***Step-by-step procedure for performing targeted pressure injections in the rat***

*Materials required:*

1. Harvard Apparatus infusion pump.
2. Polyethylene tubing.
3. Hamilton syringe (10µL or 25 µL).
4. Syringe (Volume of the syringe to be used depends on the length of the polyethylene tubing. For shorter tubing lengths, use 1 mL syringes and for longer tubing lengths, use 2.5 mL syringe).
5. Sterile water.
6. Drug (muscimol in my case) and vehicle (0.9% vol/vol saline in my case).
7. Petridish.
8. Needles (Thickness of the needles to be used depends on the thickness of the polyethylene tubing used. I use 22G X 1” needles).
9. Eppendorf tubes.
10. 50 mL falcon tubes.

*Procedure:*

1. *Dilution of drug*

Example: Dilution for muscimol

Stock solution concentration = 50mM

Stock solution volume = 10µL

Desired final concentration = 1 µg µL-1

Molar mass of muscimol = 114.1g/mol

*Calculation*

1000 mL of 50 mM muscimol solution contains (50 X 114.1) mg of muscimol.

103 mL of 50 mM muscimol solution contains (50 X 114.1 X 103) µg of muscimol.

1 mL of 50 mM muscimol solution contains (50 X 114.1) µg of muscimol.

103 µL of 50 mM muscimol solution contains (50 X 114.1) µg of muscimol.

1 µL of 50 mM muscimol solution contains (50 X 114.1 X 10-3) µg of muscimol.

We use the following expression to calculate the final volume:

where, is the stock concentration, is the desired final concentration, is the volume of stock solution and is the final volume of the solution to achieve desired dilution.

In my case,

= 50 X 114.1 X 10-3 µg µL-1

= 1 µg µL-1

= 10 µL

Therefore,

= (50 X 114.1 X 10-3 µg µL-1 \* 10 µL) / 1 µg µL-1

= (50 X 114.1 X 10-3 \* 10) µL

= (50 X 114.1 X 10-2) µL

= 57.05 µL

Therefore,

where, is the volume of the vehicle added to the stock solution .

= (57.05 – 10) µL = 47.05 µL

*Setting up the instrument for infusion:*

1. Switch ON the pressure pump after connecting it to the mains.
2. Different volumetric Hamilton syringes have different bore diameters. For example, the 10 µL syringe has a bore diameter of 0.460 µL, while the 25 µL has a bore diameter of 0.729 µL. Set the syringe diameter accordingly in the settings. Press the enter button once you have punched in the syringe diameter.
3. Put the Hamilton syringe with the appropriate needle in the holder and lock it in position.
4. Fill up the 1 or 2.5 mL syringe with sterile water and place the needle gently into one end of the polyethylene tubing.
5. Pump in the sterile water very gently through the tubing so as to wash it. Care must be taken so as to avoid inserting any air-bubble into the tubing.
6. Once the tubing is rinsed and filled up with sterile water, place an infusion cannula at the free end of the tubing.
7. Pass water gently through the tubing once more to check if the cannula is working. This step will also let you check the fluid’s flow pattern and its spread.
8. Next, tape in the cannula end of the tubing at a height which is same as that of the Hamilton syringe needle.
9. Bring the other end of the tubing at the same height and push water gently through the tubing. Slowly, slide the tubing over from the needle of the water-filled syringe and slide it over the needle of the Hamilton syringe. In this step, you need to very careful so as not to introduce any air-bubble in the tubing. In case any air-bubble is introduced in the tubing, go back to step 4 and repeat the steps till this one until you get rid of the air-bubble.
10. Next, introduce an air-bubble in the tubing at the infusion cannula end by drawing the piston of the Hamilton syringe backwards (By creating a negative pressure). An air-bubble measuring 2-3 µL in volume is good enough.
11. Dip the infusion cannula in the Eppendorf tube containing the diluted drug / vehicle for loading. Load the drug / vehicle by moving the Hamilton syringe piston backwards (creating negative pressure). Make sure the tip of the infusion cannula is dipped in the fluid being loaded while you are drawing the piston backwards. Once, the desired volume of the drug / vehicle is loaded, DO NOT immediately take the infusion cannula tip out of it. This may introduce an air-bubble at the tip. Keep the tip of the cannula immersed in the fluid for a few more seconds and then gently withdraw it from it.
12. Set a fast flow-rate on the pump to check if the fluid flow is continuous through the cannula. I generally use a flow rate of 2 µL min-1. The faster flow rate also depends on the volume of drug / vehicle loaded. If the volume is very small, say, < 1 µL, a smaller flow-rate is to be used. Wipe out the tip of the infusion cannula with a lab tissue and press the run button on the pump. Move the lab tissue in a straight line while touching it to the tip of the infusion cannula. If the flow is normal, you will see it wetting the tissue in a straight line. The straight line motion of the tissue is just an arbitrary choice. You can move the tissue in any fancy pattern of your choice. Make sure that the flow follows that fancy pattern. This step needs to be very quick, because if you run this step for long, you will exhaust all the drug loaded in to the tubing. If you are skeptical about your speed in doing this, try this step with sterile water, a day before your experiment. It is always advisable to do a dry run of your experiments a day before the actual experiments. This helps you in figuring out things which are not working. I do it at least 2-3 days before the actual experiment because sometimes a day is not long enough to troubleshoot things.
13. Once, you are done with the above steps, you are ready to do the experiments. Bring in the animal. Handle it for a few minutes. Open the guide cannula ca, and slowly insert the infusion cannula through the guide cannula. Proper insertion of infusion cannula will make a very faint click sound and effect. When the infusion cannula is properly fit, double check it by trying to take it out. If it is properly fit, it will not come out easily. Press the run button. DO NOT FORGET to change the flow-rate on the pump to the desired flow rate before you press the run button. During infusion, the height of the animal and the needle of the Hamilton syringe needs to be maintained at a similar height.