

Continuous or Repeated Sampling of Cerebrospinal Fluid (CSF) in Freely Moving Rats using a Cisterna Magna (CM) Cannula System and a Programmable Syringe Pump or Collection Port

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Abstract

Continuous or repeated sampling of CSF in freely moving rats was investigated using a CM cannula system and a programmable syringe pump or collection port. Challenges previously faced by investigators with CSF collection in rats include the disadvantage of sample collection under anesthesia, contamination of the CSF sample during a CM or lumbar puncture and the inefficiency or inability to collect serial CSF samples. A permanent cannula was placed into the rat's CM and fixed to the skull. The cannula (with connected polyurethane tubing), was tunneled subcutaneously and externalized for CSF collection. For continuous collection, a standard tether system was utilized and either gravity flow or a pump-driven collection system was implemented. Animals tolerated continuous sample collection rates between 5 and 100 μ l/hr for up to 16 hours. Serial (intermittent) samples were collected using the PinPort™ collection system. Up to 10 samples (25-100 μ l) were collected over 24 hours. In conclusion, a highly practical method has been developed to provide continuous or serial CSF collection in freely moving rats using a novel cannula system.

Cannulation

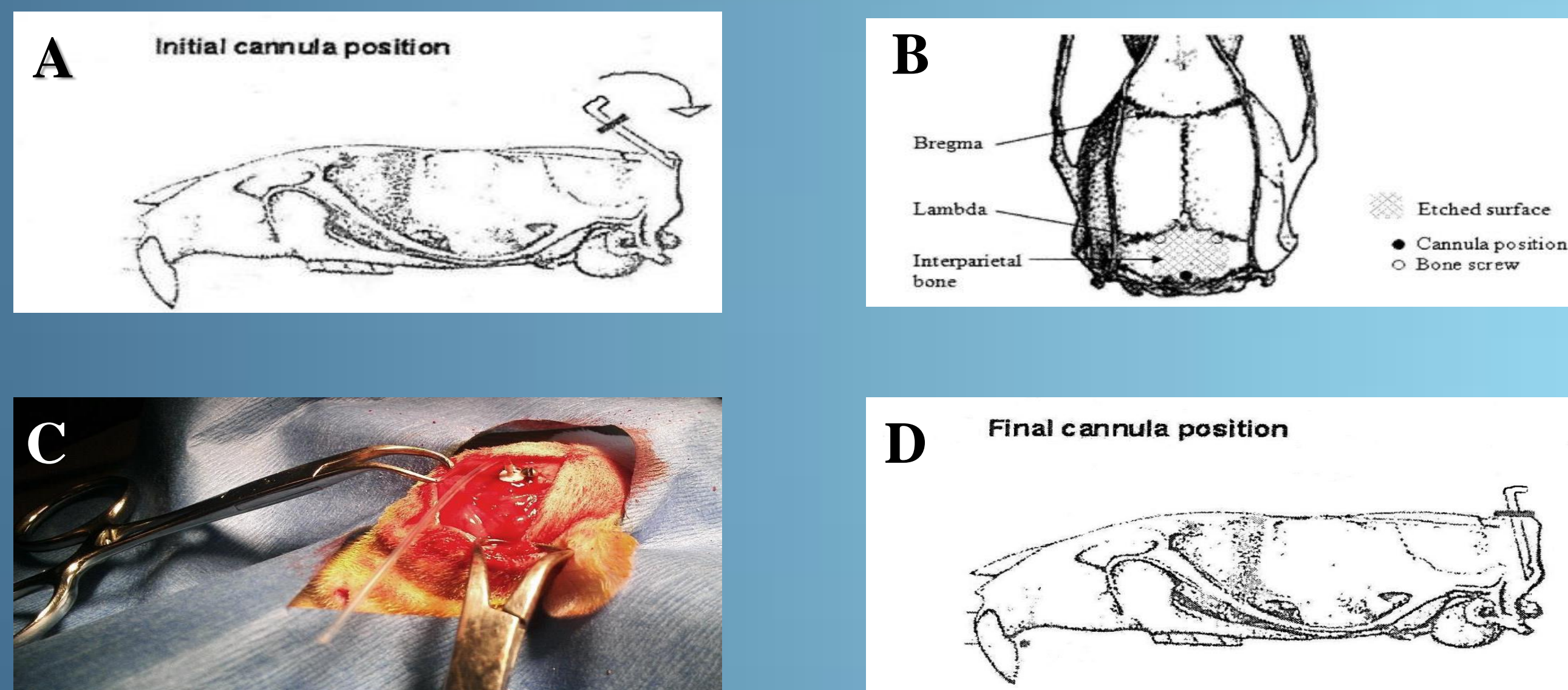


Figure 1. Rat CM Cannulation: (A) Cannula placement technique (B) Skull landmarks for cannulation (C) Cannula system at surgery (D) Final cannula position

Introduction

Major challenges previously faced by investigators with CSF collection in rats include the disadvantage of sample collection under anesthesia, contamination of the CSF sample by blood during a CM or lumbar puncture and the inefficiency or inability to collect serial CSF samples. A highly practical method has been developed to provide continuous or serial CSF collection in freely moving rats using a novel cannula system. This method is ideal for pilot investigative studies in which multiple samples or continuous CSF collection are desired over a short period of time including pharmacologic, pharmacokinetic and toxicokinetic investigations.

Materials and Methods

The surgical procedure is rapid (can be completed in approximately 15 minutes) and involves placement of a permanent cannula designed to optimize CSF flow (SAI) into the rat's CM. The cannula is guided through a midline craniotomy in the caudal aspect of the interparietal bone and fixed to the skull by anchoring screws and dental cement or cyanoacrylate. The cannula, with connected polyurethane tubing, is tunneled subcutaneously and externalized in the mid-scapular region for CSF collection. Flow of CSF from the polyurethane tubing ensures proper cannula placement.

Results

For continuous collection, a standard tether system is utilized and either gravity flow or a pump-driven system (shown to the right) can be implemented for more precise collection. Animals tolerated continuous sample rates between 5 and 100 μ l/hr for up to 16 hours. Serial (intermittent) samples, ranging from 25-100 μ l, were collected using a PinPort™ access device. Gravity flow or an attached microliter syringe was used to aid in collection. Up to 10 samples were collected at varying intervals over a 24 hour period. This method provides rapid, aseptic access to externalized catheters which is crucial for sequential CSF collection.

CSF Sample Collection

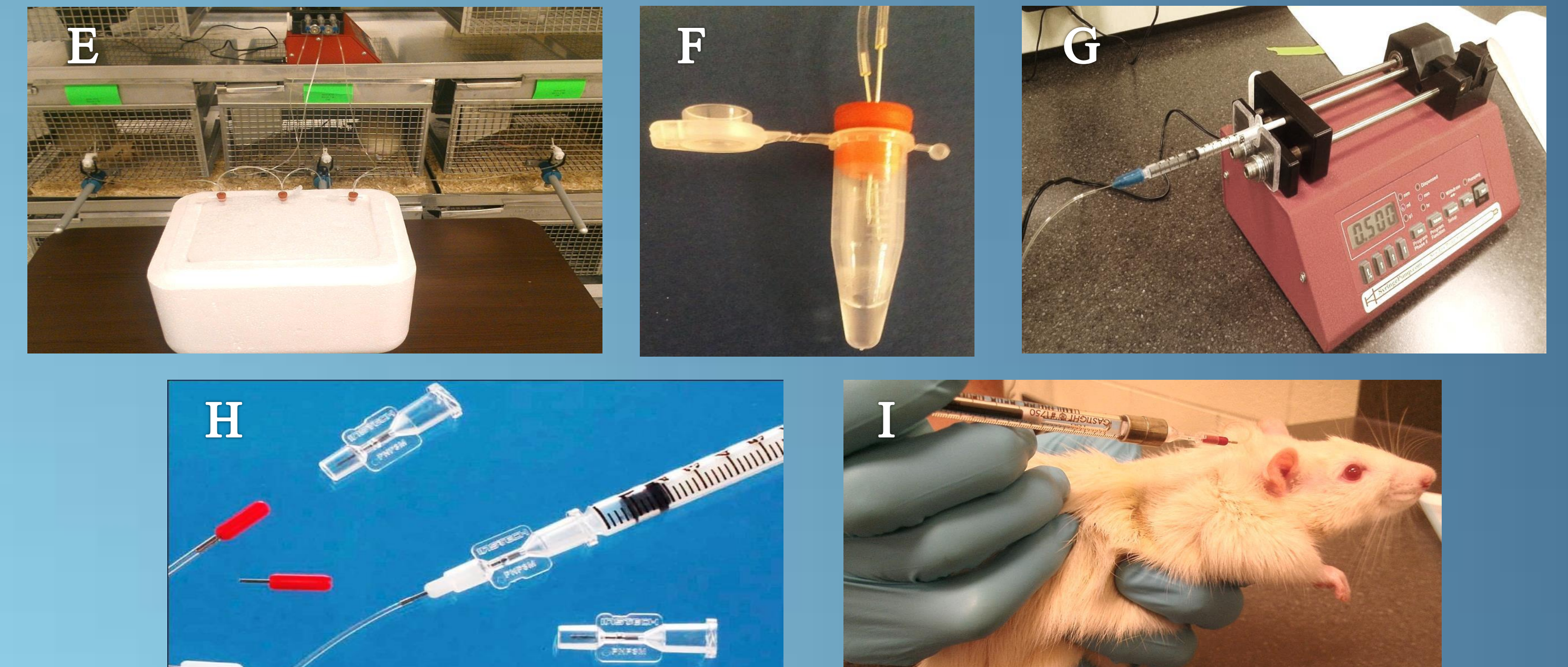


Figure 2. Continuous (E-G) and Repeated (H and I) CSF Collection: (E) Continuous CSF collection setup (F) Continuous CSF collection sample vial (G) Programmable syringe pump (H) PinPort™ and injector with recessed tip (Instech-Solomon) (I) Repeated CSF collection via PinPort™

Conclusion

This highly practical method provides continuous or serial CSF collection in freely moving rats using a novel cannula system. This method has several advantages, including sample collection with minimal manipulations to physiological conditions, collection without anesthesia and longitudinal sampling in individual animals. Regulatory compliance is also improved by reducing the number of animals needed and alleviating stressful sample collection procedures. This novel collection system allows for aseptic sampling, greatly reducing the possibility of CSF sample contamination when compared to currently accepted methods. Further investigations will be conducted to characterize CSF chemistry and total protein changes following continuous and serial collection periods. No clinical signs were observed during or following any of the collection periods.