair and put 2% Xylocaïne gel on top of the head and into the ears
- Put Vidisic on the eyes
- Put a chip under the skin to mark the animal
- Fix the animal into the stereotactic apparatus (use the mouse adaptor for mouse and only the ear clamps for rat). Put a tissue over the animal to keep it warm during surgery.
- Make sure that the left and right side of the skull is positioned as straight as possible (ears)
- Use 1% joodalcohol to clean the top of the head and make an incision with a scalpel
- Clean the skull with a spatula and saline and let dry until bregma and lambda are clearly visible
- Check volume and injection speed of the pump, rinse Hamilton syringe with RBS, ETOH and PBS. (Coat the syringe by taking a full syringe of vector and discard in eppendorf). Put vector in the syringe (± 1,5µl more than you want to inject) and make sure there are no air bubbles.
- Make sure bregma and lambda have the same height, correct the position of the head if there's a difference of more than 0,02cm
- Put the needle at the right position using bregma as a reference (find the right coordinates using the stereotactic atlas of mouse or rat)
- Drill a small hole into the skull at this position until the dura mater is visible
- Make a small hole into the dura mater using a thin needle
- Put the Hamilton needle at the right position (go down slowly to prevent tissue damage) and wait for 1 minute
- Inject vector at max. 0.25µl/min
- Wait 5 minutes after injection in order to let the vector diffuse into the brain
- Remove the needle slowly
- Close the skin and disinfect with joodalcohol
- Rinse Hamilton syringe with RBS, ETOH, PBS and AD