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DART Infusion Protocol

In 1 collection

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ABSTRACT

This protocol details how to perform a DART (drug acutely restricted by tethering) intracranial infusion.

MATERIALS

Materials:

- For Harvard Apparatus PhD Ultra Pump, using 5µL syringes (Hamilton 7634-01, fit with Hamilton 7770-01 needle)

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Prepare the pump

1 Attach infusion tubing (sized to match your infusion cannula) to the one or two Hamilton syringes, depending on whether you are doing a unilateral or bilateral infusion.

2 Soak internal cannula (fit to guide with .5mm projection) in 100% ethanol, then dry with an air can.

3 Use a 200µL pipette to backfill each syringe/attached tubing with ACSF, ensuring there are no air bubbles in the line.

4 Turn on pump.

5 Method Select -> Infuse Only and Infuse Rate -> 1µL/min, then press Play.

6 While the pump is running, forming an ACSF bubble at the tip of the tubing, carefully insert the internal cannula threads into the tubing. Tape the internal cannula to the pump, pointing downwards.

7 Continue running pump until it reaches the collar and can no longer infuse (ACSF should be coming out of the tip of the internal cannula), then press Stop.

8 Method Select -> Withdraw Only, Withdraw Rate -> 10µL/min, and Target Volume -> 0.1 µL .

9 Insert the tips of the internal cannula in ACSF, then press Play. This will ensure that the tip of the internal is filled with ACSF.

10 Change the Target Volume -> 0.6 µL .

11 Remove the internal cannula tips from the ACSF, then press Play. This will create an air bubble in both tubing lines.

12 Method Select -> Withdraw Only, Withdraw Rate -> 10µL/min, and Target Volume -> 4 µL .

13 Insert the tips of the internal cannula in your prepared DART reagent, then press Play. This will load your internal cannula with reagent.

14 Using a fine tip sharpie, mark the air bubble boundaries on the tubing.

15 Method Select -> Infuse Only, Infuse Rate -> 0.1µL/min.

Perform the infusion

1h 6m

16 Headfix the mouse, covering with a half-cylinder for comfort.

17 Prime the internal cannula: set Target Volume -> 0.2 µL, press Play, and stop when liquid is coming out of all internal tips.

18 Remove mouse's cannula dust cap and dummy, put in 100% ethanol to soak.

19 Insert internal into mouse's guide cannula and screw collar onto guide cannula until internal is stably in place.

20 For a behavior infusion:

20.1 Set Target Volume -> 0.8 µL.

20.2 Press Play, and start a timer set for the infusion time (8 minutes) plus an extra 00:06:00 6m rest time.

20.3 After the infusion is complete and the time is up, remove the internal cannula and tape back to the pump, pointing downwards.

20.4 Confirm infusion success by the movement of the tubing air bubble from your previously marked bubble boundaries. If clogged, or some other issue, repeat the infusion.

20.5 Use an air can to dry the cannula dummy and dust cap, and replace on the mouse's guide cannula

20.6 If the mouse has a unilateral cannula, i.e. for the fiber photometry dual cannula/fiber implants, only 0.8 µL was infused. To safely achieve the full 1.6 µL infusion, return mouse to its home cage and repeat infusion 01:00:00 later. 1h

21 For a unilateral infusion during electrophysiology recordings:

21.1 Set Target Volume -> 1.5 µL .

21.2 Press Play at the appropriate time during the recording.

21.3 After the recording is complete, remove the internal cannula and tape back to the pump, pointing downwards.

21.4 Confirm infusion success by the movement of the tubing air bubble from the previously marked bubble boundaries.

21.5 Use an air can to dry the cannula dummy and dust cap, and replace on the mouse's guide cannula.

Clean up

22 Use a razor to cut tubing above your boundary markings (i.e., cut off any tubing that had reagent in it). Dispose of internal and attached tubing in a sharps container.

23 Use a 200µL pipette to flush each syringe/attached tubing with DI water.

24 Use an air can to flush the remaining liquid out of each syringe/attached tubing.

25 Rinse syringe plungers with DI water, and dry with an air can, before returning to syringes.

26 Turn off pump.