Intracranial Pressure Monitoring In Nontraumatic Intraventricular Hemorrhage Rodent Model
--Manuscript Draft--

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1   **TITLE:**
2   Intracranial Pressure Monitoring In Nontraumatic Intraventricular Hemorrhage Rodent Model
3
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18
19 **KEYWORDS:**
20 cerebral perfusion pressure; femoral artery; intracranial pressure; intraventricular hemorrhage;
21 mean arterial pressure; Sprague Dawley.
22
23 **SUMMARY:**
24 Monitoring intracranial pressure in rodent models of nontraumatic intraventricular hemorrhage
25 is not common in the current literature. Herein, we demonstrate a technique for measuring
26 intracranial pressure, mean arterial pressure, and cerebral perfusion pressure during
27 intraventricular hemorrhage in a rodent animal model.
28
29 **ABSTRACT:**
30 Survivors of intraventricular hemorrhage are often left with significant long-term memory
31 impairment; thus, research utilizing intraventricular hemorrhage animal models is essential. In
32 this study, we sought out ways to measure intracranial pressure, mean arterial pressure, and
33 cerebral perfusion pressure during nontraumatic intraventricular hemorrhage in rodents. The
34 experimental design included three Sprague Dawley groups: sham, standard 200 µl
35 intraventricular hemorrhage, and vehicle control groups. By introducing an intraparenchymal
36 fiberoptic pressure sensor, precise intracranial pressure measurements were obtained in all
37 groups. Cerebral perfusion pressures were calculated with the knowledge of intracranial pressure
38 and mean arterial pressure values. As expected, the intraventricular hemorrhage and vehicle
39 control groups both experienced a rise in the intracranial pressure and subsequent decline in
40 cerebral perfusion pressure during intraventricular injection of autologous blood and artificial
41 cerebrospinal fluid, respectively. The addition of an intraparenchymal fiberoptic pressure sensor
42 is beneficial in monitoring precise intracranial pressure changes.
43
44 **INTRODUCTION:**

45 Intraventricular hemorrhage (IVH), a type of intracranial hemorrhage (ICH), is a devastating
46 disease that carries significant mortality and morbidity. IVH is characterized as the accumulation
47 of blood products inside the intracranial ventricles. Isolated IVH in uncommon and typically
48 occurs in adults[1]. It may be associated with hypertensive hemorrhage, ruptured intracranial
49 aneurysm or another vascular malformation, tumors, or trauma[1]. IVH leads to secondary brain
50 injury as well as the development of hydrocephalus[2]. Survivors of IVH are often left with
51 significant functional, memory, and cognitive impairments following their injury. These long-term
52 cognitive and memory deficits are reported in as high as 44% of survivors of ICH[3]. In subarachnoid
53 hemorrhage (SAH), another type of ICH, it is well known that approximately half of the survivors
54 will have memory deficits, and for those who have IVH in addition to SAH, outcomes tend to be
55 significantly worse[4–6].
56
57 Underlying mechanisms of memory dysfunction following IVH remain to be elucidated. *In vivo*
58 research utilizing nontraumatic IVH animal models with functional and memory dysfunction is
59 essential in order to discover potential therapeutic targets for such patients. Animal models with
60 more severe memory and functional dysfunction following IVH would be the best to study these
61 changes. The senior author's lab has also been investigating specifically the role of high
62 intracranial pressure (ICP) in the development of memory deficits in IVH rodent models. Hence,
63 methods to precisely measure ICPs during IVH were important to investigate. Herein, we report
64 on methods of precisely measuring ICPs in an IVH rodent model. Although ICP monitoring has
65 previously been used in traumatic ICH as well as subarachnoid hemorrhage animal models, ICP
66 monitoring in spontaneous IVH rodent models is not as commonly reported in the literature[7,8].
67 Hence, the experimental design presented herein included three groups of Sprague Dawley rats:
68 sham, standard 200 µl intraventricular hemorrhage, and vehicle control. For IVH group, an
69 autologous intraventricular blood injection model was used. For vehicle control animals,
70 intraventricular injection of sterile Lactated Ringer's solution was used. ICPs, mean arterial
71 pressures (MAPs), and cerebral perfusion pressures (CPPs) were recorded intraoperatively, and
72 results are reported herein.
73
74 **PROTOCOL:**
75
76 All research methods and animal care/maintenance were performed in compliance with the
77 institutional guidelines at the University of California, Davis. The Institutional Animal Care and
78 Use Committee (IACUC) of the University of California, Davis, approved all animal use protocols
79 and experimental procedures (IACUC protocol #21874).
80
81 **1.   Animal housing**
82
83 1.1.   Obtain Sprague–Dawley rats of age 8–10 months old. Prior to any experimental
84 procedure, house the rats in a vivarium and allow at least 1 week for general adaptation in their
85 cages following a 12-h light/dark cycle with food and water ad libitum.
86
87 **2.   Anesthesia and pre-operative procedures**
88

2.1.   Anesthetize the rat with 4% isoflurane for 4 min. Hang the rat by its teeth in a supine position on an intubation platform, and intubate endotracheally using an endotracheal cannula and laryngoscope.

2.2.   Place the anesthetized and intubated rat on a ventilator (2% isoflurane and O2/N2 carrier gas). The rat is adequately anesthetized if no response to a painful stimulus such as a hindleg pinch is observed.

2.3.   Insert a rectal thermometer to continuously monitor the temperature.

2.4.   Perform all operative procedures using sterile technique. Clip the hair on the head and the femoral region and prep the skin with Betadine prior to surgery.

2.5.   Aspirate any accumulated respiratory secretions by temporarily removing the rat from the ventilator and aspirating the secretions with PE-50 tubing connected to a 10 mL syringe.

2.6.   Protect the eyes with artificial eye ointment.

2.7.   Inject local bupivacaine (~0.1 mL of 0.25% solution) into the skin and subcutaneous tissues prior to the scalp incision.

3.   Surgery protocol

3.1.   Placement of intraventricular needle and intracranial pressure (ICP) monitor

3.1.1.   Place the rat in a prone position in a stereotactic frame and ear bar the rat.

3.1.2.   Make a 1.5-cm scalp incision along the midline with a 15-blade scalpel.

3.1.3.   Apply mild pressure with gauze for hemostasis.

3.1.4.   Using a sterile cotton tip applicator, separate the periosteum from the skull until the bregma landmark is visible.

3.1.5.   Locate and mark out bregma using stereotaxis and mark out the location of two bilateral burr holes, 1.4 mm lateral and 0.9 mm posterior to the bregma.

3.1.6.   Using a hand-held drill, create these two small (up to 2 mm) cranial burr holes in the right and left hemispheres. Irrigate out any excess bone chips with sterile Lactated Ringer's solution.

3.1.7.   In the right hemisphere, position a 22-G guide cannula at the level of the burr hole to insert the 28-G needle through the cannula to the depth of the right lateral ventricle (4.6 mm in relation to the bregma) in order to create IVH.

3.1.8.   Connect the fiberoptic pressure sensor to the read-out unit. Turn on the read-out unit and ensure the units selected are in mmHg. Then prime the sensor by submerging its tip into a small beaker with Lactated Ringer's solution until the read-out unit reads out zero. Once it is zeroed in the Lactated Ringers solution, it is all set to be inserted.

3.1.9.   In the left hemisphere, gently insert the pressure sensor to 2–3 mm depth into the cortex for real-time ICP monitoring.

3.2.   Femoral artery cannulation and insertion of mean arterial pressure (MAP) monitor

3.2.1.   After insertion of the ICP monitor, turn the lower trunk of the rat for easy access to the left thigh and groin area.

3.2.2.   After sterile preparation and local bupivacaine administration, make a 1.5 cm skin incision over the hindlimb with a 15-blade scalpel.

3.2.3.   Dissect out the left femoral artery first superficially with a hemostat and then deeper layers using forceps with fine tips under a microscope. Identify the deep blue femoral vein to help locate the adjacent artery.

3.2.4.   Tie off the distal femoral artery using 3-0 silk suture and place a temporary metal clip on the proximal portion of the femoral artery.

3.2.5.   Have a second fiberoptic pressure sensor connected to the read-out unit already primed. Insert the pressure sensor into the polyethylene (PE-50) tubing, which is inserted into a Tuohy Borst that is then closed. Connect the Tuohy Borst to a 3-way-stopcock connected to a 1 mL syringe at one end and a 22-G needle with PE-50 tubing at the other end.

3.2.6.   Under the microscope, make a 2 mm femoral arteriotomy with micro scissors and cannulate it with PE-50 tubing connected to the rest of the setup.

3.3.   Intraventricular injection

3.3.1.   Aspirate 500 µL of blood using a 1 mL syringe and turn the 3-way-stopcock to have the pressure sensor read MAP.

3.3.2.   Prime the 28-G intraventricular needle connected to PE-50 tubing with the aspirated blood for IVH animals and Lactated Ringer's for the vehicle control animals. Then insert this needle into the guide cannula to the depth of the right lateral ventricle.

3.3.3.   Using 100 µL/min rate, inject the blood or sterile Lactated Ringer's solution (200 µL) into the right lateral ventricle by pumping the 1 mL syringe with the thumb. Prior to this and during intraventricular injection, monitor and record ICP, arterial blood pressure, and rectal temperature.

177
178 3.3.4. Monitor and record the post-injection ICP and MAP values.
179
180 3.4. Closure
181
182 3.4.1. Following completion of the intraventricular injection, withdraw the PE-50 tubing
183 containing the pressure sensor that was inserted into the femoral artery and apply the temporary
184 clip to the femoral artery to prevent bleeding.
185
186 3.4.2. Tie off the proximal portion of the femoral artery using the 3-0 silk suture.
187
188 3.4.3. Close the femoral incision in an interrupted fashion using 3-0 silk.
189
190 3.4.4. Remove the guide cannula with the intraventricular needle and the ICP monitor.
191
192 3.4.5. Seal the burr holes with bone wax.
193
194 3.4.6. Close the cranial incision with 3-0 silk suture in an interrupted fashion.
195
196 3.4.7. Apply topical bupivacaine to the incision and inject 0.35 mL of carprofen (5 mg/kg)
197 postoperatively. Do not leave animals unattended until they have regained sufficient
198 consciousness to maintain sternal recumbency.
199
200 3.4.8. Allow rats to fully recover after surgery under supervision and return them to their home
201 cages with free access to food and water after recovery.
202
203 **4. Postoperative management**
204
205 4.1. Check all postoperative animals daily for seven postoperative days to monitor their
206 recovery, neurological status, behavior, weight, and incisions.
207
208 4.2. Administer 0.35 mL of carprofen (5 mg/kg) by subcutaneous injection at the time of
209 surgery and on the 1st and 2nd postoperative days.
210
211 4.3. Remove the sutures on the 7th postoperative day in a sterile fashion.
212
213 **REPRESENTATIVE RESULTS:**
214 ***Intracranial, mean arterial, and cerebral perfusion pressures***
215 Both ICPs and MAPs were monitored intraoperatively in all animals (**Figure 1**). Rats were of 8–10
216 months old with a mean weight of 495 ± 17 g. Real-time ICP graphs were also collected (**Figure
217 2**). Excluding the sham group, ICPs increased significantly during intraventricular injection in IVH
218 as well as vehicle control groups (**Figure 3**). ICPs peaked more in the IVH group (43 mmHg)
219 compared to the vehicle control (36.5 mmHg). The ICPs then quickly decreased and normalized
220 within five min post intraventricular injection in those animal groups. The fiberoptic sensor was

221 successfully used to monitor ICPs and MAPs in real-time. It was observed that MAPs stayed
222 similar throughout the procedure, whereas CPPs decreased during intraventricular injection of
223 either blood or Lactated Ringer's solution (**Figure 3**).
224
225 **FIGURE AND TABLE LEGENDS:**
226 **Figure 1: Experimental setup. (A)** Location of burr holes. (**B**) Depiction of the entire experimental
227 setup. Abbreviations: A-P, anterior to posterior axis; M-L, medial to lateral axis.
228
229 **Figure 2: ICP recordings**. Real-time intracranial pressure (ICP) recordings in (**A**) sham, (**B**) IVH, and
230 (**C**) vehicle control animals. Arrow denotes the start of the IVH/LR injection. N=1 in each group.
231
232 **Figure 3: ICP, MAP, and CPP graphs**. (**A**) Mean intracranial pressure (ICP), (**B**) mean arterial
233 pressure (MAP), and (**C**) mean cerebral perfusion pressure (CPP) values pre ventricular injection,
234 during ventricular injection, and post ventricular injection in IVH and vehicle control animals. N=1
235 in each group.
236
237 **DISCUSSION:**
238 This study investigated mechanisms to measure ICPs, MAPs, and CPPs in a nontraumatic IVH
239 rodent animal model. The results were recorded from the following groups: sham, IVH 200 μL,
240 and vehicle control (artificial cerebrospinal fluid intraventricular injection) group. This
241 experimental design was chosen to investigate how ICPs can be monitored during IVH injection
242 as we hypothesized that the spike in ICPs may contribute to the more significant secondary brain
243 injury and thus memory deficit in IVH animal models. Therefore, the goals of this study were to
244 establish an IVH animal model with objective monitoring of ICPs, MAPs, and CPPs following
245 nontraumatic IVH so that we can apply this further in future experiments that will focus on the
246 effects of ICPs induced by IVH on subsequent memory dysfunction. This pilot study found that
247 ICPs and MAPs can be precisely monitored using a fiberoptic pressure sensor introduced into the
248 left lateral ventricle and femoral artery, respectively. ICPs increase significantly during
249 intraventricular injection of blood and artificial cerebrospinal fluid. Additionally, the
250 corresponding CPPs decrease during the intraventricular injection.
251
252 One of the major concerns for this study was to find a way to monitor and record the very small
253 changes in pressures (ICPs and MAPs) accurately. This was done using a fiberoptic pressure
254 sensor. The fiberoptic sensor had to be small to accurately measure minimal changes in pressure.
255 The fiberoptic sensor that was used is insulated in a cable sheath for its protection. The outer
256 diameter of the sheath is 0.9 mm, and the diameter of the sensor tip itself is 420 μm. We ensured
257 that rat ICP and MAP values could fall into the normal operating range of pressures for this sensor
258 (-50 mmHg to +300 mmHg). Also, the precision of the fiberoptic sensor was ensured to be small,
259 $\pm$ 1 mmHg (Opsens Solutions).
260
261 Majority of current pre-clinical ICH models at this time use rodents with whole blood infusion
262 and collagenase (injection of collagenase enzyme in order to injure the extracellular matrix
263 resulting in IVH) models as the two most common experimental designs[9,10]. The whole blood
264 infusion model involves infusion of blood via a craniotomy or burr hole and has been reported

265   not only in rodents but also in pigs and primate species. However, no animal model is perfect,
266   and each has its own advantages and disadvantages[9,10]. With respect to outcomes, behavior,
267   cerebral edema, cell death, and hematoma size are some of the most common endpoints tested
268   in ICH studies. Of the behavioral tests assessing cognitive and memory dysfunction, the majority
269   utilize the Morris water maze test[10]. We have not found studies objectively measuring ICPs in IVH
270   nontraumatic rodent models.
271
272   A recent review by MacLellan et al. found many key issues with pre-clinical ICH literature[9].
273   MacLellan et al. found that an overwhelming majority of the studies report only on positive
274   treatment effects. Many studies with negative results are published in lower-tier journals or not
275   published at all, contributing to not an insignificant publication bias. They also found that many
276   studies do not describe the methodology such as randomization, age and sex of animals, among
277   others. Lack of blinding, lack of reporting physiological variables as well as statistical power are
278   additional weaknesses that were observed in that review. All this makes it challenging for others
279   who attempt to replicate the experiment[10]. Additionally, some studies, such as Hatman et al.,
280   demonstrated that the learning and memory deficits tend to be acute and diminish as early as in
281   8 weeks following experimental ICH in animal models[11]. Hence, these short-term memory effects
282   in animal models might not accurately reflect that long-term memory and cognitive dysfunction
283   that happens after ICH in human subjects.
284
285   This study is not without limitations. A major limitation is the low lumber of animals. This was a
286   pilot study, and future animal studies will contain a greater number of animals to solidify the
287   results observed herein. Another limitation of this study is the inability to adequately monitor
288   the MAPs for the duration of the entire surgery as the femoral artery and the Tuohy Borst system
289   both clot off easily despite the use of low heparinized saline to flush the tubing.
290
291   In conclusion, herein, we report on methods of precisely monitoring ICPs, MAPs, and CPPs in a
292   nontraumatic IVH rodent animal model. Studies such as this one will pave the path towards
293   establishing a more consistent IVH animal model and subsequently more rigorous pre-clinical
294   research. Higher quality pre-clinical research on nontraumatic IVH animal models is critical to
295   elucidate potential therapeutic options for IVH survivors in the future.
296

303   **REFERENCES:**
304   1. Gates, P. C., Barnett, H. J. M., Vinters, H. V., Simonsen, R. L., Siu, K. Primary intraventricular
305   hemorrhage in adults. *Stroke*. **17**, 872–877 (1986).
306   2. Strajle, J., Garton, H. J. L., Maher, C. O., Muraszko, K., Keep, R. F., Xi, G. Mechanisms of
307   hydrocephalus after neonatal and adult intraventricular hemorrhage. *Translational Stroke*
308   *Research*. **3** (Suppl 1), 25–38 (2012).

309   3. Murao, K., Rossi, C., Cordonnier, C. Intracerebral hemorrhage and cognitive decline. *Revue*
310   *Neurologique.* **169,** 772–778 (2013).

311   4. Al-Khindi, T., Macdonald, R. L., Schweizer, T.A. Cognitive and functional outcome after
312   aneurysmal subarachnoid hemorrhage. *Stroke.* **41**, e519–536 (2010).

313   5. Kreiter, K. T. et al. Predictors of cognitive dysfunction after subarachnoid hemorrhage. *Stroke.*
314   **33,** 200–208 (2002).

315   6. Zanaty, M. et al. Intraventricular extension of an aneurysmal subarachnoid hemorrhage is an
316   independent predictor of a worse functional outcome. *Clinical Neurology and Neurosurgery.* **170**,
317   67–72 (2018).

318   7. Gabrielian, L., Willshire, L. W., Helps, S. C., van den Heuvel, C., Mathias, J., Vink, R. Intracranial
319   pressure changes following traumatic brain injury in rats: lack of significant change in the absence
320   of mass lesions or hypoxia. *Journal of Neurotrauma.* **28**, 2103–2111 (2011).

321   8. Kolar, M., Nohejlova, K., Duska, F., Mares, J, Pachl, J. Changes of cortical perfusion in the early
322   phase of subarachnoid bleeding in a rat model and the role of intracranial hypertension.
323   *Physiological Research.* **66**, S545–S551 (2017).

324   9. Ariesen, M. J., Claus, S. P., Rinkel, G. J. E., Algra, A. Risk factors for intracerebral hemorrhage in
325   the general population. A systematic review. *Stroke*. **34,** 2060–2066 (2003).

326   10. MacLellan, C. L., Paquette, R., Colbourne, F. A critical appraisal of experimental intracerebral
327   hemorrhage research. *Journal of Cerebral Blood Flow & Metabolism.* **32**, 612–627 (2012).

328   11. Hartman, R., Lekic, T., Rojas, H., Tang, J., Zhang. J. H. Assessing functional outcomes following
329   intracerebral hemorrhage in rats. *Brain Research.* **1280**, 148–157 (2009).
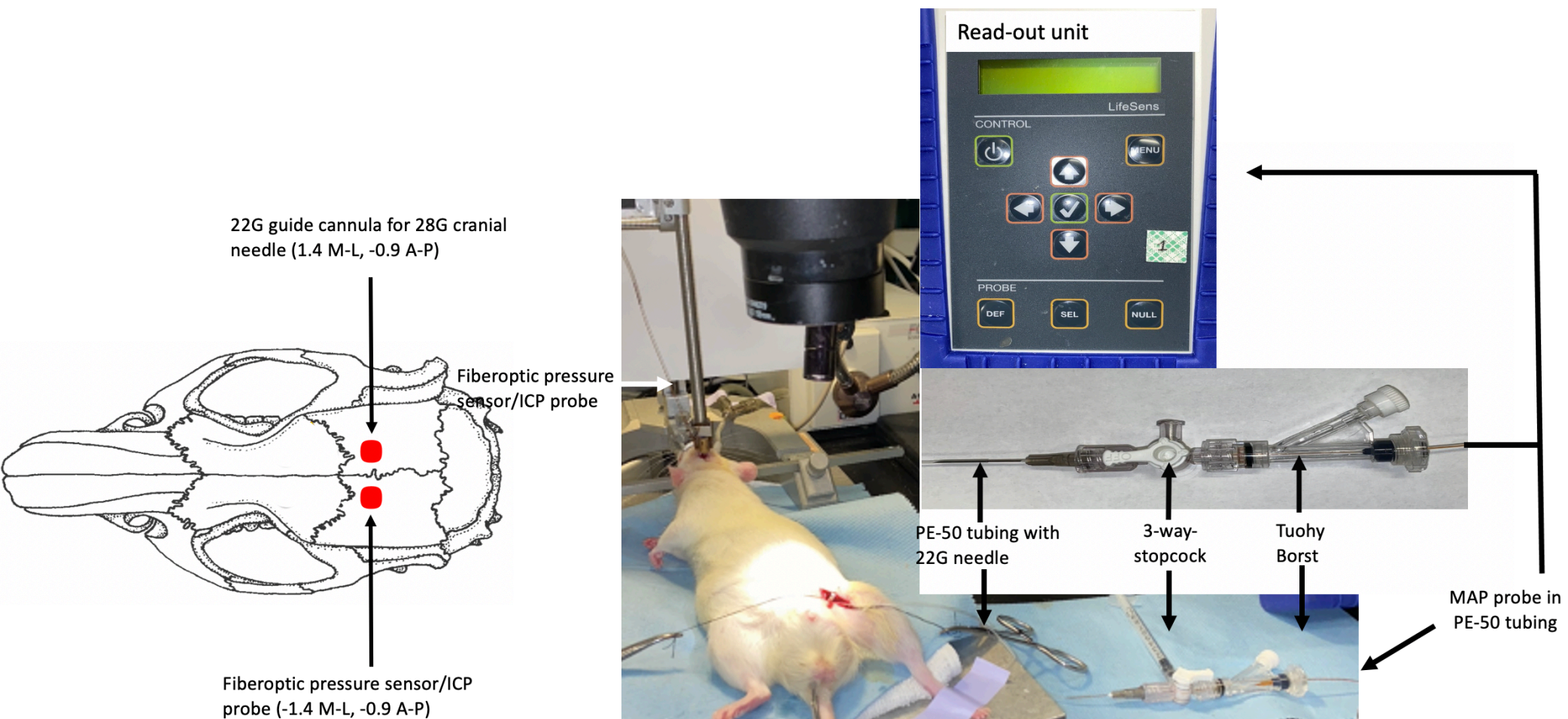
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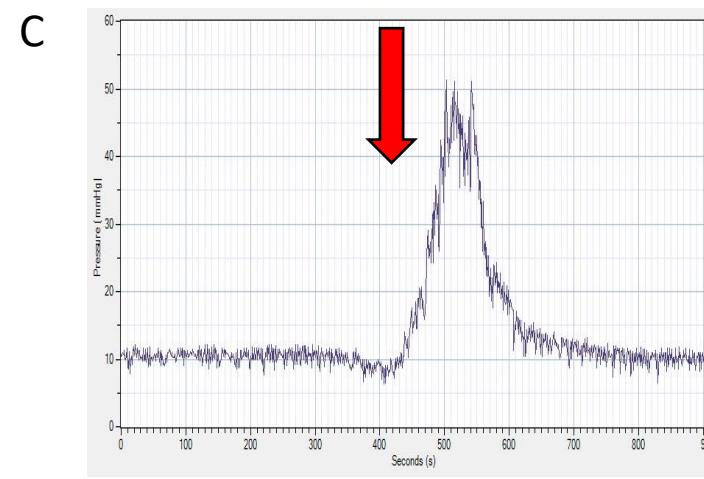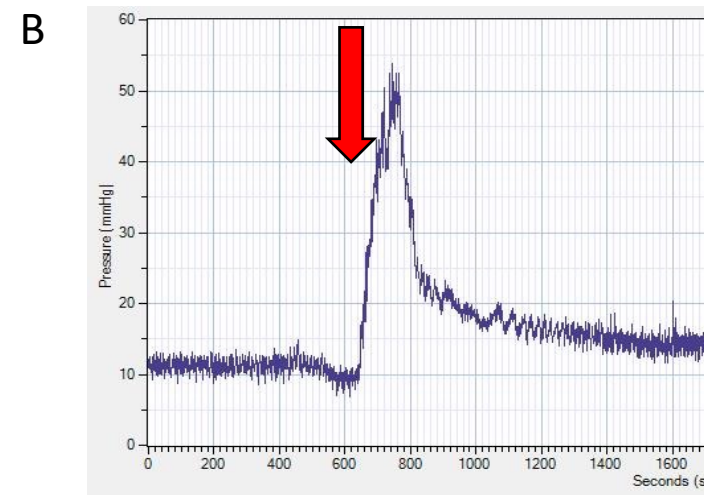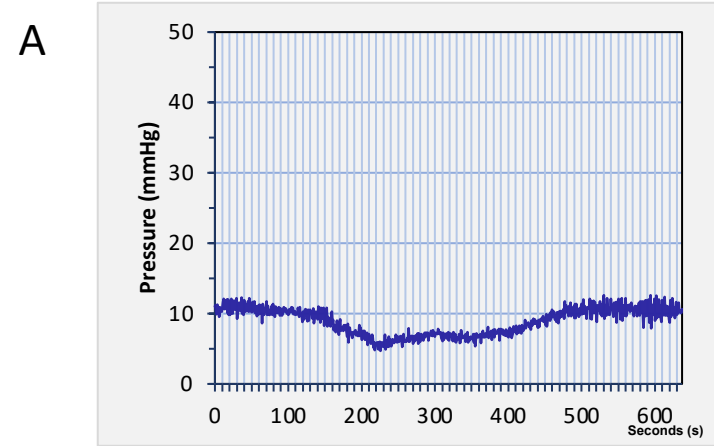Figure 1

Click here to access/download;Figure;Figure 1.pdf ⬇

22G guide cannula for 28G cranial needle (1.4 M-L, -0.9 A-P)

Fiberoptic pressure sensor/ICP probe

Fiberoptic pressure sensor/ICP probe (-1.4 M-L, -0.9 A-P)

Read-out unit

LifeSens

CONTROL

MENU

PROBE

DEF   SEL   NULL

PE-50 tubing with 22G needle

3-way-stopcock

Tuohy Borst

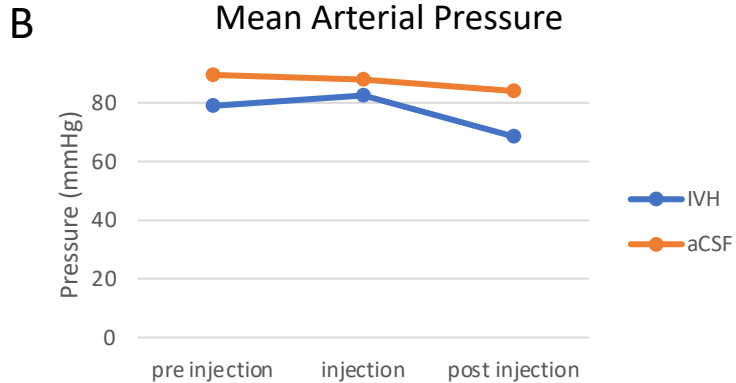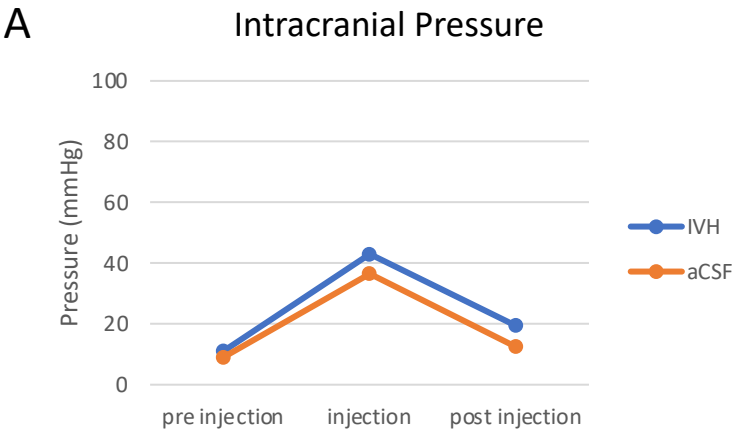MAP probe in PE-50 tubing

Figure 2

Figure 3
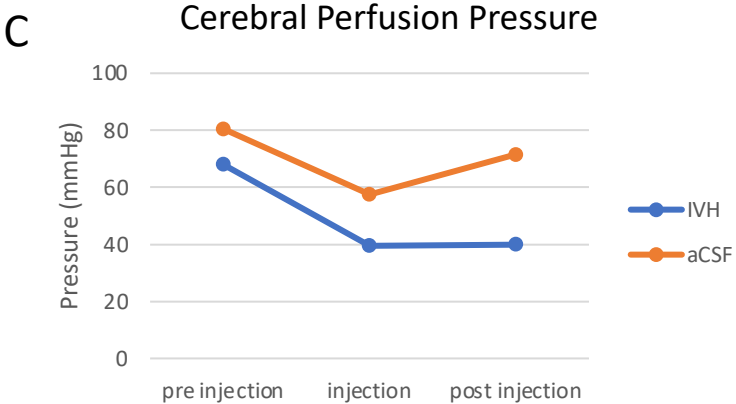
N=1 for all groups

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Table of Materials
Table of Materials-2.xlsx

Rebuttal Letter

Editorial comments:

1. Please note that the manuscript attached has been formatted to fit the journal standard. Some comments to be addressed are included in the manuscript itself. Please review and revise accordingly.

2. Figure 1: Please remove the commercial term "opSens" from the figure. Define M-L, A-P in the figure legends. Replace "gauge" with "G".

3. Please consider including the catalog numbers of the reagents, chemicals, consumables and equipment in the Table of Materials.

Response to the Editor:

1. The supplied manuscript has been reviewed and revised accordingly.

2. Commerical name has been removed and abbreviations have been defined.

3. Catalog numbers have been included when possible.

Reviewer #2 comments:

Manuscript Summary:

The authors bring a method which - despite it´s not new - can be interesting for many researchers. The authors did address all reviewer's comments.

Major Concerns:

The number of animals in each group is low - only 1 per group. On the other hand, the focus of this journal is to visualize the methods; I leave the decision on the Editor whether this is acceptable.

Minor Concerns:

line 57: space is missing between „elucidated.In"

line 135: into is missing („submerging its tip a small")

line 199: recover is missing („Allow rats to fully after surgery")

line 216: weight instead of age („mean age of 495 ⬜⬜17 g")

line 255: MAPs instead of MAPS

Please unify the naming of Lactated Ringer's solution (e.g. lines 136, 137)

Response to Review #2:

The goal of this manuscript was to describe the technique used for measuring ICPs in a nontraumatic IVH rat model. All the minor concerns have been addressed and changed in the manuscript.

Reviewer #4 comments:

I have checked the revised version sent by the authors

I am satisfied that the authors have incorporated all my suggestions

The Editor may now kindly adjudicate for publication pls

Response to Reviewer #4:

Thank you.