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Intracerebral Infusion of Neurotrophic Factors

Theo Hagg

Summary

Neurotrophic factors are among the most potent neuroprotective and neuroregenerative agents known. However, they cross the adult mammalian blood–brain barrier very poorly and can have serious peripheral side effects. These problems can be solved by using chronic infusions with small pumps to directly deliver known quantities of these proteins into selected regions of the brains of small experimental animals such as rats and mice. The method consists of commercially available Alzet osmotic pumps that are placed under the skin and are connected to commercially available metal infusion cannulas whose tip can be stereotactically placed in virtually any location of the brain. Different models of pumps that fit comfortably in rodents can be selected for infusion between 1 and 28 days and at infusion rates ranging between 8 and 0.25 μL/h, respectively. Methodological details are provided for the successful use of proteins and to minimize the time of the surgery.

[1]

Key Words: Alzet; central nervous system; chronic infusion; degeneration; growth factor; mouse; intraventricular; pharmacological; rat; regeneration.

1. Introduction

Neurotrophic factors have been widely used to promote survival of cells in the central nervous system (CNS) and to promote sprouting and regeneration of axons in adult rodent models of neurological disorders *(1–5)*. However, these sizable proteins do not normally cross the adult mammalian blood–brain barrier *(6,7)*. Moreover, as with many growth factors and cytokines, they can have serious peripheral side effects *(5)*. These are two of the reasons why systemically delivered neurotrophic factors have failed in clinical trials for

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[1]**Per Manufacturer:**
Flow rates available, 0.11-10 ul/hr
Durations available, 1-42 days

neurological disorders *(5)*. In experimental rodent models, these problems can be solved by using chronic infusions with Alzet osmotic pumps to deliver known quantities of these proteins into selected brain regions. The method can also be used to deliver other molecules and to determine whether a therapeutic agent that is effective after systemic administration acts directly through mechanisms in the CNS, rather than indirectly by inducing changes in the rest of the body.

The chronic intracerebral infusion method uses commercially available Alzet osmotic pumps that are filled with a solution containing the test reagent. Different types of pumps exist and can be used to infuse almost anywhere in the CNS from a few hours to 28 days, or longer if replaced with fresh pumps. The pump is placed under the skin, which reduces the chance of infections, and is connected to a metal infusion cannula (catheter) encased in a plastic platform. The tip of the cannula can be stereotactically placed in virtually any location of the brain, and the platform is quickly glued to the skull. At the end of the infusion period, the test reagent can be retrieved from the pump to determine the remaining biological activity. This chapter replaces an outdated version that dealt with this intracerebral infusion technology *(8)*.

2. Materials

2.1. Infusion Device

1. Alzet pumps with flow moderators (Durect Corporation, Cupertino, CA, http://www.alzet.com) (*see* **Fig. 1** and **Note 1**).
2. Brain infusion kits (Durect Corporation or Plastics One, Roanoka, VA) consisting of metal cannulas embedded in a plastic platform and their connecting lines (*see* **Note 2**).
3. If the cannulas need to be shortened (*see* **Note 2** and **Subheading 3.1., steps 3** and **4**): use Dremel drill (Dremel, Racine, WI) with fine grinding wheels (Dremel cut-off wheel no. 409), dissecting microscope, and a 30-ga needle.
4. Cyanoacrylate fluid glue also called superglue (e.g., Instant Krazy glue Advanced Formula; Elmers Products, Columbus, OH) can be purchased in most hardware stores (*see* **Note 3**).
5. Single-sided razor blade.
6. Petri dishes (9 cm) with holes in the lid (*see* **Note 4**), poster putty, and gas sterilization pouches.
7. Clear tubes (15 mL) filled with 3 mL sterile saline in a tube rack (as many as the number of pumps).

[2] **Per Manufacturer:**
Finer gauge tubing is needed if using the 30 ga Brain Infusion Kit 3 from Durect Corporation

[3] **Per Manufacturer:**
Loctite 454 (0008670) is available from Durect Corporation.

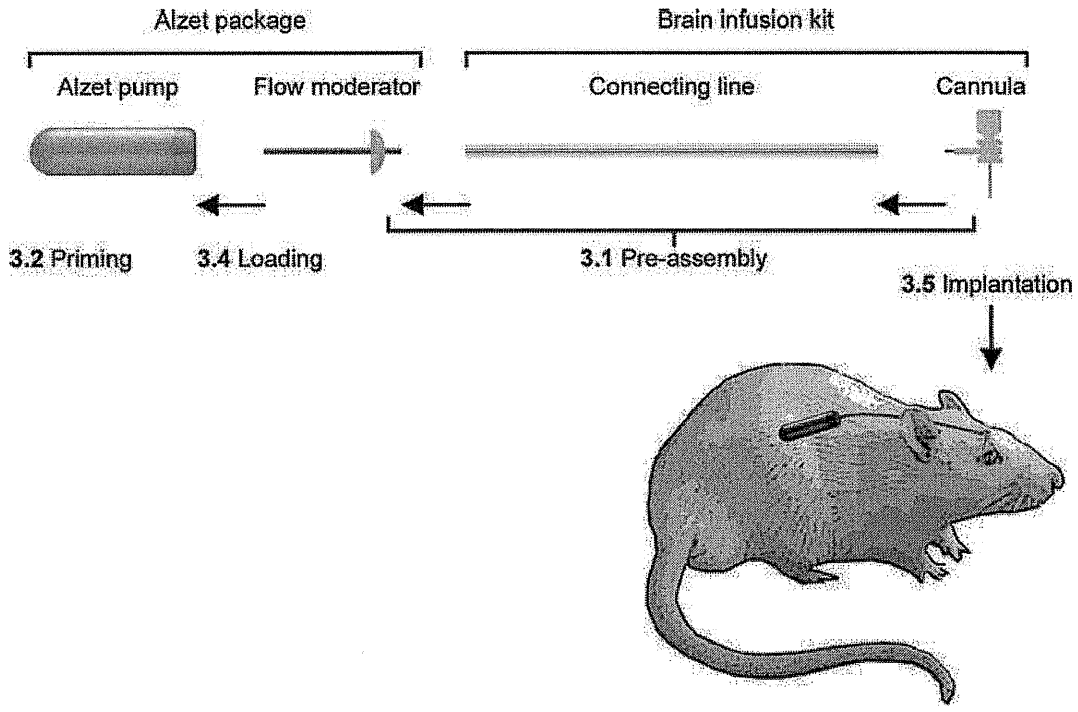

Fig. 1. Alzet pump and infusion device. Shown here are the parts of the infusion device and the steps that are described in **Heading 3.** with the corresponding section numbers. Also, shown is the final subcutaneous placement of the device in an adult rat.

2.2. Infusion Solution or Vehicle

1. Phosphate buffered saline (PBS) or appropriate other solution (depends on trophic factor; *see* **Note 5**) in a 5-mL or 15-mL polypropylene or other low-protein-binding tube.
2. Rat serum albumin (Cat. #A6272, Sigma-Aldrich, St. Louis, MO).
3. Gentamicin antibiotic solution (e.g., Cat. #G1272).
4. One's favorite sterile neurotrophic factor (*see* **Note 6**).

2.3. Filling Materials

1. Laminar tissue culture flow hood.
2. A few sterile 0.5-mL to 1.5-mL Eppendorf tubes in a small tube rack.
3. Sterile pipette tips and 20-μL and 200-μL pipetters.
4. A few 1-mL or 3-mL syringes and 0.2-μm Acrodisc low-protein-binding syringe filters (Pall Life Sciences, Ann Arbor, MI).
5. Medium-sized hemostatic forceps (e.g., FST 13009-12, Fine Science Tools, Foster City, CA) and Alzet pump filling tube from the Alzet pump package.
6. Ethanol, kimwipes, and latex or similar gloves.

2.4. Surgical Tools

1. Small animal clippers appropriate for the species (e.g., rats: Golden A5 model, Oster, McMinnville, TN; mice: GMT180TP plus CB-01, Conair Corporation, Glendale, AZ) and Betadine surgical scrub (Purdue Frederick Company, Norwalk, CT).
2. Stereotaxic apparatus with stereotaxic micromanipulator arm (*see* **Note 7**) and cannula holder (e.g., MH-300 or MH-300/SPC, Plastics One).
3. Instruments: No. 10 scalpel blade and holder (e.g., FST10003-12), blunt scissors (FST14512-15 for rats and FST14079-10 for mice), four small towel clamps (FST11095-09), low-heat electrocautery system (e.g., Gemini, Harvard Apparatus, Holliston, MA), Dremel or surgical drill with fine round-tip carbide drill burs (e.g., round HP-1 or HP-2, REF14823, SS White Burs, Lakewood, NJ), fine forceps (FST Dumont #5 and #7), sterile 30-ga needle, and two medium-sized forceps (FST11000-14 and FST110021-14).
4. Sterile surgical supplies: sterile drapes, sterile cotton applicators, gauze, small pieces of Gelfoam (Pharmacia & Upjohn, Kalamazoo, MI) presoaked in saline in Eppendorf tubes, and surgical or regular latex gloves.
5. Cyanoacrylate fluid glue.
6. Sodium bicarbonate powder (Cat. #S6014, Sigma-Aldrich) in 15-mL polystyrene or similar tube; Pasteur pipettes with rubber bulb.
7. Syringe (5 mL) filled with saline containing 0.1 mg/mL gentamicin.
8. Metal wound clips for rats (FST12040-01) with clip applying forceps (RS9290, Roboz, Gaithersburg, MD). Ethicon 5-0 silk sutures with attached needle for mice (Johnson and Johnson, Piscataway, NJ) and needle holder (e.g., FST12002-12 or hemostat FST13009-12).

3. Methods

The infusion method consists of five steps that are performed at different times and most likely in different locations of the laboratory (**Fig. 2**). These are designed to make the overall procedure efficient, to minimize the duration of the surgery, and to maintain sterility of the infusion solution. The latter is of particular importance when using proteins, which would otherwise be degraded inside the pump by organisms that grow well at the 36–37° C of the animal. The first step is to preassemble and gas-sterilize the infusion device consisting of the cannula, the connecting line, and the metal flow moderator, which will later be inserted into the Alzet pump. This preassembly can be done at any time before the surgery, and large numbers of devices can be made well in advance of large experiments. Alternatively, the assembly can be performed in a laminar flow hood just before loading the pumps. The second step is to prime the Alzet pumps by incubating them in saline, which will get the osmotic process to an equilibrium, and is needed to drive the flow. This is done overnight before the

[4] **Per Manufacturer:**
ALZET products are sold sterile. Gas sterilization of Brain Infusion Kit assembly is only necessary if aseptic technique is not followed during assembly.

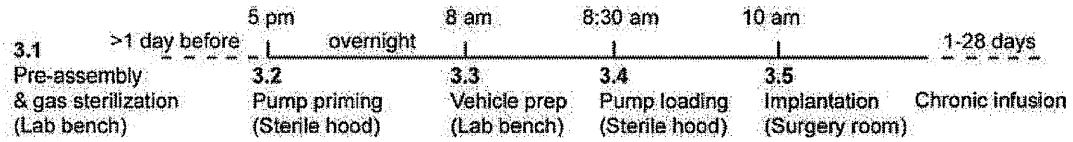

Fig. 2. Time-line of a typical experiment in the Hagg laboratory. Shown here are the steps of a typical infusion study in the laboratory as indicated by the numbers mentioned in **Heading 3.**, the locations where they are performed, and the approximate times when they are started.

day of surgery. The third step is to make the vehicle solution and is performed at the bench. The preassembled infusion device, preconditioned pumps, and vehicle solution are then taken to a laminar tissue culture flow hood for the fourth step, which consists of filling the pump and the final assembly of the infusion device. The fifth step is to implant the infusion device into the animal. Finally, if needed, the pump can be collected after the infusion period and the remaining fluid collected to measure the remaining biological activity of the neurotrophic factor.

3.1. Preassembly of the Infusion Device

1. The metal cannulas come embedded in a plastic platform that has a small cylinder attached on top to hold the platform for stereotaxic implantation. Do not remove that yet. There are two types of platforms. One has a much lower but wider profile that is well suited for mice with their less rigid skull. The wide platform can obstruct the view during surgery, and it can be helpful to cut off or trim one side of the flatter part. The other platform has a cylindrical shape and is appropriate for rats.
2. If a laboratory expects to infuse in various locations within the brain in different experiments, it is useful to purchase a large number of 11-mm cannulas and trim them to any desired length in the future. To do this, follow **steps 3–4**. If not, go to **step 5**.
3. Hold on to the platform and cut the metal cannula part to half a millimeter from the desired length. This can be done with a cut-off wheel and a Dremel or similar drill. Wear protective eye-gear. Next, using a dissecting scope, lightly bevel the tip of the cannula at a 45° angle with a fresh grinding wheel. This is best done with a sweeping motion against the direction of the spin of the cut-off wheel. If too much pressure is exerted or the wheel is old, the hole of the cannula will fill with too much metal. Afterward, any metal or other debris is removed with the tip of a 30-ga needle.
4. To test whether the cannula is open, attach the metal/plastic connector at the other end of the platform to an appropriately sized vinyl line and syringe and inject 95% ethanol through it. Afterward, remove the ethanol by injecting air through the

[5]**Per Manufacturer:**
It is suggested to prime the pumps *after* they have been filled with drug solution.

cannula. If the cannula is not open, check the opening of the metal cannula for additional debris.

5. One end of the connecting line (*see* **Note 2**) that is packaged with the cannula of the brain infusion kit (or alternative tubing) is attached to the platform via the short connector protruding from the platform and is secured with a small drop of Krazy glue (*see* **Note 8**). This glue is dry in about 10 min. For adult rats, the length of the connecting line generally needs to be 6–7 cm for placement of the Alzet pump between the shoulder blades. For mice, it is approximately 3 cm. This can more accurately be determined by measuring the required length in the particular strain used by the laboratory. The line can easily be cut with a single-sided razor blade.
6. The other end of the connecting line is attached to the short end of the flow moderator and secured by applying a small amount of Krazy glue. These moderators are the metal tubes that are located in the Alzet pump package. In some models
6 (the 200# and 2ML# series), a small removable rubber guard needs to be removed first. For some models of flow moderators (the 100# series), the white tab needs to be broken off, for example, using sturdy forceps.
7. After the glue is dry, the assembled cannula-line-moderators are placed in a 9-cm Petri dish. The platforms (as many as are needed for an experiment, up to four to six per dish) can be pushed into a strip of poster putty on the bottom of the dish, which helps in taking them out for final assembly with the pumps (*see* **Subheading 3.4.**).
8. The Petri dish with the infusion devices (not the pumps) is placed in a gas sterilization bag for gas sterilization. Do not autoclave, as this will melt the plastic of the devices and the dish.

3.2. Priming of the Alzet Pump

1. In order for the pump flow rate to equilibrate, the pumps need to be primed by placing them into saline. Therefore, fill 15-mL sterile polystyrene or other clear tubes each with 3–5 mL sterile saline in a laminar flow hood. Next, open the packages of the Alzet pumps and put the pumps into the saline with the hole facing up. To maintain the sterility of the pumps, handle them with ethanol-sterilized forceps after opening the individual pump packages. The pumps will float in the saline and fluid will not get into the inside of the pumps. Close the tubes and keep them straight up in a tube rack. (For a choice of pumps, *see* **Note 1**.)
7 2. The manufacturer recommends incubating the pumps for at least 4 h at 37° C. Alternatively, they can be incubated overnight at room temperature that helps improve the efficiency of the experiment (*see* **Note 9**).

3.3. Preparation of the Vehicle Solution

1. Measure out 1 mL more PBS than is needed for the experiment and dissolve 1 mg/mL serum albumin of the species of the experimental animal, for example, rat

[6]**Per Manufacturer:**
Hemispherical, polyethylene cap

[7]**Per Manufacturer:**
It is suggested to prime most ALZET pumps overnight to ensure delivery at specified pumping rate. The following pumps are exceptions: 2001D (3 hours); 2004 (40 hours); 1004: (48 hours); 2006 (60 hours).

serum albumin for rats (*see* **Note 10**). The same vehicle should be used to dissolve the neurotrophic factor and for the control group in the experiment.
2. To further reduce the chances of bacterial growth, add 0.1 mg/mL gentamicin to the vehicle.
3. Up to this point, the solution is not sterile and can be made at the bench on the day before loading the pumps or on the day itself. Do not dissolve the neurotrophic factor yet.

3.4. Loading of the Alzet Pump and Final Assembly

1. Decontaminate the working area in a laminar tissue culture hood with 70% ethanol using a spray bottle and kimwipes. Throughout the loading procedure, wear latex or similar gloves and decontaminate them after touching nonsterile surfaces. Arrange materials in the hood for convenient access while maintaining sterile areas for later placement of the pumps and infusion devices.
2. Filter-sterilize the vehicle through a 0.2-μm low-protein-binding Acrodisc filter into sterile 0.5-mL or 1.5-mL Eppendorf tubes using a 1-mL or 3-mL syringe.
3. Connect the sterile filling tube that came with the Alzet pump package to a sterile 1-mL syringe and fill it with sterile vehicle to prime it for 2 min (*see* **Note 5**). Afterward, express the vehicle back into the Eppendorf tube.
4. Take up more than enough fresh vehicle to fill one or more of the pumps of the vehicle group plus what is required to fill the infusion device. This amount depends on the volume of the pump and the length of the connecting line.
5. Take the Alzet pump out of the saline with forceps and while holding it between the fingers with its opening facing up, insert the filler tube, and slowly inject the vehicle until the pump is filled. This is noticeable by the slight darkening of the shell, by the appearance of a fluid bubble at the top, or by the ending of small air bubbles that may form on the top of the pump during loading. Place the pump horizontal on a sterile surface.
6. Pick up a sterile infusion device at the metal part of the flow moderator end using the hemostatic forceps and keep the device horizontal. Insert the filler tube all the way into the moderator and fill the device up to approximately 3 mm from the cannula platform. Withdraw the filler tube while holding the moderator almost vertical with the opening pointing down.
7. Insert the flow moderator approximately four-fifths into the Alzet pump (*see* **Note 11**) and place the Alzet pump back into the tube with saline, with the rest of the infusion device positioned above the saline. Close the tube. This will keep the assembled infusion device pump sterile until use at the surgery later in the day.

3.5. Stereotaxic Implantation Into Rats or Mice

1. After anesthetizing the animal (*see* **Note 12**), the head and dorsal neck area are shaved and the surgical area cleaned with Betadine. The head of the animal is placed securely and straight in the stereotaxic apparatus with the tooth bar set at a

level appropriate for the stereotaxic atlas that was used to determine the coordinates for the tip of the cannula, for example, rats *(9)* and mice *(10)* (*see* **Note 7**).

2. Make a mid-sagittal (in the middle along the long axis of the animal) skin incision from behind the level of the eyes to the back of the skull. Insert a pair of blunt medium-sized scissors through the back of the incision under the skin of the neck area up to one-third or halfway along the length of the body. Spread the scissors and withdraw in that position to create a subcutaneous pocket for later placement of the Alzet pump.
3. Cover the animal with a sterile drape and make a slit in the middle over the skull area to access the surgical area. Attach the sides of the drapes to the skin using the towel clamps, which are retracted to the sides to open up the surgical field for easy access to the skull.
4. Remove the membrane covering of the skull by scraping it to the sides with the back of the scalpel holder. Clean the skull by lightly scraping it with the sharp edge of the scalpel blade held at a 45° angle. Remove all blood with cotton applicators and stop any remaining bleeding from the skull with the low-heat cautery device (*see* **Note 13**).
5. Measure out the mediolateral and rostrocaudal coordinates from Bregma (*see* **Note 14** and **Fig. 3**) using a pin that is inserted into the cannula holder attached to the micromanipulator. Drill a small (approximately 1 mm) burr hole through the skull without penetrating the dura. Make a small incision in the dura with a bent 30-ga needle.

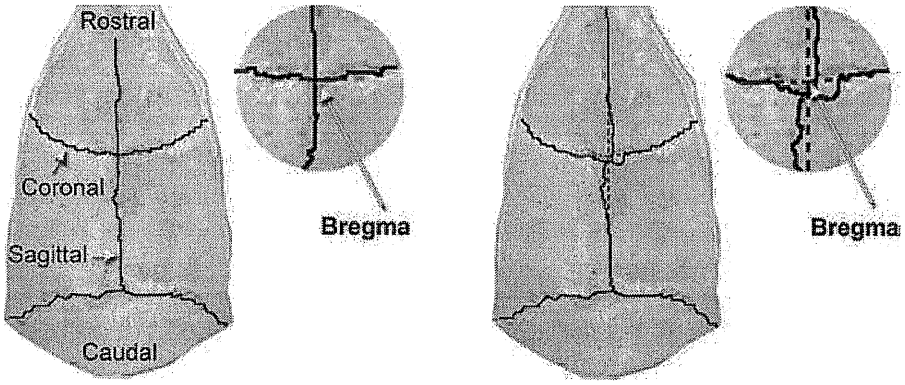

Fig. 3. Two examples of Bregma. The intersection between the sagittal and coronal sutures between the skull plates is named Bregma and is used as a reference point to determine the coordinates where the tip of an infusion cannula is inserted. On the left is the ideal Bregma; on the right, one variation that is often seen. The broken lines indicate the imaginary lines that can be drawn from the caudal and rostral portions of the sagittal suture, which are more consistent, and the projection of the coronal suture, to determine Bregma in cases where the sutures are aberrant.

6. Clean and completely dry (*see* **Note 15**) the skull area where the platform will touch, by scraping with the scalpel blade or cotton applicators and/or by cauterizing blood vessels. If bleeding occurs from the burr hole, fill it with a small piece of Gelfoam for a minute, remove the Gelfoam, and clean off the skull again. Do not cauterize the burr hole.
7. Ensure that the glue can be expressed from the tip of its container. Next, secure the removable tab of the plastic platform of the infusion device in the cannula holder of the micromanipulator, with the connecting line and attached Alzet pump located over the midline of the animal and on top of the drape. Insert the rest of the flow moderator into the Alzet pump and check that a droplet forms at the tip of the cannula. Wipe this off with a sterile cotton applicator. Ensure that the cannula is positioned vertical by optically aligning it with the vertical parts of the stereotaxic apparatus by looking from the back and the side.
8. Again measure out the mediolateral and rostrocaudal coordinates, now using the tip of the cannula, without touching the skull. Ensure that the skull is still dry and the burr hole is filled with a small amount of fluid (*see* **Note 16**). Insert the cannula through the burr hole until the platform touches the skull, making sure the cannula does not touch the side of the burr hole (*see* **Note 17**).
9. Apply a drop of cyanoacrylate in the corner between the platform and the skull on one side, enough for the glue to penetrate to the other side of the platform. Next, deposit a small amount of sodium bicarbonate powder all around the sides and front (not the back) of the platform, using a Pasteur pipette (*see* **Note 18**). This will immediately polymerize (harden) the glue. Repeat application of glue and powder if needed to build up a small rim of glue around the sides of the platform. A small amount of glue and powder can be applied underneath the caudal end of platform while ensuring that the clear part of the connecting line remains free.
10. Place the Alzet pump under the skin all the way between the scapula or farther, using forceps to pull up the skin of the neck and another forceps to hold on the Alzet pump. Ensure that the connecting line does not have kinks by palpating through the skin.
11. Open the cannula holder and raise the micromanipulator. Cut off the removable tab of the platform using the cauterizer. Clean the wound by removing debris and flushing it with sterile saline containing 0.1 mg/mL gentamicin. Inject 0.1 mL (mice) or 1 mL (rats) of this saline–gentamicin solution around the pump through the open neck area.
12. Close the skin of rats with removable wound clips or metal staples or of mice with silk sutures (*see* **Note 19**). Return animals to a cage placed on a heating pad for recovery. Remove clips or sutures after 7–10 days.
13. At the end of the experiment, the pump can be retrieved, the flow moderator removed, and the remaining fluid collected with a syringe and filler tube to measure remaining biological activity, if necessary. Please note, the Alzet pump cannot be reused.

4. Notes

1. Alzet pumps have a hard semipermeable outer shell filled with a gel-like substance that attracts water from the subcutaneous location in the animal through osmosis. As the osmotic substance swells, it compresses an internal bag containing the infusion fluid to expel the latter through the opening of the pump into the infusion device. There are currently 10 different types of Alzet pumps available. Because of their size, the 100# series is most suitable for mice and the 200# series for rats. They readily fit under the skin. The 2ML# series is large and fits under the skin only with some difficulty in adult rats or can be placed into the peritoneal cavity of larger rats (preferably over 300 g). Some of the pumps have a seemingly high infusion rate, for example, the 2001D infuses at 8.0 μL/h to achieve a maximal delivery time of 1 day. This may be considered too high for intraparenchymal infusions. If a 1-day infusion is required, one can use a 3-day 1 μL/h pump and process the animal after 1 day. The only disadvantage in case of expensive neurotrophic factors is the wasted fluid.
2. Durect offers two precut cannula lengths that will place the tip in the lateral ventricle of rats or mice. Plastics One offers customized lengths up to 11 mm to fit the particular depth needed for any particular location in the brain. The length should be measured from the top of the skull. There are two types of platforms. One has a much lower but wider profile that is intended for mice. The other platform has a cylindrical shape and is appropriate for rats. If a laboratory expects to use various locations, it is useful to purchase a large number of 11-mm cannulas and trim them to any desired length in the future using the Dremel drill and cut-off wheel (*see* **Subheading 3.1., step 3**). The lines that come with the platform may not be kink resistant (e.g., polyethylene), meaning the line can fold in the animal and thus obstruct the flow. In most cases, the line will be straight in the animal and this would not be a problem. When infusing agents into the fourth ventricle or intrathecally into or around the spinal cord, the placement of the pump might require bending the line. In such cases, one can purchase kink-resistant polyvinyl tubing (V-3A, Scientific Commodities Inc., Lake Havasu City, AZ).
3. Cyanoacrylate glue comes in many types, including fluid and gel. I prefer the fluid, as it can enter into small spaces easier and faster, which is particularly important for the surgery.
4. The lid of the dish should have holes, which can be made with the hot (flamed) thick end of a glass Pasteur pipette.
5. The choice of vehicle is essential for the success of the experiment. For most neurotrophic factors, a pH-neutral solution like PBS is suitable. If not, this most likely is indicated on the data sheet that came with the factor. If unsure, the easiest is to check what others have done. Alzet maintains a bibliography of agents tested and the vehicles (carriers) used. Like most proteins, neurotrophic factors have the potential to "stick" to charged surfaces they come into contact with, such as the tubes, loading syringes, and infusion device. The albumin helps protect the

[8] **Per Manufacturer:**
Twelve pump models currently available

[9] **Per Manufacturer:**
Durect has three Brain Infusion Kits available

much less abundant trophic factor. Albumin should not sequester the neurotrophic factors. Some neurotrophic factors or other proteins might require nonphysiological vehicles. If so, it is important to include another control group, in addition to the vehicle group, which is infused with a physiological solution.

6. Most commercially available neurotrophic factors are already filter-sterilized and come as a single amount. If the factor will be used for different experiments or at different times, it is best to aliquot it into sterile Eppendorf tubes with as much per tube as is expected to be used for one animal. Freeze them at −70 to −80° C. If the factor is not sterile, filter it through a 0.2-μm low-protein-binding Acrodisc filter, which could first be primed with a solution containing 1 mg/mL serum albumin.
7. We use a David Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA), which is sufficiently precise and reliable. For rats, we use the standard ear bars and penetrate the eardrums to ensure a precise centering of the skull. For mice, there are two options. One is to use the same rat ear bars, but this requires a skilled person to be able to feel the superficial entry into the ear canal. The other is to use mouse-specific ear bars that are positioned externally over the lower caudal end of the skull. These are less precise but can achieve a reasonably centered position of the skull. The toothbar for the rat is standard. For mice, a smaller toothbar is needed and is preferable over bars that are not placed into the mouth.
8. When applying the fluid glue, take off the excess with a kimwipe leaving only a small amount at the connection between the line and the platform. This will prevent the fluid from going into the platform in the rare cases that the line does not fit tightly. Too much glue also can make the polyvinyl lines brittle. Do not glue your fingers together.
9. The manufacturer's recommendation is based on their findings that the pump rate accelerates from zero to a constant rate over 4 h at 37° C, the approximate core temperature of an adult rodent. Some types of pumps require a longer preincubation (see Alzet recommendations on their website). In the laboratory, we incubate them overnight at room temperature for several reasons. First, this increases the efficiency of larger experiments; for example, pumps can be loaded early in the morning without having to wait another 4 h for the pumps to get ready. Second, when changing the pump from a 37° C to a room temperature environment for filling, the fluid in the pump shrinks. If this happens after the final assembly of the pump, this results in air being drawn into the cannula and connecting line. Third, in our approach, when going from room temperature to the animal, the flow rate increases for a while, which will deliver a small "burst" of fluid, which reduces the potential backflow of blood into the cannula.
10. Measuring out small amounts of albumin can be challenging, as it can stick to the spatula and can readily be blown away. Steady hands help. To maintain accuracy, we do not measure out less than 1 mg.
11. Small air bubbles may appear in the connecting line. These will be resorbed into the vehicle or will not harm the nervous tissue if infused. If air pockets larger than

3 mm form, remove the flow moderator from the pump, remove the fluid from the infusion device, and refill the device. This is most likely caused by inadvertently injecting air into the moderator or because the moderator is not held up straight while withdrawing the filler tube. The remaining one-fifth of the moderator is inserted just before implantation in the animal. This ensures that the entire infusion device is filled in case the fluid level withdraws between filling and implantation.

12. A good anesthetic for rats is an intramuscular injection of 3 mL/kg of a mixture containing 25 mg/mL ketamine hydrochloride (e.g., Abbott Laboratories, North Chicago, IL), 1.2 mg/mL acepromazine maleate (e.g., The Butler Company, Columbus, OH), and 0.25 mg/mL xylazine (e.g., The Butler Company) in 0.9% saline. This provides deep anesthesia within 15 min and lasts for at least 1 h. A 0.2-mL booster shot can be given after 45 min to ensure deep anesthesia for a longer time. For mice, we use an intraperitoneal injection of Avertin (0.4 mg per gram body weight; 2,2,2-tribromoethanol in 0.2 mL of 1.25% [v/v] 2-methyl-2-butanol in saline, Sigma-Aldrich), which induces anesthesia within 10 min and also lasts for at least an hour. Avertin should be stored in the dark. Both anesthetics should be stored in the refrigerator and should be replaced at least every 2 weeks to maintain their effectiveness.
13. When using the cautery device, do not place it too long in one place, as this will heat up the cortex through the skull, causing damage. For the same reason, do not use it close to exposed nervous tissue.
14. Bregma can be recognized by the sutures between the skull plates (**Fig. 3**). These will become clearly visible after scraping the skull or can be enhanced by applying a dye such as toluidine blue. These sutures often are aberrant and may not form a perfect cross at the intersection that defines Bregma. It is therefore necessary to double-check the position of Bregma by moving the tip of the measuring device (e.g., a pin) caudal and rostral over the sagittal suture, whose caudal and rostral ends are more consistent. In case the coronal suture (in the mediolateral plane) is aberrant, the position of Bregma can be determined by projecting imaginary lines through the more lateral portions of the suture toward the midline. Using a surgical microscope will greatly enhance the precision of the measurement and subsequent implantation of the infusion device.
15. The skull needs to be dry for the glue to bond well. There is often some bleeding and build up of fluid in the wound pockets on the side, which can be repeatedly removed by cotton applicators to prevent the fluids from entering the region where the platform will touch. This is easier than trying to stop all bleeding from the sides, which most often is difficult to locate.
16. Cyanoacrylate is toxic for the cortex, and we therefore leave a small amount of fluid in the burr hole, which will prevent the glue from entering. This most often occurs spontaneous with some cerebrospinal fluid.
17. There might be a small space on one side of the platform, if the cannula was not entirely vertical. This is not a problem, as the glue will fill the space. This is one

of the reasons for using the fluid form of the cyanoacrylate. If the cannula touches the sides of the burr hole anywhere along its length, the tip of the cannula will not end up at the intended coordinates, as it will be pushed to the opposite side when lowered further. The wide platforms with the low profile obstruct the view from above making it difficult to see when the platform touches the skull. If this is the case, one side of the flat part of the platform can be trimmed during preassembly (*see* **Subheading 3.1., step 1**).

18. Sodium bicarbonate changes the pH of the glue and causes its immediate polymerization. This is a great time-saver. Repeated layering can be used to build up substantial and strong structures, which can be useful. Note that the powder needs to be applied by very gentle squeezing of the bulb to prevent a large amount from being deposited onto the skull. Also, ensure that the powder does not touch fluid that may have built up in the sides of the wound, as the powder will quickly absorb the fluid. The next layer of glue will not bond. If this happens, remove the wet powder, dry the surface, and reapply.
19. We use metal clips/staples from Fine Science Tools for rats, as they are affordable and reliable. Make sure not to tighten the skin too much, which would prevent the eyes from closing which would cause unnecessary animal morbidity. For this reason, the staples are too big for mice. It is advised to check the sutures every couple of days.

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