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Original Research

Development of a Cerebrospinal Fluid Lateral Reservoir Model in Rhesus Monkeys (*Macaca mulatta*)

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Rapid, serial, and humane collection of cerebrospinal fluid (CSF) in nonhuman primates (NHP) is an essential element of numerous research studies and is currently accomplished via two different models. The CSF reservoir model (FR) combines a catheter in the 4th ventricle with a flexible silastic reservoir to permit circulating CSF flow. The CSF lateral port model (LP) consists of a lateral ventricular catheter and an IV port that provides static access to CSF and volume restrictions on sample collection. The FR model is associated with an intensive, prolonged recovery and frequent postsurgical hydrocephalus and nonpatency, whereas the LP model is associated with an easier recovery. To maximize the advantages of both systems, we developed the CSF lateral reservoir model (LR), which combines the beneficial features of the 2 previous models but avoids their limitations by using a reservoir for circulating CSF flow combined with catheter placement in the lateral ventricle. Nine adult male rhesus monkeys were utilized in this study. Pre-surgical MRI was performed to determine the coordinates of the lateral ventricle and location of choroid plexus (CP). The coordinates were determined to avoid the CP and major blood vessels. The predetermined coordinates were 100% accurate, according to MRI validation. The LR system functioned successfully in 67% of cases for 221 d, and 44% remain functional at 426 to 510 d postoperatively. Compared with established models, our LR model markedly reduced postoperative complications and recovery time. Development of the LR model was successful in rhesus macaques and is a useful alternative to the FR and LP methods of CSF collection from nonhuman primates.

Abbreviations: CP, choroid plexus; FR, CSF 4th ventricular reservoir model; LP, CSF lateral port model; LR, CSF lateral reservoir model; SER, successful establishment rate.

Serial ventricular CSF sampling in NHP is a frequent and critical requirement for a wide variety of studies and is predominantly accomplished by using either of 2 models. The 4th ventricle (FR) model, previously referred to as an Ommaya reservoir,[6] and lateral port (LP) models[2] are closed, indwelling, subcutaneous systems that allow for serial, rapid, and humane collection of CSF, as well as intraventricular drug administration, in unanesthetized and restrained NHP.

The FR model (Figure 1 A) consists of a catheter that is placed in the 4th ventricle and attached to a silastic reservoir that is implanted subcutaneously over the occipital bone. The silastic reservoir is depressed repetitively prior to and after sampling to circulate the CSF throughout the ventricles and catheter system to provide an unbiased sample without volume loss to dead space. The reservoir is accessed percutaneously to obtain a CSF sample via aspiration or to administer drug. The FR model initially was developed in 1977[6] and continues to be used for pharmacokinetic studies. This system continues to demonstrate a low rate of successful establishment, but once established, the FR model remains patent for prolonged periods without evidence of neurologic sequelae or bleeding from the choroid plexus (CP) in rhesus macaques. The decreased establishment rate of this model is attributed, at least in part, to postsurgical development of hydrocephalus, given that the catheter, which is routed through the aqueduct of Magendie to the 4th ventricle, can obstruct the flow of CSF. In addition, maintaining catheter patency is problematic due to CP bleeding during the recovery period. Postsurgical care and recovery after creating the FR are extensive, frequently requiring prolonged analgesics and steroid administration, with many days needed for complete recovery.

The LP model (Figure 1 B) consists of a catheter that is implanted in the lateral ventricle and attached to a subcutaneous intravenous access port. The port is accessed percutaneously to obtain the CSF sample or to administer a drug. The LP is a static model, because the CSF is not circulated or mixed through the ventricles, and CSF is obtained via unidirectional flow. The LP model was developed in 1990 for intrathecal drug administration[3] and has been used subsequently for CSF collection[4] by several investigators. CSF sampling with the LP model is restrictive: the volume of the system (that is, the dead space) must be removed

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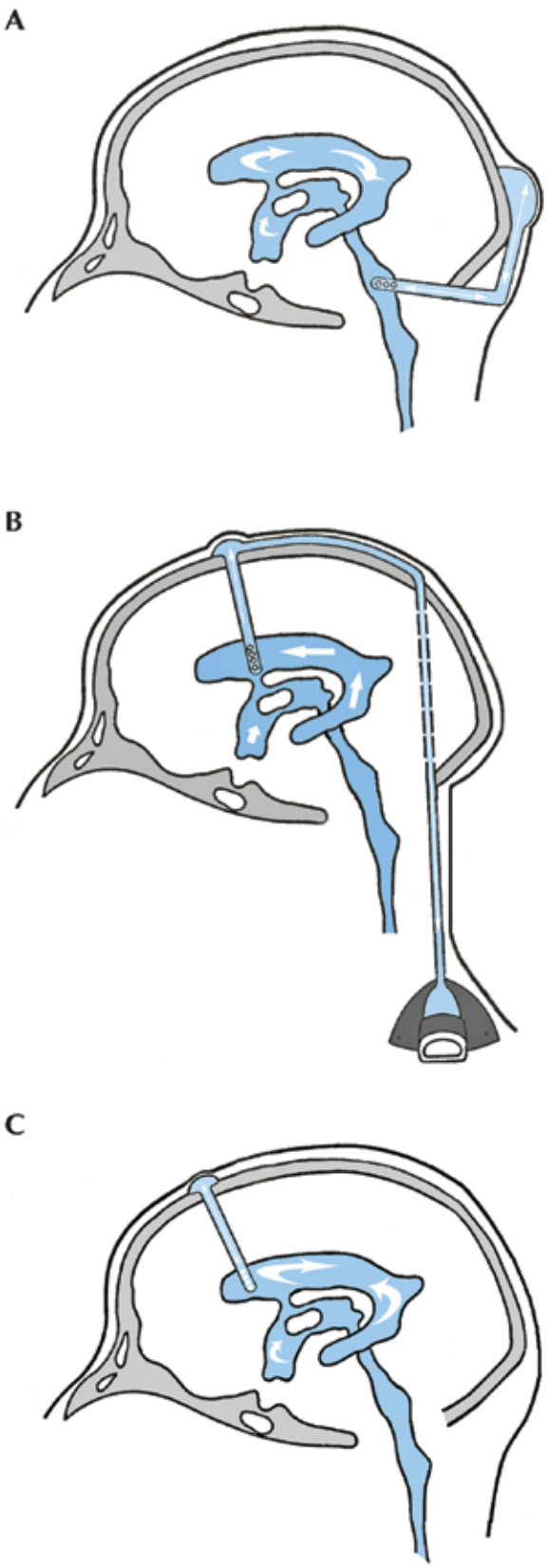

Figure 1. Evolution of CSF ventricular models, with flow dynamics. (A) Sagittal diagram of original FR (Ommaya) model, with placement in the 4th ventricle. Developed in 1977. Arrows indicate the circulating flow of CSF. (B) Original LP model, with catheter placement in the lateral ventricle and attachment to an IV access port. Developed in 1990. Arrows indicate the static, unidirectional flow of CSF. (C) The LR model, a composite of the 2 earlier CSF models. Arrows indicate the circulating flow of CSF.

at each collection to obtain an unbiased sample, collection is accomplished via gravitational flow and not aspiration, and the collection frequency is dependent on the rate of CSF replacement. In addition, the potential for sample contamination from blood due to CP bleeding remains problematic for the duration of LP implantation. However, the use of the lateral ventricle avoids the postsurgical complication of hydrocephalus. This system demonstrated a high rate of successful establishment with a reduction in the necessary analgesic and steroid administration as well as days to complete recovery, as compared with the FR model. Analysis of our clinical records from 2003 to 2013 revealed a successful establishment rate (SER) of 39% for the FR model; 33% of these systems remained functional for 3 to 7.5 y (Table 1). For the LP model, review of our data from 1987 to 1996 demonstrated a SER of 91%, and 82% remained patent for 1 to 9.8 y (Table 1). The LP model was used solely for drug administration.

To combine the functionality of the FR model with the higher SER and ease of recovery associated with the LP model, we developed the lateral ventricular reservoir (LR) model (Figure 1 C). This new model provides a closed, indwelling, and subcutaneous system with circulating CSF flow and unrestrictive sampling and avoids the potential postsurgical complications of hydrocephalus and nonpatency, extensive postsurgical care, and prolonged recovery.

Materials and Methods

The National Cancer Institute's IACUC approved this study. Nine adult male rhesus macaques (weight, 8.6 to 13.6 kg) were used. The macaques were socially housed and cared for in accordance with the *Guide for the Care and Use of Laboratory Animals*.[2]

Preoperative evaluation. A presurgical MRI was performed on each macaque to determine individual coordinates,[5] the criteria for which included the angle of surgical approach (determined by the length of the catheter, size of the ventricle, and the need to avoid the CP and major blood vessels), distance of the catheter off the midline, distance and direction from the ear bars (the mathematical difference of the slice number of the ear bars [stereotactic zero] from the slice number of the area of the left lateral ventricle that displays negligible CP), depth (the distance from the top of the skull to the distal end of the catheter), and presence of CP within the lateral ventricle. The macaques were anesthetized with ketamine (10 mg/kg IM) and dexmedetomidine (1000 µg/m^2 IM), maintained with isoflurane (1% to 2%) and oxygen (2 L/min), placed in a custom MRI-compatible stereotaxic unit (model 1530M, David Kopf Instruments, Tujunga, CA), and imaged by using a 3T MRI (Philips Healthcare, Best, The Netherlands). All scans were 3D T1-weighted images. A vitamin E capsule was used as an external fiducial marker on the left side of the head at stereotactic zero. OsiriX imaging software (version 3.9.4, Antoine Rosset, Bernex, Geneva, Switzerland) was used to calculate the coordinates from the presurgical scans for each animal.

Surgical procedure. Fasted macaques were anesthetized with ketamine (10 mg/kg IM), glycopyrrolate (0.015 mg/kg IM), and propofol (1 to 2.5 mg/kg IV) and intubated. Anesthesia was

Table 1. Average successful establishment rate and duration of various CSF reservoir models

	Successful establishment rate (%)		Duration		
	4 mo	1 y or more	No. of days	No. of months	No. of years
LP (n = 11)	91	82	1075	35.3	2.9
LR (n = 9)	67	44	292.9	9.6	0.8
FR (n = 18)	39	33	637.6	21.0	1.8

maintained via isoflurane (1% to 2%) and oxygen at a total flow rate of 2 L/min. Temperature, heart rate, oxygen saturation, electrocardiographic response, and end-tidal partial pressure of CO_2 were monitored. The macaques were positioned in ventral recumbency, with the head centered and secured in a stereotaxic unit (model 1504, David Kopf Instruments). Surgery was performed as an aseptic procedure. The ventricular catheter and reservoirs were sterilized with ethylene oxide. CNS pediatric antiblock ventricular catheters (length, 15 cm; inner diameter, 1.2 mm; outer diameter, 2.5 mm; Sophysa USA, Crown Point, IN) and Side-Inlet Integra Flat Bottom CSF reservoirs (volume, 0.31 mL; Medtronics, Goleta, CA) were used (Figure 2).

Implantation of LR system. A custom metal guide that had holes at the proximal end and polyethylene tubing (PE50) attached to the distal end was designed. The guide was used for stereotactic attachment, placement verification, and positioning of the catheter cuff. The guide–catheter assembly was calibrated to zero by using the stereotactic anterior–posterior zeroing bar and then attached to the stereotactic arm, and the tubing was filled with normal sterile saline. A skin incision was made over the frontal and parietal bones. The periosteum and temporalis muscles were retracted laterally off the bone, and a 4-mm burr hole was created off the midline according to the predetermined coordinates. Three or four 2-mm holes were created in the skull and surrounded the burr hole, for placement of nylon screws to secure the catheter to the skull. The lateral catheter was implanted according to the predetermined coordinates. Correct placement of the catheter in the ventricle was confirmed by the gravitational flow of saline in the tubing–guide assembly. The catheter cuff and screws were positioned and used with bone cement (Zimmer, Warsaw, IN) to secure the catheter. The ventricular reservoir was attached to the catheter and implanted subcutaneously on the parietal bone.

Postsurgical treatment and evaluation. The macaques received enrofloxacin (10 mg/kg IM daily) for 7 d, ketorolac (30 mg IM divided into 2 doses daily) for 1 to 2 d, sustained-release burprenorphine (0.1 to 0.2 mg/kg SC) postoperatively, and dexamethasone (1.0 mg/kg decreasing to 0.25 mg/kg IM BID) for 2 to 3 d. Daily neurologic assessment[1] of each macaque was based on the criteria in Figure 3.

The macaques were anesthetized with ketamine (10 mg/kg IM) and the reservoir was accessed percutaneously once daily for 7 d and then once weekly for 4 wk. A volume of 0.5 to 1.0 mL CSF was collected via gravitational flow for the first 5 d and then via aspiration after several depressions of the reservoir. The turbidity of and presence of blood in the CSF was noted. Pupillary size and reflex, as well as vital signs, were recorded. In addition to the flow of CSF in the catheter–reservoir system, postsurgical MRI, as previously described, was performed in 4 of the 9 cases, to confirm catheter placement in the lateral ventricle.

The criteria for successful functioning of the LR model were based on the absence of any neurologic sequelae (Figure 3) and successful collection of 0.5 to 1.0 mL CSF (after repeated depression of the reservoir) without contamination by blood for 4 mo (average, 122 d).

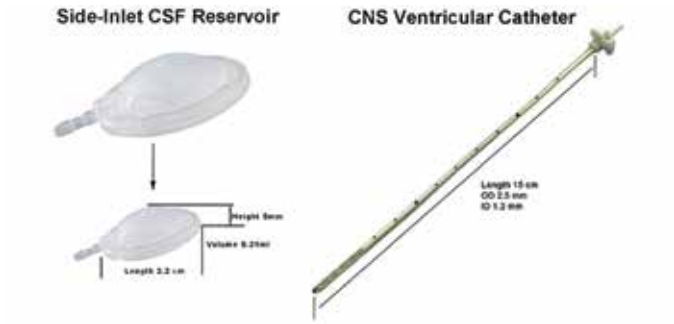

Figure 2. Lateral CNS ventricular reservoir and catheter system.

The duration of postoperative abnormal food intake, abnormal CSF turbidity or blood contamination, analgesia, steroids, postoperative care (daily evaluation of the incision; neurologic and clinical assessments; continued provision of antibiotics, analgesics, or steroids as determined by the veterinarian) and case resolution (macaque cleared by veterinarian for study use) were evaluated for each macaque in which an LR system was implanted.

Results

For all cases, a left-sided approach and an anterior direction from the ear bar were used. Table 2 illustrates the individual coordinates, identifiable CP, success rate, and duration of successful maintenance of the LR in each of the 9 cases. The coordinates for the placement of the LR were 100% accurate (Figure 4).

The LR system functioned successfully for 221 d in 6 of the 9 cases (67%). Due to a clinical illness unrelated to the study, one macaque was lost from the cohort at this time point. Of the remaining cases, 56% retained functionality for 352 d, and 44% had continuing functionality at 426 to 510 d (Table 2).

The LR system did not function successfully in 33% of the cases. These animals (macaques 2, 8, and 9) all had identifiable and unavoidable CP at or near the implantation site, resulting in bleeding and nonpatency (Figure 5). Macaques with these failed systems did not exhibit any neurologic sequelae or clinical symptoms and were retained within our colony for other, noninvasive studies.

The durations of postoperative abnormal food intake, abnormal CSF turbidity or blood contamination, analgesia, steroids, and postoperative care were all markedly decreased and case resolution was greatly increased (Table 3) for the LR model compared with the FR model.

To demonstrate the benefit of the LR model relative to the FR model, we determined the percentage improvement (calculated as $[FR_{value} - LR_{value}] / FR_{value} \times 100\%$) for several important parameters. Compared with the FR model, use of the LR model was

Score	Normal signs present	Abnormal signs present
0	Eating appropriately Bright, alert, and reactive Normal locomotion No change in pupil size; animal gazing directly Normal hand–eye coordination; animal tracks treat without difficulty No head tilt No appearance of or change in the level of abnormal behaviors (such as circling, weaving, pacing, saluting, hair plucking)	None
1	Normal locomotion Eyes normal Normal hand–eye coordination No head-tilt No additional abnormal behaviors	Off food Depressed or lethargic
2	Normal locomotion Eyes normal Normal hand–eye coordination No head-tilt	Off food Not bright, alert, or reactive Slight to moderate increase in appearance of abnormal behaviors
3	Eyes normal Normal hand–eye coordination No head tilt	Off food Not bright, alert, or reactive Slight to moderate increase in appearance of abnormal behaviors Changes in locomotion but still ambulatory
4	None	Off food Not bright, alert, or reactive Abnormal or lack of locomotion; animal may be partially or completely nonambulatory Lack of hand–eye coordination Eyes abnormal; both pupils dilated or constricted, or one dilated and one constricted; animal staring in the wrong direction Marked appearance of or increase in abnormal behaviors Head tilt

Figure 3. NHP neurologic assessment criteria. One or more conditions may be exhibited in each level.

Table 2. Results from individual macaques with LR model

	Angle (°)	Distance from ear bars (mm)	Depth (mm)	Distance off midline (mm)	Target verified?	Choroid plexus identifiable?	Successful?	Duration (d)
1	4	30	25	3	Yes	No	Yes	221[a]
2	4	28	37	3	Yes	Yes	No	112[b]
3	4	28	26.5	3	Yes	No	Yes	510[c]
4	0	27	28	2	Yes	No	Yes	352
5	0	30	25	2	Yes	No	Yes	489[c]
6	4	28	24	3	Yes	No	Yes	482[c]
7	6	27	22	3	Yes	No	Yes	426[c]
8	4	30	25	3	Yes	Yes	No	29[b]
9	6	30	28.5	3	Yes	Yes	No	15[b]

[a]Animal with functional system lost due to an unrelated clinical illness.
[b]Criteria for successful functioning of the LR model were not achieved for a minimum of 4 mo (average, 122 d).
[c]Ongoing functionality.

associated with a 72% improvement in the SER at 4 mo, 33% improvement in SER at 1 y, 90% reduction in the number of days of abnormal feed intake, 46% reduction in the duration of postoperative analgesia, 38% reduction in duration of steroid treatment, 39% decrease in overall steroid dose, 62% decrease in the number of days of abnormal CSF (for example, excess turbidity, blood present), and 53% reduction in the duration of postoperative recovery.

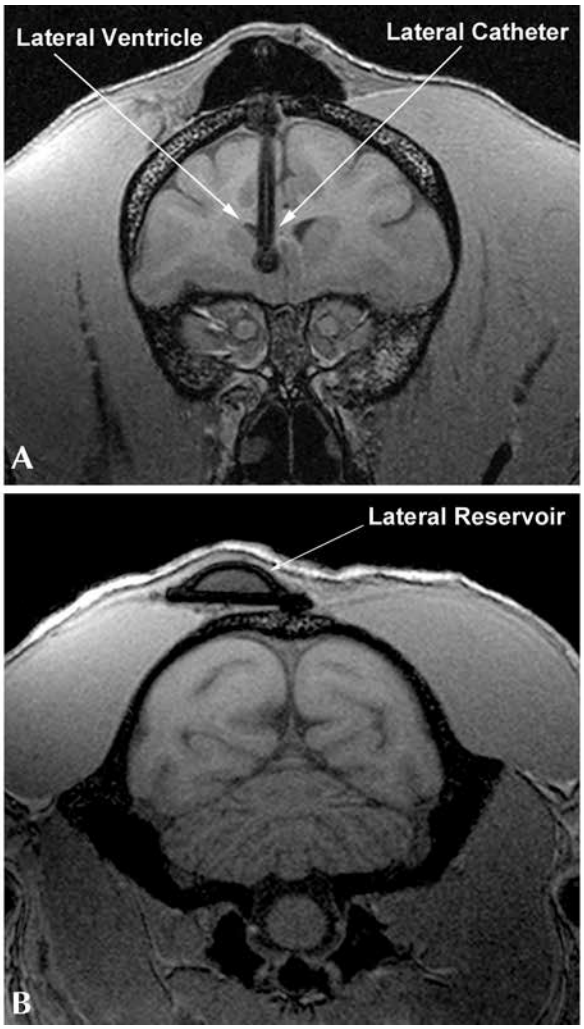

Figure 4. Coronal T1-weighted image from 3T MRI of LR model. (A) Catheter in the lateral ventricle. (B) Ventricular reservoir.

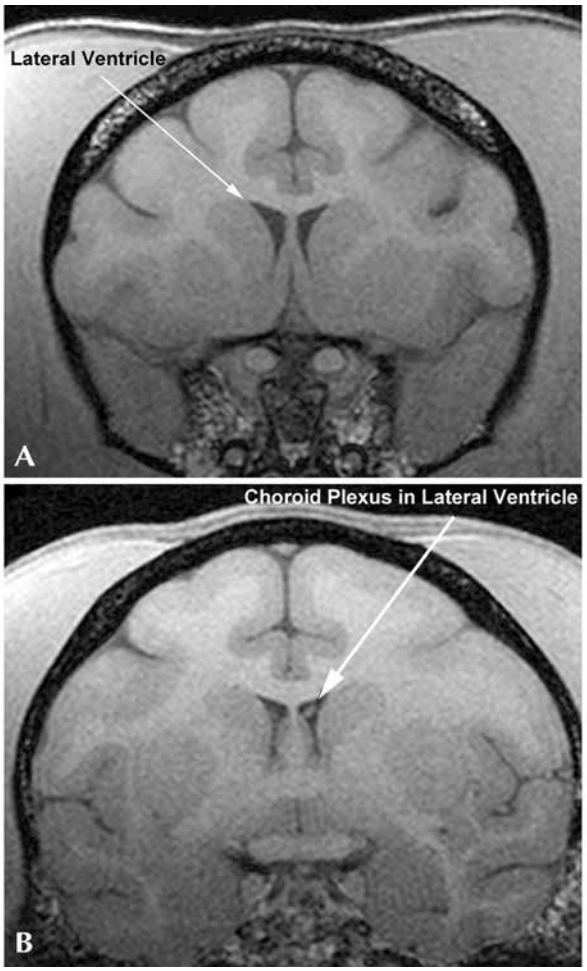

Figure 5. Coronal T1-weighted image from 3T MRI of rhesus macaque showing (A) negligible and (B) identifiable amounts of choroid plexus in lateral ventricles.

Table 3. Average postsurgical treatment and recovery

CSF model	Abnormal food intake (d)	Analgesia provided (d)	Steroids provided (d)	Steroid dose (mg/kg daily)	Abnormal turbidity or blood present in CSF (d)	Time until recovery (d)
LP (n = 11)	0	1.4	3.5	1.6	2.8	8.0
LR (n = 9)	1.3	2.4	5.9	1.2	3.8	18.2
FR (n = 18)	13. 6	4.5	9.6	2.0	9.8	38.4

Discussion

Access to CSF is critical to translational research aimed at the CNS. This access is particularly important for evaluating the pharmacokinetics, including CSF penetration, of agents being developed for CNS indications, such as brain tumors. Reliable and repetitive access to CSF, which is limited in human studies, is achieved by using the NHP models previously described; our goal in the current study was to advance these models to minimize complication rates and necessary interventions. The development of our hybrid LR model accomplished our goal by incorporating the advantages of the FR and LP existing systems (that is, ability to mix CSF) while minimizing their disadvantages (for example, postoperative complications).

Table 1 illustrates an overview of the average SER and durations of all 3 systems. Using our new LR model achieved a 71.8% improvement in SER at the initial 4-mo time point, decreasing to a 33% improvement at 1 y or more. Although the SER of the LR model did not approach that of the LP model, the LP model was developed and typically used for drug administration only, and the historic SER reflects this type of use. We attribute the reduction in the SER for the LR model to the collection of CSF at the lateral ventricular site. The LR model, although it has not been available for use as long as have the other 2 systems, has demonstrated prolonged functionality once the system is established in an animal.

The loss of functionality from the 4-mo time point to the 1-y mark was greater for the LR model than the FR and LP models

(34.3% 15.4%, 9.9%, respectively). LR model failure during this time period was due to nonpatency rather than to the presence of blood in the CSF. On average, the postoperative treatment and animal recovery times for the LR model were similar to those for the LP model (Table 3) but demonstrated a 38% to 90% improvement when compared with those for the FR model.

The LR model, combining the circulating CSF flow and unrestrictive sampling with a high establishment rate and ease of postoperative recovery, was successfully established in rhesus macaques for CSF collection and drug administration. It is therefore and advantageous alternative to the fourth FR and LP models.

Presurgical MRI and the coordinate criteria were invaluable tools for accurate placement of the ventricular catheter and for identifying whether CP was present in the implantation area. Using macaques with negligible CP near the implantation site dramatically improves the SER of the LR model.

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