



Effects of perfusion volume and delivery technique on rat brain histological quality: a comparison of transcordial perfusion protocols

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Abstract

Transcordial perfusion is widely used to preserve rodents brain tissue. However, standardized protocols regarding parameters such as fixative volume and delivery technique that ensure optimal histological quality have not yet been established. This study aims to evaluate the effects of fixative volume and delivery technique on the histological quality of rats brain tissues. Sixteen male Speague-Dawley rats were allocated to four groups: manual-200 ml, manual-650 ml, pump-200 ml, and pump-650 ml. Transcordial perfusion was performed using specific methods for each group. Brains were processed with Hematoxylin-Eosin staining and photographed. Quantitative assessment of staining intensity in nuclei & cytoplasm was performed using Fiji. Histological artifacts (e.g., blood pooling) were scored. Group differences were analyzed using IBM SPSS Statistics. The staining intensity of nuclei was the highest in the manual-200 mL group (103.31 ± 19.29) and lowest in the manual-650 mL group (156.10 ± 14.94). Welch ANOVA confirmed a statistical difference ($P < 0.001$) between the manual-200 mL and manual-650 mL groups, and between the pump-200 mL and manual-650 mL groups. The staining intensity of cytoplasm showed the same trend. Tukey HSD indicates significance ($P < 0.001$) between the same groups, as well as manual-200 mL and pump-650 mL. Artifcat counts were diescripviely higher in the pump groups, but showed no significant association ($p = 0.362$). A lower volume of 200 mL and manual administration resulted in better histological quality during transcordial perfusion.

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Introduction

In neurological research, the use of animal models is indispensable (1). Animal models enable investigators to observe biomedical mechanisms, disease progression, and pathological trajectories that cannot be observed in clinical studies alone (2). Among these models, rodents, particularly rats, are the most widely used for brain research because of their anatomical, histological, and genetic similarity to the human brain (3).

The accuracy of brain-based research is determined not only by how well the model replicates clinical disease

conditions, but also by how effectively the harvested tissue is preserved to represent its in vivo state (4). Tissue fixation is therefore required to prevent decay by halting autolysis and stabilizing cellular components, thereby allowing microscopic examination in a state approximating that of living tissue. Among available techniques, transcordial perfusion fixation (TP) is widely employed. The method uses the circulatory system to deliver fixative throughout the body of a live animal. This method ensures uniform fixative distribution while simultaneously inducing a controlled and ethically appropriate form of euthanasia (5-7). The procedure involves anesthesia, a thoracotomy to expose the heart, and

vascular cannulation to perfuse the heart with fixative at specific volumes and pressures. Such systemic delivery is particularly advantageous for the brain, a large and densely innervated organ that requires rapid and homogeneous fixative penetration to maintain structural integrity (7).

Transcardial perfusion is time-dependent and operator-sensitive, and its success relies heavily on fixative volume and perfusion pressure. However, despite its long-standing use and numerous available protocols, existing protocols show substantial variability in these parameters, which can affect perfusion pressure (8). Reported fixative volumes range widely from 200 to 650 mL. This may significantly influence tissue penetration and the quality of preservation (7,9). Variations in administration techniques are also observed. Wu et al employed manual administration with a 20-mL syringe fitted with a 25-gauge needle. In contrast, protocols by Gage, Kwan, and Paul utilized automated delivery via peristaltic pumps, which provide more stable perfusion pressures (7-11). However, the availability of tools such as peristaltic pumps can be a problem in resource-limited settings, such as low- and middle-income countries (LMICs) (12). Thus, the need for pumps requires evaluation.

Insufficient fixation volume can lead to uneven fixative distribution, eventually to the degradation of genetic and protein materials. This degradation progresses from nuclear condensation and shrinkage (pyknosis) to atomic fragmentation (karyorrhexis) and ultimately to nuclear dissolution (karyolysis) (13). Conversely, excessive fixative volume may result in excessively high perfusion pressures, damaging tissue morphology, and causing cytoplasmic protein loss. Such consequences are reflected in Hematoxylin-Eosin (H&E) staining, as hematoxylin intensity depends on the preservation of nucleic acids, whereas eosin binds to cytoplasmic proteins (14). Poor fixation, therefore, results in reduced nuclear and cytoplasmic staining intensity, alongside histological artifacts that obscure microscopic interpretation (15-17).

Staining alterations can be quantified objectively using the software ImageJ. The software assesses pixel-level intensity within each chromatic channel following H&E color deconvolution. It computes area, mean gray value, and integrated density as quantitative indicators of fixation and staining quality (18).

Although transcardial perfusion is considered a standard procedure, no study has evaluated the effects of fixative volume and perfusion technique on H&E staining quality (8). This study aims to assess the impact of different perfusion volumes and administration techniques on the histological quality of rat brain tissue, assessing morphological preservation, staining intensity, and the minimization of microscopic artifacts.

Materials and methods

Animals and Housing Conditions

All experimental procedures were approved by the Research Ethics Committee of Universitas Pembangunan Nasional “Veteran” Jakarta (Approval No: 316/XII/2025/KEP) and conducted in accordance with the principles of Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 guidelines (see appendix 1a and 1b) as well as the principles of Replacement, Reduction, and Refinement (3Rs) to ensure transparent reporting and ethical treatment of animal subjects (19).

Due to the invasive nature of transcardial perfusion, which requires vascular cannulation and controlled euthanasia, animal models are used. Various species have been used in neurological studies, including primates, sheep, pigs, and rodents. Non-human primates show the most remarkable neuroanatomical similarity to humans, but raise significant ethical issues, high costs, and access restrictions (4,20). Rats, on the other hand, offer advantages in terms of similarities and cost-effectiveness. Therefore, Sprague-Dawley mice were selected due to their well-defined neuroanatomy, tame behavior, and widespread use in neurological research (2).

Sixteen healthy male Sprague-Dawley rats (200–350 g) were included. Animals presenting with traumatic injury, clinical evidence of infection, or systemic illness before the procedure were excluded. Male rats were used to minimize the biological variability associated with fluctuations in hormones related to the estrous cycle in female rats, which can influence neuronal morphology (21–23). The rats were housed in cages approximately 35 cm in length, 32 cm in width, and 25 cm in height (3 rats per cage) under controlled environmental conditions. Standard polypropylene cages used wood-shaving bedding, with a controlled room temperature ranging from 22 to 24 degrees Celsius. Room temperature was maintained by continuous operation of the air-conditioning system. During the 7-day acclimatization period, rats had *ad libitum* access to food and water. Sixteen male Sprague-Dawley rats were directly acquired from the Indonesian FDA Laboratory Services (INFALABS).

The Animal Transfer Form was issued by the Biological Product Testing Center of the Indonesian Food and Drug Authority (BPOM RI) (No. 048/LHP/IX/2024).

Experimental design and group allocation

A 2×2 factorial experimental design was employed to investigate the effects of perfusate volume (200 mL vs 650 mL) and perfusion technique (manual vs peristaltic pump) on histological outcomes. The sample size was calculated using the Resource Equation Approach (24). Rats were randomly assigned to four groups (n = 4 per group): manual perfusion with 200 mL (manual-200mL), manual perfusion with 650

mL (manual-650mL), pump perfusion with 200 mL (pump-200mL), and pump perfusion with 650 mL (pump-650mL).

Transcardial perfusion procedure

Before perfusion, the rats were administered with an overdose of ketamine (80 mg/kg; Ket-A-100®) and xylazine (10 mg/kg; Xyla®) to induce deep terminal anesthesia. After complete deep anaesthesia is achieved (as evidenced by no *toe-pinch response*), the operators perform transcardial perfusion. Thoracotomy is done by making a 5 to 6 cm incision laterally in the thorax, followed by incising the diaphragm and along the ribs. After the heart was exposed, a 21-gauge intravenous cannula (Terumo®, Terumo Corporation, Japan) was placed into the left ventricle. A 20 ml syringe (Terumo®, Terumo Corporation, Japan) was used for manual perfusion, and the operator controlled the flow to administer either 200 mL or 650 mL of fixative. Meanwhile, a peristaltic pump (INTLLAB®, China) with volumes of 200 mL or 650 mL and a steady flow rate of approximately 8–10 mL/min was used for pump perfusion. The pump was connected to the cannula with a disposable infusion set (Onemed®, Indonesia). After perfusion began, the right atrium was cut to allow effluent to exit. Then, brain extraction is performed by incising the skin covering the skull, and the skull is opened by creating a midline sagittal cut along the sagittal suture. The cranial bones were separated and removed using forceps. Once the cranial cavity was fully exposed, the brain was gently freed from the skull by severing the cranial nerves and the spinal cord at the level of the foramen magnum. A 24-hour post-fixation in 4% paraformaldehyde (PFA) in PBS (TissuePro Technology®, USA) (7,9–11). Remaining animal carcasses were categorised as biological waste, placed in sealed containers, and temporarily stored in a freezer facility at -20°C. In compliance with institutional biosafety and ethical guidelines, the institution's biological waste management unit carried out the final disposal (Figure 1).

Tissue processing and hematoxylin-eosin staining

Brains that had been fixed underwent standard tissue processing, were oriented in the coronal plane, and were trimmed to obtain representative sections of the cerebral hemispheres. Gross sectioning was performed using a scalpel blade to produce coronal brain slices of approximately 3mm thickness. The selected brain slices were then processed through a standard dehydration series. Paraffin blocks were sectioned using a rotary microtome to obtain serial sections with a thickness of 3 µm. The sections were mounted onto glass slides, dried, and subsequently stained with hematoxylin and eosin (H&E) in the Anatomical Pathology Laboratory, Dr Cipto Mangunkusumo National General Hospital (RSCM), Jakarta, Indonesia, for histological evaluation.

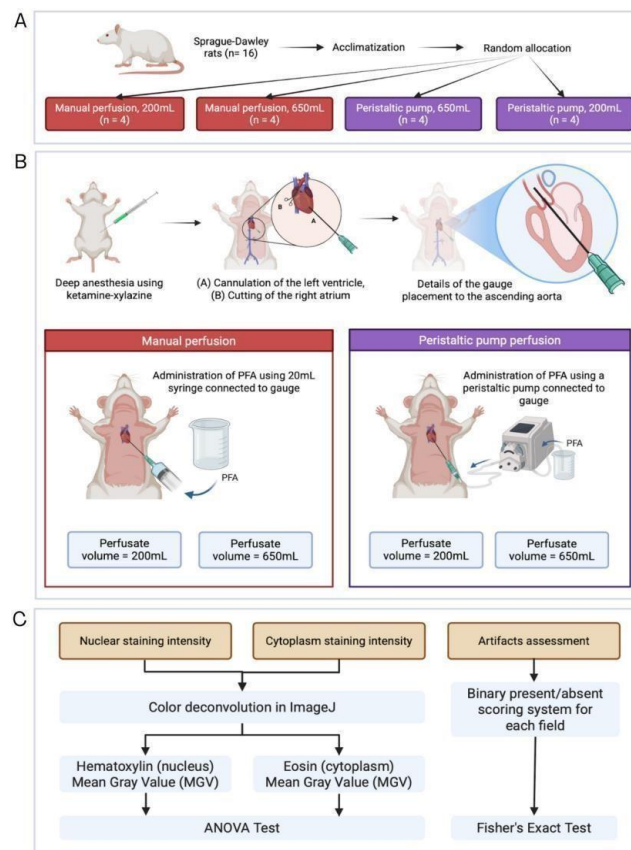


Figure 1: Detailed schematic of the experiment workflow. (A) Group Allocation of Subjects. Sixteen Sprague-Dawley rats are acclimatised under controlled environmental conditions, then are randomly assigned into four groups ($n = 4$ each) based on perfusion method (manual or peristaltic pump) and perfusate volume (200 mL or 650 mL). (B) Detailed schematic of the transcardial perfusion protocol. The procedure starts with the induction of deep anaesthesia with ketamine-xylazine, cannulation of the left ventricle with a gauge directed toward the ascending aorta, incision of the right atrium for outflow, and delivery of paraformaldehyde fixative using either manual syringe perfusion or a peristaltic pump. Two perfusate volumes (200 mL and 650 mL) were employed to assess the combined effects of perfusion method and volume on tissue fixation quality. (C) Workflow of histological analysis. Histological evaluation consisted of a quantitative assessment of nuclear and cytoplasmic staining intensity using colour deconvolution in ImageJ to separate the hematoxylin and eosin channels, followed by measurement of the Mean Gray Value (MGV) for each component. Then, the artifacts were scored in each field. The artefact scores are analysed using Fisher's exact test. The illustrations were created using BioRender (<https://BioRender.com>).

Quantitative histological assessment

A Moticam 2.0 MP digital camera (Motic Instruments Inc., Xiamen, China) mounted on a light microscope was used to capture all micrographs, with the same exposure, illumination, and white balance settings maintained for each sample. Five randomly selected, nonoverlapping fields from the cerebral cortex with large pyramidal neurons were examined at 40x and 100x total magnification for each specimen.

ImageJ/Fiji (National Institutes of Health, USA), an open-source software freely available from the official Fiji distribution, was used to quantify nuclear and cytoplasmic staining intensities. Hematoxylin and eosin channels were separated using the Colour Deconvolution plugin. To ensure uniformity across samples, standardised square regions of interest (ROI; 8 x 8 pixels) were applied consistently to both nuclei and cytoplasm across all samples. The Mean Grey Value (MGV) was calculated for each ROI using the Measure plugin, with lower values indicating more intense staining (18,25,26). Staining intensity was quantified by calculating MGV, which ranges from 0 to 255 in 8-bit grayscale images. A lower MGV corresponds to higher staining intensity (darker staining), whereas a higher MGV indicates weaker staining (lighter staining). This approach allows objective and reproducible quantification of both nuclear (hematoxylin) and cytoplasmic (eosin) staining. For each field, a binary present/absent scoring system was used to assess the artifacts (blood pooling, incomplete vascular clearance, tearing, and tissue swelling), as histological artifacts represent discrete qualitative events (18).

Statistical Analysis

All the statistical analyses were done using the application Statistical Package for the Social Sciences (SPSS), with version 29.0 (SPSS v29.0, IBM Corp., United States of America). The staining intensities of the nucleus and cytoplasm are analysed using Analysis of Variance (ANOVA) or Welch ANOVA, depending on the assumptions of normality and homogeneity of variance. Then, the appropriate post hoc test, such as Tukey HSD or Games-Howell, is used to identify statistical differences among the 4 groups. For the artefact dichotomous data, Fisher's Exact test was used to evaluate the significance of the difference between the groups. The results of all data were then visualised using the GraphPad Prism Version 10.6.1 (GraphPad Software, San Diego, CA, USA).

Results

Procedural observation during the transcatheter perfusion

During manual perfusion, continuous operator involvement was required to maintain flow consistency. Minor adjustments of needle position were frequently necessary to ensure stable vascular access to the rat's aorta.

In contrast, pump-assisted perfusion provided a more stable, continuous delivery of fixative, with minimal need for real-time adjustment once the system was set correctly.

Observable physiological responses differed between perfusion volumes. In the 650 mL groups, macroscopic signs of tissue overperfusion, including generalised swelling and increased tissue rigidity, were observed during the later perfusion phase. These changes were less apparent in the 200 mL groups, in which perfusion was completed more quickly. Transient involuntary movements were occasionally observed during the early phase of perfusion, corresponding to residual spinal reflex activity prior to complete circulatory arrest. No prolonged motor activity was noted following completion of perfusion. All animals reached irreversible circulatory cessation during the procedure.

Macroscopic and microscopic view of brain tissue

The brains in all groups were well-preserved, with no signs of damage or haemorrhage. The brains were then processed for Hematoxylin & Eosin staining and showed standard cortical architecture, including pyramidal neurons and surrounding glia (Figure 2).

Staining intensity of nuclei

The staining intensity of nuclei was quantitatively assessed using Mean Gray Value (MGV) analysis with ImageJ (National Institutes of Health, USA). With the ImageJ application, color deconvolution was applied using the "Color Deconvolution" plugin with the H&E vector to separate hematoxylin and eosin staining components. The hematoxylin channel was used for nuclear analysis. Regions of interest (ROIs) of uniform size (8 × 8 pixels) were manually selected using the selection tools, and staining intensity was measured using the "Measure" function under the Analyze menu (Figure 2A). Quantitative assessment of hematoxylin staining demonstrated distinctions among the groups. The 200 ml manual group (Group 1) exhibited the lowest mean grey value (MGV) of 103.31 ± 19.29 , reflecting the most pronounced staining of cell nuclei. This was succeeded by the 200 ml pump group (Group 3) (115.31 ± 23.21) and the 650 ml pump group (Group 4) (136.18 ± 24.17). The least intense staining (maximum MGV) was noted in the 650 ml manual group (Group 2) (156.10 ± 14.94) (Table 1). The Shapiro-Wilk test indicates that the data are approximately normally distributed. The Levene test, on the other hand, shows that the groups have different variances. Therefore, a Welch ANOVA was performed, which revealed a statistically significant overall effect of the groups ($***P \leq 0.001$). A post hoc comparison using the Games-Howell method showed significant differences between the manual-200 ml and manual-650 ml groups, and between the manual-200 mL and pump-650 ml groups. These results show that both the perfusion technique and the volume significantly influence the effectiveness of nuclear staining.

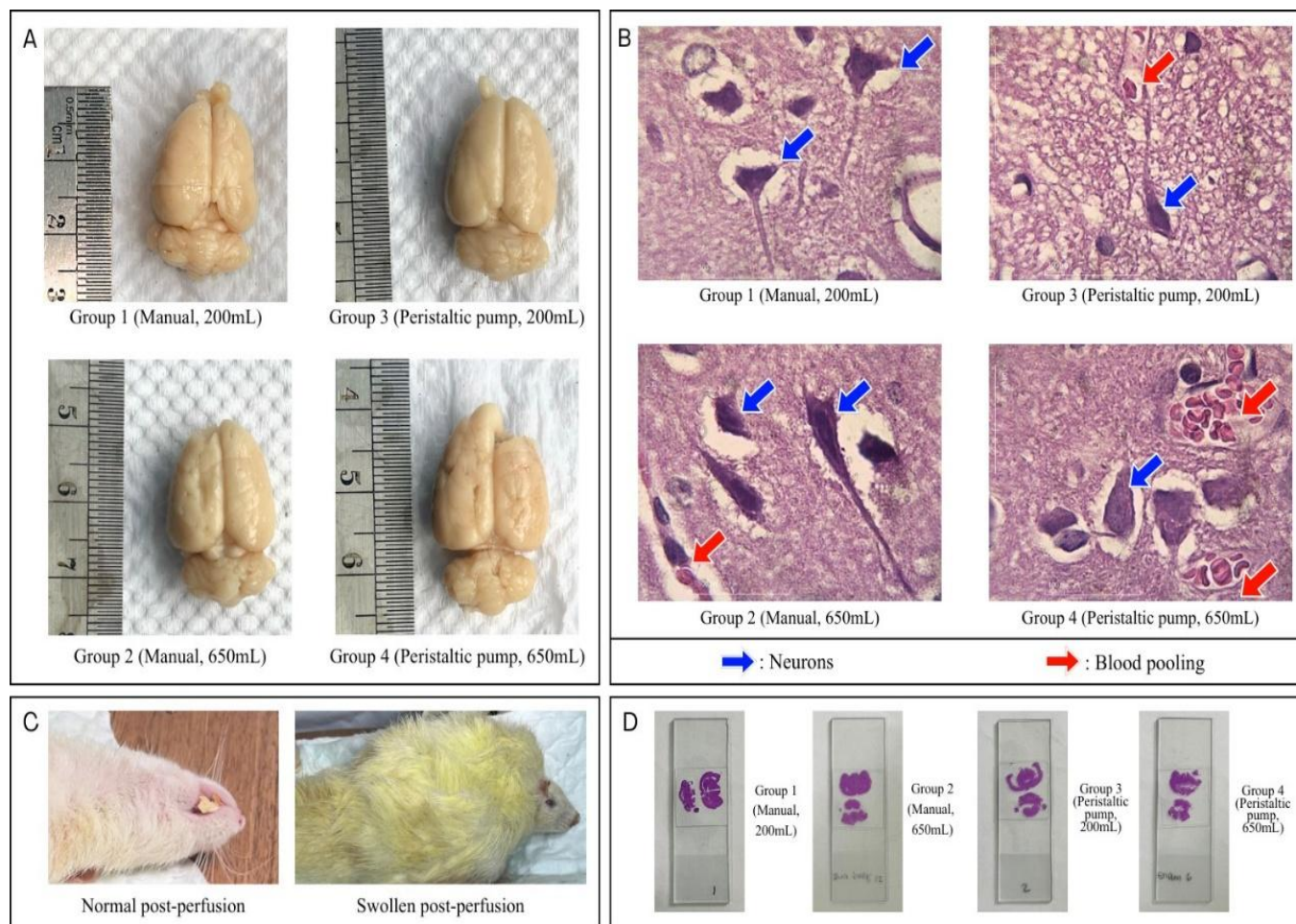


Figure 2: Macroscopic and microscopic views of brain tissue. (A) The macroscopic view of the brain of a Sprague-Dawley rat after transcardial perfusion shows the general morphology and integrity of the tissue. The scale is given in centimetres (cm). The morphology of the brains was well preserved in all groups, with no signs of structural distortion, haemorrhage, or tissue collapse. The surface of the cortex remained intact and showed no congestion. (B) Microscopic examination of brain tissue stained with hematoxylin and eosin (HE) revealed neuronal morphology within the brain parenchyma. The sections demonstrated preserved cortical architecture across all samples, with clearly identifiable neurons. Histological artifacts were observed in the manual-650 ml, pump-200 ml, and pump-650 ml groups, whereas no artifacts were detected in the manual-200 ml group. Images were obtained at 100x magnification with a scale bar of 100 μ m. representative areas of the cerebral cortex (grey matter) showed well-defined pyramidal neurons. The regions used for staining intensity measurement are indicated, including representative ROI applied to both nuclei and cytoplasm. (C) Standard view of the rat after perfusion versus a swollen rat after the manual-650 mL perfusion, possibly indicating excessive volume and rigidity. (D) Macroscopic view of histological slides from the four groups.

Staining intensity of cytoplasm

The staining intensity of nuclei was quantitatively assessed using Mean Gray Value (MGV) analysis with ImageJ (National Institutes of Health, USA). After the color deconvolution, the eosin channel was used for cytoplasmic analysis. Regions of interest (ROIs) of uniform size (8×8 pixels) were manually selected using the selection tools, and staining intensity was measured using the “Measure”

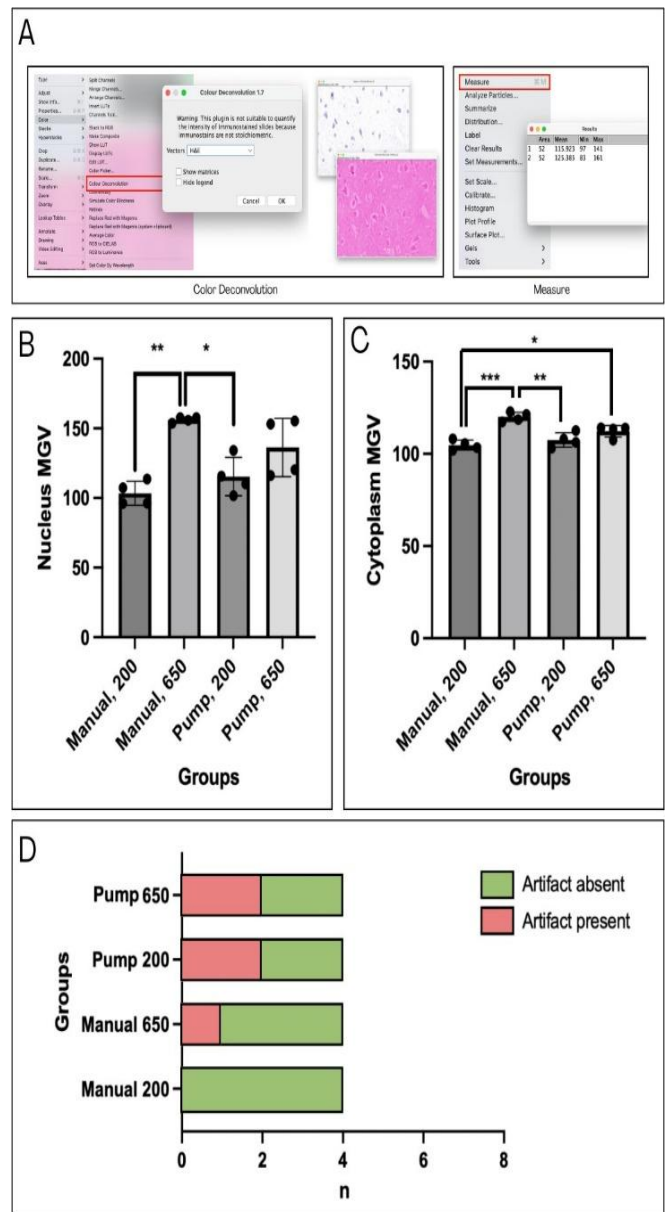
function under the Analyze menu (Figure 3). The staining power of eosin follows a similar pattern. The manual 200 ml group showed the most pronounced staining in the cytoplasm (104.72 ± 17.85), followed by the 200 ml pump group (107.46 ± 14.32) and the 650 ml pump group (112.20 ± 19.94). The group with 650 ml of manual technique (120.01 ± 18.13) showed the least color change.

Table 1: Quantitative analysis of staining intensity and histological artifacts across perfusion groups

Groups	N	Nuclear Mean Gray Value ($\bar{x} \pm SD$)	Cytoplasm Mean Gray Value ($\bar{x} \pm SD$)	Amounts of artifacts (n)
Manual perfusion, 200 mL	4	103.31 $\pm$ 19.29	104.72 $\pm$ 17.85	0
Manual perfusion, 650 mL	4	156.10 $\pm$ 14.94	120.01 $\pm$ 18.13	1
Peristaltic pump, 200 mL	4	115.31 $\pm$ 23.21	107.46 $\pm$ 14.32	2
Peristaltic pump, 650 mL	4	136.18 $\pm$ 24.17	112.20 $\pm$ 19.94	2
<i>p-value</i>		< 0.001 ^{a***}	<0.001 ^{b***}	0.362 ^c

Values are presented as mean $\pm$ SD. Mean comparisons were performed using Welch ANOVA^a or one-way ANOVA^b for continuous variables and Fisher's exact test^c for categorical variables. Statistically significant variables were denoted as * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$. Statistically insignificant variables were denoted as (').

Figure 2: Extraction of mean gray value and bar plot of the variables. (A) The use of ImageJ to extract the mean gray value of nucleus and cytoplasm with a fixed region of interest. Digital images of hematoxylin and eosin (H&E)-stained sections were captured using the Moticam 2.0 MP digital camera (Motic Instruments Inc., Xiamen, China) mounted on a light microscope. Image preprocessing was performed by converting images to 8-bit grayscale format using the "Type" function. Color deconvolution was applied using the "Color Deconvolution" plugin with the H&E vector to separate hematoxylin and eosin staining components. The hematoxylin channel was used for nuclear analysis, while the eosin channel was used for cytoplasmic analysis. Regions of interest (ROIs) of fixed size (8 x 8 pixels) were selected using the selection tool, and the staining intensity was measured using the "Measure" function under the Analyze menu. The MGv values ranged from 0 to 255, where lower values indicate stronger staining intensity. Measurements were performed in multiple non-overlapping fields, and the mean value was calculated for analysis. (B) Bar chart showing nuclear mean gray value (MGv) across experimental groups. Data are presented as mean $\pm$ standard deviation with individual data points. Due to unequal variances, Welch's ANOVA was applied, followed by Games-Howell post hoc analysis. Significant differences were observed between the manual 200 mL and manual 650 mL groups, as well as between the manual 200 mL and pump 650 mL groups. (C) Bar chart showing cytoplasmic mean gray value (MGv) across the same groups. Data are presented as mean $\pm$ standard deviation with individual data points. One-way ANOVA followed by Tukey's HSD post hoc test revealed significant differences between the manual 200 mL and manual 650 mL groups, as well as between the manual 200 mL and pump 650 mL groups. (D) Stacked bar chart illustrating the distribution of histological artifacts across groups. Artifact presence was recorded in binary form and analyzed using Fisher's exact test, which showed no statistically significant differences between groups. The degrees of significance are shown by * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.



The data were normally distributed. Therefore, a one-way ANOVA was performed, revealing a significant effect of group ($p \leq 0.05$). Tukey's HSD post hoc analysis demonstrated significant differences between the manual-200 ml group and the manual-650 ml group, the manual-200 ml group and the pump-200 ml group, and the manual-200 ml group and the pump-650 ml group. These findings indicate that cytoplasmic staining intensity is significantly influenced by variations in perfusion method and perfusion volume. The manual-200 ml group exhibited the lowest mean gray value, indicating the strongest cytoplasmic staining intensity among all groups. Similar to nuclear analysis, cytoplasmic staining intensity was interpreted inversely to MGV values, with lower values indicating stronger eosin staining.

Artifacts

The number of artifacts is relatively low in all groups. The number of artifacts is higher in the pump-perfused tissues than in the manual group. The pump-200 ml and pump-650 ml groups each had 2 artifacts, whereas the manual-650 mL group had 1, and the manual-200 ml group had none. Nonetheless, Fisher's Exact Test indicated that these discrepancies were not statistically significant ($p = 0.362$). As a result, the perfusion technique and volume did not significantly affect artefact creation within this sample size.

Discussion

This study demonstrated that modifications in the perfusion technique and the volume of the perfusate significantly influenced the intensity of nuclear and cytoplasmic staining in rat brain tissue, indicated by the staining intensity of both the nucleus and the cytoplasm, as well as artifact count. We use the mean gray value (MGV) from image analysis to objectively measure this effect. The image can convert histological images into 8-bit grayscale images with values ranging from 0 to 255, where 0 is the darkest and 255 is the lightest color. This suggests an inverse relationship between MGV and staining intensity. Staining adheres more strongly when the MG value is lower and more weakly when the MG value is higher. This quantitative approach reduces subjectivity and provides an objective framework for evaluating fixation quality under different perfusion conditions (18,25,26).

The intensity of nuclear staining differs significantly between groups, suggesting that both the perfusion technique and the volume influence hematoxylin binding to chromatin. Hematoxylin interacts with basophilic components such as DNA and chromatin, and the staining effectiveness is strongly influenced by the speed and uniformity of fixative penetration and cross-linking throughout the tissue (27). Meanwhile, eosin binds to structural proteins (actin

filaments, myosin, and other cytoskeletal components). The staining power is strongly influenced by how tightly these proteins bind and by how well the cells balance their osmotic pressure (14,16). Rapid fixation is essential for preserving cell integrity and inhibiting premature autolysis, thereby maintaining tissue affinity for the dye. Tissues with darker, more homogeneous nuclei and cytoplasm take fixatives better. On the other hand, lighter nuclei and cytoplasm may indicate that fixation was performed too late or unevenly, that the chromatin broke down too quickly, or that the staining binding sites were less accessible (28).

The perfusate volume strongly affected staining intensity in both the nucleus and the cytoplasm. The present study indicates that the group administered 200 mL showed greater staining intensity than the group receiving 650 mL, suggesting that a reduced volume produces higher staining quality. This impact is due to the ratio of tissue to fixative volume, which is an important factor in specimen fixation. Most of the time, a 10:1 ratio is best (29). It is thought that adult rats have a total circulating blood volume of about 64 mL/kg of body weight (30). Assuming a circulating blood volume of roughly 60–70 mL per kilogram, this corresponds to approximately 15–24 mL of intravascular volume. Applying the conventional tissue-to-fixative ratio of 10:1 suggests a volume of 150–240 mL of fixative is required. These results support the idea that larger perfusion volumes are not always needed, as good fixation can be achieved once vascular flushing is complete. This notion is consistent with our finding that a volume of 200 ml yielded improved histological results compared to much larger volumes.

Furthermore, larger amounts of fixative can prolong perfusion duration, which is known to affect fixation quality; prolonged fixation time can lead to over-crosslinking (31). The manually perfused rats with 650 mL volume also show a swollen appearance (Figure 2), possibly indicating the use of too much volume. The manual-650mL groups also had the longest duration, up to 30 minutes per rat. In line with this, the group showed worse nuclear and cytoplasmic staining, indicating that excess volume leads to lower staining quality. Whereas the 200mL groups in both manual and pump perfusion show better quality, with shorter times up to 10 minutes per rat.

Peristaltic pumps are designed to maintain a stable flow, but their pressure cycles are repetitive and unchanging, which can lead to shear stress in capillaries. This makes the endothelium more porous and triggers interstitial edema (32,33). These

microstructural changes can hinder the penetration of the fixative, reduce chromatin density, and impair hematoxylin binding. In addition, increased perfusion pressure can lead to chromatin dilution and inadequate nuclear staining, which favors this process (31,34,35).

On the other hand, manual perfusion allows for more natural and adaptive pressure control, with pressure controlled in real time by the operator. This is consistent with the results of this study, which show that manually perfused tissue yields better staining quality. However, despite its histological advantages and resources, manual perfusion still depends on the availability of experienced operators. In the present study, two operators were involved, and perfusion was performed simultaneously. The operators must have knowledge and skills in performing transcatheter perfusion and in handling animals. Therefore, the availability of operators is a decisive factor in choosing a perfusion technique. In addition, manual perfusion requires a long operating time (depending on the volume used, this can take about 10 to 30 minutes per sample), which can be inefficient for larger samples. Operator safety must also be considered, as sustained manual syringe compression involves repetitive forceful exertion and static muscle loading of the hand and forearm, conditions that have been shown to contribute to muscle fatigue and increased risk of musculoskeletal strain during prolonged manual tasks (36,37). These ergonomic considerations are relevant in studies that require repeated perfusion procedures or larger sample sizes.

On the other hand, the use of peristaltic pumps reduces the time and energy required in this study. These pumps offer practical advantages for larger sample volumes. However, this study showed that the artifacts in the group using pumps were higher on average. It should also be noted that the artifacts found in this study were perfusion artifacts (e.g., blood accumulation) and not fixation artifacts (e.g., pyknosis, karyolysis). This shows that, despite the greater number of foreign bodies in the pump group, the use of peristaltic pumps does not result in inadequate fixation. Therefore, pump perfusion can be used for efficiency reasons when artifacts from foreign bodies are negligible and when operator availability is limited. However, the use of a peristaltic pump also requires strict monitoring, as adverse events may occur during the procedure (e.g., displacement of the catheter from the aorta). Therefore, the use of a pump does not eliminate the need for personnel.

Artifacts are structural changes that do not fully reflect the tissue's original state (15). These changes can occur at any stage of the fixation or perfusion process. The most common signs include cell shrinkage or swelling, vacuolization, nuclear distortion, loss of membrane integrity, and blood clotting. Poor fixation can also lead to autolysis, making it challenging to preserve cell shape. These changes make it challenging to interpret histology and can alter the pathology or make it appear different, leading to a less accurate diagnosis (17,38).

The most common artifacts are blood accumulations or pools, indicating that cleansing of the blood vessels is not yet complete. This tendency is particularly noticeable in the pump-perfused group, but not in the manual-perfused group.

Although these abnormalities could indicate insufficient perfusion dynamics, they are not consistent with autolysis of the cell nucleus. The absence of pyknosis, karyorrhexis, and karyolysis in all groups suggests that the fixative injection was performed quickly enough to prevent enzymatic degradation, indicating adequate fixation. The brain's significant susceptibility to postmortem autolysis underscores the adequacy of overall perfusion in all treatments (6,10). The evaluation of artifacts covers five areas per slide to ensure representativeness across the entire tissue area. The specimens were classified as "artifact present" or "artifact not present," a methodology consistent with established histotechnological criteria for assessing fixation quality. The corresponding Fisher test revealed no statistically significant difference between the pump and manual perfusion groups, although a trend toward a difference was observed ($p = 0.362$). The small sample size made it harder to detect differences between the groups using statistical tests. As a result, even if there were some artifact tendencies between the groups, these results do not show a cause-and-effect relationship at this time.

These results are significant for histology laboratories, especially in low- and middle-income countries (LMICs). In these situations, restricted resources affect the availability of equipment (12). In this study, the 4% paraformaldehyde (PFA) solution was prepared in the laboratory according to standard protocols (39). The estimated material cost for laboratory-prepared PFA was approximately USD 1.20 for 200 mL and USD 3.89 for 650 mL (PBS). In comparison, commercially available 4% PFA solutions are priced at approximately USD 25–38 per 250 mL, resulting in a substantially higher cost when larger volumes are required. When calculated using market price, a perfusion volume of 650 mL would be disproportionately more expensive, particularly for studies involving larger sample sizes. Importantly, this increased cost is not accompanied by improvements in histological staining quality, as higher perfusion volumes were associated with inferior staining outcomes in the present study. Therefore, the use of excessive perfusion volumes offers no practical or economic benefit, regardless of whether laboratory-prepared or commercially sourced fixatives are used. Not only in terms of volume, but international standards are also increasingly promoting the use of peristaltic pumps to achieve regulated, uniform perfusion (8). However, existing statistics show that using a pump does not necessarily improve fixation. On the other hand, manual perfusion produces better staining of the nucleus and cytoplasm. This suggests that the physiological adaptability that comes with manually controlled pressure may be better than the consistent, rhythmic output of mechanical pumps.

Despite its histological and resource advantages, manual perfusion still depends on the availability of an experienced operator. The operator must be familiar and skilled in

transcardial perfusion and animal handling. Thus, the availability of human resources is a critical consideration when choosing a perfusion technique. In addition, manual perfusion requires a long operation time (approximately 15-30 minutes per sample, depending on the volume used), which can be ineffective for larger samples. The operator's safety must also be considered, as manual perfusion requires significant energy to push the syringe for extended periods, which could strain the operator's muscles. In addition, applying high pressure to deliver large volumes of fixative through a narrow cannula increases the risk of fluid splashing, potentially exposing the operator to chemical hazards. On the other hand, the use of a peristaltic pump significantly lessens the burden of time and energy. It offers great practicality in conditions of a higher sample size. However, it is apparent in this study that the artifacts are descriptively higher in the pump groups. It is also important to note that the artifacts found in the study are perfusion artifacts (e.g., blood pooling), not fixation artifacts (e.g., pyknosis, karyolysis). This indicates that, although a greater number of foreign bodies are found in the pump groups, the use of a peristaltic pump does not result in inadequate fixation. Thus, pump perfusion can be used for effectiveness reasons if foreign body artifacts can be ignored. However, peristaltic pumps also require close monitoring, as adverse events may occur during the process (e.g., displacement of the cannula from the aorta). Therefore, the use of pumps does not ultimately erase the need for human resources. However, pump perfusion still offers benefits, as it facilitates the operator's workflow and ensures a consistent process for all samples. This study highlights the importance of controlling pressure and understanding how the pump affects it, as excessively high or low pressure can compromise fixation quality.

This study shows how important it is to control the pressure and understand how the pump works. Furthermore, the adverse effects on quality when using excessive volume, especially with a pump, demonstrate that a larger volume does not always lead to better results. Optimizing perfusion volume to minimize reagent consumption while ensuring effective staining results can significantly reduce operating costs, especially in resource-limited settings (12). Overall, these results show that laboratories should allocate resources to improve the quality of tissue visible during examination and to simplify decision-making regarding perfusion techniques (pump vs. no pump) and volumes, based on data.

This study still has some limitations, as it is an exploratory study evaluating the effects of perfusion method and volume on the histological quality of brain tissue. Essentially, the volumes of 200 ml and 650 ml reflect only two extreme values in the protocol. In future studies, intermediate volumes could be tested to determine the optimal parameters. Second, the limited sample size may

reduce statistical significance, as seen in the results for the number of artifacts, where differences are only visible descriptively and cannot be statistically proven. Third, manual perfusion is highly dependent on the user. This study faced some effectiveness challenges, as only two perfusionists with sufficient experience were available. Future studies could focus on a broader volume range, a larger sample size, and different parameters, such as fixation artifacts, to further validate this issue. Despite these limitations, this study's results provide valuable initial insights into the factors that determine histological quality in transcardial perfusion.

Conclusion

This study demonstrates the influence of volume and perfusion technique on histological quality in rat brains. Manual perfusion with 200 mL yielded the best histological quality, with better staining intensity of the cell nucleus and cytoplasm, and the fewest artifacts. In contrast, the 650 mL pump group showed the poorest histological quality. Statistical analysis shows that the staining intensity of the cell nucleus and cytoplasm is strongly influenced by perfusion volume, with a higher volume (650 mL) resulting in lower staining quality. There was no significant difference in artifact frequency between the groups.

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Conflict of interest

There is no conflict of interest.

Data Acknowledgment

The dataset supporting the findings of this study is available in Zenodo under the Creative Commons Attribution 4.0 International License (CC by 4.0): <https://doi.org/105281/zenodo.18297949>.

Authors Contributors

The authors' contributions were carried out in a structured and collaborative manner. D.P. and F.A. were responsible for the conceptualization and overall supervision of the study. The methodology was developed by D.P., F.A., and M.A. The investigation was conducted by D.P., F.A., M.A., D.L., E.P., and G.R. Data curation was performed by D.P., F.A., and G.R., who also contributed to formal analysis and data interpretation. The manuscript was written by D.P. and F.A. All authors have read and approved the final version of the manuscript.

References

1. Abed MA. A comprehensive examination of human brain disorders. *J Biomed Sustain Healthc Appl*. 2023;141:141–152. DOI: [10.53759/0088/jbsha202303014](https://doi.org/10.53759/0088/jbsha202303014).
2. Neziri S, Köseoglu AE, Deniz Köseoglu G, Özgültekin B, Özgentürk NÖ. Animal models in neuroscience with alternative approaches: evolutionary, biomedical, and ethical perspectives. *Anim Model Exp Med*. 2024;7(6):868–880. DOI: [10.1002/ame2.12487](https://doi.org/10.1002/ame2.12487).
3. Breschi A, Gingeras TR, Guigó R. Comparative transcriptomics in human and mouse. *Nat Rev Genet*. 2017;18(7):425–440. DOI: [10.1038/nrg.2017.19](https://doi.org/10.1038/nrg.2017.19).
4. Domínguez-Oliva A, Hernández-Ávalos I, Martínez-Burnes J, Olmos-Hernández A, Verduzco-Mendoza A, Mota-Rojas D. The importance of animal models in biomedical research: current insights and applications. *Animals*. 2023;13(7):1223. DOI: [10.3390/ani13071223](https://doi.org/10.3390/ani13071223).
5. Kasukurthi R, Brenner MJ, Moore AM, Moradzadeh A, Ray WZ, Santosa KB. Transcardial perfusion versus immersion fixation for assessment of peripheral nerve regeneration. *J Neurosci Methods*. 2009;184(2):303–309. DOI: [10.1016/j.jneumeth.2009.08.012](https://doi.org/10.1016/j.jneumeth.2009.08.012).
6. McFadden WC, Walsh H, Richter F, Soudant C, Bryce CH, Hof PR. Perfusion fixation in brain banking: a systematic review. *Acta Neuropathol Commun*. 2019;7(1):146. DOI: [10.1186/s40478-019-0804-z](https://doi.org/10.1186/s40478-019-0804-z).
7. Wu J, Cai Y, Wu X, Ying Y, Tai Y, He M. Transcardiac perfusion of the mouse for brain tissue dissection and fixation. *Bio Protoc*. 2021;11(5):e3923. DOI: [10.21769/BioProtoc.3923](https://doi.org/10.21769/BioProtoc.3923).
8. Wahyudi DP, Makkiyah FA, Raja Buana G. A comparative analysis of transcardial perfusion techniques for brain fixation in rodents: a call for standardization of transcardial perfusion. *Health Res J*. 2025;2(01):7–13. [\[available at\]](#)
9. Gage GJ, Kipke DR, Shain W. Whole animal perfusion fixation for rodents. *J Vis Exp*. 2012;(65):e3564. DOI: [10.3791/3564](https://doi.org/10.3791/3564).
10. Paul CA, Beltz B, Berger-Sweeney J. Perfusion of brain tissues with fixative. *Cold Spring Harb Protoc*. 2008;2008(4):pdb.prot4802. DOI: [10.1101/pdb.prot4802](https://doi.org/10.1101/pdb.prot4802).
11. Kwan R, Wright C, Cottle L, Rangel A, Prasad A. Transcardial perfusion of mouse tissues. *protocols.io*. 2022. DOI: [10.17504/protocols.io.rm7vzywnxlx1/v1](https://doi.org/10.17504/protocols.io.rm7vzywnxlx1/v1).
12. Nkengasong JN, Yao K, Onyebujoh P. Laboratory medicine in low-income and middle-income countries: progress and challenges. *Lancet*. 2018;391(10133):1873–1875. DOI: [10.1016/S0140-6736\(18\)30451-3](https://doi.org/10.1016/S0140-6736(18)30451-3).
13. Hade AC, Phillips MA, Promet L, Jagomäe T, Hanumantharaju A, Salumäe L. A cost-effective and efficient ex vivo, ex situ human whole brain perfusion protocol for immunohistochemistry. *J Neurosci Methods*. 2024;404:110059. DOI: [10.1016/j.jneumeth.2024.110059](https://doi.org/10.1016/j.jneumeth.2024.110059).
14. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. *Cold Spring Harb Protoc*. 2008;2008(5):pdb.prot4986. DOI: [10.1101/pdb.prot4986](https://doi.org/10.1101/pdb.prot4986).
15. Rastogi V. Artefacts: a diagnostic dilemma—a review. *J Clin Diagn Res*. 2013;7. DOI: [10.7860/jcdr/2013/6170.3541](https://doi.org/10.7860/jcdr/2013/6170.3541).
16. Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. In: Turksen K, editor. *Histopathology*. New York: Humana Press; 2014. 31–43 p. DOI: [10.1007/978-1-4939-1050-2_3](https://doi.org/10.1007/978-1-4939-1050-2_3).
17. Taqi SA, Sami SA, Sami LB, Zaki SA. A review of artifacts in histopathology. *J Oral Maxillofac Pathol*. 2018;22(2):279. DOI: [10.4103/jomfp.JOMFP_125_15](https://doi.org/10.4103/jomfp.JOMFP_125_15).
18. Jayadi GT, Khristian E, Friliansari LP. Penggunaan metanol sebagai larutan fiksatif alternatif dalam proses fiksasi. *Technol Med Lab Environ Dis*. 2021. [\[available at\]](#)
19. MacArthur Clark J. The 3Rs in research: a contemporary approach to replacement, reduction and refinement. *Br J Nutr*. 2018;120(S1):S1–S7. DOI: [10.1017/S0007114517002368](https://doi.org/10.1017/S0007114517002368).
20. Obafemi OT, Ayeleso AO, Adewale OB, Unuofin J, Ekundayo BE, Ntwasa M. Animal models in biomedical research: relevance of *Drosophila melanogaster*. *Heliyon*. 2025;11(1):e41605. DOI: [10.1016/j.heliyon.2025.e41605](https://doi.org/10.1016/j.heliyon.2025.e41605).
21. Prendergast BJ, Onishi KG, Zucker I. Female mice liberated for inclusion in neuroscience and biomedical research. *Neurosci Biobehav Rev*. 2014;40:1–5. DOI: [10.1016/j.neubiorev.2014.01.001](https://doi.org/10.1016/j.neubiorev.2014.01.001).
22. Beery AK, Zucker I. Sex bias in neuroscience and biomedical research. *Neurosci Biobehav Rev*. 2011;35(3):565–572. DOI: [10.1016/j.neubiorev.2010.07.002](https://doi.org/10.1016/j.neubiorev.2010.07.002).
23. Gutierrez-Castellanos N, Husain BF, Dias IC, Lima SQ. Neural and behavioral plasticity across the female reproductive cycle. *Trends Endocrinol Metab*. 2022;33(11):769–785. DOI: [10.1016/j.tem.2022.08.005](https://doi.org/10.1016/j.tem.2022.08.005).
24. Wan Mohammad WM. Sample size calculation in animal studies using resource equation approach. *Malays J Med Sci*. 2017;24(5):101–105. DOI: [10.21315/mjms2017.24.5.11](https://doi.org/10.21315/mjms2017.24.5.11).
25. Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*. 2017;18(1):529. DOI: [10.1186/s12859-017-1934-z](https://doi.org/10.1186/s12859-017-1934-z).
26. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden CT, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri KW, Tomancak P, Cardona A. Fiji: an open-source platform for biological-image analysis. *Nat Methods*. 2012;9(7):676–682. DOI: [10.1038/nmeth.2019](https://doi.org/10.1038/nmeth.2019).
27. Ma ZY, Zhang XF, Hu YZ, Zhu MD, Jin J, Qian P. Comparison of staining quality between rapid and routine hematoxylin and eosin staining of frozen breast tissue sections: an observational study. *J Int Med Res*. 2024;52(6). DOI: [10.1177/03000605241255381](https://doi.org/10.1177/03000605241255381).
28. Pawlina W, Ross MH. *Histology: a text and atlas with correlated cell and molecular biology*. 8th ed. USA: Wolters Kluwer; 2018.
29. Hassannia H. Characterization of fixatives and their application in histopathology. *Biomed J Sci Tech Res*. 2023;47(5). DOI: [10.26717/bjstr.2023.47.007563](https://doi.org/10.26717/bjstr.2023.47.007563).
30. Shah JS, Macaitis J, Lundquist B, Johnstone B, Coleman M, Jefferson MA, Bunnell BA, Cox CS. Evaluating Thera-101 as a low-volume resuscitation fluid in a model of polytrauma. *Int J Mol Sci*. 2022;23(20):12664. DOI: [10.3390/ijms232012664](https://doi.org/10.3390/ijms232012664).
31. Singh H, Bishen KA, Garg D, Sukhija H, Sharma D, Tomar U. Fixation and fixatives: roles and functions—a short review. *Dent J Adv Stud*. 2019;7(2):51–55. DOI: [10.1055/s-0039-1693514](https://doi.org/10.1055/s-0039-1693514).
32. Ferretti P, Pagliari C, Montalti A, Liverani A. Design and development of a peristaltic pump for constant flow applications. *Front Mech Eng*. 2023;9. DOI: [10.3389/fmech.2023.1207248](https://doi.org/10.3389/fmech.2023.1207248).

33. Saunier J, Khzam A, Yagoubi N. Impact of mechanical stress on flexible tubing used for biomedical applications: characterization of the damages and impact on the patient's health. J Mech Behav Biomed Mater. 2022;136:105477. DOI: [10.1016/j.jmbbm.2022.105477](https://doi.org/10.1016/j.jmbbm.2022.105477)
34. Deng Y, Rowe KJ, Chaudhary KR, Yang A, Mei SH, Stewart DJ. Optimizing imaging of the rat pulmonary microvasculature by micro-computed tomography. Pulm Circ. 2019;9(4):1–9. DOI: [10.1177/2045894019880538](https://doi.org/10.1177/2045894019880538)
35. Higashi Y, Homma J, Sekine H, Yago H, Kobayashi E, Shimizu T. External pressure dynamics promote kidney viability and perfusate filtration during ex vivo kidney perfusion. Sci Rep. 2022;12(1):21564. DOI: [10.1038/s41598-022-26035-z](https://doi.org/10.1038/s41598-022-26035-z)
36. Fan X, Rydgård J, Yang L, Johansson P. Assessing forearm exertion in manual tasks with surface EMG: a comparative analysis of through-forearm vs muscle-specific EMG placements. Appl Ergon. 2026;132:104699. DOI: [10.1016/j.apergo.2025.104699](https://doi.org/10.1016/j.apergo.2025.104699)
37. Yi C, Zuo H, Zhao C, Li KW, Hu H, Tang F, Long T. Fatigue and recovery of muscles for pulling tasks. Int J Environ Res Public Health. 2022;19(22):15159. DOI: [10.3390/ijerph192215159](https://doi.org/10.3390/ijerph192215159)
38. Bindhu P, Krishnapillai R, Thomas P, Jayanthi P. Facts in artifacts. J Oral Maxillofac Pathol. 2013;17(3):397. DOI: [10.4103/0973-029X.125214](https://doi.org/10.4103/0973-029X.125214)
39. R&D Systems. Making 4% formaldehyde solution in phosphate-buffered saline (PBS) [Internet]. Minneapolis: R&D Systems. [available from]

تأثيرات حجم التروية وتقنية التوصيل على الجودة النسيجية لدماغ الفئران: مقارنة بين بروتوكولات التروية عبر القلب

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الخلاصة

يُستخدم الإرواء عبر القلب على نطاق واسع لحفظ أنسجة دماغ الجرذان. ومع ذلك، لم يتم بعد وضع بروتوكولات موحدة فيما يتعلق بمعايير مثل حجم المثبت وتقنية التوصيل التي تضمن جودة نسيجية مثلى. تهدف هذه الدراسة إلى تقييم تأثير حجم المثبت وتقنية التوصيل على جودة الأنسجة الدماغية للجرذان. تم توزيع ستة عشر جرذاً ذكراً من سلالة سبراغ-داولي على أربع مجموعات: المجموعة الأولى (200 مل يدوي)، والمجموعة الثانية (650 مل يدوي)، والمجموعة الثالثة (200 مل بال مضخة)، والمجموعة الرابعة (650 مل بال مضخة). أُجري إرواء القلب باستخدام طرق محددة لكل مجموعة. عُولجت أدمغة الجرذان بصيغة الهيماتوكسيلين-إيوسين. وصُورت. أُجري تقييم كمي لشدة تلوين الأنوية والهولي باستخدام برنامج فيجي. سُجّلت التشوهات النسيجية (مثل تجمع الدم). حُللت الفروق بين المجموعات باستخدام برنامج SPSS Statistics. IBM. كانت شدة تلوين الأنوية أعلى ما يمكن في المجموعة الأولى (200 مل يدوي) (103.31 ± 19.29)، وأدنى ما يمكن في المجموعة الثانية (650 مل يدوي) (156.10 ± 14.94). أكد تحليل التباين باستخدام ويلش وجود فرق إحصائي دال ($p < 0.001$) بين مجموعتي الحقن اليدوي بحجم 200 مل والحقن اليدوي بحجم 650 مل، وكذلك بين مجموعتي الحقن بال مضخة بحجم 200 مل والحقن اليدوي بحجم 650 مل. وأظهرت شدة تلوين الهولي نفس الاتجاه. يشير اختبار توكي HSD إلى دلالة إحصائية ($p < 0.001$) بين المجموعات نفسها، وكذلك بين الحقن اليدوي بحجم 200 مل والحقن بال مضخة بحجم 650 مل. كانت أعداد الخلايا المشوهة أعلى باستمرار في مجموعات الحقن بال مضخة، لكنها لم تُظهر فرقاً ذا دلالة إحصائية ($p = 0.362$). أدى استخدام حجم أقل (200 مل) والحقن اليدوي إلى جودة نسيجية أفضل أثناء الإرواء عبر القلب.