Video Article

An Improved Method for Collection of Cerebrospinal Fluid from Anesthetized Mice

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Abstract

The cerebrospinal fluid (CSF) is a valuable body fluid for analysis in neuroscience research. It is one of the fluids in closest contact with the central nervous system and thus, can be used to analyze the diseased state of the brain or spinal cord without directly accessing these tissues. However, in mice it is difficult to obtain from the cisterna magna due to its closeness to blood vessels, which often contaminate samples. The area for CSF collection in mice is also difficult to dissect to and often only small samples are obtained (maximum of 5-7 µL or less). This protocol describes in detail a technique that improves on current methods of collection to minimize contamination from blood and allow for the abundant collection of CSF (on average 10-15 µL can be collected). This technique can be used with other dissection methods for tissue collection from mice, as it does not impact any tissues during CSF extraction. Thus, the brain and spinal cord are not affected with this technique and remain intact. With greater CSF sample collection and purity, more analyses can be used with this fluid to further aid neuroscience research and better understand diseases affecting the brain and spinal cord.

Video Link

The video component of this article can be found at https://www.jove.com/video/56774/

Introduction

The CSF is a valuable body fluid for analysis in neuroscience research. The CSF is primarily made from blood plasma, containing few cells (no red blood cells and only a few white blood cells) and is almost protein-free. It is one of the fluids in close contact with the central nervous system (CNS) and it can pass many electrolytes out from the brain and spinal cord to the peripheral system. In humans, CSF samples can be collected to aid in diagnosing disease or for research purposes in clinical trials, as a spinal tap (or lumbar puncture) is a minor, invasive procedure: the CSF fluid can reflect changes in the CNS without having to directly access these tissues. Thus, in recent years, for research purposes in the clinic, CSF samples have been obtained from patients of neurodegenerative diseases such as Alzheimer's disease and other dementias[1,2,3]. There are many biomarker assays which have been developed using CSF samples to potentially aid in diagnosing diseases in the clinic[2,3]. However, there is much debate on the reliability of these assays to produce consistent, sensitive results to specifically diagnose disease[4,5]. So, there is a great need for the development of better assays and targets, which can be found in the CSF, to aid in producing a standard technique to diagnose neurodegenerative diseases with greater sensitivity and specificity. Due to the potential importance of human CSF samples in disease, the collection of CSF from rodents in neuroscience research is also of interest.

Mice are important animals in biological and medical research and allow for the testing of potential therapeutic compounds and proof-of-concept studies before human clinical trials. However, in mice it is difficult to obtain CSF samples due to its closeness to the brain in a small animal, as the usual method of CSF collection in mice is to obtain it via the cisterna magna, an opening between the cerebellum and dorsal surface of the medulla oblongata. This causes difficulty in collecting CSF samples as this area is difficult to dissect to and in close proximity to blood vessels, increasing the risk of contamination from blood cells. Due to these difficulties, most researchers can only obtain a small amount of CSF for analysis (usually stated as 5-7 µL) and the contamination of CSF samples by blood cells is a primary concern for analyses[6,7,8,9]. Blood contamination can obscure results and not truly reflect the state of the CNS. Furthermore, limited sample collected can impact research as the usual amount collected from mice is enough for only one measurement (in duplicate or triplicate) using enzyme-linked immunosorbent assay (ELISA). Thus, CSF samples are usually pooled from multiple mice in order to have enough sample to run multiple assays. Developing a protocol for the abundant, uncontaminated collection of CSF from mice is greatly desired and will be beneficial in improving neuroscience research using rodents.

In this protocol, a technique for the abundant (an average of 10-15 µL) collection of CSF from anesthetized mice is described in detail and improves on a currently known method of CSF collection to minimize contamination from blood[10]. A robust protocol for CSF collection will aid in the development of CSF-based biomarker assays, which could be used to aid in diagnosing disease, as well as improve research into the mechanisms that underlie diseases affecting the CNS.

Protocol

All animal experiments were performed in accordance with the policies of the Society for Neuroscience (USA) and Fudan University (Shanghai, China) ethical committees. This procedure is for a non-survival surgery.

1. Setup of CSF Collection Apparatus

1. Pull the glass capillary (inner diameter 0.75 mm, outer diameter 1.0 mm) using a micropipette puller (as shown in Liu *et al.*[10]; sharpened capillary is shown in **Figure 1**).
2. Place a sharpened glass capillary into the capillary holder firmly mounted on a micromanipulator (**Figure 1A**).
3. Break the tip of the sharpened capillary with straight forceps under a dissecting microscope, so that the inner diameter of the broken tip is about 10-20 µm (**Figure 1C**).
4. Attach a thin tube and a syringe to the other end of the capillary holder, and connect the thin tube and the syringe with a three-way valve.
5. Shift the three-way valve to open and plunge the syringe to blow air from the syringe through the tubing to the glass capillary to expel any contaminants and create positive pressure in the capillary tube.
6. Repeat this a few times by dis-connecting and re-connecting the syringe to the three-way valve, and drawing the syringe up ~ 100-200 µL before the last re-connection of the syringe with the three-way valve (**Figure 1B**).
7. Move the micromanipulator with the glass capillary to the side to prevent damage during mouse dissection.

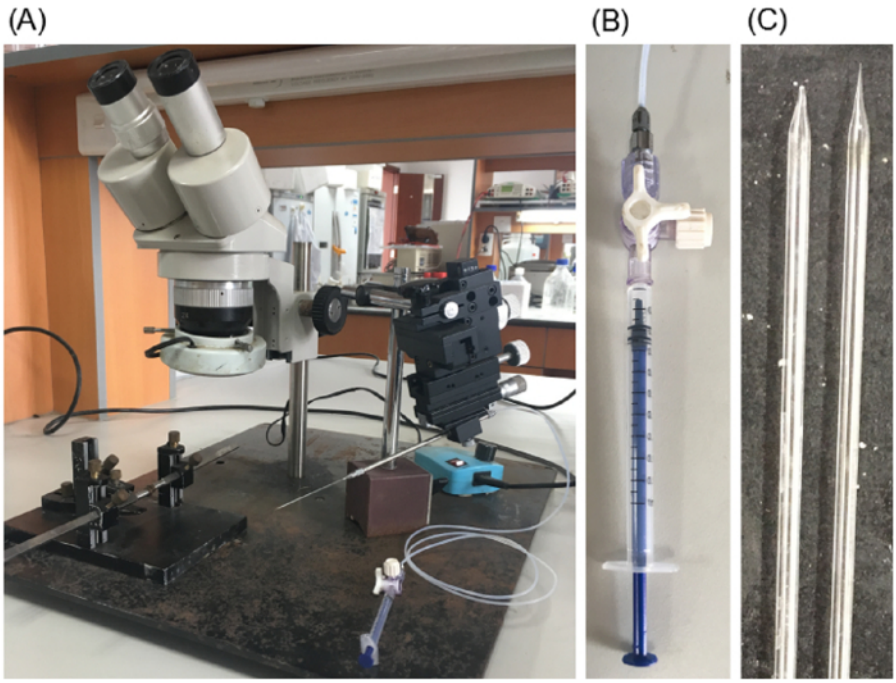

Figure 1. Microscope, micromanipulator, and capillary setup. (**A**) Microscope and micromanipulator with capillary attached setup, as well as a closer image of (**B**) the connected syringe and three-way valve to open (shown here) or close the piping, and (**C**) an unbroken (right) and broken (left) capillary tip ready for CSF collection. Once broken, the tip of the capillary should have an inner diameter of 0.75 mm, outer diameter of 1.0 mm. Please click here to view a larger version of this figure.

2. Mouse Dissection

1. Anesthetize the mouse.
   NOTE: For this protocol, C57BL/6 and amyloid precursor protein/presenilin 1 (APP/PS1) transgenic (Alzheimer's disease mouse model) mice were used and anesthetized with 4% chloral hydrate (in dH_20) at 10 µL/g of mouse weight via intraperitoneal injection.
2. When fully anesthetized, ensure that the mouse is adequately anesthetized. The withdrawal response to toe pinch, by slightly extending the hind limbs and pinching the toes and watching for a withdrawal response, is performed to assure adequate anesthesia. Check by pinching all four limbs; if there is no reaction the mouse is properly anesthetized. Cut and trim the hair at the back of the head of the mouse from above the eyes, between the ears to the bottom of the neck using dissecting scissors (**Figure 2A**). Alternatively, clippers or depilatory cream can be used to aid in hair removal.

3. Secure the head of the mouse using a mouse adapter (back of the head facing up with the nose pointed down at ~ 45 °, **Figure 2A**). Fix ear bars between the ears and eyes of the mouse and tighten. Make sure the head cannot move and is secured tightly in the adapter by gently pushing down onto the head of the mouse.
4. Recheck withdrawal response to toe pinch prior to making surgical incision and regularly thereafter to assure surgical plane of anesthesia. Visually note any change in respiratory rate during the procedure to indicate changes in planes of anesthesia. A clean, non-aseptic, technique can be used for this short non-survival surgery. Using scissors and curved forceps, cut through the skin and the first layer of muscle until the base of the skull is exposed with only a thin layer of muscle.
   NOTE: Cut through the mouse skin across the back of the neck and then cut the skin down the middle of the skull between the ears and eyes of the mouse. Skin and tissue can then be pulled apart and out of the area over the cisterna magna.
5. While viewing through the dissecting microscope, carefully dissect away the last thin layers of muscle over the base of the skull using forceps and move these layers of muscle to the side and out of the area of interest. If there is bleeding, use cotton buds to remove and help stop the bleeding.
6. Once the dura over the cisterna magna is exposed (triangular in shape with usually 1-2 large blood vessels running through the area; either side of or between the blood vessels is optimal for capillary insertion and CSF collection, **Figure 2B**), use a wet cotton bud to wipe the membrane clean and then use a dry cotton bud to dry the area.

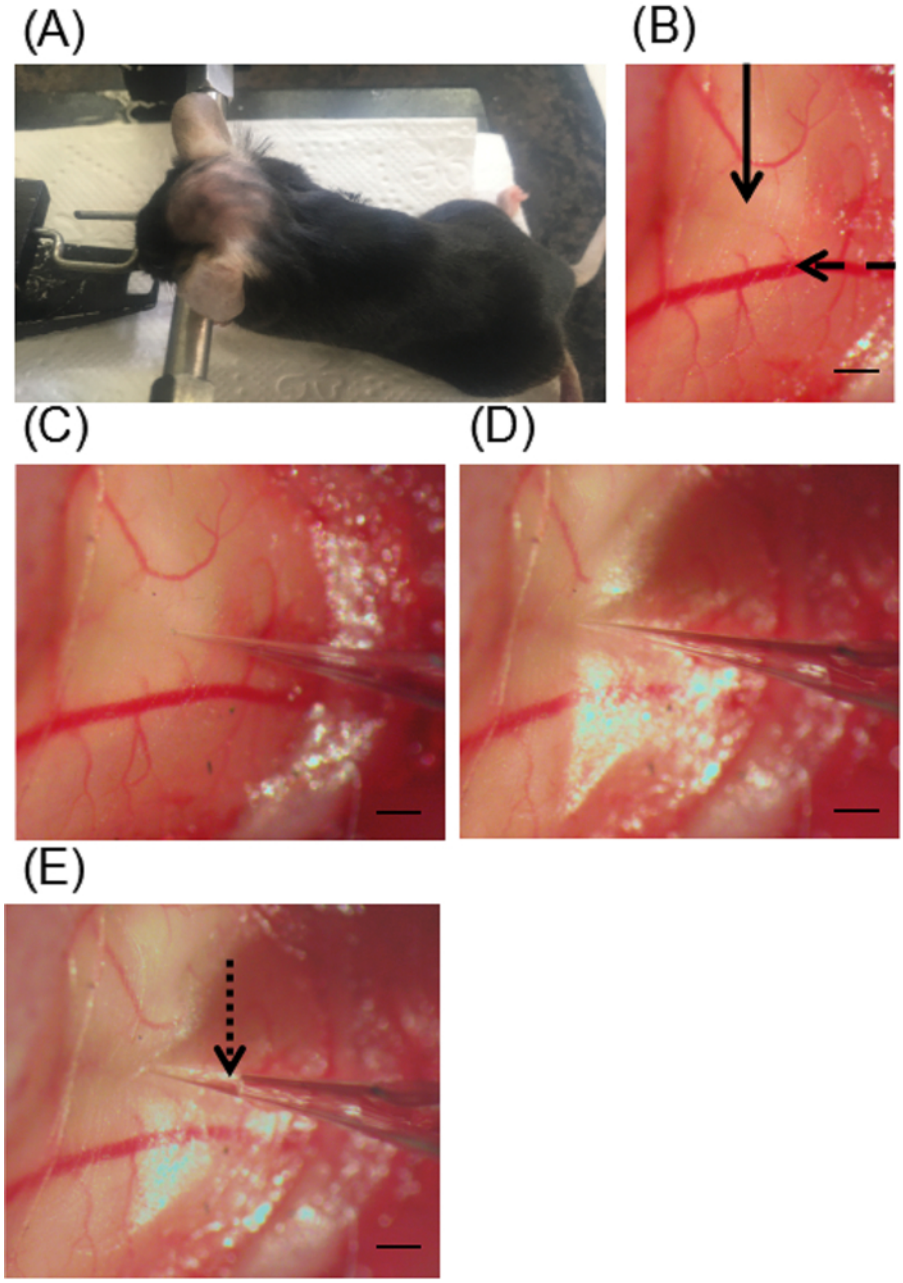

Figure 2. Mouse and dissection setup with representative images of capillary insertion for CSF collection. Setup of mouse ready for CSF extraction with (**A**) shaved head clamped in place for dissection and (**B**) detailed image (10x view) of a dissected mouse dura covering the cisterna magna (dashed arrow shows a blood vessel running through the area and solid arrow shows area optimal for capillary insertion). Detailed images (10x view) of (**C**) sharpened tip of glass capillary aligned against dura of cisterna magna, (**D**) the point at which the capillary almost punctures the dura, with some resistance from dura, and (**E**) the capillary tip tapped through the dura to collect CSF. CSF should be a clear liquid collected in the glass capillary (dotted arrow). All scale bars at the bottom right of each microscope image indicate 100 µm. Please click here to view a larger version of this figure.

3. CSF Collection from Anesthetized Mouse

1. Align the glass capillary to the back of the mouse head so that the sharpened point is just behind the membrane at a ~ 30-45 ° angle (**Figure 2C**).
2. Plunge the syringe once or a few times and draw the syringe up ~ 100-200 µL (to make sure there are no contaminants and there is negative pressure in the glass capillary).
3. Using the micromanipulator, move the capillary tip closer to the membrane until resistance can be seen, but not puncturing it (**Figure 2D**).
4. Once resistance is seen between the tip of the capillary and the membrane, gently tap the capillary tube through the membrane by knocking on the micromanipulator controls. Use the microscope to see the sharpened point of the capillary puncture into the cisterna magna. CSF should automatically be drawn into the capillary tube once the opening has been punctured (**Figure 2E**).

5. Leave the capillary to slowly collect CSF. If the CSF has stopped drawing out, draw the syringe up a small amount slowly (~ 50 µL) to create more negative pressure in the capillary to extract out the CSF.
   NOTE: Alternatively, the capillary can be gently and slowly pulled out of the brain using the fine micromanipulator controls to allow CSF to flow into the capillary again. A total amount of ~ 10-15 µL of CSF (~ 3-4 cm in the capillary) takes ~ 10-30 min, and occasionally 20 µL or more can be collected. Although not absolutely necessary, it is recommended to use a heat pad during CSF collection. CSF collection was performed at room temperature (~ 23 °C).
6. Once the amount of desired CSF is collected, close the tubing by shifting the three-way valve to close (to stop drawing out the CSF).
7. Take out the syringe connected to the tubing, and open and then close the tubing (to create equalized pressure in the capillary, tubing, and the area outside the capillary). Reconnect the syringe with the three-way valve, but do not plunge the syringe.
8. Gently remove the glass capillary from the mouse using the micromanipulator.
9. Place the collection tube (1.5 mL microtubes with 1 µL of 20x protease inhibitor) under the sharpened point of the glass capillary.
10. Open the tubing and gently plunge the syringe: the CSF should flow out of the capillary and into the collection tube.
11. After collection, quickly centrifuge (pulse spin for 5 s at maximal speed using a mini centrifuge) the CSF to mix the sample with the protease inhibitor at the bottom of the collection tube.
    NOTE: CSF samples can be aliquoted and then stored for further analysis at -80 °C. The rest of the mouse can be dissected for other tissues, such as brain and spinal cord. The mouse is still alive at this stage and thus blood can also be collected, if needed.

Representative Results

Using the procedure outlined here (**Figure 1** and **Figure 2**), the CSF immediately collected in the capillary should be clear (**Figure 2E**), not pink or red. If there is a pink to red tinge to the fluid collected in the capillary, then there was contamination with blood.

As an example of the application of the CSF sample collected with this method, levels of the protein amyloid-beta (Aβ) was measured using ELISA. Levels of Aβ in CSF is commonly measured in Alzheimer's disease research[7]. CSF is mostly protein-free. In a mouse model of Alzheimer's disease (APP/PS1 mice), levels of human $A\beta_{42}$ are significantly increased, as these mice overexpress human APP. This can be observed with the samples of CSF collected from 12-month-old mice using the methods described here (0 pg/mL in the CSF of wild-type (WT) mice compared to 1,303 pg/mL in the CSF of APP/PS1 mice, **Figure 3**).

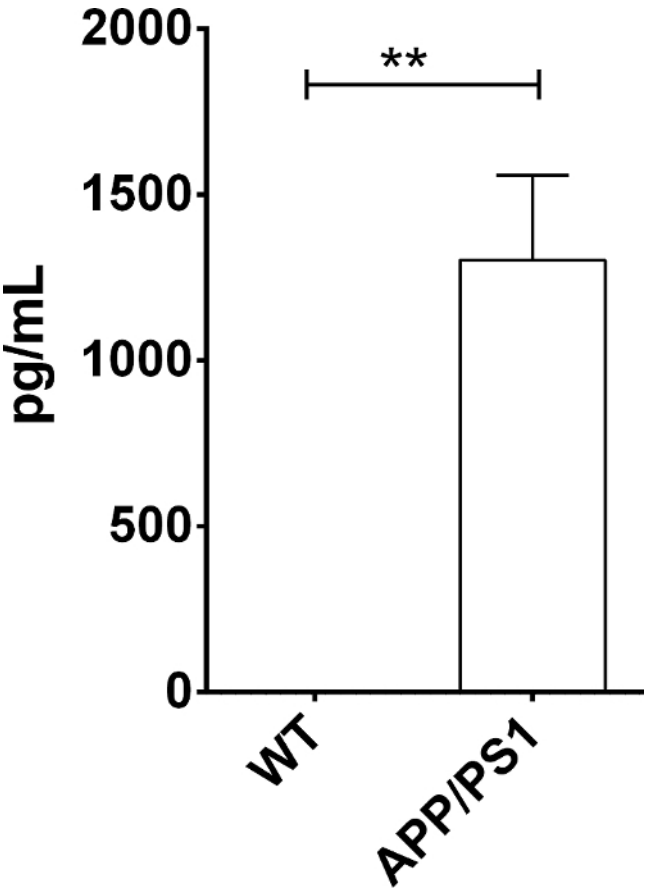

Figure 3. Representative results for CSF sample collected. ELISA analysis of CSF sample collected. In a mouse model of Alzheimer's disease (APP/PS1), there is a significant increase in levels of human $A\beta_{42}$ in the CSF, as these mice overexpress human $A\beta_{42}$ (1,303 pg/mL). In wild-type (WT) mice there should be no human $A\beta_{42}$ detected (0 pg/mL). An unpaired *t*-test was used to measure significance, ** $p < 0.01$, error bars represented as SEM, n = 3 and 4 mice at 12 months of age respectively. Please click here to view a larger version of this figure.

Discussion

This protocol describes in detail a technique which improves on current methods[10] of CSF collection to minimize contamination from blood and allow for the abundant collection of CSF (on average ~ 10-15 µL can be obtained) from mice. When breaking the capillary tip, the tip of the capillary should not be too small (as then the CSF will be extracted very slowly) or too big (will not be fine enough to collect the CSF and tissue can become lodged in the capillary). Care should be taken when dissecting the area for CSF collection: any bleeding should be stopped and the area for insertion of the glass capillary cleaned from blood with dH_20 to avoid contamination with the sample. The anatomy of each mouse is different, but most mice have 1-2 major blood vessels running through the area where the capillary needs to be inserted for CSF collection. Choosing the correct place for insertion of the capillary is important, as inserting too close to major blood vessels will contaminate the CSF with blood. As shown in **Figure 2**, an area clear of blood vessels, such as between or on either side of the major blood vessels, is optimal for capillary insertion to avoid contamination. If the CSF does not flow out at a steady rate and stops after some time, drawing the syringe a little (~ 50 µL) can help to restart the flow of CSF into the capillary. Alternatively, the capillary itself can be moved gently and slowly in or out of the mouse with the micromanipulator to restart the flow of CSF extraction. Care must be taken when doing these steps as blood can flow into the capillary as well. This technique works well due to the differences in pressure from the brain and glass capillary to draw out CSF from the mouse. Thus, the mouse can only be punctured once, as after the capillary is inserted into the mouse and taken out, there is a loss of pressure in the brain and CSF will no longer be drawn into the capillary automatically if it is inserted again. Therefore, practice will perfect this technique to produce no blood contaminants and obtain adequate CSF collection on the first insertion of the capillary into the mouse (from experience, 10-20 practice mice will be enough to reach this stage).

This protocol is an improved and efficient method for extracting CSF from mice and if done correctly, minimizes contamination from blood (**Figure 1**, **Figure 2**, **Figure 3**). This technique is limited by the time taken for CSF to be collected. Normally, 30 min is needed to collect ~ 15 µL or more of CSF at room temperature (~ 23 °C). During this time, the mouse can be kept warm with cotton wool or tissue, but without a covering or heat pad, CSF can still be successfully collected. Addition of a heat pad or warming the mouse before the procedure may improve this technique by increasing the flow of CSF out of the brain and reduce the time needed for collection, as described in other methods[10,11]. But in the protocol described here, no heat pad or warming of the mouse was used and between mice there was no difficulty in the amount of CSF that could be collected. As an example of the application this technique can be used for, levels of human $A\beta_{42}$ were measured using ELISA. Aβ is known to be sticky and can adhere to glass and the collection tubes. In this protocol, borosilicate glass capillaries and polypropylene tubes were used to collect the CSF from mice. While some Aβ may stick to the glass capillary and walls of the collection tubes, the ELISA results show that Aβ levels can be measured (**Figure 3**). Nonetheless, it is a possibility that Aβ levels measured from the CSF collected in this protocol are less than that described by other studies, but the glass capillaries are necessary to puncture through the dura of the mouse to collect the CSF. Besides measuring Aβ, other proteins of interest can also be measured using the CSF collected.

While the previously published method of CSF collection by Liu *et al.*[10] was meant for the continuous collection of CSF from living mice, the method described here is for the collection of CSF from mice to be sacrificed for tissue collection and analysis. Thus, this technique can be used along with other dissection methods for mouse tissue collection as it does not affect the brain and spinal cord, and after CSF collection (*i.e.*, after 10-30 min for ~ 10-15 µL of CSF), the mouse should still be breathing and under anesthesia. Therefore, other tissues such as blood, brain, spinal cord, and liver are still viable for collection and biochemical analysis. Although the Liu *et al.* method collected CSF very quickly (10 min per mouse for 3-7 µL of CSF), the method described here takes a longer time but can collect more CSF[10]. Another method for CSF collection has also been described by Maia *et al.*[11] Both the Liu *et al.* and Maia *et al.* methods describe collecting CSF from mice by hand-holding glass capillaries or gel loader tips and puncturing into the cisterna magna. The method described here differs by fixing the capillary to a micromanipulator. This ensures that the capillary is kept immobile and decreases contamination from blood during CSF collection. The micromanipulator also allows for greater precision and accuracy in puncturing the cisterna magna to obtain CSF. Although the Maia *et al.* method was also able to obtain 15-20 µL of CSF per mouse, it requires puncturing the cisterna magna repeatedly by hand with gel loader tips, which may increase the risk of contamination from blood or tissue. Furthermore, the Maia *et al.* method may decrease pressure in the brain with each sequential puncturing of the cisterna magna so that there is less and less CSF collected each time. The method described here involves only one puncture and leaving the capillary in the cisterna magna to further collect CSF as it refills with CSF while the mouse is under anesthesia. Thus, the protocol described here continuously collects CSF and minimizes possible contamination from repeated punctures into the cisterna magna as well as disruption of the surrounding tissue.

With greater CSF sample collection and purity, more analyses can be used with this fluid to further aid in neuroscience research. It is well known among researchers that the collection of CSF from mice can be tedious and only a limited sample can be obtained (usually stated as a maximum of 5-7 µL[6,7,8,9]). In the literature, there are not many detailed protocols published for CSF extraction and this protocol improves on a previously published method[10] to minimize contamination from blood, while maintaining the abundant collection of CSF. CSF has many applications in neuroscience research and as one of the closest fluids to the tissues of the CNS, it is a valuable sample for research. It has been previously identified that an adult mouse has a total volume of 40 µL of CSF[12]. Thus, this protocol is able to obtain 25% and possibly more of the total amount of CSF in an adult mouse. Besides the inbred strains C57BL/6 and APP/PS1 mice, the outbred strain imprinting control region (ICR) mice have also been used to successfully collect CSF using this protocol. CSF from mice of various ages (4 to 18 months) has been successfully collected using this protocol. Thus, there should be no difficulty in collecting CSF from different strains or ages of mice with this method. This detailed protocol will hopefully be used by many to improve the development of CSF assays and other analysis techniques to aid in research to understand neurodegenerative diseases that affect the brain and spinal cord.

It is critical that the head of the mouse be fastened tightly, so it does not move during the dissection and CSF collection (Section 3 of the Protocol). The site for the initial puncture into the cisterna magna is important for obtaining abundant, non-contaminated CSF. The capillary should not be inserted too close to any blood vessels to avoid contamination of the sample collected. Furthermore, adjustment of the capillary is critical for optimal CSF collection. The use of the micromanipulator allows for fine adjustments to be made without greatly disrupting the surrounding tissue and contaminating the CSF. Using the fine controls of the micromanipulator, the capillary can be slowly moved in or out to allow for CSF to flow out from the mouse and into the capillary for collection.

Disclosures

The authors have nothing to disclose.

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