

SCIENCE AND ART OF PRESENTING
MEDICAL TESTIMONY

Anna Samuel

DOCUMENTS FROM SCIENCE AND ART OF PRESENTING MEDICAL TESTIMONY

Anna Samuel

1. Itiel Dror – cognitive bias
2. Article explaining Ischemia by Mestre
3. SUIDI form
4. Sample SDT for histology slides
5. Sample SDT and documents for Newborn Blood screening
 - Letter to next of kin and DOH
 - Declaration to court confirming notice compliance
 - SDT
6. Sample SDT and documents for Blood Splotch
 - Letter to next of kin and medical examiner's office
 - Declaration to court confirming notice compliance
 - SDT
7. Sample SDT for deposition transcripts
8. Sample SDT for case notes of medical examiner
9. Public Health contact for viral testing information. King County risk manager might get involved. Karen Baker was the person I contacted to help interview the director of public health.

Paul Swenson
Lab Mgr. Public Health
206-744 8950
Harborview Medical Center
325 Ninth Avenue, Room 003
Box 359973
Seattle, WA 98104-2499

10. If you want to identify particular virus if there is tracheal swab available, here's some information:
Why antibody testing on blood doesn't help figure out viruses: Antibody testing of blood for infections in the lung has little or no evidentiary value at all when blood is old and because of various biological factors.

There are also issues involving the sheer number of viruses that can cause pneumonia - there are 100s of them. Apparently adenovirus alone has about 50 variations. It's more like taking an x-ray of your foot when you are suffering from malaria. It has no use to identify what virus might have infected (child's) lungs and other tissues.

Antibody testing blood is insufficient to detect what a patient might have had. UW microbiology lab does not test for antibodies for respiratory viruses because it gives no information about what illness a patient might be currently suffering. Similarly, it won't tell what infection caused death because the body does not create antibodies after death.

Scientists test for RNA and DNA to identify viruses. That's what they do with the tracheal swabs. Another option is to test tissue samples for infection in tissues. They need sufficient tissue to extract DNA/RNA.

UW microbiology lab does RNA testing.

They do caution that RNA degrades over time, improper preservation (UW uses minus 80 degrees), freezing and thawing process, formalin fixing and even the paraffin embedding process. Apparently the RNA is less stable than DNA, but most respiratory viruses seem to be RNA based. But DNA testing for the one or more is also a possibility. There are also hundreds of them.

Test codes are BSEQID and REVSQF. There are very detailed specifications about the size of samples such as "10 sections of 10 micro thickness in a DNA free tube with the patient identifier".

Test code BSEQID to test for sequencing for paraffin embedded tissue.

<https://testguide.labmed.uw.edu/view/BSEQID?>

<https://depts.washington.edu/molmicdx/mdx/tests/bctseq.shtml>

<https://depts.washington.edu/molmicdx/mdx/submit.shtml>

Another private lab is the National Autopsy Assay Group:

They can process quickly.

Rates quoted were \$5000-30,000.

Check with them at:

Kasey Thompson

Director of Operations

National Autopsy Assay Group

Voice: 1-800-985-5346

www.autopsyassay.com

INSTRUCTIONS FOR HISTOLOGY SLIDES

1. Authorization: (a) permission from next of kin, (2) permission from legal representative or (c) legal order or subpoena
2. Items requested: (a) exact list of slides created for the ME
(b) If doctor wants specific ones for staining, they will send a list
3. Defense subpoena to KCMEO
Signed by a judge ex parte
Address: King County Medical Examiner's Office
908 Jefferson Street
Seattle, WA 98104
4. Content: Name and d.o.b. of child
Medical examiner's case number
Items requested
Name and address of where you want it sent
5. Contact them by email and/or phone to decide whether you want FedEx or USPS. USPS doesn't have tracking when done through Harborview
6. Contact: In King County, Admin.meo@kingcounty.gov; Colleen Wayne at Colleen.wayne@kingcounty.gov; also Nathan Geerdes, at Nathan.geerdes@kingcounty.gov
7. Cost in 2022 - \$25 a slide/approximately 24 slides plus shipping
8. If overseas, remember customs may have fees assessed.

Attached: Sample SDT

INSTRUCTIONS FOR BLOOD SPLOTCH

1. Authorization: (a) permission from next of kin, (2) permission from legal representative or (c) legal order or subpoena
2. Identify a lab where you want material tested. Work with your expert.
3. Step 1: Draft SDT
Step 2: set court date
Step 3: notice via mail with copy of SDT and letter to next of kin and court date
Step 4: Notice to prosecutors as well
Step 5: After 14 days, send SDT to judge with declaration to sign the SDT
Step 6: Serve the SDT on Medical Examiner's office
4. Item requested: Blood Splotch

Address for King County ME: Address:
King County Medical Examiner's Office
908 Jefferson Street
Seattle, WA 98104

5. Details to include: Medical examiner case number, initials and dob of child, address of lab where it should be sent, name of doctor expert who will be notified by the lab.
6. Sample attached

INSTRUCTIONS FOR NEW-BORN SCREENING BLOOD

1. Maintained at Department of Health
2. Kept till child turns 21
3. Authorization: (a) permission from next of kin, (2) permission from legal representative or (c) legal order or subpoena
4. Items requested: (a) Newborn screening blood card

DO NOT USE THIS AREA

WASHINGTON STATE NEWBORN SCREENING

For use on newborns born in Washington State
 (Do not use for newborns born outside Washington State)

XXXXXX Expires YYYY-MM-DD 903TM
 LC WXXX

MOTHER/LEGAL GUARDIAN'S NAME		CHILD'S INFORMATION	
LAST NAME FIRST NAME Mother/Legal Guardian's Birth Date _____ ☐ Maternal Steroids (within 7 days) Date last _____ ☐ Surrogacy/Adoption	Mo Day Yr Military Time Birth: _____ Collection: _____ Name: _____ First Last Med Rec #: _____ Sex: ☐ F ☐ M ☐ Ambiguous Gestational Age _____ weeks Birth Order: ☐ single if multiple ☐ A ☐ B ☐ _____ Birthweight: _____ grams Race: (Fill in all that apply) ☐ Asian ☐ Black ☐ Hawaiian/Pacific Islander ☐ Hispanic ☐ Native American ☐ Other ☐ Unknown ☐ White ☐ NICU ☐ HA/TPN (within 24 hours) ☐ Steroids (within 7 days) ☐ Antibiotics (within 24 hours) ☐ Meconium ileus ☐ Transfused (RBC) _____ Military _____ Date last ____/____/____ Time _____		
MISCELLANEOUS INFORMATION		FOLLOW-UP CARE	
BIRTH FACILITY Facility ID (born at): _____	Collected at (facility): Follow-Up Clinic ID: ☐ Same as Birth Facility ☐ Same as Submitter		
☐ REFUSED: Signature required on back			

SN XXXXXXXX

SEE DIRECTIONS ON BACK. PLEASE PRINT.

SATURATE EACH CIRCLE COMPLETELY BEFORE MOVING TO THE NEXT

XXXXXX
LC
WXXX
SN

Newborn Screening Cards | Washington State Department of Health

5. Identify a lab where you want it sent. Work with your expert.
6. Step 1: Draft SDT
Step 2: set court date
Step 3: notice via mail with copy of SDT and letter to next of kin and court date
Step 4: Notice to prosecutors as well
Step 5: After 14 days, send SDT to judge with declaration to sign the SDT
Step 6: Serve the SDT on Department of Health
7. Address of DOH: see sample SDT
8. Information to include: child's initials and dob, address of lab for mailing, name of defense expert requesting it.
9. Sample memo to court and subpoena attached.

SUBPOENA FOR DEPOSITION TRANSCRIPTS

1. Determine if there are depositions
2. Determine the court reporter on the case for the witness
3. Ex parte SDT to judge
4. Sample SDT attached
5. Serve SDT on court reporter
6. Since civil attorneys pay per copy
7. Court order avoids this

SUBPOENA FOR MEDICAL EXAMINER'S CASE NOTES

1. Identify ME
2. Identify autopsy number, child's initials and date of birth
3. Serve on Medical Examiner
4. Sample attached

1. ITIEL DROR ARTICLE

PAPER

General

Cognitive bias in forensic pathology decisions

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Abstract

Forensic pathologists' decisions are critical in police investigations and court proceedings as they determine whether an unnatural death of a young child was an accident or homicide. Does cognitive bias affect forensic pathologists' decision-making? To address this question, we examined all death certificates issued during a 10-year period in the State of Nevada in the United States for children under the age of six. We also conducted an experiment with 133 forensic pathologists in which we tested whether knowledge of irrelevant non-medical information that should have no bearing on forensic pathologists' decisions influenced their manner of death determinations. The dataset of death certificates indicated that forensic pathologists were more likely to rule "homicide" rather than "accident" for deaths of Black children relative to White children. This may arise because the base-rate expectation creates an *a priori* cognitive bias to rule that Black children died as a result of homicide, which then perpetuates itself. Corroborating this explanation, the experimental data with the 133 forensic pathologists exhibited biased decisions when given identical medical information but different irrelevant non-medical information about the race of the child and who was the caregiver who brought them to the hospital. These findings together demonstrate how extraneous information can result in cognitive bias in forensic pathology decision-making.

KEYWORDS

bias, cognitive bias, confirmation bias, contextual influence, decision-making, expertise, forensic pathology, forensic science, stereotypes, task-relevance

1 | INTRODUCTION

The biases that may have led to unjustified deaths by police actions have received much attention and have been extensively discussed (e.g., [1–3]), but are the deceased also subject to bias after their death by scientists examining their bodies? Are scientists biased by race or other irrelevant contextual information [4]?

Forensic pathologists play a critical part in administering justice because of their role in criminal investigations and court

proceedings, as they determine whether the manner of death was homicide vs. something else (e.g., accidental or suicide). Despite bias plaguing many forensic domains [5], forensic scientists often deny that bias can impact their decisions (the *bias blind spot* [6], and the fallacies of *expert immunity* and *illusion of control* [7]).

Especially acute has been the resistance to adopt policies that minimize bias, which has "been met with stern resistance from the forensic pathology community" ([8] p. 261). However, cognitive bias

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in forensic science decisions has led to erroneous conclusions with devastating consequences (e.g., [9,10]).

Cognitive biases are not intentional discriminatory biases [11], and they can emerge from various sources [7]. They can emerge from the specific case at hand and how it was examined, from the specific person conducting the examination and organizational factors, as well as from human nature --see sources of bias in Figure 1. Indeed, research has demonstrated the *forensic confirmation bias* ("the class of effects through which an individual's pre-existing beliefs, expectations, motives, and situational context influence the collection, perception, and interpretation of evidence during the course of a criminal case" [12] p. 45). Such cognitive bias has been shown to influence DNA mixture interpretations, fingerprint comparisons, toxicology, and other forensic science judgments [7,13].

Documented biases emerge, for example, from contextual influences, such as 'an eyewitness identified the suspect', or 'the suspect confessed.' Past research has revealed racial bias in medical diagnosis and treatment of living patients (e.g., [14–16]), including children (e.g., [17]) —but no research has explored biases in manner of death determinations among forensic pathologists, the principle medical arm of the death investigation system.

To examine bias in manner of death decisions, the obvious data are death certificates, which document the decisions made in real deaths by medically or legally qualified experts (depending on the case and jurisdiction, the manner of death may be determined by medical examiners, forensic pathologists, or coroners who frequently rely on the opinion of the forensic pathologist in determining the manner of death —in this paper we use them interchangeably).

We therefore examined all death certificates of children under the age of six issued in the State of Nevada over a ten year period, comparing the recorded manners of death of "accident" vs. "homicide" across White and Black children.

Although death certificates reflect real death cases, each case is different, and the ground truth of the real manner of death is unknown. Hence, to complement these archival data, we also conducted an experiment in which we presented 133 forensic pathologists with identical medical information about a child's death, but randomly assigned them with differing medically irrelevant contextual information, and asked them to determine the manner of death. It is important to note that non-medical contextual information may not only be considered when determining the manner of death, but in fact, must be considered. By its very nature of being a circumstantial explanation, the manner of death is dependent on the investigation, which provides the circumstances (and as necessary, the broader background, e.g., medical history) surrounding the death.

The issue of cognitive bias is not trying to ignore or deny the need for circumstances, that is, contextual information, to determine a manner of death, but rather identifying and recognizing what contextual information is irrelevant to that decision-making process. The specific contextual information chosen in the experimental study was purposely (and properly) designed to be always irrelevant to determining the manner of death, allowing the data to reflect

Highlights

- Two data sets revealing cognitive bias in forensic pathologists' decisions about manner of death.
- Death certificate data show racial disparity in judging child deaths as homicide vs. accidental.
- Experimental data reveal forensic pathology contextual bias by irrelevant non-medical information.
- Both data sets show extraneous information, for example, race, cognitively biasing forensic pathologists.
- Cognitively informed training and policies must be in place to minimize forensic pathology biases.

whether cognitive bias impacts the decisions and conclusions of the forensic pathologists.

2 | DEATH CERTIFICATE DATA

We examined all death certificates of children under the age of six from a dataset of all death certificates issued in the state of Nevada between 2009 and 2019. Our focus was on Black and White children in which a qualified expert concluded that the death was unnatural, that is, neither "natural" nor "undetermined" ("suicide" was inapplicable to young children), thus focusing on "accident" and "homicide" determinations ($N = 1024$).

Overall, the percentage of unnatural child deaths (i.e., that medical examiners categorized as "accident" or "homicide," combined) was comparable across White and Black children: 23.3% vs. 23.5%, respectively. However, when separating "homicide" vs. "accident," a very different picture emerged: coroners more often attributed death to homicide when the child was Black (8.5%) vs. White (5.6%) and more often ruled the death accidental when the child was White (17.7%) vs. Black (15.0%). See Figure 2, $\chi^2(1) = 4.02$, $p < .05$, $OR = 1.81$ [95% CI: 1.01, 3.25].

Stated otherwise, the data revealed that forensic pathologists ruled a White child's unnatural death as "homicide" 24% of the time, vs. as "accident" 76% of the time, yielding an approximate ratio of 1:3. In contrast, forensic pathologists ruled a Black child's unnatural death as "homicide" 36% of the time, vs. as "accident" 64% of the time, resulting in a ratio of about 1:2.

We must be careful in drawing conclusions about bias from these archival data, especially given that the ground truth of how these children actually died is unknown. For example, it is possible that Black children die from homicide more often than White children.

3 | EXPERIMENTAL DATA

To complement the death certificate data, we conducted an experiment with a sample of qualified forensic pathologists, who examined

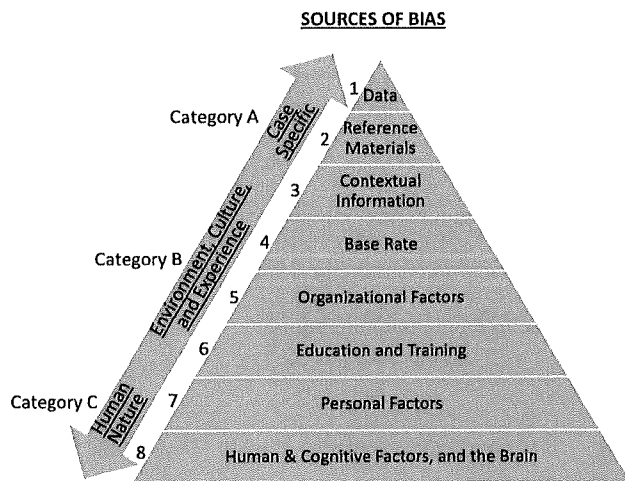


FIGURE 1 Eight sources of bias that may impact observations and conclusions, even by expert scientists. They are organized in a taxonomy with three categories: starting at the top, with sources relating to the specific case at hand (Category A); sources relating to the specific person conducting the analysis (Category B); and at the bottom, sources that relate to human nature (Category C). Taken from [7]

a hypothetical death case of a young child, with identical medical information but different extraneous medically irrelevant contextual information (the child was either Black and the mother's boyfriend was the caretaker, or the child was White and the grandmother was the caretaker).

4 | METHOD

4.1 | Participants

All participants ($N = 133$) were American Board of Pathology Board-certified members of the National Association of Medical Examiners (NAME, their premier professional organization, founded in 1966). The participants consisted of 50 females and 79 males (4 left this information blank), and 10 were under the age of 35 years, 30 were between the ages of 35–45, 39 were between 46–55, 28 between 56–65, 17 between 66–75, and 6 over 75 (3 left age information blank). The participants were recruited through an email sent to the NAME mailing list of 713 pathologists (18.6% response rate).

4.2 | Materials

Participants read a vignette describing a not straightforward or simple case in which a 3.5-year-old child was presented to an Emergency Department with diminished vital signs and who died shortly after arrival. In the vignette, the caretaker described finding the toddler unresponsive on the floor of a living room. Postmortem examination determined that the toddler had a skull fracture and subarachnoid

hemorrhage of the brain. The vignette further briefly described scene and ancillary investigation findings, as well as the results of the medical examination (details about bruising on the head, neck and extremities, as well as the fractures and brain injury).

By random assignment, each pathologist read one of two vignettes, which were identical apart from two pieces of information: some were told that the child was African-American and that the caretaker was the mother's boyfriend (henceforth referred to as the "Black condition", $n = 65$), whereas the other pathologists were told that that child was White and that the caretaker was the child's grandmother (henceforth referred to as the "White condition", $n = 68$). To be consistent with typical medical information, the race of the child was stated, but the race of the caretaker was not explicitly stated (the caretakers explicitly differed only in their relations to the child as well as their sex; implicitly they may have differed in their age and race).

4.3 | Procedure

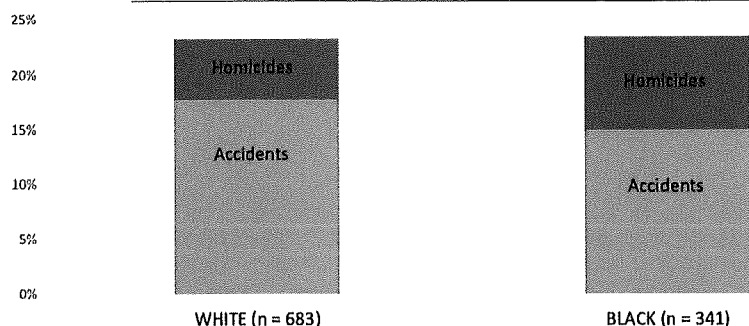
Medical examiners who responded to the recruitment email were randomly assigned to either the Black or White condition. They were given the information and asked to examine the case and to determine the manner of death—they were not directed to do it differently than they do ordinarily. The standard death certificate format mandates that the manner of death be determined as either "natural," "accident," "suicide," "homicide," or "undetermined." Because of the autopsy findings and circumstances described in the case, "natural" and "suicide" were not viable options, leaving realistically only the options of "accident" or "homicide", or that the manner of death was "undetermined."

5 | RESULTS

None of the participants determined the manner of death as "natural" or "suicide," and 78 participants ruled the manner of death as "undetermined." The "undetermined" decisions were comparable across both groups of contextual information (38 vs. 40, for the Black vs. White conditions, respectively).

Of interest were the remaining 55 participants who reached a conclusive determination about the manner of death, with 23 ruling it an "accident" and 32 ruling it a "homicide." The medically irrelevant information had a significant impact on manner of death determinations, revealing a clear contextual effect. In the Black condition, the pathologists were about five times more likely to rule the death as a "homicide" rather than an "accident" (35.4% vs. 6.2%), but in the White condition, the results were the opposite: The pathologists ruled the death as a "homicide" only about half as often as they ruled it an "accident" (13.2% vs. 27.9%). See Figure 3, $X^2(1) = 15.89$, $p < .0001$, $OR = 12.14$ [95% CI: 3.23, 45.68].

As presented in Figure 3, with all medical data identical, forensic pathologists were biased in their decisions. The data do not allow us to ascertain whether they were biased by the race of the child or/and

Determined Manner of Death that is Unnatural for Ages 0-5 (n = 1,024)**FIGURE 2** Forensic pathologists were more likely to attribute the deaths of Black children to homicide, relative to White children; whereas the deaths of White children, relative to black, were more likely deemed accidental**FIGURE 3** With identical medical findings, the proportion of forensic pathologists determining that the manner of death was an "accident" (left panel) vs. a "homicide" (right panel). White bars are for White children with the grandmother as a caretaker; Black bars are for Black children with the mother's boyfriend as a caretaker. When the forensic pathologists could not reach a decision (an "accident" or "homicide") with confidence, they concluded that the manner of death was "undetermined"

characteristics of the caretaker. The important finding is that *their decisions were noticeably affected by medically irrelevant contextual information* (information that should not have any bearing on the decision). Moreover, their decisions were made with confidence, given that pathologists also had the option to decide that the manner of death was undetermined (indeed, 78 participants did not reach a conclusive manner of death).

Most important is the phenomenon identified in this study, namely demonstrating that biases by medically irrelevant contextual information do affect the conclusions reached by medical examiners. The degree and the detailed nature of these biasing effects require further research, but establishing biases in forensic pathology decision-making—the first study to do so—is not diminished by the potential limitation of not knowing which specific irrelevant information biased them (the race of the child, or/and the nature of the caretaker). Also, one must remember that the experimental study is complemented and corroborated by the data from the death certificates. These, together, are discussed below.

6 | DISCUSSION

A dataset of death certificates revealed that Black children, relative to White children, were more often judged as victims of homicides rather than accidents. We then complemented these archival data

with an experiment that directly manipulated medically irrelevant contextual information, and found that forensic pathologists' decisions were dramatically influenced and biased by such information.

Cognitive bias is not about denying the need for certain relevant contextual information, but rather about the need to identify and recognize what contextual information is relevant to the decision-making process. The race of the child and who was the caretaker were chosen because they are irrelevant to determining the manner of death in our experimental study, thus allowing the experimental data to reveal whether or not cognitive bias impacts forensic pathologists.

The experimental data, along with the death certificate data, taken together, show that even highly trained professional scientists can be biased in their decisions. When considering the possibility of bias, it is important to consider its possible source. Cognitive bias can emerge from factors related to the particular case itself (see Figure 1, Category A)—for example, the race of the child in a given case activates implicit racial bias. However, the bias may not emerge from racial bias *per se*, but instead originate from the second category of sources of bias, namely base rate (see Figure 1, Category B). That is to say, the forensic pathologists may "learn" over time, regardless of accuracy, that more Black children than White children die as a result of homicide. As a result, the forensic pathologists develop an *a priori* expectation, high prior odds, that a Black child has died as a result of homicide rather than accident.

With time, the pathologists are thus exposed to more rulings that Black children die from homicide, which strengthens the base-rate bias and prompts even more "homicide" findings—thereby creating a bias that perpetuates itself, resulting in *bias cascade* and *bias snowball* effects [5,7]. It may even be that Black children were in the past indeed more likely to die from homicide, and that is no longer the case (now, or in the future), but the bias has already established itself, feeding and reinforcing itself—a self-fulfilling prophecy.

This source of cognitive bias relates to the medical examiner's experience and other factors connected to their work environment (Category B) and is not mutually exclusive to implicit racial bias *per se* (Category A). These biases—Categories, A, B, and C—can unconsciously impact experts, and therefore, we do not insinuate that forensic pathologists intentionally discriminate and knowingly conduct their work in a racially biased manner.

As per the limitations of our studies, given that the death certificates were from the state of Nevada in the United States, it does not allow us to necessarily generalize our findings to other states in the United States or to other countries. Also, the experimental contextual manipulation included both the race of the child and the nature of the caregiver, not allowing to determine which (or if both) contributed to the biasing effect. Furthermore, the caretakers were not only explicitly different in their relation to the child, but also in their sex (as well as perhaps implied differences about their age and race).

Further research can compare the Nevada death certificates to those in other locations, and examine what factors may impact the level of racial bias in determining the manner of death of children, as well as explore other factors that may bias manner of death decisions. Also, further experimental research can elucidate and tease apart the different cognitive biases at play in forensic pathology (e.g., what we found in our experimental study, the race of the child vs. nature of caregiver, as well as other contextual information). Nevertheless, the critical finding from our experimental study is that it is the first study to examine and demonstrate that medically irrelevant contextual information does bias forensic pathology decision-making.

Our two data sets are the first step in examining biases in post-mortem decision-making, and they do not answer all the questions. For example, were the forensic pathologists aware that the medically irrelevant contextual information impacted their decisions? And, what, if any, contextual information is required for their medical decision-making? These are important questions that need further research. Even with answers to the above research questions, there is a need to examine if such cognitive bias impacts how experts perceive information, their testing strategies, or/and how they interpret the findings [7,18].

The data presented in our study is the first to establish cognitive bias in forensic pathology decisions and it has important implications to policies. A critical step is to develop and implement policies that mitigate bias. In many police and forensic domains efforts are made to have policies to minimize bias, however, such policies have yet to be accepted or implemented in the forensic pathology domain [8].

Hence, our study is critical in demonstrating cognitive bias in forensic pathology and should drive discussions, further research and

policies in forensic pathology. Rather than denying the existence of bias, there needs to be a cognitively informed discussion on what (as well as how and when) contextual information should be appropriately used in forensic pathology decision-making [19]. Our concerns about cognitive bias in forensic pathology decision-making do not call for removing *all* contextual information, nor do we claim that context cannot be (and is often) important and relevant.

Furthermore, contextual information is not always a simple dichotomy of relevant vs. non-relevant, but more of a continuum [8]. In addition, an added complexity is that the level of relevance (or lack thereof) can be decision and case dependent, thus changing from case to case and between different types of decisions. The relevance continuum starts with the most relevant, the body of evidence itself—the deceased. Then there is a variety of contextual information that can play a role in interpreting that body of evidence. Moving along the continuum of relevance is the medical history, then findings from the death scene, and moving to social history and various other leads and findings from the police investigation [8]. Further down along the continuum, moving further and further away from the actual body of evidence, is the wider circumstantial context, such as who brought the deceased to the hospital and the socio-economic status of the deceased.

Given this continuum:

1. Forensic pathology decisions and conclusions about the cause of death should be based solely on medically relevant information. The manner of death, being a broader circumstantial explanation for how the cause of death occurred, often relies on some investigative, that is, non-medical, information. However, that does not mean that *all* contextual information is task relevant to the assessment and determination of the manner of death. Therefore, it must be recognized that some types of circumstantial information should not be considered in that decision-making process. The two factors that were manipulated in our experimental study were task irrelevant to the decision on the manner of death, and thus should not have affected the decisions, yet they clearly did affect the conclusions, demonstrating cognitive bias in forensic pathology decision-making.
2. Since there is no clear cutoff place along the relevance continuum which applies to all cases, there needs to be a discussion and debate about the (mis)use of contextual information by forensic pathologists. Such a discussion should take into account research and findings from cognitive science, especially those about the nature of decision-making, sources of bias and contextual influences (e.g., [7,11]). The forensic pathology community should not deny the existence of cognitive bias and potential effects of contextual information and should consider and explore ways to mitigate and minimize such biases. What is clear is that at some point along the relevant-irrelevant continuum, the context is so far removed and is so task irrelevant, that it should never be used. The forensic pathology community needs to consider and explore the (mis)use of contextual information in light of our data showing



that such extraneous information can cognitively bias forensic pathologists. Hence, the need for debate and to take action regarding bias in forensic pathology.

3. Medical relevant information should be the primary driver of pathology decisions, supplemented by the less medically relevant when needed and justified. To achieve this, the forensic pathology community must explore and adopt procedures that minimize bias. Procedures such as Linear Sequential Unmasking (LSU), minimize bias in forensic DNA and fingerprinting by optimizing the sequence and timing of exposure to potentially biasing information and making sure to document their potential effects. The forensic pathology community should consider LSU approaches for context management (as well as compartmentalization and case managers) in forensic pathology, which will optimize the use of contextual information in a way that minimizes bias. These context management policies will be there to ensure that the medical information, rather than context, drives the forensic pathology decision-making [5,7].
4. It is ideal when the medical pathology decisions are based primarily (if not totally) on the medical relevant information. As the decision is more and more supplemented by, and dependent on, contextual information that is less and less medical, the medical decision weakens. It is warranted and we fully support when non-medical information *independently* corroborates medical decisions (that strengthens the medical decision), but not when the non-medical information is the major (and implicitly hidden) driver of the conclusions.
5. There is a difference when non-medical context and investigative findings are used to suggest seeking medical information, such as which toxicology tests to run, vs. when they implicitly influence and bias a medical decision. Consider, for example, a decision whether a death is suicide or homicide. Does the pathologist need to know that next to the body was an empty bottle of pills? Yes, and they should also know which pills they were, that is not disputed. However, this context is not to be used to determine the manner of death *per se*, but more to trigger which toxicology tests to run, which can help determine the manner of death. Non-medical context can be used to trigger such tests, or theories, as well as requests for additional information—that is part of the legitimate, not necessarily biased, pathology inquiry. However, when the non-medical contextual information and investigative findings cognitively bias the pathologists' decisions, and do so implicitly without transparency about the factors that actually underpin the decision, that is a major concern.
6. There should be transparency [20] about if and what contextual information was relied upon, that is, that the pathologists' reports explicitly state what non-medical contextual information was known and used in their decisions, and how it has impacted their conclusions. It is important to distinguish and make very clear when decisions are medical (and based on medical information) and when decisions are non-medical (and are based on contextual and non-medical information). Most dangerous is when juries and other fact finders take the non-medical decisions as being

medical and medically based decisions. To avoid that non-medical decisions be unintentionally disguised as medical, the forensic pathology reports and testimony must make it explicitly clear what is medical and what is not medical. The cognitive challenge and problem is that when one is exposed to non-medical irrelevant context, it can unconsciously impact the medical decisions. Given that there is no awareness of such cognitive bias, pathologists cannot account and report what factors actually influenced their decisions. The point is that there needs to be a discussion about how to deal with these issues, rather than just falling into the *bias blind spot* [6] and dismissing the entire issue under the false belief and pretense that bias does not exist. Two options to consider are adopting LSU kind of context management tools (that blind some information and optimize the exposure and timing of other information), and detailing, documenting and reporting what (as well as when and by whom) information was given to the forensic pathologist.

It is also important to note that in many domains it is relatively easy to have policies that blind scientists to irrelevant contextual information, but in forensic pathology this is not always feasible (e.g., during an autopsy, the race of the deceased is present). However, context management policies, such as LSU, compartmentalization and proper blind reviews [5,7,21] can be developed and considered in forensic pathology (e.g., race-blind peer reviews in complex and not straightforward cases).

"Acknowledging that bias can influence forensic science experts would be a substantial step toward implementing countermeasures that could greatly improve forensic evidence and the fair administration of justice" [5]. Thus, policies that mandate proper cognitive education and training in forensic pathology are important, so they properly understand that no one is immune to cognitive bias nor that it can be controlled by mere willpower [7], and therefore mitigating actions are required.

The findings about bias in forensic pathology have implications that go beyond police investigations, court proceedings, training, and best practices, as they touch upon wider issues, such as stereotyping and prejudice, unconscious processes, motivation, cognitive and perceptual confirmation biases, and a whole host of psychology and law, and socially important issues [22].

REFERENCES

1. National Academies of Sciences. Racial bias and disparities in proactive policing. In: Proactive policing: effects on crime and communities. Washington, DC: The National Academies Press; 2018. p. 251–301.
2. Correll J, Park B, Judd CM, Wittenbrink B. The police officer's dilemma: using ethnicity to disambiguate potentially threatening individuals. *J Pers Soc Psychol*. 2002;83(6):1314–29. <https://doi.org/10.1037/0022-3514.83.6.1314>.
3. DeGue S, Fowler KA, Calkins C. Deaths due to use of lethal force by law enforcement. *Am J Prev Med*. 2016;51:S173–87. <https://doi.org/10.1016/j.amepre.2016.08.027>.
4. Thorp HH. Time to look in the mirror. *Science*. 2020;368(6496):1161. <https://doi.org/10.1126/science.abd1896>.

5. Dror IE. Biases in forensic experts. *Science*. 2018;360(6386):243. <https://doi.org/10.1126/science.aat8443>.
6. Kukucka J, Kassin S, Zapf P, Dror IE. Cognitive bias and blindness: a global survey of forensic science examiners. *J Appl Res Mem Cogn*. 2017;6(4):452–9. <https://doi.org/10.1016/j.jarmac.2017.09.001>.
7. Dror IE. Cognitive and human factors in expert decision making: six fallacies and the eight sources of bias. *Anal Chem*. 2020;92(12):7998–8004. <https://doi.org/10.1021/acs.analchem.0c00704>.
8. Simon D. Minimizing error and bias in death investigations. *Seton Hall Law Rev*. 2018;49:255–305.
9. Garrett BL. *Convicting the innocent: where criminal prosecutions go wrong*. Cambridge, MA: Harvard University Press; 2012. p. 1–376.
10. Garrett BL. *Autopsy of a crime lab*. Berkeley, CA: University of California Press; 2021. p. 1–264.
11. Kahneman D, Sibony O, Sunstein CR. *Noise: a flaw in human judgment*. Glasgow, Scotland: William Collins Publishers; 2021. p. 1–384.
12. Kassin SM, Dror IE, Kukucka J. The forensic confirmation bias: problems, perspectives, and proposed solutions. *J Appl Res Mem Cogn*. 2013;2(1):42–52. <https://doi.org/10.1016/j.jarmac.2013.01.001>.
13. Cooper GS, Meterko V. Cognitive bias research in forensic science: a systematic review. *Forensic Sci Int*. 2019;297:35–46. <https://doi.org/10.1016/j.forsciint.2019.01.016>.
14. FitzGerald C, Hurst S. Implicit bias in healthcare professionals: a systematic review. *BMC Med Ethics*. 2017;18:1–18. <https://doi.org/10.1186/s12910-017-0179-8>.
15. Chapman E, Kaatz A, Carnes M. Physicians and implicit bias: how doctors may unwittingly perpetuate health care disparities. *J Gen Intern Med*. 2013;28:1504–10.
16. Anderson KO, Green CR, Payne R. Racial and ethnic disparities in pain: causes and consequences of unequal care. *J Pain*. 2009;10:1187–204. <https://doi.org/10.1016/j.jpain.2009.10.002>.
17. Goyal MK, Kuppermann N, Cleary SD, Teach SJ, Chamberlain JM. Racial disparities in pain management of children with appendicitis in emergency departments. *JAMA Pediatr*. 2015;169(11):996–1002. <https://doi.org/10.1001/jamapediatrics.2015.1915>.
18. Dror IE. A hierarchy of expert performance. *J Appl Res Mem Cogn*. 2016;5(2):121–7. <https://doi.org/10.1016/j.jarmac.2016.03.001>.
19. Thompson WC. Determining the proper evidentiary basis for an expert opinion: what do experts need to know and when do they know too much? In: Robertson C, Kesselheim A, editors. *Blinding as a solution to bias: Strengthening biomedical science, forensic science, and law*. Cambridge, MA: Elsevier; 2015. p. 133–50.
20. Almazrouei MA. Comment on "Cognitive and human factors in expert decision making: six fallacies and the eight sources of bias". *Anal Chem*. 2020;92(18):12725–6. <https://doi.org/10.1021/acs.analchem.0c03002>.
21. Mattijssen EJAT, Witteman CLM, Berger CEH, Stoel RD. Cognitive biases in the peer review of bullet and cartridge case comparison casework: a field study. *Sci Justice*. 2020;60(4):337–46. <https://doi.org/10.1016/j.scijus.2020.01.005>.
22. Eberhardt J. *Biased: uncovering the hidden prejudice that shapes what we see, think, and do*. New York, NY: Penguin Random House; 2020. p. 1–368.

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2. ISCHEMIA

RESEARCH ARTICLE

NEUROSCIENCE

Cerebrospinal fluid influx drives acute ischemic tissue swelling

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Stroke affects millions each year. Poststroke brain edema predicts the severity of eventual stroke damage, yet our concept of how edema develops is incomplete and treatment options remain limited. In early stages, fluid accumulation occurs owing to a net gain of ions, widely thought to enter from the vascular compartment. Here, we used magnetic resonance imaging, radiolabeled tracers, and multiphoton imaging in rodents to show instead that cerebrospinal fluid surrounding the brain enters the tissue within minutes of an ischemic insult along perivascular flow channels. This process was initiated by ischemic spreading depolarizations along with subsequent vasoconstriction, which in turn enlarged the perivascular spaces and doubled glymphatic inflow speeds. Thus, our understanding of poststroke edema needs to be revised, and these findings could provide a conceptual basis for development of alternative treatment strategies.

Stroke is among the leading causes of death worldwide, affecting 10 million patients annually (1, 2). Recent strides in treatment have improved these statistics, but stroke remains a principal cause of long-term disability (1, 3). A detrimental complication is cerebral edema, the abnormal accumulation of fluid that leads to secondary ischemia, additional tissue loss, and potential death (4, 5). Targeting edema represents a promising therapeutic strategy, because the severity of swelling predicts long-term functional outcomes (6–8). Although edema also develops

in a range of other central nervous system diseases (e.g., trauma, tumors, and infections), treatment options remain limited, and those available are suboptimal. Traditionally, cerebral edema is divided into two distinct phases: an early cytotoxic phase and a later vasogenic phase (9). Cytotoxic edema occurs within minutes of an ischemic insult and is triggered by spreading depolarizations (SDs) that result from dysregulated ion homeostasis causing cell swelling (6, 7, 10–14). During the subsequent phase, vasogenic edema, fluid from the blood enters the brain as a result of blood-brain barrier (BBB) breakdown (15). Ions and fluid can cross the BBB via transcellular and paracellular routes (12). The transcellular pathway allows for the early entry of plasma proteins and other osmotically active solutes facilitating fluid entry, whereas the paracellular pathway takes 2 days to become active (16, 17). However, edema actually develops several hours before significant BBB dysfunction (18). This terminology used for staging edema was defined in the 1960s, and the conceptual framework for understanding and treating edema is still based on these early observations (8, 9, 19). The term ionic edema was introduced to explain this intermediary phase, during which tissue Na^+ content increases (5, 18). This generates an osmotic gradient with plasma, allowing water to move into the brain across an intact BBB (20, 21). However, the source of the excess Na^+ has been attributed exclusively to influx from the intravascular compartment (5). Yet, the brain is bathed in cerebrospinal fluid (CSF), which has a high Na^+ concentration and accounts for almost 10% of intracranial fluid (22). CSF has not been recognized as a

source of edema fluid (8), but the notion that perivascular spaces (PVSs) provide a conduit for CSF influx prompted us to reassess this idea. Glymphatic exchange of CSF with the interstitial fluid (ISF) provides a means by which CSF in large quantities may rapidly enter the brain and drive tissue swelling (23, 24). Here, we found that CSF can indeed provide a key source for the initial rise in brain water content in the ischemic brain.

CSF flows into the brain after stroke, driving acute tissue swelling

To determine whether CSF contributes to edema in the early phases of acute ischemic stroke, the middle cerebral artery (MCA) was occluded by injecting microspheres into the right common carotid artery in wild-type mice (Fig. 1, A and B) (25). Embolic MCA occlusion (MCAO) resulted in an immediate, steep reduction in relative cerebral blood flow (rCBF: $92.5 \pm 1.2\%$; Fig. 1C). Mice exhibited severe neurological deficits the following day, and the infarct occupied most of the vascular territory of the MCA (fig. S1). We used this model to map influx of CSF tagged with a fluorescent tracer immediately after MCAO. The CSF tracer was injected 15 min before occlusion to ensure that it had distributed along the circle of Willis but not yet transported up along the MCA (Fig. 1D). Imaging revealed no difference in CSF tracer entry between ipsilateral and contralateral hemispheres before stroke, but MCAO resulted in a sharp threefold increase in ipsilateral CSF tracer influx compared with the contralateral hemisphere (Fig. 1, E and F). The temporal derivative of the fluorescence curves identified that CSF influx occurred at two separate time points: an early peak (11.4 ± 1.8 s) and a second peak (5.24 ± 0.48 min) after MCAO (Fig. 1, F and G). We next asked whether this CSF influx contributed to edema. The tissue water content of the ischemic cortex increased rapidly 15 min after occlusion, whereas the water content in the nonischemic hemisphere remained constant (Fig. 1H). To assess the contribution of blood versus CSF to the rapid increase in water content, the two fluid compartments were tagged by either intravenous or intracisternal injection of ^{22}Na and ^3H -mannitol. Early ischemic Na^+ accumulation is an indirect measure of edema (5, 18, 26); mannitol, on the other hand, is a small BBB-impermeable tracer whose accumulation reflects opening of the barrier (Fig. 1I) (27). ^{22}Na and ^3H -mannitol accumulation in the ischemic and nonischemic hemispheres was directly comparable after intravenous injection, suggesting that the vascular compartment was not the source of edema fluid in early times (Fig. 1, J and K). By contrast, both ^{22}Na and ^3H -mannitol accumulated to a greater degree in the ischemic hemisphere when the tracers were delivered to the CSF (Fig. 1, L and M). To determine which pool of CSF

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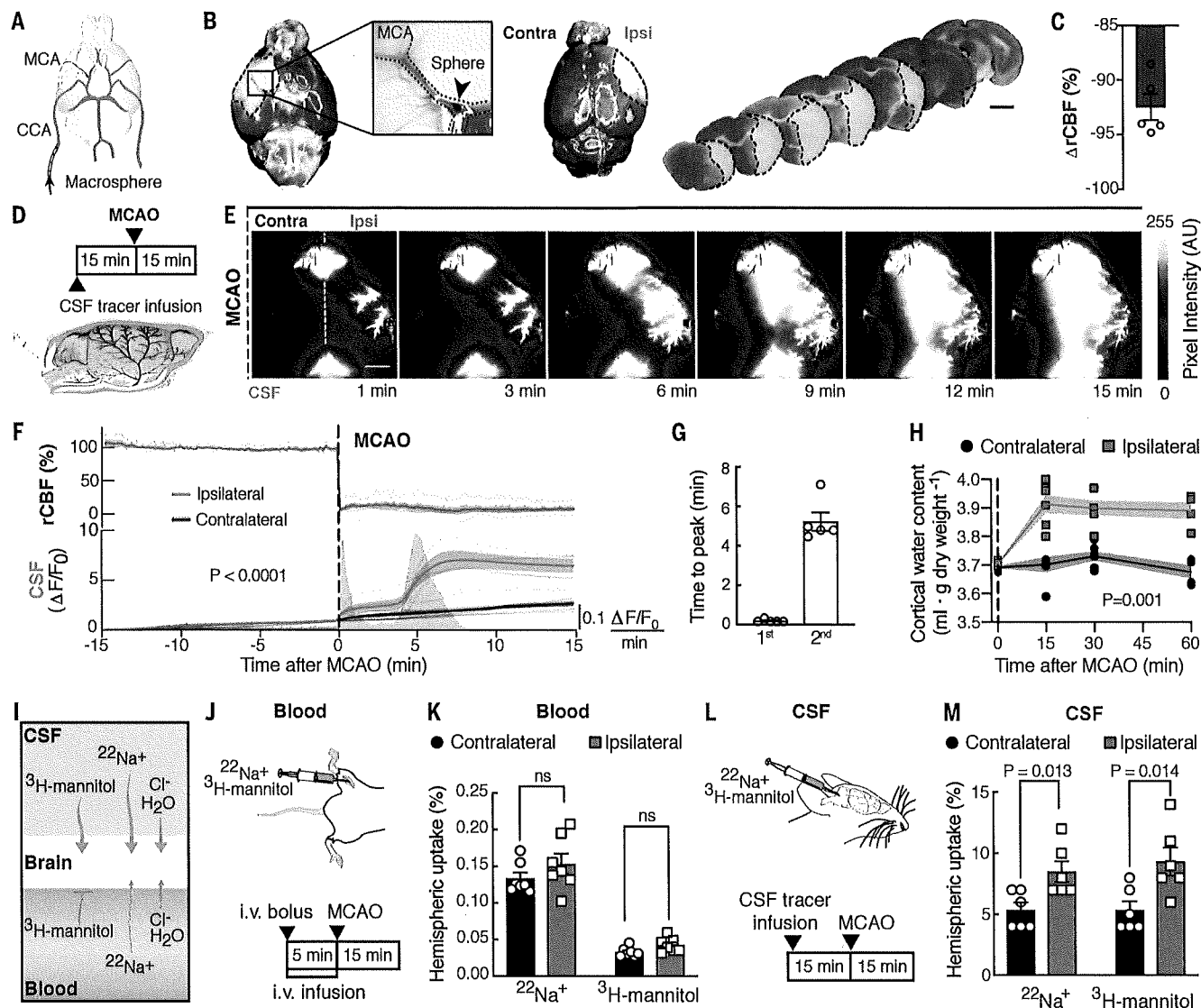


Fig. 1. CSF rapidly enters the brain after stroke, resulting in edema.

(A) Macrospheres (arrow) were infused into the common carotid artery (CCA), occluding the MCA. (B) Triphenyltetrazolium chloride staining after MCAO. Contra, contralateral; ipsi, ipsilateral. (C) Ipsilateral rCBF after MCAO. (D) Intracisternal Alexa Fluor647-conjugated bovine serum albumin (BSA-647) was infused 15 min before MCAO. (E) Ipsilateral and contralateral tracer influx was imaged while measuring rCBF. (The white line indicates the sagittal suture and fiber optic probe.) AU, arbitrary units. (F) Time series of rCBF and fluorescence intensity ($\Delta F/F_0$) in each hemisphere. Gray peaks are the temporal derivative of fluorescence intensity. Repeated measures two-way analysis of variance (ANOVA) was performed; interaction P value < 0.0001 ; $n = 5$ mice. (G) Timing of the first and second peaks. (H) Water content of the cortex.

Mixed-effects repeated measures two-way ANOVA with Sidak's multiple comparisons test was performed; $n = 4$ to 7 mice per time point. The shaded regions above and below the plot lines indicate SEM. (I) The source of the edema fluid was identified by labeling either the blood or CSF compartment. (J) $^{22}\text{Na}^+$ and ^3H -mannitol were delivered intravenously (i.v.). (K) Percentage of the total injected radiation found in each hemisphere after intravenous delivery. Student's t tests with Holm-Sidak correction were performed; $n = 7$ mice. ns, not significant. (L) Isotopes were delivered into CSF. (M) Percentage of the total injected radiation in each hemisphere after intracisternal delivery. Student's t tests with Holm-Sidak correction were performed; $n = 6$ mice. In (B) and (E), scale bars are 2 mm. In (C), (G), (K), and (M), error bars represent SEM.

was the primary source of edema fluid, we performed CSF cisternography using three-dimensional (3D) fast imaging employing steady-state acquisition (3D-FIESTA) magnetic resonance imaging (MRI). The CSF compartment—including the lateral, third, and fourth ventricles and the cisterna magna—accounted for a total of $13 \pm 1 \text{ mm}^3$ (Fig. 2A). Smaller collections of fluid ($7.6 \pm 1.3 \text{ mm}^3$)

were observed along the PVS of the large arteries of the circle of Willis, and the rest of the intracranial volume was occupied by the brain tissue, including the intravascular compartment ($509 \pm 11 \text{ mm}^3$). After MCAO, CSF in the lateral ventricle (Fig. 2B and Movie 1) of the ischemic hemisphere and in the cisterna magna (Fig. 2C) slowly disappeared. Total CSF volume in the ventricular system

and cisterna decreased by $9.0 \pm 2.5\%$ at 15 min and $17 \pm 3\%$ at 29 min poststroke (Fig. 2, D and E). PVS fluid volume began decreasing at around 15 min (Fig. 2F), several minutes after the CSF compartment started shrinking (~ 5 min). Combined, these results add additional support to the notion that CSF is the primary source of early edema fluid after ischemic stroke.

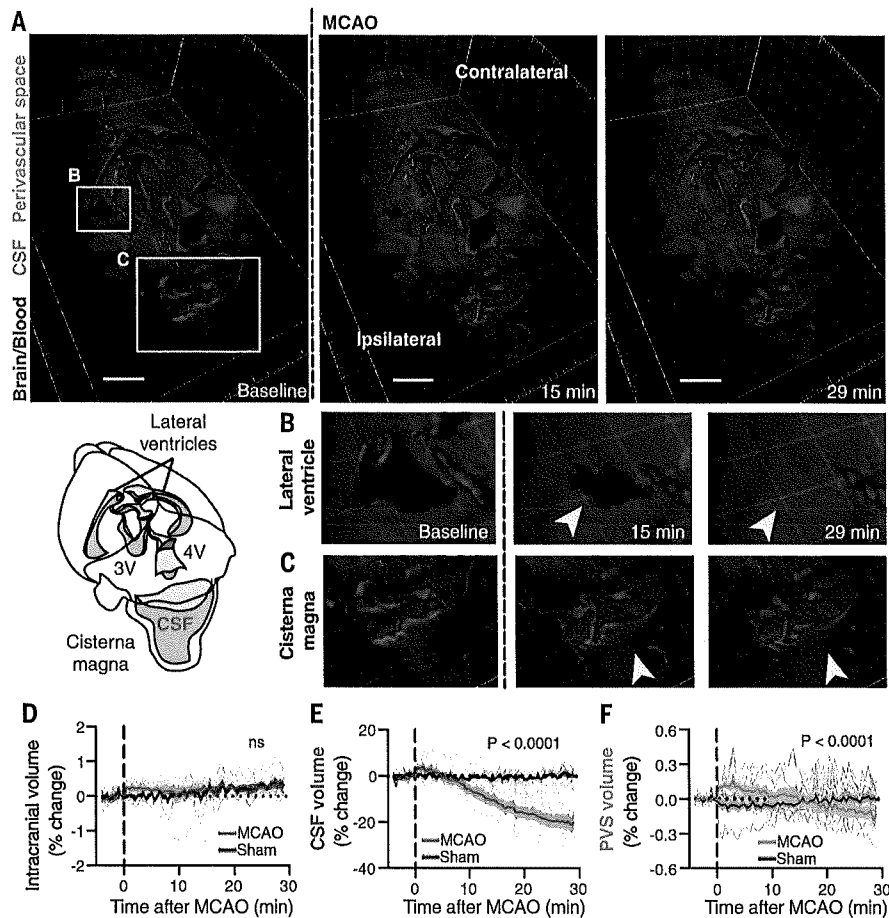
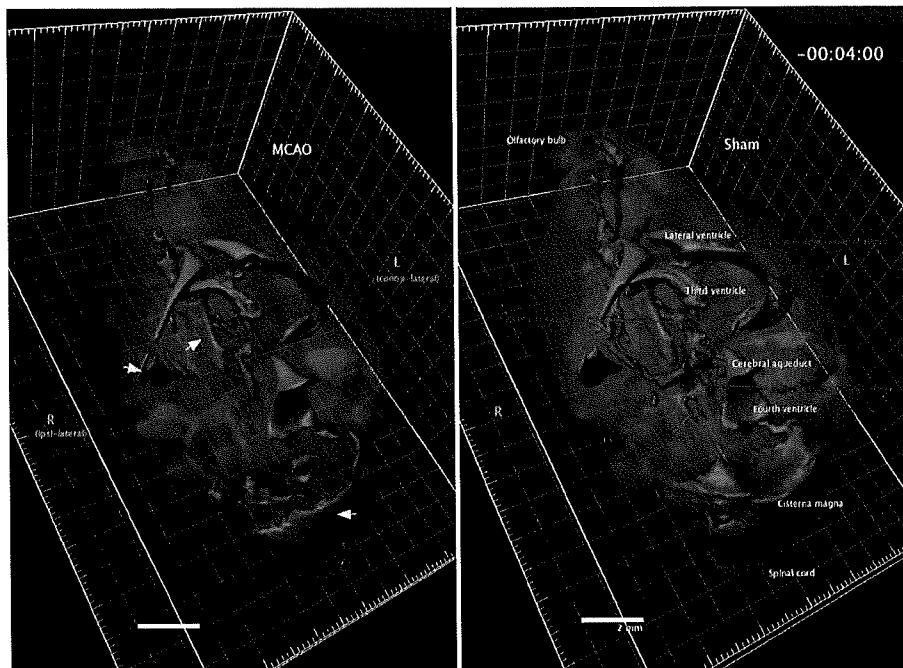


Fig. 2. After MCAO, CSF shifts into other intracranial compartments. (A to C) 3D-FIESTA shows three main compartments: (i) intraventricular CSF in the lateral, third (3V), and fourth (4V) ventricles and cisternal CSF in the cisterna magna (blue); (ii) free fluid in the PVSs (red) of the circle of Willis along the anterior, middle, posterior cerebral, and basilar arteries; and (iii) intracranial content primarily composed of brain tissue and cerebral blood volume (green). 3D-FIESTA at baseline before MCAO and 15 and 29 min later is shown. Scale bar, 2 mm. Insets in (A) of the ventral anterior horn of the ipsilateral lateral ventricle (B) and the cisterna magna (C) demonstrate the loss of free water at 15 and 29 min after MCAO, most notably in the lateral ventricles and the cisternal magna (yellow arrows). (D) Percent change of intracranial volume after MCAO and in sham animals. Repeated measures two-way ANOVA was performed; interaction P value not significant (ns). (E) Percent change of CSF volume after MCAO. Repeated measures two-way ANOVA was performed; interaction P value < 0.0001 . (F) Percent change of PVS volume after MCAO. Repeated measures two-way ANOVA was performed; interaction P value < 0.0001 . Time-lapse measurements from $n = 7$ mice in the sham group and 8 mice in the MCAO group were collected. The shaded regions above and below the plot lines indicate SEM.

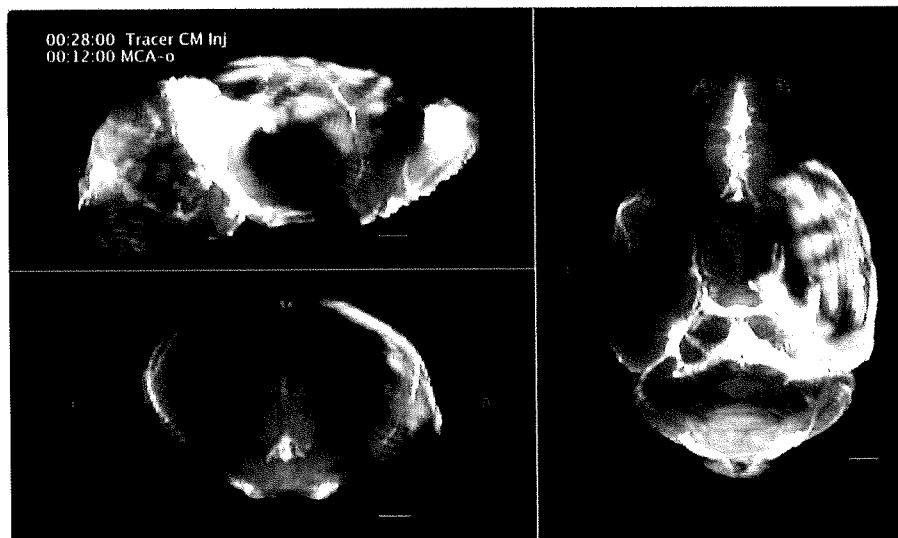
SDs trigger CSF flow into the ischemic brain
MRI suggested that the volume of CSF rapidly declined upon artery occlusion, so we next used dynamic contrast-enhanced MRI to determine the most likely location of CSF brain entry (Movie 2 and fig. S2A). MRI analysis confirmed an abrupt increase in ipsilateral perivascular influx of a CSF-delivered contrast agent (gadobutrol) within the first 5 min after occlusion (fig. S2B). Significant contrast enhancement was found at all measured sites along the perivascular trajectory of the ipsilateral MCA: ventral segment of the MCA [region of interest 1 (ROI1): 2.9-fold compared with the contralateral hemisphere], convexity

MCA (ROI2: 4.3-fold), and far from the main MCA trunk in the parenchyma (ROI3: 5.5-fold; fig. S2C). Tracer signal was seen deep below the cortical surface of the ipsilateral hemisphere, reaching $450 \pm 43 \mu\text{m}$ at 14 min and $580 \pm 58 \mu\text{m}$ at 28 min after MCAO, indicating that CSF enters the brain tissue (fig. S3, A to E). The entry route was along the glymphatic pathway, with tracer influx occurring primarily along penetrating arterioles (97.7%, versus 2.3% along venules; $P < 0.0001$; fig. S4, A to D) (28). Tracers moved along PVSs of the branching vascular network, including capillaries, and were found deep within parenchyma 30 min after MCAO (fig. S4B). Thus, several independent sets of

analyses point to acceleration of glymphatic influx into the ipsilateral brain in the setting of focal ischemia (6, 8). To identify the driver of CSF influx, we speculated that a loss in blood flow after stroke could cause a hydrostatic pressure gradient that would facilitate CSF flow into the brain. To test this, we measured intracranial pressure (ICP) changes during MCAO (fig. S5A). ICP dropped from 5.49 ± 0.85 to 4.63 ± 0.88 mmHg after MCAO (fig. S5, A and B), but this response was variable between mice and, in some cases, returned to baseline within a few minutes. Thus, the ICP decrease coincided with the first influx peak but could not explain the second, larger peak in CSF influx (Fig. 1F and fig. S5C). We next asked whether this large influx of CSF contributed to the cytotoxic phase of edema. To address this question, cytotoxic edema was detected as a decrease in the apparent diffusion coefficient (ADC) using diffusion-weighted MRI (12). A large ADC lesion first appeared in the primary somatosensory cortex after MCAO and then slowly expanded in the form of a spreading wave across the ipsilateral hemisphere (Movie 3 and fig. S2, D and E). ADC dropped by $17.5 \pm 1.3\%$ within the lesion and spread over $22 \pm 2\%$ of the brain volume within the first 4.8 ± 0.9 min after stroke (fig. S2, F and G). We generated a registered average of the contrast-enhanced and ADC datasets to compare the evolution of cytotoxic edema with CSF influx (Movie 4). The aligned contrast-enhanced and ADC data and the onset times of both indicated that the ADC changes happened simultaneously with the second peak of CSF entry ($P = 0.70$; Fig. 1, F and G). Does the ADC drop trigger the large CSF influx? The wave-like kinetics of the ADC decrease is caused by a SD (29). Ischemic SD consists of waves of sustained, mass depolarizations of cells in the gray matter of the central nervous system that result from the near-complete loss of cellular transmembrane ion gradients (29). Ischemic SD begins in the barrel cortex of the primary somatosensory cortex in rodents (Movie 3) (30) and is thought to trigger cytotoxic edema after ischemia (12). Genetically encoded calcium indicators can be used to visualize the mass depolarization caused by the SD wave (31). To directly test the hypothesis that SD drives glymphatic influx after stroke, we used mice that express GCaMP7 under the *Glt1* promoter (GCaMP) (32). GCaMP mice received an intracisternal tracer injection 15 min before MCAO and were imaged using dual-channel macroscopic imaging (Fig. 3A and Movie 5). Several minutes after MCAO, a spreading wave of GCaMP fluorescence was seen, starting at the primary sensory cortex and traveling slowly over the cortical surface, with CSF tracer following closely behind. The area covered by the SD and the expansion kinetics were consistent between mice, but the onset time varied (Fig. 3B). Aligning the data to the



Movie 1. CSF cisternography during MCAO. Mice that received a right MCAO (left) or sham controls (right) were imaged using 3D-FIESTA MRI. Cisternography revealed that ventricular and subarachnoid CSF (blue) in the ventricles and cisterna magna (yellow arrows) disappeared after stroke, primarily in the ipsilateral hemisphere. Perivascular CSF (red) volume decreased at later time points. The brain tissue and blood compartment (green) increased in volume at the expense of CSF volume decreasing, suggesting that CSF shifts into this compartment. There were no time-dependent changes in the sham mice. Scale bar, 2 mm.

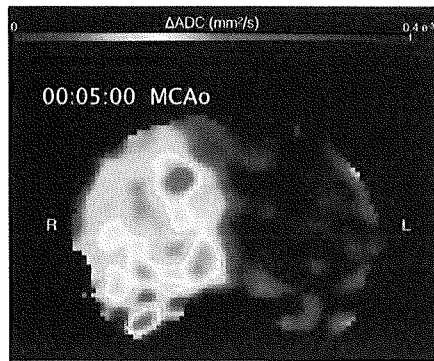


Movie 2. Dynamic contrast-enhanced MRI of intracisternal contrast after stroke. A gadolinium-based tracer (gadobutrol) was injected into the cisterna magna (CM inj) of wild-type mice 15 min before MCAO (labeled MCA-o). Whole-volume sagittal (top left), coronal (bottom left), and dorsoventral (right) projections are shown. Within the first 5 min after MCAO, the tracer is rapidly transported along the perivascular network of the MCA on the ipsilateral hemisphere. Contrast enhancement can be seen over most of the cortical surface and then is seen being distributed in the brain tissue. At later time points, contrast is found outlining the ventricular system, suggesting that labeled cisternal CSF enters the ventricles. R, ipsilateral hemisphere; L, contralateral hemisphere. Scale bar, 2 mm

peak of GCaMP coverage revealed that the SD preceded a 5.6-fold increase in the area covered by CSF tracer (Fig. 3C). The SD peak coincided with maximal CSF influx, and SD onset time was a near-perfect predictor of the time of CSF influx [coefficient of determination (R^2) = 0.94; Fig. 3, D and E]. To gain insight into the spatiotemporal dynamics of the SD wave and CSF influx, we used a front-tracking algorithm (33). The SD wave propagated at a maximum speed of $36 \pm 5 \mu\text{m/s}$, consistent with an isotropic reaction-diffusion mechanism (Fig. 3, F to I) (12). The CSF tracer front sped up after the onset of the SD wave but reached a much slower maximum speed of $16 \pm 1 \mu\text{m/s}$, indicating that the SD might not directly drive the CSF front. To quantify this delay, we computed the time it took CSF tracer to reach each given pixel after the SD wave had passed and found that 30 ± 6 s separated the SD and the tracer fronts (Fig. 3, J and K). Thus, the ischemic SD triggers the onset of glymphatic influx after stroke.

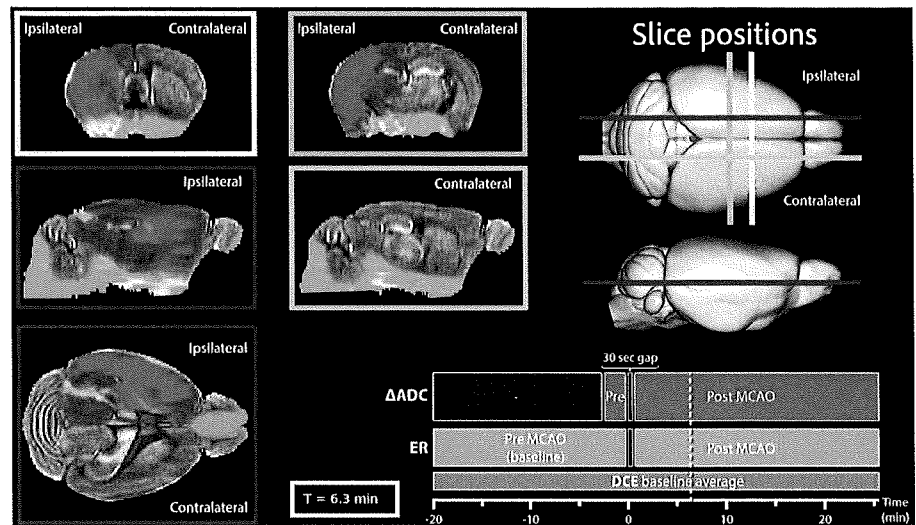
Spreading ischemia drives a rapid increase in perivascular CSF flow

A phenomenon termed spreading ischemia (SI) has been shown to take place 10 to 30 s after SD, which would temporally coincide with glymphatic influx (34–36). Pronounced constriction of parenchymal and pial arterioles occurs after SD, owing to release of K^+ and other vasoactive substances and the depletion of nitric oxide in the endothelium (35, 37). CSF flow within PVSs is highly dependent on arterial diameter changes (38, 39). Could vasoconstriction drive the increase in CSF entry? To address the role of SI in CSF flow, we used two-photon (2P) microscopy (Fig. 4A and Movie 6) (23, 24). Immediately after occlusion, there was a transient loss of intravascular flow as rCBF dropped (Fig. 4, B and C). Despite intravascular dye returning a few seconds later owing to collateral flow, the hemisphere remained severely hypoperfused (Fig. 1F). Several minutes after MCAO, a SD wave propagated across the imaging field, followed by a potent vasoconstriction of the pial and penetrating arterioles, culminating in an intense increase in CSF tracer signal (Fig. 4, D to F, and Movie 7). The propagation speed of the SD wave was comparable to that seen in macroscopic experiments ($42 \pm 2 \mu\text{m/s}$), and the delay between SD and the maximal vasoconstriction (30 ± 2 s) was temporally consistent with the delay between the SD wave and the CSF tracer front from the previous analysis ($P = 0.395$; Fig. 4, G to I). The vasoconstrictive response was variable between mice and arteriolar location, with penetrating arterioles constricting more ($79 \pm 10\%$) and earlier than pial arterioles ($45 \pm 10\%$; Fig. S6, A to D). The variability of this vasomotor response has been attributed to differences in the intravascular perfusion pressure and the location of vessels relative to the infarct core



Movie 3. ADC maps after focal ischemia. Delta ADC (ΔADC) maps were generated from diffusion-weighted MRI. Coronal (top), dorsal (bottom left), and sagittal projections of the right ipsilateral cortical surface (lower right) are shown. A large decrease in ADC can first be seen in the primary sensory cortex at 1:50 min after MCAO (labeled MCAO) and slowly spreads over the entire ipsilateral hemisphere by 4:00 min. The decrease in ADC marks the onset of cytotoxic edema. R, ipsilateral; L, contralateral; Ro, rostral; C, caudal.

(35, 37). Constriction of both the penetrating and pial arterioles was followed by a parallel increase in the space occupied by the PVS tracer (Fig. 4F and fig. S6, B, E, and F) (39). PVSs are believed to form an interconnected hydraulic network, where flow is transported by means of perivascular pumping generated by the cardiac cycle (38). However, we hypothesized that an increase in the size of the distal PVSs owing to vasoconstriction would create a pressure gradient that could effectively speed up perivascular flow. To test the hypothesis that diameter changes in the perivascular network could drive flow, we simulated the event using a topological network from mice pial MCAs (Fig. 5, A and B, and Movie 8) (40). The simulation showed that as a result of the SD, the constricting arteries increased the PVS size, drawing fluid into the tissue owing to reduced pressure in the expanding PVS. This influx of fluid resulted in a 2.2-fold increase in baseline flow speed at the MCA, which eventually decreased (Fig. 5C). To determine whether we could detect a similar increase in CSF flow speed at the inlet of the network, we performed particle tracking velocimetry (38). CSF flow speed was analyzed for changes in pulsatile velocity ($v_{\text{pulsatile}}$) or net flow velocity ($v_{\text{downstream}}$) to determine if flow speed variations were caused by changes in pulsatility or by an overall pressure gradient (Movie 9 and fig. S7, A and B). After MCAO, there was a large, instantaneous increase in $v_{\text{downstream}}$ that quickly returned to baseline. But $v_{\text{pulsatile}}$ never recovered to baseline owing to the occlusion. This first component was consistent with the first peak of CSF influx. At the onset of SD (3.79 ± 0.44 min after MCAO), the large pial MCA also constricted, albeit to a



Movie 4. Co-registered contrast-enhanced and ADC maps from group data. Owing to limitations in temporal resolution, both sequences cannot be collected simultaneously after MCAO. An average was generated from enhancement ratios (ERs) of dynamic contrast-enhanced MRI from four animals (green) and the ΔADC from six animals (red) and overlaid on a population-based average of the dynamic contrast enhancement baseline. After the spreading of cytotoxic edema, the tracer is rapidly distributed throughout the ipsilateral hemisphere. This suggests that CSF influx occurs after the onset of cytotoxic edema. Color-coded frames denote the anatomical position of the slice (top right). The 30-s gap corresponds to the angiography performed immediately after MCAO to confirm successful occlusion. High ΔADC signal change in the ventricles is due to the ventricular space shrinking and being replaced by swollen tissue in the later time points.

lesser degree ($27 \pm 6\%$), and was accompanied by a ~ 2.5 -fold increase in $v_{\text{downstream}}$, whereas $v_{\text{pulsatile}}$ remained below baseline (fig. S7, C and D). The degree of vasoconstriction was a significant predictor of $v_{\text{downstream}}$, with every 10% decrease in diameter causing a twofold increase in flow, but no such correlation was seen with $v_{\text{pulsatile}}$ (fig. S7E). Thus, postischemic CSF influx is not driven by changes in pulsatility but instead by an overall pressure gradient caused by vasoconstriction. The simulation also supported the notion that vasoconstriction is a sufficient mechanism to explain water accumulation in the tissue. However, this does not exclude the possibility that ionic or osmotic gradients secondary to the SD might also play a role in driving flow. In conclusion, both simulations and experimental evidence indicate that SI after SD abruptly accelerates perivascular CSF influx into the brain after stroke.

Spreading edema depends on aquaporin-4 expression

We next asked whether blocking the SD using a glutamate *N*-methyl-D-aspartate receptor antagonist (MK-801) or inhibiting SI using a cocktail of vasodilators (nimodipine, papaverine, and *S*-nitroso-*N*-acetylpenicillamine) would prevent the rapid entry of CSF (fig. S8). Both approaches have been shown to modulate either the spatiotemporal dynamics of SD or the severity of SI, but neither has been shown to inhibit completely the effects of the first depola-

rization after an ischemic insult (35, 36, 41–44). In our model, pretreatment with MK-801 or infusion of a vasodilator cocktail was ineffective in blocking the SD or SI (fig. S8, A to J). As before, MCAO was followed by a three- to four-fold increase in CSF influx in all groups (fig. S8, K to N; $P = 0.39$). However, residual CBF after SI was a significant predictor of the degree of CSF influx, suggesting that targeting reperfusion and SI might be a more feasible approach than trying to block the SD (fig. S8O). Alternatively, CSF transport into the brain has been shown to be facilitated by aquaporin-4 (AQP4) water channels expressed on the endfeet of astrocytes that form the outer wall of the penetrating PVS (23, 45). Because AQP4 facilitates the transport of CSF out of the PVS, we hypothesized that AQP4-null mice (*Aqp4*^{−/−}) might have reduced CSF influx after MCAO (Fig. 6). This is in line with evidence demonstrating that knockout animals and wild types treated with AQP4 inhibitors are protected from edema after stroke (46–49). Hence, we tested the effect of AQP4 knockout on CSF tracer entry after MCAO using transcranial macroscopic imaging (Fig. 6, A and B). As with wild types (*Aqp4*^{+/+}), *Aqp4*^{−/−} mice had a SD followed by SI after occlusion (Fig. 6C). Likewise, CSF tracer also distributed in the ipsilateral cortex but to a reduced degree in *Aqp4*^{−/−} mice compared with wild type ($P = 0.018$; Fig. 6D). Could the absence of AQP4 at the perivascular endfoot reduce the entry of CSF to the tissue? To test this,

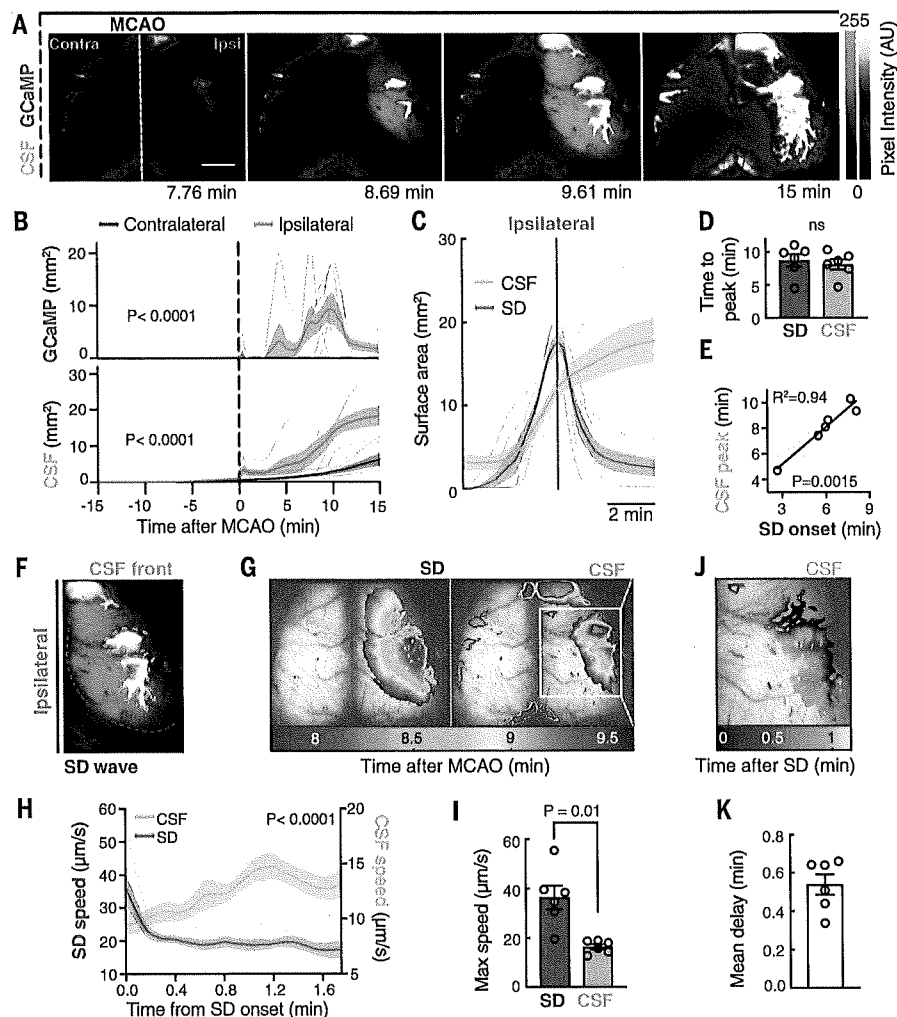
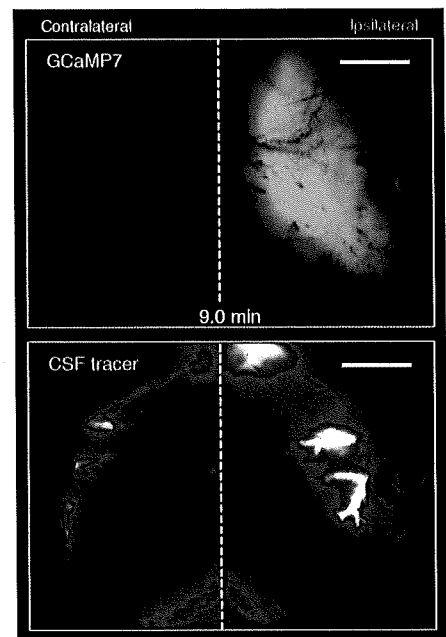


Fig. 3. CSF influx is triggered by SDs after focal ischemia. (A) A fluorescent CSF tracer (BSA-594) was delivered into the cisterna magna of *Glt1*-GCaMP7 mice 15 min before MCAO and imaged using a dual-channel macroscope for 15 min, as was done in Fig. 1D. Scale bar, 2 mm. (B) Area covered by GCaMP and CSF tracer fluorescence. Repeated measures two-way ANOVA with Sidak's multiple comparisons test was performed; interaction P value < 0.0001; $n = 6$ mice. (C) CSF tracer surface area aligned to the SD (GCaMP) peak. (D) Time to peak influx rate for the SD (GCaMP) and the CSF tracer. Paired Student's t test was performed. (E) Linear regression of CSF peak influx and SD onset time with 95% confidence intervals. (F) The fronts of the ipsilateral SD wave and the CSF tracer were tracked. (G) Area covered by the SD (left) and the CSF tracer (right) over time. (H) Front speed of the SD wave and the CSF front. Repeated measures two-way ANOVA with Sidak's multiple comparisons test was performed; interaction P value < 0.0001. (I) Maximum speed of the SD and CSF front. Paired Student's t test was performed. (J) Delay time between the SD wave and the CSF tracer front. (K) Mean delay between the SD and CSF front. In (B), (C), and (H), the shaded regions above and below the plot lines indicate SEM. In (D), (I), and (K), error bars represent SEM.

we acquired 2P imaging after intracisternal infusion of a small CSF tracer (3-kDa dextran; Fig. 6E). During SI, tracer entered the penetrating PVS in large quantities, reaching depths of up to 200 μ m below the cortical surface and distributing into the ISF in wild type (Fig. 6F). *Aqp4*^{-/-} mice have been shown to have abnormal SD kinetics, so we first determined whether differences in the vasodynamics of penetrating arterioles during SI could be responsible for the differential response in CSF influx. Knockout

animals exhibited slower arteriolar constrictions but to a similar degree as *Aqp4*^{+/+} animals, which would not explain the observed results (Fig. 6, G to J). We then looked to see how much traced CSF actually reached the penetrating PVS and how much of it exchanged with the ISF. Less tracer entered the penetrating PVSs of *Aqp4*^{-/-} mice compared with their wild-type counterparts ($P < 0.0001$; Fig. 6K). At the same time, less of the CSF tracer could be found in the neighboring ISF of the



Movie 5. Transcranial macroscopic imaging of CSF tracer in GCaMP mice after MCAO. Dual-channel transcranial macroscopic imaging in a mouse that expresses GCaMP7 under the *Glt1* promoter (top left) after intracisternal injection of a fluorescent CSF tracer 15 min before MCAO (BSA-594; bottom left). After MCAO (dashed line), there is a rapid drop in rCBF. The surface area covered by the fluorescent front was quantified in the ipsilateral and contralateral hemispheres. Several minutes after occlusion, there is a SD in the ipsilateral hemisphere, triggering CSF influx. Scale bar, 2 mm.

penetrating PVS in the knockouts, as in previous reports (Fig. 6L) (23). Global tracer quantification in ex vivo brain sections confirmed a significant reduction in fluorescence intensity in the ipsilateral and contralateral hemisphere of *Aqp4*^{-/-} mice ($P = 0.005$ and 0.001 , respectively; Fig. 6, M to O). In line with these results, AQP4 knockout animals did not develop edema within the first 15 min after MCAO, as opposed to *Aqp4*^{+/+} mice (Figs. 6P and 1H). Thus, deletion of AQP4 significantly suppresses ischemia-induced CSF tracer influx.

Discussion

Historically, brain swelling has been assumed to develop entirely through the accumulation of fluid from the intravascular compartment (15). The present study demonstrates that CSF influx, initiated by ischemic SD, plays an important role in driving acute tissue swelling (Movie 10) (16, 50–52). This new finding is further supported by a detailed examination of edema formation at CSF-ISF exchange zones, which we observed in cortex adjacent to leptomeningeal vessels as well as in periventricular zones 30 min after MCAO in mice and during the acute phase of human cerebral ischemic

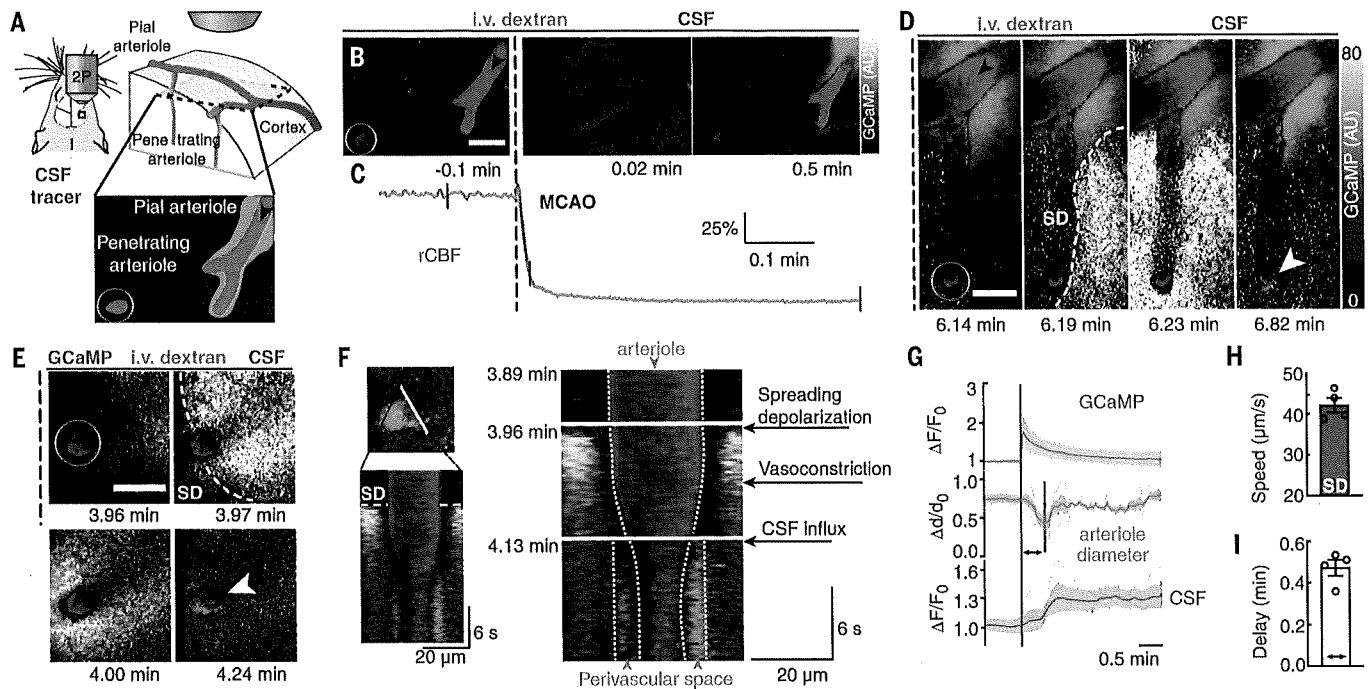


Fig. 4. SI after SD drives perivascular CSF influx. (A) Pial (black arrows) and penetrating arterioles (red circles, 40 to 50 μm below surface), which are branches of the ipsilateral MCA, were imaged using 2P microscopy. (B) Pial and penetrating arteriole (i.v. dextran) in a *Glt1-GCaMP7* mouse after receiving an intracisternal tracer injection (BSA-647) during MCAO. (C) rCBF measurements. The ticks align with images in (B). (D) SD after MCAO. Normalized GCaMP fluorescence ($\Delta F - F_{\text{average}}$) is color coded for pixel intensity and displayed in arbitrary units. (E) Constriction of penetrating arteries after SD causes tracer influx into the brain (white arrow). (F) Line

scan over the penetrating arteriole in (E) depicting the appearance of the SD, the subsequent vasoconstriction, and the CSF tracer influx filling the PVS left by the constricted arteriole. (G) Quantification of GCaMP and CSF tracer fluorescence ($\Delta F/F_0$) and arteriole diameter ($\Delta d/d_0$) aligned to the onset of the SD; $n = 4$ mice. The shaded regions above and below the plot lines indicate SEM. (H) SD wave speed. (I) Delay time between SD onset and minimum arteriolar diameter. The double-headed arrow is a visual representation of the delay shown in the second plot in (G). In (H) and (I), error bars represent SEM. Scale bars in (B) to (E), 50 μm .

infarction (fig. S9 and table S1). Edema was primarily seen in brain regions that are known flow pathways for CSF-ISF rather than in deeper structures such as striatum that are farther away. Is glymphatic edema a previously unrecognized contributor to secondary ischemic injury? The entry of CSF through PVSs is likely to facilitate swelling of astrocytic endfeet and, together with pericyte constriction, would further reduce blood flow and cause infarct expansion (53, 54). However, this study does not exclude the possibility that vascular fluid is a substantial source of swelling in later stages after stroke, because transvascular ion and fluid influx are increasingly important at 24 hours after MCAO when the BBB is already open (fig. S10, A to D). Several hours after CSF influx, de novo expression of the nonselective monovalent cation channel SUR1-TRPM4 in brain endothelial cells contributes to additional Na^+ influx, and blockade of these transporters with glibenclamide reduces edema (5, 55). Nonetheless, CSF production continues to be a significant source of Na^+ and water after stroke (fig. S10, E to H). Our results posit that CSF is the earliest source of Na^+ and fluid, driving tissue swelling. This early phase is likely to play an additive role to

the later stages of edema formation. Perhaps even more important is the fact that SDs continue for several days after stroke and are also observed in other acute conditions, including subarachnoid hemorrhage, intracerebral hemorrhage, and traumatic brain injury (29). Our study predicts that SDs will worsen edema and possibly explains why the frequency of depolarizations correlates with secondary injury and poor outcome (29, 39, 56–58). Thus, targeting glymphatic edema may offer a therapeutic strategy for treatment of a broad range of acute brain pathologies.

Materials and Methods

Animals

All experiments were approved by the University Committee on Animal Resources of the University of Rochester and the Danish Animal Experiments Inspectorate. Efforts were taken to minimize the number of animals used. Male C57BL/6 mice (Charles River) or *Glt1-GCaMP7* mice (RIKEN BioResource Research Center, Japan) on a C57BL/6 background 8 weeks of age were used for all the experiments. *Glt1-GCaMP7* mice express GCaMP7 in 95.2% of cortical astrocytes with minimal expression

in other glial cell types and about 50% of cortical neurons in L4 and L6 (32). Male 8-week-old NG2-dsRed (Jackson Laboratory, stock no. 008241) mice were used to identify perivascular inflow routes. *Aqp4*-knockout (*Aqp4*^{−/−}) mice on a C57BL/6 background between 8 and 16 weeks old were used (45). *Aqp4*^{−/−} mice have been shown to have slower SD, delayed $[\text{K}]_e$ increase and uptake kinetics (59). The knockouts also exhibit shorter extracellular glutamate increases compared with wild type (60). Some of these studies have attributed this difference to the larger extracellular space volume observed in these mice, causing a slower increase in $[\text{K}]_e$ during depolarization (59). Other studies have instead credited it to AQP4-dependent changes in volume-regulated anion channels (60). *Aqp4*^{−/−} mice also show significant differences in O_2 diffusion, especially to remote areas away from microvessels (61). All experiments performed in this study were done on mice anesthetized with ketamine and xylazine (100 and 10 mg/kg, intraperitoneally). The vasomotor response to SDs in naïve cortex is different in mice compared with other species (29). However, in ischemic cortex, as seen in our model of MCAO, SI is

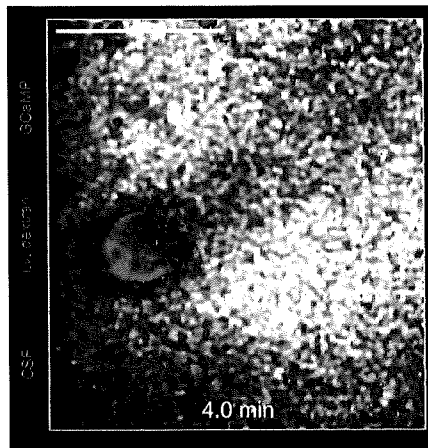


Movie 6. 2P imaging of a SD at a pial and penetrating arteriole after stroke. An anesthetized *Glt1-GCaMP7* (purple) mouse received intravenous tetramethylrhodamine isothiocyanate (TRITC)-dextran injection (i.v. dextran, 2000 kDa, red) and intracisternal BSA-647 tracer (CSF, green). At early time points, CSF tracer can first be seen around the pial arterioles. At ~6.2 min after MCAO, a SD can be seen crossing over the imaging field (GCaMP fluorescence, $\Delta F/F_0$). Then at ~6.5 min, there is a pronounced vasoconstriction of the penetrating arteriole, $\Delta d/d_0$, and a parallel increase in the amount of tracer in the penetrating PVS. At ~6.8 min, there is a smaller constriction of the pial arteriole. Both vasomotor events cause the CSF tracer fluorescence to intensify several minutes after MCAO (CSF tracer fluorescence, $\Delta F/F_0$). Scale bar, 50 μm .

the predominant vasomotor response after SD across a variety of diseases and animal models [i.e., mice (35), rats (62), cats (34), swine (63), and humans (64–67)].

Intracisternal injections

Animals were fixed in a stereotaxic frame. A 30-gauge needle was connected to PE-10 tubing filled with artificial CSF (aCSF) into the cisterna magna as described here (68). For intracisternal



Movie 7. 2P imaging of SI at a penetrating arteriole after stroke. An anesthetized *Glt1-GCaMP7* (purple) mouse received intravenous TRITC-dextran injection (i.v. dextran, 2000 kDa, red) and intracisternal BSA-647 tracer (CSF, green). At ~3.9 min after MCAO, a SD covers the field of view (GCaMP fluorescence, $\Delta F/F_0$). Several seconds later, the penetrating arteriole constricts to ~80% of its pre-MCAO diameter, $\Delta d/d_0$. In response to this, CSF tracer fills the penetrating PVS (CSF tracer fluorescence, $\Delta F/F_0$). Scale bar, 50 μm .

injections, 10 μl of CSF tracer was injected at a rate of 2 $\mu\text{l}/\text{min}$ over 5 min with a syringe pump (Harvard Apparatus).

CSF tracers

Alexa Fluor647- or Alexa Fluor594-conjugated bovine serum albumin (BSA-647 or BSA-594, 66 kDa, Invitrogen) and Cascade Blue-conjugated 3-kDa dextran (Invitrogen) were diluted in aCSF (0.5 or 1% m/v) and used as a fluorescent CSF tracer. Radio-labeled ^{22}Na (0.5 μCi , Perkin Elmer) and ^3H -mannitol (1 μCi , American Radiolabeled Chemicals) were dissolved in aCSF. For particle tracking velocimetry experiments, red fluorescent polystyrene microspheres (FluoSpheres 1.0 μm , excitation 580 nm, emission 605 nm, 0.25% solids in aCSF, Invitrogen) were briefly sonicated and infused as before (38).

Drugs

MK-801 (5 mg/kg in 0.9% NaCl; Tocris) was administered by intraperitoneal injection 15 min before MCAO. The vasodilator cocktail contained nimodipine (2 $\mu\text{g}/\text{kg}/\text{min}$; Tocris), papaverine (3 mg/kg/min; Sigma Aldrich) and *S*-nitroso-*N*-acetylpenicillamine (3 $\mu\text{mol}/\text{kg}/\text{min}$ in 0.9% NaCl; Sigma-Aldrich) and was delivered intravenously through a PE-10 tubing catheter in the femoral vein using an infusion pump (Harvard Apparatus).

Physiological recordings

Heart rate and respiratory rate were acquired using an animal physiological monitoring device (Harvard Apparatus). ICP was measured

through a 30-gauge needle connected to a PE-10 catheter filled with aCSF into the cisterna magna. The line was connected to a pressure transducer and monitor (World Precision Instruments). rCBF was measured using laser Doppler flowmetry (PF5010 Laser Doppler Perfusion Module, PR 418-1, Perimed). The fiber optic probe (MT500-0, Perimed) was fixed onto the skull above the MCA vascular territory (5 mm lateral and 1 mm posterior to bregma) on the right hemisphere with cyanoacrylate glue. For 2P experiments, rCBF was measured at the lateral aspect of the skull over the right temporal bone in order to place the fiber optic probe below the headplate and shield it from laser irradiation. All the signals were collected using a 1440A digitizer and AxoScope software (Axon Instruments) and analyzed using Matlab.

MCAO

The anesthetized animal was laid on its back on a heating pad. A surgical midline incision was made from the clavicle to the chin to expose the right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA). Then the distal side of ECA and proximal side of CCA were ligated by 7-0 silk suture. The distal side of the ICA was clamped using a micro clamp (B-1, Fine Science Tools). A small incision was subsequently made in the proximal portion of the CCA. A PE-10 polyethylene tube (PE-10, Beckton-Dickinson) filled with heparinized saline and six macrospheres, 200 μm in diameter, was inserted into the CCA and the clamp was subsequently removed. The tubing was secured in position by 7-0 silk suture. The macrospheres were advanced into the ICA via an injection of 0.02 ml heparinized saline. Ischemia was confirmed by laser Doppler flowmetry. The incision was closed with a 5-0 suture. All mice were monitored using laser Doppler flowmetry during the macrosphere infusion. If rCBF did not decrease >70% of baseline or if rCBF returned to baseline, the animal was excluded.

Behavior testing

Twenty-four hours after MCAO or sham, mice were placed in a 45-cm-by-61-cm open-field apparatus. Movement of the mice was recorded for 30 min and then analyzed on ANY-maze video tracking software. MCAO and sham mice were placed on an accelerating rota-rod (Ugo Basile) that accelerated from 5 to 40 rpm. The mice were allowed to remain on the rota-rod until they could no longer continue, and a final time was recorded once they fell off the rotating cylinder. MCAO and sham mice were allowed to grasp a wire bar suspended 43 cm above the surface with only their forepaws before being released. Each mouse was tested for three 20-s trials and given a score from 0 to 3 depending on how well it was able to stay on the string.

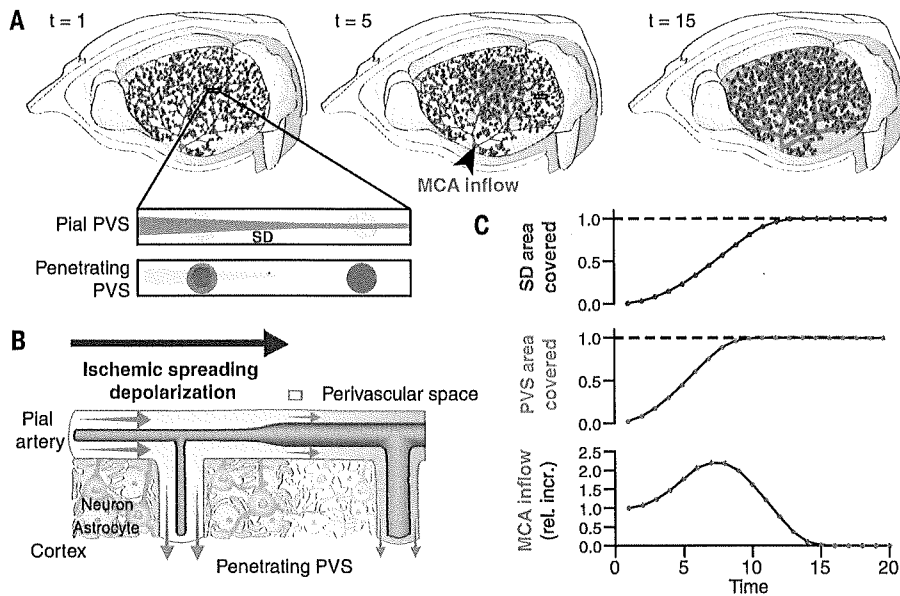


Fig. 5. Topological glymphatic network model of PVSs around the mouse MCA. (A) Network model representing a system of interconnected PVSs surrounding the mouse pial MCA at different time points (*t*) during an ischemic SD (green). Pial PVSs are depicted as blue lines that get wider during SI. Penetrating arteries are depicted as red circles, and the size of the surrounding blue circle is proportional to the flow rate into the penetrating PVS as the arteriole constricts after SD. The simulation evaluated the relative increase in baseline flow at the inlet of the MCA (MCA inflow; black arrow). (B and C) Pial and penetrating PVS area increases as the arteries constrict owing to the passage of a SD, thus increasing the fluid volume in the network (B). Conservation of mass controls the resulting MCA inflow (C), resulting in a net increase in fluid volume in the network. The dashed line represents the tissue border of the cortical surface. The SD travels over the entire cortex, spanning an area larger than that covered by the MCA network. rel. incr., relative increase.

A score of 3 was given to mice who would immediately fall from the string when released, a 2 if the mouse was able to hang on the string and attempted to climb, and a 1 was given to mice that were able to put both forepaws and one or both hind paws onto the string; if the mouse placed all four paws and the tail onto the string and made lateral movement, a score of 0 was given (69).

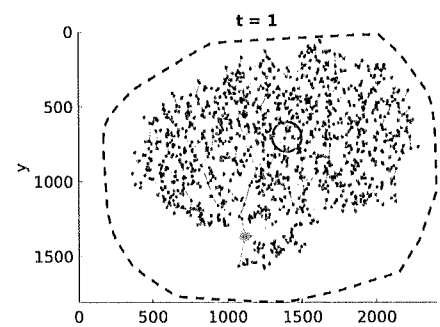
Transcranial optical imaging

Mice were fixed to either a MAG-1 or MAG-2 stereotaxic holding device (Narishige) and injection, via a cisterna magna cannula, of 10 μ l of BSA-conjugated Alexa Fluor-594 or -647 (Invitrogen) in aCSF, occurred over a period of 5 min using a syringe pump (Harvard Apparatus). Whole-cortical macroscopic imaging was performed using either an Olympus MVX10 with a PRIOR Lumen LED and Hamamatsu ORCA-Flash4.0 V2 Digital CMOS camera using Metamorph software or a Leica M205 FA fluorescence stereomicroscope equipped with an Xcite 200DC light source and A12801-01 W-View GEMINI (Hamamatsu) for simultaneous GFP (excitation 480 nm, emission 510 nm, Leica) and Cy3 (excitation 560 nm, emission 630 nm, Leica) acquisition. Images were acquired with a Hamamatsu ORCA-Flash4.0 V2 Digital CMOS

camera using LAS X Leica software, with a 512-pixel-by-512-pixel, 16-bit resolution, at 20 Hz.

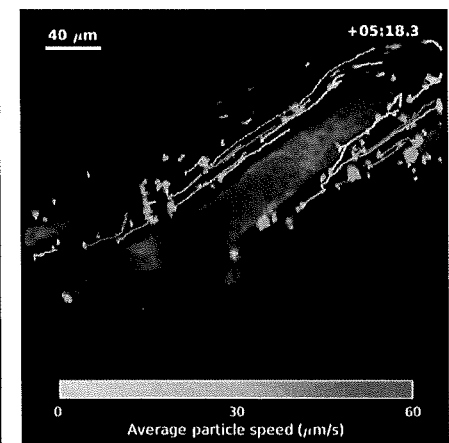
MRI scans

MRI was performed using a 9.4-tesla animal scanner (BioSpec 94/30 USR, Bruker BioSpin, Ettlingen, Germany) equipped with a cryogenically cooled quadrature-resonator (CryoProbe, Bruker BioSpin, Ettlingen, Germany). Mice were placed on a magnetic resonance (MR)-compatible stereotaxic holder with ear bars to minimize head movement during scanning. Body temperature was maintained at 37°C and monitored along with the breathing rate by a remote monitoring system (SA Instruments, NY, USA). All required adjustments and T2-weighted scans [2D TurboRARE: TR/TE: 8000/36 ms, Matrix 384 by 256, field of view (FOV) 19.2 mm by 12.8 mm, NEX 1, 48 horizontal slices, slice thickness = 0.2 mm] were performed within 15 min after CCA catheter placement for visualization of the geometry before the baseline dynamic contrast enhancement (DCE), diffusion-weighted imaging (DWI), or cisternography scans. Ischemia was confirmed by flow-compensated 2D time-of-flight MR angiography scans (2D-GEFC: TR/TE 10/2.4 ms, Matrix 192 by 128, FA 80, FOV 19.2 mm by 12.8 mm, NEX 1, 10 horizontal slices, slice



Movie 8. Topological glymphatic network

simulation of perivascular CSF influx. A network was generated from a mouse pial MCA. Nodes were located at bifurcations (black squares) and penetrating arteries (red squares). Edges represent the perivascular flow pathways following the trajectory of the MCA (blue). The SD (green) starts at the proximal MCA (blue circle) and spreads outward toward the cortical tissue border (dashed line). As the SD propagates isotropically, there is a delayed vasoconstrictive response of the pial and penetrating arterioles, causing the area of the PVSs to increase and fluid volume within the network to follow. This spreading vasoconstriction causes flow speed at the MCA inlet to increase.



Movie 9. Particle tracking velocimetry in the

PVS of the MCA. Fluorescent 1- μ m microspheres were injected into the cisterna magna of an anesthetized wild-type mouse after labeling the vasculature with a fluorescent dextran [fluorescein isothiocyanate (FITC)-dextran, 2000 kDa, i.v.]. 2P imaging of the particles in the PVS of the MCA (left) is shown. Particle tracks are color coded to the average particle speed (μ m/s). The average speed of all the particles (v_{mean}) is plotted simultaneously to quantification of $v_{\text{downstream}}$ and $v_{\text{pulsatile}}$, rCBF (in percent pressure units, p.u.), and artery diameter (μ m). Around 300 s after MCAO, there is marked vasoconstriction and CSF flow speed increases. Time denotes minutes after MCAO. Scale bar, 40 μ m.

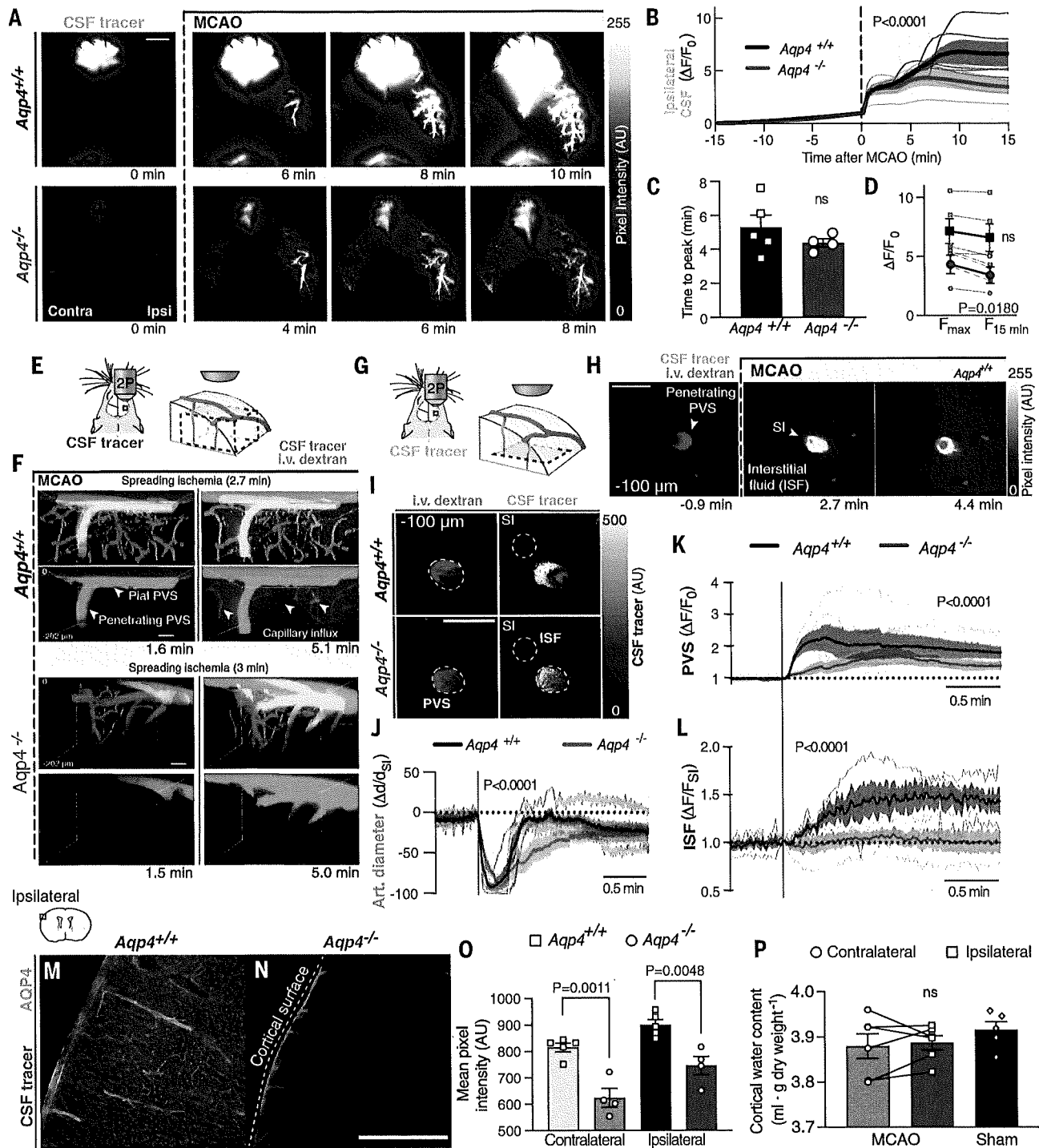
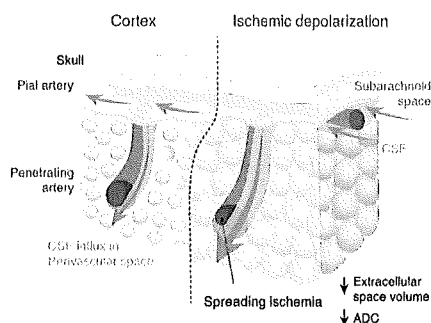


Fig. 6. CSF-mediated edema after MCAO is dependent on AQP4 expression. (A) Transcranial imaging after intracisternal tracer injection (BSA-647) in *Aqp4*^{+/+} and *Aqp4*^{-/-} mice. (B) Quantification of ipsilateral CSF tracer influx ($\Delta F/F_0$). Two-way repeated measures ANOVA was performed; P value from interaction term < 0.0001 ; $n = 4$ or 5 mice per genotype. (C) Time of CSF influx peak. Unpaired Student's t test was performed. (D) Change in maximum $\Delta F/F_0$ ($F_{\max}$) to 15 min after MCAO ($F_{15 \text{ min}}$). Paired Student's t test was performed. (E and F) Volumetric 2P imaging shows CSF tracer (3-kDa dextran) entering the brain via the surface and penetrating PVSs in *Aqp4*^{+/+} and *Aqp4*^{-/-} mice. After SI, the tracers were also found surrounding capillaries only in *Aqp4*^{+/+}. (G) Time-lapse 2P imaging of a penetrating arteriole 100 μm below the cortical surface. (H) The tracer enters the ISF after SI. (I) CSF tracer entered the penetrating PVS of both *Aqp4*^{+/+} and

Aqp4^{-/-} mice. The dashed circles indicate the regions of interest used for the quantifications in (K) and (L). (J to L) Penetrating arteriole (art.) diameter changes after MCAO (J). Tracer influx was quantified in the penetrating PVS (K) and the ISF neighboring the same PVS (L). One PVS per mouse was analyzed; $n = 4$ or 5 mice per genotype. (M and N) Immunohistochemical labeling for AQP4 from ipsilateral dorsal cortex of *Aqp4*^{+/+} (M) and *Aqp4*^{-/-} (N) after CSF tracer injection. (O) Mean pixel intensity from six coronal sections from 4 or 5 mice per genotype. (P) Cortical water content 15 min after MCAO or bilateral cortices from sham-treated *Aqp4*^{-/-} mice. Paired Student's t test was performed; $n = 5$ or 6 mice per group. Scale bars, 2 mm (A), 50 μm (F) to (I), and 500 μm (M) and (N). In (B), (J), (K), and (L), the shaded regions above and below the plot lines indicate SEM. In (C), (O), and (P), error bars represent SEM.



Movie 10. Spreading glymphatic edema animation. SDs propagate over the cortex, followed by SI. As the penetrating and pial arterioles constrict, the volume of the perivascular network increases, driving flow into the parenchyma. Cytotoxic edema and CSF influx cause cells to swell and the extracellular space to shrink, seen as a decrease in the ADC.

thickness = 0.3 mm) 30 s immediately after macrosphere infusion: If angiography did not show occlusion of MCA, the animal was excluded.

DCE MRI

Pre- and postcontrast T1-weighted (T1W) images were collected with 3D-FLASH sequence (TR/TE 17.7/3.1 ms, FA = 15°, Matrix 192 by 128 by 96, FOV 19.2 mm by 12.8 mm by 9.6 mm, NEX 1). T1W imaging of the entire mouse brain was performed in 2 min at an isotropic spatial resolution of 100 μ m as a T1-enhancing contrast agent, gadobutrol (Gadovist, Bayer Pharma AG, Leverkusen, Germany), was injected into cisterna magna. The time series T1W scanning protocol was composed of three baseline scans (6 min) followed by intracisternal infusion of 12.5 mM gadobutrol at a constant rate of 1.0 μ l/min for 10 min. Considering the dead space within the catheter, the actual infused volume of the contrast was 9 μ l. Scans continued over 50 measurements, and MCAO started at 24 min after the first T1W scan.

DWI

DWI was performed with 2D single-shot echo-planar DWI sequence with parameters set as follows: TR/TE 2500/20 ms, NEX 1, FOV 18 mm by 15 mm, Matrix 108 by 96, eight axial slices, slice thickness 0.8 mm. Z-direction motion probing gradient was acquired with multiple b values (0, 100, 400, and 1000 s/mm²). For the DWI study, 30 baseline images before MCAO were acquired, followed by a series of images acquired over time after MCAO. Scans continued over 360 measurements, with each measurement lasting 10 s. Maps of the mean diffusivity (ADC map) were derived using the standard algorithm of Paravision 6.0.1 software (Bruker).

MR CSF cisternography

CSF cisternography was performed with 3D-FIESTA sequence (TR/TE 17.7/3.1 ms, FA = 15°,

Matrix 192 by 128 by 96, FOV 19.2 mm by 12.8 mm by 9.6 mm, NEX 1). For CSF volumetric measurements, 10 baseline images were acquired before MCAO, followed by a series of images acquired over time after MCAO. Scans continued over 60 measurements, with each measurement lasting 30 s. Ventricular compartments were segmented, and temporal volume change was measured by IMARIS (v. 9.2.1, Bitplane, Concord, MA, USA).

In vivo 2P laser scanning microscopy

A cranial window was prepared over the right anterolateral parietal bone above the MCA vascular territory. The dura mater was left intact and, to prevent intracranial depressurization, the window was sealed with agarose (1.1% at 37°C) and a glass coverslip (8 mm diameter). Unsealed craniotomies have been shown to affect glymphatic function (45). 2P imaging was performed using a resonant scanner Bergamo scope (Thorlabs) and a Chameleon Ultra II laser (Coherent) with a water-immersion 20 $\times$ objective (1.0 NA, Olympus). Intravascular FITC-dextran (2000 kDa, 2.5%, Sigma-Aldrich) and either red microspheres or BSA-647 were excited at an 820-nm wavelength. In GCaMP mice, vessels were labeled with TRITC-dextran (2000 kDa) and CSF with BSA-647 and excited at 860 nm. Emission was filtered at 525, 607, and 647 nm. Images were acquired using ThorImage software and synchronized with physiological recordings (3 kHz, ThorSync software).

Quantification of CSF production rate

CSF production rate was quantified as previously described in rats (70, 71). Anesthetized mice were fixed on a stereotactic apparatus, and a 0.5 mm burr hole was made over the left lateral ventricle (anterior-posterior = -0.1 mm, medial-lateral = -0.85 mm). A 30-gauge needle connected to PE-10 tubing was lowered through the burr hole to -2.00 mm dorsal-ventral. The mouse then received a MCAO as before. The rCBF drop was confirmed with laser Doppler flowmetry for 5 mins, and then the mouse was placed back in a stereotactic head frame and the cisterna magna was surgically exposed with the mouse's neck flexed at 90°. A 30-gauge needle connected to PE10 tubing filled with mineral oil (Sigma Aldrich, M5904) was inserted into the cisterna magna and advanced gently 2 mm through the foramen of Magendie into the fourth ventricle. One microliter of mineral oil was infused at a rate of 1 μ l/min for 1 min with a syringe pump (Harvard Apparatus) to block outflow from the ventricular system. The location of CSF within the intraventricular PE-10 tubing was marked at 10-min intervals. The volume of CSF was calculated as: CSF volume = $\pi \times (\text{internal radius: } 0.14 \text{ mm})^2 \times \text{length}$. The rate of CSF production (μ l/min) was calculated as the slope of the linear regression from each mouse.

Brain water content measurements

Anesthetized animals were decapitated immediately, and the cortex was dissected and weighed (W_{wet} ; g). The tissue was dried at 65°C until it reached a constant weight (~48 hours) and brains were reweighed (W_{dry}). For calculations of the tissue water content (ml/g dry weight), the following formula was used: $(W_{\text{wet}} - W_{\text{dry}})/W_{\text{dry}}$.

Radioisotope influx

To evaluate CSF influx into the brain, radio-labeled tracers ²²Na (0.5 μ Ci, Perkin Elmer) and ³H-mannitol (1 μ Ci, Perkin Elmer) were injected into the cisterna magna at 2 μ l/min for 5 min in aCSF and allowed to circulate for 15 min before MCAO. After 15 min, the animals were rapidly decapitated, the skull and dura were removed, and the brain was harvested. Brains were cut into six sections for processing. In a separate set of experiments, the radio-labeled tracers were injected intravenously first as a 2 μ l bolus and then a 2 μ l/min infusion for 5 min. Immediately after the end of the infusion, MCAO was induced, and 15 min later, animals were rapidly decapitated, the skull and dura were removed, and the brain was harvested. All brain tissue was weighed and solubilized in 0.5 ml of tissue solubilizer (Solvable, PerkinElmer) overnight. Upon solubilization, 5 ml of scintillation cocktail was added (Ultima Gold, PerkinElmer). The injectate controls were treated in the same way as the tissue samples. All samples were analyzed by liquid scintillation spectrometry using a scintillation counter (LS 6500 Multipurpose Scintillation Counter, Beckman Coulter). The radioactivity (counts per minute) remaining in the brain after injection (percentage of injected dose) was determined as $R_b/R_i \times 100$, where R_b is the radioactivity remaining in the brain at the end of the experiment and R_i is the radioactivity in the injectate controls for each experiment. Only cerebrum was considered, and cerebellum was excluded from analysis because ischemia does not reach this region. All samples were background subtracted to blank samples with 0.5 ml of tissue solubilizer and 5 ml of scintillation cocktail.

Triphenyltetrazolium chloride staining

Triphenyltetrazolium chloride (TTC) was prepared at 2% in phosphate-buffered saline (PBS) and kept in a 37°C water bath. MCAO and sham mice were anesthetized and perfused with 5 to 10 ml of ice-cold PBS. Mice were immediately decapitated, and the brains were extracted and put into TTC. Brains remained in the TTC solution for 15 min and then imaged under an Olympus SZX12 bright-field microscope. In a separate experiment, the brains were sectioned into slices 1 mm thick on a vibratome (Leica VT1200S) in ice-cold PBS. The coronal slices were placed into TTC for

15 min and then 4% paraformaldehyde (PFA) for an additional 30 min. The coronal sections were then imaged under the microscope for infarct volume quantification. The area of the infarct volume was calculated in each slice by tracing the infarct core using the polygon selection tool in Fiji. Area (mm^2) of the infarct was then converted to volume (mm^3) accounting for the 1 mm slice thickness. The sum of all the coronal infarct volumes was calculated to give whole brain infarct volume. The edema-corrected infarct volume was calculated as previously described (72) and expressed as a percentage of the contralateral hemisphere.

Arteriole and venule labeling experiments

NG2-dsRed mice were anesthetized and received an intravenous injection of 0.2 ml of 1 mg/ml wheat-germ agglutinin lectin (Invitrogen Alexa Fluor 647 conjugated) into the saphenous vein. Next, a fluorescent CSF tracer (0.5% 3-kDa dextran Cascade Blue; Invitrogen) was infused into the cisterna magna and allowed to circulate for 15 min before MCAO. Thirty min later, the mouse was perfusion-fixed with PBS (100 μg lectin) followed by 4% PFA, and then the brains were harvested. The following day, the brains were sectioned into 100- μm slices using a vibratome (Leica VT1200S) and mounted onto slides with ProLong Gold antifade mounting medium (Invitrogen). Images were acquired using a Leica TCS SP8 confocal system. A total of 8 to 10 Z-stacks at 20 $\times$ magnification from cortex were taken from four to six coronal sections from each mouse. The percentage of tracer-positive arterioles and venules was calculated from the total number of blood vessels with CSF tracer labeling (42 to 49 vessels per mouse).

Tissue histology

Mouse brains were obtained after sacrifice, after sham or MCAO, and fixed in 4% PFA. Patients who died with acute cerebral ischemia documented between January 2018 and December 2018 and who underwent autopsy were identified retrospectively by reviewing the autopsy records of the Department of Pathology at the University of Rochester Medical Center. With these constraints, five focal cerebral infarct specimens from patients were identified. As controls, brain samples were also evaluated from adult patients with documented absence of an ischemic brain lesion who died from acute cardiorespiratory collapse. In all cases, the presence or absence of an ischemic brain lesion was confirmed by a neuropathologist. Standard tissue fixation protocols (7 to 10 days in formalin) were applied. Paraffin-embedded tissue blocks from frontal cortex, basal ganglia, and periventricular regions were obtained in each case by an expert neuropathologist (RIM). Blocks were sectioned at 6 μm thickness, stained with

hematoxylin and eosin (HE), and imaged at 1000 $\times$ (oil immersion) using a Nikon NIU Microscope and Nikon Microscope Solutions Imaging Software (NIS-Elements AR Version 4.30.01). Edema area was quantified by a blinded rater using Fiji. HE images were automatically thresholded to include the highest-intensity pixels (Otsu's method), which consisted of the white pixels of the fluid accumulation. The edema area was measured as a percentage of the total tissue area from a representative image from each animal or subject. In the case of mouse cortex (fig. S9B), each biological replicate was an average from four separate images from the same mouse. For coronal sections in Fig. 6, M to O, brains were sliced into 100- μm sections using a vibratome (Leica VT1200S) and mounted onto slides with ProLong Gold antifade mounting medium (Invitrogen). Images were acquired with an Olympus MVX10 stereomicroscope and a PRIOR Lumen LED and Hamamatsu ORCA-Flash4.0 V2 Digital CMOS camera using Metamorph software. Images were analyzed as previously described (33). Mean pixel intensity for six sections starting at the anterior aspect of the corpus callosum and skipping 400- μm intervals moving posteriorly was quantified using ROIs for the ipsilateral and contralateral hemispheres in Fiji. An average of the six coronal sections was computed for each mouse.

Transcranial optical imaging analysis

An ROI was manually drawn around the skull, and fluorescence intensity of CSF tracer influx was quantified using Fiji software (33). Mean fluorescence intensity was normalized to the time of MCAO (F_0), and the derivative of the curve was computed to calculate the mean rate of change in fluorescence intensity over time. The time at which a peak in the rate of change occurred was determined to calculate time to peak influx. To extract more quantitative information, we tracked fronts using an automated Matlab algorithm previously developed at the University of Rochester (33, 73). Fronts are curves that separate bright regions from dark regions in videos of murine brains. Local front speeds quantify the local speeds of CSF influx and SD. We tracked fronts only within the brain hemispheres, sometimes treating the ipsilateral and contralateral hemispheres separately. We located those hemispheres by calculating the time-averaged GCaMP brightness of each video, then finding the two largest bright regions. Front tracking requires choosing a brightness threshold: Each front separates a region brighter than the threshold from a region dimmer than the threshold. To calculate thresholds, we calculated the mean brightness of each channel in each hemisphere, varying over time. We chose the thresholds as the brightness values halfway between the initial, dim values and the final, saturated val-

ues. From the thresholded dataset, we calculated the surface area in mm^2 over time in an ipsilateral and contralateral hemisphere ROI. The surface area data for the CSF tracer and the GCaMP channels were aligned in time using the maximum area of the GCaMP channel. We next calculated the mean rate of change in the surface area covered by CSF tracer over time. The maximal rate of change was used to compute the time to CSF influx peak and was compared with the time at which maximal GCaMP surface area occurred. SD onset time was measured as the first nonzero value of the thresholded GCaMP channel after MCAO. We next measured front speeds in a smaller ROI over the MCA territory. The average front speed for all pixels was plotted over time. To reduce noise caused by outlier pixels, we smoothed front speeds with a sliding line fit and a 20-s smoothing window. The maximum value of front speeds after the onset of the SD was considered the max speed. To determine the delay time between the SD and the arrival of CSF tracer, we evaluated the time elapsed between a pixel having GCaMP fluorescence and CSF tracer fluorescence; an average of all the pixels in the ROI was calculated to obtain mean delay time.

MRI postprocessing and analysis

Both DCE and ADC-map time series data were motion corrected using Advanced Normalization Tools normalization software (74, 75). DCE-MRI time series were motion corrected and converted into percent change from baseline time series, calculated as the percent signal change from the averaged signal of baseline images. To avoid biases in tracer calculations from availability of tracer to the glymphatic system, we then normalized percent change maps to the peak average. A population-based average of the baseline scans was created by an iterative registration process with two rigid, two affine, and five nonlinear registration-and-averaging steps. To allow unbiased ipsilateral-contralateral comparisons, original and left-right-flipped images were both used in the template creation. Registration was carried out using Advanced Normalization Tools v. 2.1.0. The average template was skull-stripped manually using ITK-SNAP and was then segmented into brain structures by registering the Waxholm Space Atlas of the C57BL/6J Mouse Brain (76) to the template. Normalized percent-change maps were finally transformed to the average space for analysis. We drew lines extending from the MCA at the level of the temporal ridge and 1 mm orthogonal to the brain surface in the coronal slice. Tracer penetration depth was defined as the distance from the MCA to the deepest voxel with normalized tracer signal >1 . For DWI analysis, edema was defined as the ADC value in a pixel dropping three standard deviations below baseline. Analysis of images was performed in ITK-SNAP

(version 3.6.0, www.itk-snap.org), ImageJ (version 2.00, NIH, Bethesda, MD, USA), and Matlab (version 9.3.0, MathWorks, Natick, MA, USA). Circular ROIs were drawn in the images both for the DCE enhancement ratio calculations and ADC calculations. 3D maximum intensity projection and multiplanar reformation for DCE data visualization was generated using IMARIS (v. 9.2.1, Bitplane, Concord, MA, USA). To create the registered average of the DCE and ADC time series, the time series were registered to the average template described above, and from those, different slices of the volumes were processed into videos using Python 3.6.

2P imaging analysis

Images obtained were 16 bit, with either two or three channels depending on the experiment, each with spatial dimensions of 512 by 512 and an average frame rate of 15 Hz. The 525-nm channel captured the vascular FITC-dextran or GCaMP7 fluorescence and the 607-nm channel the vascular Texas Red-dextran or the red microspheres in CSF for particle tracking, whereas the 647-nm channel captured the fluorescent BSA CSF tracer. Images were analyzed using Fiji and Matlab. The GCaMP channel was processed by converting it to 8 bit and generating a Z-projected average intensity of the entire imaging session and subtracting the average image from each frame ($\Delta F - F_{\text{average}}$). A Gaussian blur filter with a sigma of 1 was applied to the resulting stack and was subsequently color coded using the lookup table `mpl-inferno`. A mask of the CSF tracer and vascular channel was applied, and then all channels were merged. Line scans of the XYT data were generated using Fiji. For quantification purposes, GCaMP and CSF tracer fluorescence were quantified as $\Delta F/F_0$, where F_0 is the first frame of the experiment before MCAO. Wave speed was calculated by drawing two ROIs 100 μm apart, along the direction of wave propagation. The time between the GCaMP fluorescence appearing in the first and second ROIs was considered the speed of propagation. PVSs were defined as any fluid compartment following a blood vessel. Pial PVS followed leptomeningeal vessels at the brain surface, and penetrating PVS dove into cortical parenchyma with penetrating arterioles. PVS area was compared with arteriolar area for both pial and penetrating PVS. Images for both the CSF and intravenous dextran channel were thresholded using an automated method (Otsu), and the diameter of the vessel was measured. In early time points after intracisternal infusion, tracers had not reached the penetrating arteriole PVS, so CSF tracer area was normalized to the penetrating arteriolar area ($\Delta A/A_{\text{art}}$). Tracer was found in the pial PVS starting at 15 min after infusion and was therefore normalized to the area covered by tracer before MCAO before normalizing to the pial arteriolar

area ($\Delta A_{\text{norm}}/A_{\text{art}}$). For experiments in Fig. 6, E to G, time-lapse Z-stacks were acquired and processed using IMARIS (v. 9.2.1, Bitplane, Concord, MA, USA). To quantify the arteriolar diameter changes in $Aqp4^{-/-}$ and $Aqp4^{+/+}$ mice, the diameter was normalized to the beginning of the SI-induced constriction ($\Delta d/d_{\text{SI}}$). An ROI was placed over the penetrating arteriole and the surrounding PVS, and fluorescence intensity was normalized to the background intensity before MCAO ($\Delta F/F_0$). For ISF tracer quantification, an ROI was placed next to the PVS, making sure to avoid any surrounding capillaries or the overlying pial arteriole. The same size ROI was used for all animals. To determine the amount of PVS tracer that entered the ISF, fluorescence intensity was normalized to the time of onset of the SI ($\Delta F/F_{\text{SI}}$).

Particle tracking velocimetry

To directly measure the speed of CSF flowing through PVSs, we performed particle tracking velocimetry using time series of images obtained from in vivo 2P laser scanning microscopy. Our procedure is similar to one described previously (38). The particle tracking velocimetry analysis is performed using automated Matlab software (77, 78), in which tens of thousands of microspheres are tracked with sub-pixel accuracy through the time series of images to obtain spatially and temporally resolved velocities. We use an improved algorithm in which dynamic masking is performed to remove stagnant particles from the measurements. Specifically, before identifying and tracking particles, we first subtract a unique background image from each frame that is obtained by averaging the nearest 300 frames (150 before and after). To calculate heatmaps of average flow speed (fig. S7A), the spatial domain was divided into 5-pixel-by-5-pixel bins, the velocities were time-averaged in each bin using measurements from 1 min or less (centered on the indicated time), and the speed was computed from the average velocity. To calculate the time series of $v_{\text{downstream}}$, first the downstream velocity component of each particle in each frame is computed as $\mathbf{u} \cdot \hat{\mathbf{u}}_{\text{avg}}$, where $\mathbf{u}$ is the instantaneous particle velocity and $\hat{\mathbf{u}}_{\text{avg}}$ is a field of unit vectors computed from time-averaging the velocity field over the entire time series. We then obtain $v_{\text{downstream}}$ by calculating the spatial average of all the downstream velocity components in 1-s bins and normalizing the time series by the baseline value. The baseline value is the average value of $v_{\text{downstream}}$ over the initial 2 min of the experiment before the MCAO occurs. To calculate $v_{\text{pulsatile}}$, we first obtained the time series of v_{mean} by computing the spatially averaged speed for each frame. We then defined the time series of $v_{\text{pulsatile}}$ as $\max(v_{\text{mean}}) - \min(v_{\text{mean}})$ over each cardiac cycle, divided by the baseline value of this quantity. The car-

diac cycle is defined using sequential peaks of the R wave in synchronized electrocardiogram measurements. The normalized artery diameter time series was measured using custom software developed in Matlab. The measurements were performed by subsampling the time series of images obtained from in vivo 2P laser scanning microscopy by a factor of 10 and analyzing a user-defined ROI centered on a straight segment of the artery in each frame using an automated algorithm. The algorithm first separates the artery segment from the background by binarizing the ROI based on a user-defined threshold. The major and minor axes of the artery segment are then measured using the built-in Matlab function "regionprops." By ensuring that the segment length is longer than the artery diameter, the major and minor axes then correspond to the segment length and artery diameter, respectively. The normalized artery diameter time series then corresponds to the length of the minor axis in each frame, divided by the baseline value. The baseline value is the average artery diameter over the initial 2 min of the experiment before the MCAO occurs.

Glymphatic network model

Transport of water follows a network composed of PVSs, bifurcations, and terminal nodes (penetrating PVS). In mathematical language, we call the pial PVS the "edges" along the network, collected in the set E , and the terminals and bifurcations are collected in the set of "nodes," N . Thus, the network is given by nodes $i \in N$ and edges $(i, j) \in E$. The edges of the network have the property of an inverse resistance, or conductance C_{ij} . To simplify the model, we assume that each PVS is a straight pipe segment of length L_{ij} with cross-sectional area A_{ij} . The conductance is then

$$C_{ij} = \frac{kA_{ij}^2}{L_{ij}} \quad (1)$$

where $k = \pi/(8\mu)$ and μ is the dynamic viscosity, in agreement with Hagen-Poiseuille flow.

We assume that the SD wave propagates from (x_0, y_0) across a two-dimensional domain on the cortical surface with radius $R(t)$ growing at a constant speed c , so that $R(t) = ct$. The presence of the wave at node i with coordinates (x_i, y_i) is then defined as $w(\delta) = 1$ for $\delta \leq 1$ or $w(\delta) = 0$ otherwise, where the relative distance from node to the wave's origin is $\delta_i := \sqrt{(x_i - x_0)^2 + (y_i - y_0)^2}/R(t)$, or from the farthest end of an edge to the wave's origin, $\delta_{i,j} = \max(\delta_i, \delta_j)$.

The arrival of the SD wave at a penetrating arteriole located at site $i \in N$ triggers a dilation process

$$\frac{d}{dt} V_i = k_V \cdot w(\delta_i) \cdot V_i \cdot \left(1 - \frac{V_i}{V_i(\infty)}\right) \quad (2)$$

where $V_i(\infty)$ are the final PVS volumes. The wave covering pial surface vessels (i, j) $\in E$ triggers an analogous dilation response

$$\frac{d}{dt}C_{ij} = k_C \cdot w(\delta_{ij}) \cdot C_{ij} \cdot \left(1 - \frac{C_{ij}}{C_{ij}(\infty)}\right) \quad (3)$$

leading to an increase of the PVS's conductance C_{ij} , and $C_{ij}(\infty)$ are the final cross-sectional PVS conductances. Mass balance demands

$$\rho \frac{d}{dt}S_i + \sum_j Q_{ij}(t) = q_i(t) \quad (4)$$

where ρ is the mass density, q_i is the flow rate in or out of a terminal node, Q_{ij} is the flow rate along an edge (i, j), and $S_i = 1/2 \sum_j A_{ij} L_{ij}$ is the volume of the PVS connected to the bifurcating node i . Summation of eq. 4 over all nodes i yields the following relation: $\rho \frac{d}{dt} \sum_i S_i(t) + \sum_{i,j} Q_{ij}(t) = \sum_i q_i(t) = \sum_{i \in P} q_i(t) + Q_{MCA}$, in which the sum over the edge flows cancels (i.e., over forward and backward directions). Furthermore, the pial surface volume is defined as $S_{pial} = \sum_j S_j$ and $P \subseteq N$ is defined as the subset of nodes corresponding to penetrating arterioles which we use to split up the sum over terminal nodes into inflows (Q_{MCA}) and outflows. Thus, we can compute the inflow at the MCA

$$Q_{MCA} = -\sum_i q_i(t) + \rho \frac{d}{dt}S_{pial} \quad (5)$$

To determine this flow rate, we first need to compute $\frac{d}{dt}S_{pial}$. To obtain an expression for the rate of change of the cross-sectional area A_{ij} , we simply differentiate eq. 1, resulting in $\frac{d}{dt}A_{ij} = \frac{1}{2}k^{-1} \cdot \frac{L_{ij}}{A_{ij}} \cdot \frac{d}{dt}C_{ij} = \frac{1}{2}k^{-1/2} \cdot L_{ij}^{1/2} \cdot C_{ij}^{-1/2} \cdot \frac{d}{dt}C_{ij}$, where we in the last step again use eq. 1 or $A_{ij} = k^{-1/2} \cdot L_{ij}^{1/2} \cdot C_{ij}^{-1/2}$. Second, we need to express the terminal flow rates $q_i(t)$. The following relation holds: $q_i + \rho \frac{d}{dt}S_i = 0$. Hence, we can fully determine the value of eq. 5 through computation of eqs. 3 and 2. We set the initial values $V_i(t=0) = V_0 > 0$, $S_i(t=0) = S_0 > 0$ and $C_{ij}(t=0) = C_0 > 0$, consistent with a constant downstream flow (inflow-outflow of the network) that terminates at the time of occlusion. For the network graph, we used data from (40) and data to reflect realistic vessel diameters, adapted algorithmically to optimize power dissipation losses inside the network (79). The algorithm was implemented in Matlab using a simple Euler forward method.

Estimated diffusion calculations

The diffusion of tracer presented in fig. S3E was estimated as previously described (80, 81). To our knowledge, the diffusion coefficient of gadobutrol in live rodent brain has not been reported in the literature, so we estimated the degree of penetration of a similar sized tracer (3-kDa dextran). Calculations were done using the error function solution for plane diffusion

into a half-space: $C = C_0 \operatorname{erfc}[x/2\sqrt{Dt}]$ and an effective diffusivity $D^* = 5.36 \times 10^{-7} \text{ cm}^2/\text{s}$ (82). Because the D^* value was calculated for the cortex of live normoxic rats, we also used a value $D^* = 0.284 \times 10^{-7} \text{ cm}^2/\text{s}$ after 1 min of terminal ischemia induced by intracardiac 1M KCl to better reflect the reduced extracellular space after the SD, as seen in fig. S2, D to F.

Statistical analysis

All statistical analyses were done in GraphPad Prism 8. Data in all graphs are plotted as mean $\pm$ standard error of the mean (SEM) over the individual data points and lines from each mouse. Parametric and nonparametric tests were selected based on normality testing and are reported in the figure legends. Normality tests were chosen depending on the sample size (D'Agostino Pearson omnibus test where possible and Shapiro-Wilk if the n was too small). Sphericity was not assumed; in all repeated measures, two-way ANOVAs and a Geisser-Greenhouse correction were performed. All hypothesis testing was two-tailed, and significance was determined at $\alpha = 0.05$.

REFERENCES AND NOTES

1. E. J. Benjamin *et al.*, Heart disease and stroke statistics—2019 update: A report from the American Heart Association. *Circulation* **139**, e56–e528 (2019). doi: 10.1161/CIR.0000000000000659; pmid: 30700139
2. V. L. Feigin *et al.*, Update on the global burden of ischemic and hemorrhagic stroke in 1990–2013: The GBD 2013 Study. *Neuroepidemiology* **45**, 161–176 (2015). doi: 10.1159/000441085; pmid: 26505981
3. C. Iadecola, J. Anrather, Stroke research at a crossroad: Asking the brain for directions. *Nat. Neurosci.* **14**, 1363–1368 (2011). doi: 10.1038/nrn.2953; pmid: 22030546
4. R. L. Rungta *et al.*, The cellular mechanisms of neuronal swelling underlying cytotoxic edema. *Cell* **161**, 610–621 (2015). doi: 10.1016/j.cell.2015.03.029; pmid: 25910210
5. J. M. Simard, T. A. Kent, M. Chen, K. V. Tarasov, V. Gerzanich, Brain oedema in focal ischaemia: Molecular pathophysiology and theoretical implications. *Lancet Neurol.* **6**, 258–268 (2007). doi: 10.1016/S1474-4422(07)70055-8; pmid: 17303532
6. D. Liang, S. Bhatta, V. Gerzanich, J. M. Simard, Cytotoxic edema: Mechanisms of pathological cell swelling. *Neurosurg. Focus* **22**, E2 (2007). doi: 10.3171/foc.2007.22.5.3; pmid: 17613233
7. A. J. Hansen, M. Nedergaard, Brain ion homeostasis in cerebral ischemia. *Neurochem. Pathol.* **9**, 195–209 (1988). pmid: 3247069
8. J. A. Stokum, V. Gerzanich, J. M. Simard, Molecular pathophysiology of cerebral edema. *J. Cereb. Blood Flow Metab.* **36**, 513–538 (2016). doi: 10.1177/0271678X15617172; pmid: 26661240
9. I. Klatzo, Neuropathological aspects of brain edema. *J. Neuropathol. Exp. Neurol.* **26**, 1–14 (1967). doi: 10.1097/00005072-196701000-00001; pmid: 5336776
10. A. Van Harreveld, Changes in the diameter of apical dendrites during spreading depression. *Am. J. Physiol.* **192**, 457–463 (1958). doi: 10.1152/ajplegacy.1958.192.3.457; pmid: 13520934
11. A. van Harreveld, S. Ochs, Cerebral impedance changes after circulatory arrest. *Am. J. Physiol.* **187**, 180–192 (1956). doi: 10.1152/ajplegacy.1956.187.1.180; pmid: 13362612
12. J. P. Dreier, C. L. Lemale, V. Kola, A. Friedman, K. Schoknecht, Spreading depolarization is not an epiphenomenon but the principal mechanism of the cytotoxic edema in various gray matter structures of the brain during stroke. *Neuropharmacology* **134**, 189–207 (2018). doi: 10.1016/j.neuropharm.2017.09.027; pmid: 28941738
13. J. P. Dreier, The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease. *Nat. Med.* **17**, 439–447 (2011). doi: 10.1038/nm.2333; pmid: 21475241
14. M. Nedergaard, J. Astrup, Infarct rim: Effect of hyperglycemia on direct current potential and [^{14}C]2-deoxyglucose phosphorylation. *J. Cereb. Blood Flow Metab.* **6**, 607–615 (1986). doi: 10.1038/jcbfm.1986.108; pmid: 3760045
15. I. Klatzo, Pathophysiological aspects of brain edema. *Acta Neuropathol.* **72**, 236–239 (1987). doi: 10.1007/BF00691095; pmid: 3564903
16. D. Knowland *et al.*, Stepwise recruitment of transcellular and paracellular pathways underlies blood-brain barrier breakdown in stroke. *Neuron* **82**, 603–617 (2014). doi: 10.1016/j.neuron.2014.03.003; pmid: 24746419
17. E. J. Kang *et al.*, Blood-brain barrier opening to large molecules does not imply blood-brain barrier opening to small ions. *Neurobiol. Dis.* **52**, 204–218 (2013). doi: 10.1016/j.nbd.2012.12.007; pmid: 23291193
18. W. Young, Z. H. Rappaport, D. J. Chalif, E. S. Flamm, Regional brain sodium, potassium, and water changes in the rat middle cerebral artery occlusion model of ischemia. *Stroke* **18**, 751–759 (1987). doi: 10.1161/01.STR.18.4.751; pmid: 3603602
19. T. W. Battey *et al.*, Brain edema predicts outcome after nonlacunar ischemic stroke. *Stroke* **45**, 3643–3648 (2014). doi: 10.1161/STROKEAHA.114.006884; pmid: 25336512
20. S. Hatashita, J. T. Hoff, S. M. Salamat, Ischemic brain edema and the osmotic gradient between blood and brain. *J. Cereb. Blood Flow Metab.* **8**, 552–559 (1988). doi: 10.1038/jcbfm.1988.96; pmid: 3392116
21. S. Hatashita, J. T. Hoff, Brain edema and cerebrovascular permeability during cerebral ischemia in rats. *Stroke* **21**, 582–588 (1990). doi: 10.1161/01.STR.21.4.582; pmid: 1691534
22. A. S. Thrane, V. Rangroo Thrane, M. Nedergaard, Drowning stars: Reassessing the role of astrocytes in brain edema. *Trends Neurosci.* **37**, 620–628 (2014). doi: 10.1016/j.tins.2014.08.010; pmid: 25236348
23. J. J. Iliff *et al.*, A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β . *Sci. Transl. Med.* **4**, 147ra111 (2012). doi: 10.1126/scitranslmed.3003748; pmid: 22896675
24. L. Xie *et al.*, Sleep drives metabolite clearance from the adult brain. *Science* **342**, 373–377 (2013). doi: 10.1126/science.1241224; pmid: 24136970
25. Y. Inoue *et al.*, Detection of necrotic neural response in super-acute cerebral ischemia using activity-induced manganese-enhanced (AIM) MRI. *NMR Biomed.* **23**, 304–312 (2010). pmid: 19950123
26. O. Gotoh, T. Asano, T. Koide, K. Takakura, Ischemic brain edema following occlusion of the middle cerebral artery in the rat. I: The time courses of the brain water, sodium and potassium contents and blood-brain barrier permeability to 125I-albumin. *Stroke* **16**, 101–109 (1985). doi: 10.1161/01.STR.16.1.101; pmid: 3966252
27. W. B. Sisson, W. H. Oldendorf, Brain distribution spaces of mannitol-3H, inulin-14C, and dextran-14C in the rat. *Am. J. Physiol.* **221**, 214–217 (1971). doi: 10.1152/ajplegacy.1971.221.1.214; pmid: 5555788
28. J. J. Iliff *et al.*, Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. *J. Clin. Invest.* **123**, 1299–1309 (2013). doi: 10.1172/JCI67677; pmid: 23434588
29. J. P. Dreier, C. Reiffurth, The stroke-migraine depolarization continuum. *Neuron* **86**, 902–922 (2015). doi: 10.1016/j.neuron.2015.04.004; pmid: 25996134
30. V. B. Bogdanov *et al.*, Susceptibility of primary sensory cortex to spreading depolarizations. *J. Neurosci.* **36**, 4733–4743 (2016). doi: 10.1523/JNEUROSCI.3694-15.2016; pmid: 27122032
31. R. Enger *et al.*, Dynamics of ionic shifts in cortical spreading depression. *Cereb. Cortex* **25**, 4469–4476 (2015). doi: 10.1093/cercor/bhv054; pmid: 25840424
32. H. Monai *et al.*, Calcium imaging reveals glial involvement in transcranial direct current stimulation-induced plasticity in mouse brain. *Nat. Commun.* **7**, 11100 (2016). doi: 10.1038/ncomms11100; pmid: 27000523
33. B. A. Plog *et al.*, Transcranial optical imaging reveals a pathway for optimizing the delivery of immunotherapeutics to the brain. *JCI Insight* **3**, 120922 (2018). doi: 10.1172/jci.insight.120922; pmid: 30333324
34. A. J. Strong *et al.*, Peri-infarct depolarizations lead to loss of perfusion in ischemic gyrencephalic cerebral cortex. *Brain* **130**, 995–1008 (2007). doi: 10.1093/brain/awl392; pmid: 17438018
35. H. K. Shin *et al.*, Vasoconstrictive neurovascular coupling during focal ischemic depolarizations. *J. Cereb. Blood Flow Metab.* **26**, 1018–1030 (2006). doi: 10.1038/sj.jcbfm.9600252; pmid: 16340958

36. J. P. Dreier *et al.*, Nitric oxide scavenging by hemoglobin or nitric oxide synthase inhibition by *N*-nitro-L-arginine induces cortical spreading ischemia when K^+ is increased in the subarachnoid space. *J. Cereb. Blood Flow Metab.* **18**, 978–990 (1998). doi: 10.1097/00004647-199809000-00007; pmid: 9740101
37. J. Chuquet, L. Hollender, E. A. Nimchinsky, High-resolution in vivo imaging of the neurovascular unit during spreading depression. *J. Neurosci.* **27**, 4036–4044 (2007). doi: 10.1523/JNEUROSCI.0721-07.2007; pmid: 17428981
38. H. Mestre *et al.*, Flow of cerebrospinal fluid is driven by arterial pulsations and is reduced in hypertension. *Nat. Commun.* **9**, 4878 (2018). doi: 10.1038/s41467-018-07318-3; pmid: 30451853
39. A. J. Schain, A. Melo-Carrillo, A. M. Strassman, R. Burstein, Cortical spreading depression closes paravascular space and impairs glymphatic flow: Implications for migraine headache. *J. Neurosci.* **37**, 2904–2915 (2017). doi: 10.1523/JNEUROSCI.3390-16.2017; pmid: 28193695
40. P. Blinder, A. Y. Shih, C. Raffie, D. Kleinfeld, Topological basis for the robust distribution of blood to rodent neocortex. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 12670–12675 (2010). doi: 10.1073/pnas.1007239107; pmid: 20616030
41. J. P. Dreier *et al.*, Ischemia triggered by spreading neuronal activation is inhibited by vasodilators in rats. *J. Physiol.* **531**, 515–526 (2001). doi: 10.1111/j.1469-7793.2001.05151.x; pmid: 11230523
42. T. Iijima, G. Mies, K. A. Hossmann, Repeated negative DC deflections in rat cortex following middle cerebral artery occlusion are abolished by MK-801: Effect on volume of ischemic injury. *J. Cereb. Blood Flow Metab.* **12**, 727–733 (1992). doi: 10.1038/jcbfm.1992.103; pmid: 1506440
43. R. Gill, P. Andin , L. Hillered, L. Persson, H. Hagberg, The effect of MK-801 on cortical spreading depression in the penumbral zone following focal ischemia in the rat. *J. Cereb. Blood Flow Metab.* **12**, 371–379 (1992). doi: 10.1038/jcbfm.1992.54; pmid: 1314840
44. M. Lauritzen, A. J. Hansen, The effect of glutamate receptor blockade on anoxic depolarization and cortical spreading depression. *J. Cereb. Blood Flow Metab.* **12**, 223–229 (1992). doi: 10.1038/jcbfm.1992.32; pmid: 1312539
45. H. Mestre *et al.*, Aquaporin-4-dependent glymphatic solute transport in the rodent brain. *eLife* **7**, e40070 (2018). doi: 10.7554/eLife.40070; pmid: 30561329
46. X. Yao, N. Derugin, G. T. Manley, A. S. Verkman, Reduced brain edema and infarct volume in aquaporin-4 deficient mice after transient focal cerebral ischemia. *Neurosci. Lett.* **584**, 368–372 (2015). doi: 10.1016/j.neulet.2014.10.040; pmid: 25449874
47. G. T. Manley *et al.*, Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nat. Med.* **6**, 159–163 (2000). doi: 10.1038/72256; pmid: 10655103
48. H. Igarashi, V. J. Huber, M. Tsujita, T. Nakada, Pretreatment with a novel aquaporin 4 inhibitor, TGN-020, significantly reduces ischemic cerebral edema. *Neuro. Sci.* **32**, 113–116 (2011). doi: 10.1007/s10072-010-0431-1; pmid: 20924629
49. I. Pirici *et al.*, Inhibition of aquaporin-4 improves the outcome of ischemic stroke and modulates brain paravascular drainage pathways. *Int. J. Mol. Sci.* **19**, 46 (2017). doi: 10.3390/ijms19010046; pmid: 29295526
50. A. L. Betz, R. F. Keep, M. E. Beer, X. D. Ren, Blood-brain barrier permeability and brain concentration of sodium, potassium, and chloride during focal ischemia. *J. Cereb. Blood Flow Metab.* **14**, 29–37 (1994). doi: 10.1038/jcbfm.1994.5; pmid: 8263055
51. S. Ishimaru, K. A. Hossmann, Relationship between cerebral blood flow and blood-brain barrier permeability of sodium and albumin in cerebral infarcts of rats. *Acta Neurochir. Suppl.* **51**, 216–219 (1990). doi: 10.1007/978-3-7091-9115-6_73; pmid: 2089898
52. W. D. Lo, A. L. Betz, G. P. Schielke, J. T. Hoff, Transport of sodium from blood to brain in ischemic brain edema. *Stroke* **18**, 150–157 (1987). doi: 10.1161/01.STR.18.1.150; pmid: 3810748
53. U. Ito, Y. Hakamata, E. Kawakami, K. Oyanagi, Temporary cerebral ischemia results in swollen astrocytic end-feet that compress microvessels and lead to delayed focal cortical infarction. *J. Cereb. Blood Flow Metab.* **31**, 328–338 (2011). doi: 10.1038/jcbfm.2010.97; pmid: 20588315
54. L. Khennouf *et al.*, Active role of capillary pericytes during stimulation-induced activity and spreading depolarization. *Brain* **141**, 2032–2046 (2018). doi: 10.1093/brain/awy143; pmid: 30053174
55. J. M. Simard *et al.*, Glibenclamide in cerebral ischemia and stroke. *Neurocrit. Care* **20**, 319–333 (2014). doi: 10.1007/s12028-013-9923-1; pmid: 24132564
56. T. Gaberel *et al.*, Impaired glymphatic perfusion after strokes revealed by contrast-enhanced MRI: A new target for fibrinolysis? *Stroke* **45**, 3092–3096 (2014). doi: 10.1161/STROKEAHA.114.006617; pmid: 25190438
57. J. L ckl *et al.*, Peri-infarct flow transients predict outcome in rat focal brain ischemia. *Neuroscience* **226**, 197–207 (2012). doi: 10.1016/j.neuroscience.2012.08.049; pmid: 22986160
58. J. A. Hartings *et al.*, The continuum of spreading depolarizations in acute cortical lesion development: Examining Le o's legacy. *J. Cereb. Blood Flow Metab.* **37**, 1571–1594 (2017). doi: 10.1177/0271678X16654495; pmid: 27328690
59. X. Yao *et al.*, Aquaporin-4 regulates the velocity and frequency of cortical spreading depression in mice. *Glia* **63**, 1860–1869 (2015). doi: 10.1002/glia.22853; pmid: 25944186
60. R. Enger *et al.*, Deletion of aquaporin-4 curtails extracellular glutamate elevation in cortical spreading depression in awake mice. *Cereb. Cortex* **27**, 24–33 (2017). doi: 10.1093/cercor/bhw359; pmid: 28365776
61. A. S. Thrane *et al.*, In vivo NADH fluorescence imaging indicates effect of aquaporin-4 deletion on oxygen microdistribution in cortical spreading depression. *J. Cereb. Blood Flow Metab.* **33**, 996–999 (2013). doi: 10.1038/jcbfm.2013.63; pmid: 23611872
62. Z. Bere, T. P. Obrenovitch, F. Bari, E. Farkas, Ischemia-induced depolarizations and associated hemodynamic responses in incomplete global forebrain ischemia in rats. *Neuroscience* **260**, 217–226 (2014). doi: 10.1016/j.neuroscience.2013.12.032; pmid: 24365459
63. J. A. Hartings *et al.*, Subarachnoid blood acutely induces spreading depolarizations and early cortical infarction. *Brain* **140**, 2673–2690 (2017). doi: 10.1093/brain/awx214; pmid: 28969382
64. J. Woltzik *et al.*, Propagation of cortical spreading depolarization in the human cortex after malignant stroke. *Neurology* **80**, 1095–1102 (2013). doi: 10.1212/WNL.0b013e3182886932; pmid: 23446683
65. J. M. Hinzman *et al.*, Inverse neurovascular coupling to cortical spreading depolarizations in severe brain trauma. *Brain* **137**, 2960–2972 (2014). doi: 10.1093/brain/awu241; pmid: 25154387
66. J. P. Dreier *et al.*, Cortical spreading ischemia is a novel process involved in ischemic damage in patients with aneurysmal subarachnoid hemorrhage. *Brain* **132**, 1866–1881 (2009). doi: 10.1093/brain/awp102; pmid: 19420089
67. J. L ckl *et al.*, The negative ultraslow potential, electrophysiological correlate of infarction in the human cortex. *Brain* **141**, 1734–1752 (2018). doi: 10.1093/brain/awy102; pmid: 29668855
68. A. L. R. Xavier *et al.*, Cannula implantation into the cisterna magna of rodents. *J. Vis. Exp.* **2018**, 57378 (2018). doi: 10.3791/57378; pmid: 29889209
69. O. Albayram *et al.*, CIs P-tau is induced in clinical and preclinical brain injury and contributes to post-injury sequelae. *Nat. Commun.* **8**, 1000 (2017). doi: 10.1038/s41467-017-01068-4; pmid: 29042562
70. J. K. Karimiy *et al.*, Inflammation-dependent cerebrospinal fluid hypersecretion by the choroid plexus epithelium in posthemorrhagic hydrocephalus. *Nat. Med.* **23**, 997–1003 (2017). doi: 10.1038/nm.4361; pmid: 28692063
71. J. K. Karimiy *et al.*, A novel method to study cerebrospinal fluid dynamics in rats. *J. Neurosci. Methods* **241**, 78–84 (2015). doi: 10.1016/j.jneumeth.2014.12.015; pmid: 25554415
72. D. W. McBride, D. Klebe, J. Tang, J. H. Zhang, Correcting for brain swelling's effects on infarct volume calculation after middle cerebral artery occlusion in rats. *Transl. Stroke Res.* **6**, 323–338 (2015). doi: 10.1007/s12975-015-0400-3; pmid: 25933988
73. T. D. Nevins, D. H. Kelley, Front tracking for quantifying advection-reaction-diffusion. *Chaos* **27**, 043105 (2017). doi: 10.1063/1.4979668; pmid: 28456164
74. B. B. Avants *et al.*, A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage* **54**, 2033–2044 (2011). doi: 10.1016/j.neuroimage.2010.09.025; pmid: 20851191
75. B. Avants *et al.*, Multivariate analysis of structural and diffusion imaging in traumatic brain injury. *Acad. Radiol.* **15**, 1360–1375 (2008). doi: 10.1016/j.acra.2008.07.007; pmid: 18995188
76. G. A. Johnson *et al.*, Waxholm space: An Image-based reference for coordinating mouse brain research. *Neuroimage* **53**, 365–372 (2010). doi: 10.1016/j.neuroimage.2010.06.067; pmid: 20600960
77. N. T. Ouellette, H. T. Xu, E. Bodenschatz, A quantitative study of three-dimensional Lagrangian particle tracking algorithms. *Exp. Fluids* **40**, 301–313 (2006). doi: 10.1007/s00348-005-0068-7
78. D. H. Kelley, N. T. Ouellette, Using particle tracking to measure flow instabilities in an undergraduate laboratory experiment. *Am. J. Phys.* **79**, 267–273 (2011). doi: 10.1119/1.3536647
79. E. A. Martens, K. Klemm, Transitions from trees to cycles in adaptive flow networks. *Front. Phys.* **5**, 62 (2017). doi: 10.3389/fphy.2017.00062
80. E. Syk v , C. Nicholson, Diffusion in brain extracellular space. *Physiol. Rev.* **88**, 1277–1340 (2008). doi: 10.1152/physrev.00027.2007; pmid: 18923183
81. G. Ringstad *et al.*, Brain-wide glymphatic enhancement and clearance in humans assessed with MRI. *JCI Insight* **3**, e121537 (2018). doi: 10.1172/jci.insight.121537; pmid: 29997300
82. R. G. Thorne, C. Nicholson, In vivo diffusion analysis with quantum dots and dextrans predicts the width of brain extracellular space. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 5567–5572 (2006). doi: 10.1073/pnas.0509425103; pmid: 16567637

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SUPPLEMENTARY MATERIALS

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References

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RESEARCH ARTICLE SUMMARY

NEUROSCIENCE

Cerebrospinal fluid influx drives acute ischemic tissue swelling

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INTRODUCTION: Cerebrospinal fluid (CSF) covers and protects the brain from mechanical injury. CSF also flows along an interconnected network of perivascular spaces surrounding blood vessels and communicates with interstitial fluid permeating brain tissue, aiding in the removal of metabolic waste produced by cells. This glial cell-mediated lymphatic (glymphatic) function of CSF represents a continuous source of fluid and ions for the brain. When a cerebral artery is occluded, nearby brain tissue is abruptly deprived of blood flow, oxygen, and glucose. This process, known as acute ischemic stroke, is a leading cause of morbidity and mortality worldwide. After stroke, fluid accumulates in ischemic tissue, and the brain becomes edematous and begins to swell, a dangerous complication of the disease. In the first hours after occlusion, the degree of swelling correlates with the net gain of cations, primarily sodium,

and this gain draws in fluid from surrounding sources.

RATIONALE: Because the brain is already encased by CSF, we asked if glymphatic flow could play a role in early edema formation. To test this, we evaluated CSF dynamics using in vivo magnetic resonance (MR) and multimodal optical imaging after occluding the middle cerebral artery in mice. Edema was assessed using diffusion-weighted MR, and edema fluid sources were labeled using radionuclides. Changes in the flow of CSF in perivascular spaces were explored using a network model of the mouse middle cerebral artery. Histology was used to evaluate edema formation in regions adjacent to CSF inflow routes in mouse and human autopsy tissue.

RESULTS: We found that within minutes of ischemic stroke, CSF flowed rapidly into brain

tissue along perivascular spaces. Its entry coincided with the onset of swelling and increased brain water content. Radionuclides and multimodal imaging confirmed that CSF was the earliest contributor of both fluid and ions. Calcium imaging in transgenic mice expressing GCaMP7 in cortical neurons and astrocytes revealed that this process was initiated

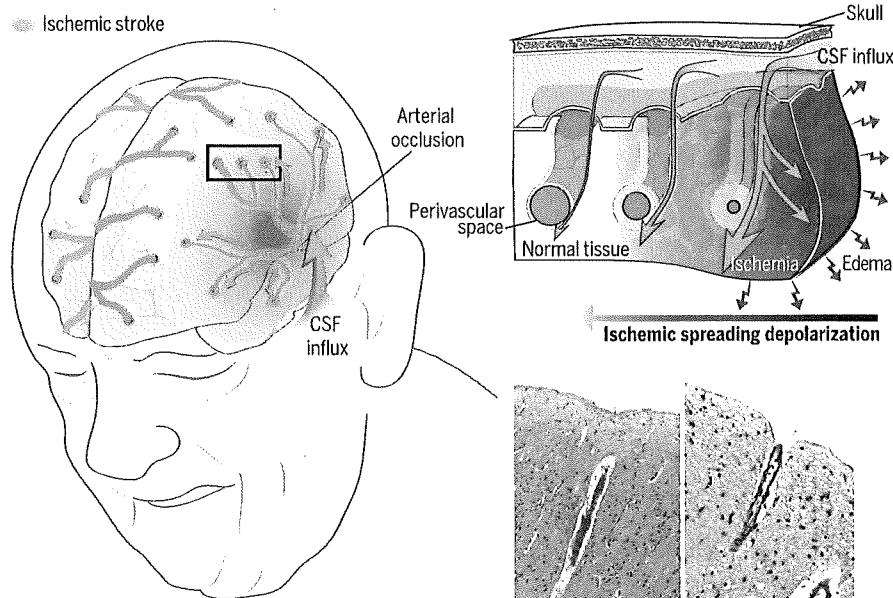
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by spreading depolarizations that were triggered when tissue was deprived of blood flow. Diffusion-weighted MR imaging showed that this was the earliest phase of edema

formation. This aberrant CSF inflow was found to be caused by spreading ischemia, the pathological constriction of cerebral blood vessels that follows spreading depolarizations. We present a network model that predicts that the space left unoccupied after vessels constrict would be filled by an inrush of CSF that nearly doubles flow speed. That prediction was confirmed experimentally using particle tracking velocimetry of CSF flow in live mice. Inflow depended on the aquaporin-4 water channel that is highly expressed by glial cells (astrocytes), which is a key contributor to glymphatic function. Postmortem examination of rodent and human brains showed increased fluid accumulation in tissue surrounding perivascular spaces and the cerebral ventricles compared with regions deep in the brain that were far from large CSF reservoirs.

CONCLUSION: Here, we demonstrate that CSF can provide a source of ischemic edema. Glymphatic inflow of CSF appears to be the primary initial event driving tissue swelling. This finding challenges our current understanding of edema formation after stroke and may provide a basis for treatment of acute ischemic stroke. Spreading depolarizations continue several days after stroke and are also present in many other neurological conditions, ranging from traumatic brain injury to migraine; therefore, it will be important to determine if spreading edema is also a feature of these diseases and whether CSF influx contributes to worsening at more delayed time points. It is also intriguing to speculate that abnormal CSF inflow could be a source of edema fluid in other types of chronic cerebrovascular disease, such as small-vessel disease characterized by enlarged perivascular spaces and transient accumulations of fluid in periventricular white matter. ■



CSF influx is responsible for early tissue swelling after stroke. (Left) Spreading ischemia accelerates CSF inflow to the region deprived of blood flow. (Top right) Spreading ischemia constricts the cortical vessels, increasing the perivascular space and resulting in CSF inflow. (Bottom right) Histology of postmortem tissue shows fluid accumulation in the ischemic human brain (diffuse white empty space, right) that is not present in a control brain (left).

ILLUSTRATION: DAN XUE

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Science

Cerebrospinal fluid influx drives acute ischemic tissue swelling

Humberto Mestre, Ting Du, Amanda M. Sweeney, Guojun Liu, Andrew J. Samson, Weiguo Peng, Kristian Nygaard Mortensen, Frederik Filip Stæger, Peter A. R. Bork, Logan Bashford, Edna R. Toro, Jeffrey Tithof, Douglas H. Kelley, John H. Thomas, Poul G. Hjorth, Erik A. Martens, Rupal I. Mehta, Orestes Solis, Pablo Blinder, David Kleinfeld, Hajime Hirase, Yuki Mori and Malken Nedergaard

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Spreading edema after stroke

The brain is enveloped in a cushion of cerebrospinal fluid (CSF), which normally provides protection and helps to remove metabolic waste. CSF transport has also recently been shown to play unexpected roles in neurodegeneration and sleep. Mestre *et al.* used multimodal in vivo imaging in rodents and found that, after a stroke, an abnormally large volume of CSF rushes into the brain, causing swelling (see the Perspective by Moss and Williams). This influx of CSF is caused by constrictions of arteries triggered by a well-known propagating chemical reaction-diffusion wave called spreading depolarization. CSF transport can thus play a role in brain swelling after stroke.

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3. SUIDI FORM



Sudden Unexpected Infant Death Investigation Reporting Form

For use during the investigation of infant (under 1 year of age) deaths that are sudden, unexpected, and unexplained prior to investigation.

INFANT DEMOGRAPHICS

1. Infant information. Full name: _____ Date of birth: (mm/dd/yyyy) _____
- Age: _____ SS#: _____ Case number: _____
- Primary residence address: _____
- City: _____ State: _____ Zip: _____
2. Race: ☐ White ☐ Black/African Am. ☐ Asian/Pacific Islander ☐ Am. Indian/Alaskan Native ☐ Hispanic/Latino ☐ Other
3. Sex: ☐ Male ☐ Female

PREGNANCY HISTORY

1. Birth mother information. ☐ Unavailable Full name: _____
- Maiden name: _____ Date of birth: (mm/dd/yyyy) _____ SS#: _____
- Current address: _____
- ☐ Same as infant's primary residence address above City: _____
- State: _____ Zip: _____ Email address: _____
2. How long has the birth mother been at this address? Years: _____ Months: _____ Days: _____
3. Previous address(es) (cities/counties/states) in the past 5 years: _____
4. Did the birth mother receive prenatal care? ☐ Yes ☐ No ☐ Unknown
- If yes: At how many weeks or months did prenatal care begin? _____ Weeks _____ Months
- How many prenatal care visits were completed? _____
5. Where did the birth mother receive prenatal care? Physician/Provider: _____
- Hospital or Clinic: _____ Phone: _____
- Address: _____
- City: _____ State: _____ Zip: _____
6. Did the birth mother have any complications, medical conditions, or injuries during her pregnancy?
(e.g., high blood pressure, bleeding, gestational diabetes, fall, or accident) ☐ Yes ☐ No ☐ Unknown
- If yes, describe:

CS310043

7. During her pregnancy, did the birth mother use any of the following?

Substance	Use	Specify Type	Frequency
Over the counter medications	☐ Yes ☐ No ☐ Unknown		
Prescribed medications	☐ Yes ☐ No ☐ Unknown		
Herbal remedies	☐ Yes ☐ No ☐ Unknown		
Alcohol	☐ Yes ☐ No ☐ Unknown		
Illicit drugs (e.g., heroin)	☐ Yes ☐ No ☐ Unknown		
Tobacco (e.g., cigarettes or e-cigarettes)	☐ Yes ☐ No ☐ Unknown		
Other	☐ Yes ☐ No ☐ Unknown		

INFANT HISTORY

1. Source of infant medical history information. (check all that apply)

- ☐ Doctor ☐ Other health care provider ☐ Medical record ☐ Parent or primary caregiver ☐ Other family member
☐ Other, specify: _____

2. Were there any complications during delivery or at birth? (e.g., emergency C-section, or infant needed oxygen)

☐ Yes ☐ No ☐ Unknown If yes, describe: _____

3. Did the infant have abnormal newborn screening results? ☐ Yes ☐ No ☐ Unknown

If yes, describe: _____

4. Infant's length at birth: _____ ☐ IN ☐ CM5. Infant's weight at birth: _____ ☐ LBS and OZ ☐ GM

6. Compared to the due date, when was the infant born?

☐ Early (before 37 weeks) ☐ Late (after 41 weeks) ☐ On time How many weeks? _____ Infant's due date: (mm/dd/yyyy) _____

7. Was the infant a singleton or multiple birth? ☐ Singleton ☐ Twin ☐ Triplet ☐ Quadruplet or higher8. Was the infant born with Neonatal Abstinence Syndrome (NAS)? (NAS is a drug withdrawal syndrome in newborns exposed to substances, like opioids, before birth) ☐ Yes ☐ No ☐ Unknown

If yes, did the infant need pharmacologic treatment? ☐ Yes ☐ No ☐ Unknown

9. Fill out the contact information for the infant's regular pediatrician and birth hospital.

Item	Regular Pediatrician	Birth Hospital
Date	Of last visit: _____	Of discharge: _____
Name of hospital or clinic		
Address		
Phone number		

10. Describe the two most recent times the infant was seen by a health care provider.
(include ER and clinic visits, hospital admissions, observational stays, regular pediatrician, and phone calls)

Visit type	1 st most recent visit	2 nd most recent visit
Reason for visit		
Action taken		
Date		
Physician's name		
Hospital or clinic		
Address		
Phone number		

11. Did the infant have any of the following?

Symptom	Within 72 hrs of incident
Fever	☐ Yes ☐ No ☐ Unknown
Cough	☐ Yes ☐ No ☐ Unknown
Diarrhea	☐ Yes ☐ No ☐ Unknown
Excessive sweating	☐ Yes ☐ No ☐ Unknown
Stool changes	☐ Yes ☐ No ☐ Unknown
Lethargy or sleeping more than usual	☐ Yes ☐ No ☐ Unknown
Difficulty breathing	☐ Yes ☐ No ☐ Unknown
Fussiness or excessive crying	☐ Yes ☐ No ☐ Unknown
Exposure to anyone who was sick (e.g., at home or at daycare)	☐ Yes ☐ No ☐ Unknown
Decrease in appetite	☐ Yes ☐ No ☐ Unknown
Falls or injuries	☐ Yes ☐ No ☐ Unknown
Other, specify: _____	☐ Yes ☐ No ☐ Unknown

Symptom	Within 72 hrs of incident	At any time
Allergies or allergic reactions (food, medication, or other)	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Abnormal growth, weight gain, or weight loss	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Apnea (stopped breathing)	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Cyanosis (turned blue or gray)	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Seizures or convulsions	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Cardiac (heart) abnormalities	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Colic (frequent prolonged crying/chronic inconsolable fussiness)	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Feeding issues (e.g., reflux)	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Vomiting	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Choking	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Other, specify: _____	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown

If yes to any of the above, describe:

12. Infant exposed to second hand smoke? (*environmental tobacco smoke*) ☐ Yes ☐ No ☐ Unknown
 If yes, how often? ☐ Frequently (*several times a week*) ☐ Occasionally (*several times a month*) ☐ Unknown

13. In the 72 hours before death, was the infant given any vaccinations or medications? (*include any home remedies, herbal medications, prescription medications, over-the-counter medications*)

Vaccine or medication name	Dose last given	Date given (mm/dd/yy)	Approx. time given	Reasons given or comments

14. Was the infant last placed to sleep with a bottle? ☐ Yes ☐ No ☐ Unknown
 If yes, was the bottle propped? (*object used to hold bottle while infant feeds*) ☐ Yes ☐ No ☐ Unknown

If yes: What object propped the bottle? _____

Could the infant hold the bottle? ☐ Yes ☐ No ☐ Unknown

15. Who was the last person to feed the infant? (*name and familial relationship to infant*)

16. Did the death occur during feeding? ☐ Breastfeeding ☐ Bottle-feeding ☐ Eating solids ☐ Not during feeding

17. Was the infant ever breastfed? ☐ Yes ☐ No ☐ Unknown If yes, for how many months? _____

18. What did the infant consume in the 24 hours prior to death?

Consumed?	If yes, describe	If yes, newly introduced?	If yes, was this the last thing consumed prior to incident?	If last fed, indicate quantity	If last fed, indicate date and time?
☐ Breastmilk		☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No		
☐ Formula		☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No		
☐ Water		☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No		
☐ Other liquids		☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No		
☐ Solids		☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No		
☐ Other		☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No		

19. Among the infant's blood relatives (*siblings, parents, grandparents, aunts, uncles, or first cousins*) was there any...

Sudden or unexpected death before the age of 50? ☐ Yes ☐ No ☐ Unknown

Heart disease? (*e.g., cardiomyopathy, Marfan or Brugada syndrome, long or short QT syndrome, or catecholaminergic polymorphic ventricular tachycardia*)
☐ Yes ☐ No ☐ Unknown

If yes to either, describe: (*include relation to infant*) _____

20. Did the infant have any birth defect(s)? ☐ Yes ☐ No ☐ Unknown

If yes, describe: _____

21. Was the infant able to roll over on his or her own? (check all that apply) ☐ Front to back ☐ Back to front

22. Indicate the infant's ability to lift or hold his or her head up. ☐ Unable ☐ 1 second ☐ 5 seconds ☐ ≥10 seconds ☐ Unknown

23. Was the infant meeting or not meeting growth and developmental milestones? (e.g., sitting up, crawling, rolling over, or feeding well. Include if the caregiver, supervisor, or medical professional had any concerns.)

24. Is there anything else that may have affected the infant that has not yet been documented? (e.g., exposed to fumes, infant unusually heavy, placed with positional support or wedge, or international travel)

INCIDENT SCENE INVESTIGATION

1. Incident scene (place infant found unresponsive or dead). Type of location? (e.g., primary residence, daycare, or grandmother's house)

Address: _____ City: _____

State: _____ Zip: _____

2. Was the infant in a new or different environment? (not part of the infant's normal routine) ☐ Yes ☐ No ☐ Unknown

If yes, describe: _____

3. Did the death occur at a daycare? ☐ Yes ☐ No ☐ Unknown

If yes: How many children younger than 18 years of age were under the care of the provider at the time of the incident?

(including their own children) _____

How many adults aged 18 years or older were supervising the child(ren)? _____

How long has the daycare been open for business? _____

Is the daycare licensed? ☐ Yes ☐ No ☐ Unknown

If yes: License number? _____ Licensing agency? _____

4. How many people live at the incident scene? **Children** (younger than 18 years) _____ **Adults** (18 years or older) _____

5. What kind of heating or cooling sources were being used at the incident scene? (e.g., A/C window unit, wood-burning fireplace, or open window)

6. Was there a working carbon monoxide (CO) alarm at the incident scene? ☐ Yes ☐ No ☐ Unknown

7. Indicate the temperature of the room where the infant was found unresponsive, and the surrounding area. (fill in temperatures)

Thermostat setting: _____ Thermostat reading: _____ Incident room: _____ Outside: _____ Time of reading: _____

8. Which of these devices were operating in the room where the infant was found unresponsive? (check all that apply)

☐ Fan ☐ Apnea monitor ☐ Humidifier ☐ Vaporizer ☐ Air purifier ☐ None ☐ Unknown

☐ Other, specify: _____

9. What was the source of drinking water at the incident scene? (check all that apply)

☐ Public or municipal water ☐ Bottled water ☐ Well water ☐ Unknown

☐ Other, specify: _____

10. Which of the following were present at the incident scene? *(check all that apply)*

- ☐ Insects ☐ Mold growth ☐ Smokey smell ☐ Pets ☐ Dampness ☐ Peeling paint ☐ Visible standing water
☐ Presence of alcohol containers ☐ Rodents or vermin ☐ None
☐ Odors or fumes, describe: _____
☐ Presence of prescription drugs, describe: _____
☐ Presence of illicit drugs or drug paraphernalia, describe: _____
☐ Other, describe: _____

11. Describe the general appearance of incident scene. *(e.g., cleanliness, hazards, or overcrowding)*

12. Is there anything else that may have affected the infant that has not yet been documented? *(e.g., drug or alcohol use at scene, history of domestic violence, or child abuse or neglect)*

INCIDENT CIRCUMSTANCES

1. Who was the usual caregiver(s)? *(name(s) and familial relationship to infant)* _____

2. Who was the caregiver(s) at the time of the incident? *(name(s) and familial relationship to infant)*

3. Who found the infant unresponsive? *(If caregiver is same as birth mother Skip question #3)*

Full name: _____

Address: _____ City: _____

State: _____ Zip: _____ Date of birth: _____

Email address: _____ Phone number: _____

Work address: _____

Familial relationship to infant? *(e.g., birth mother, grandfather, or adoptive or foster parent)*

4. Describe what happened. *(include details about how the infant was found)*

5. Was there anything different about the infant in the last 24 hours? ☐ Yes ☐ No ☐ Unknown

If yes, describe:

6. What was the temperature in the incident room? ☐ Hot ☐ Cold ☐ Normal ☐ Other

7. Was there a crib, bassinet, or portable crib at the place of incidence? ☐ Yes ☐ No ☐ Unknown

If yes, was it in good or usable condition? (e.g., not broken or not full of laundry) ☐ Yes ☐ No ☐ Unknown

If no, explain:

INCIDENT CIRCUMSTANCES, *continued*

8. Where was the infant (P)laced before death, (L)ast known alive, (F)ound, and (U)sually placed? (*write P, L, F, or U, leave blank if none*)

☐ Crib ☐ Portable Crib ☐ Waterbed ☐ Stroller ☐ Playpen/play area (*not portable crib*)
☐ Bassinet ☐ Sofa/couch ☐ Swing ☐ Futon ☐ Bouncy chair
☐ Bedside sleeper ☐ Chair ☐ Baby box ☐ Floor ☐ Rocking sleeper
☐ Car seat ☐ Unknown ☐ Held in person's arms ☐ In-bed sleeper

☐ Other, specify: _____

☐ Adult bed — *If yes, what type?* ☐ Twin ☐ Full ☐ Queen ☐ King ☐ Unknown

☐ Other, specify: _____

9. Describe the condition and firmness of the surface where the infant was found.

10. Was the infant wrapped or swaddled? ☐ Yes ☐ No ☐ Unknown

If yes: Describe the arm position. ☐ Arms free and out ☐ Arms in ☐ One arm in and one arm out

Describe swaddle. (include blanket type and tightness) _____

11. What was the infant wearing? (*e.g., t-shirt or disposable diaper*) _____

12. What was the infant's usual sleep position? ☐ Sitting ☐ Back ☐ Stomach ☐ Side ☐ Unknown

13. Describe the circumstances of infant when last placed by caregiver, last known alive, and found.

	Placed	Last known alive	Found
Date			
Time			
Location (<i>e.g., living room or bedroom</i>)			
Position (<i>e.g., sitting, back, stomach, side, or unknown</i>)			
Face position (<i>e.g., down, up, left, right, or unknown</i>)			
Neck position (<i>e.g., hyperextended or head back, hyperextended or chin to chest, neutral, or turned</i>)			

14. Was the infant's airway obstructed by a person or object when found? (*includes obstruction of the mouth or nose, or compression of the neck or chest*)

☐ Unobstructed ☐ Fully obstructed ☐ Partially obstructed ☐ Unknown

If fully or partially, what was obstructed or compressed? (check all that apply) ☐ Nose ☐ Mouth ☐ Chest ☐ Neck

15. Indicate the items present in the sleep environment and their positional relation to the infant when the infant was found.

Item	Present?	If yes, position in relation to infant?	If yes, did object obstruct the infant's mouth, nose, chest, or neck?
Adult(s) (18 years or older)	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Other child(ren) (younger than 18 years)	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Animal(s)	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Mattress	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Comforter, quilt or other	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Fitted sheet	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Thin blanket	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Pillow(s)	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Cushion	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Nursing or u-shaped pillow	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Sleep positioner (wedge)	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Bumper pads	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Clothing (not on a person)	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Crib railing or side	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Wall	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Toy(s)	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Other, specify: _____	☐ Yes ☐ No ☐ Unknown	☐ Over ☐ Under ☐ Next to ☐ Unknown	☐ Yes ☐ No ☐ Unknown

If yes to adult(s) or child(ren) sharing sleep surface with the infant, complete table below. ☐ NA

Name of individual(s) sharing sleep surface with infant	Relationship to infant	Age	Height	Weight	Impaired by drugs or alcohol?	Fell asleep feeding infant?
					☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
					☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
					☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown

If yes to impaired, describe: _____

16. Were there any secretions present at the scene? ☐ Yes ☐ No ☐ Unknown

If yes, describe: (include where they were found) _____

17. Was there evidence of wedging? (wedging is an obstruction of the nose or mouth, or compression of the neck or chest as a result of being stuck or trapped between inanimate objects) ☐ Yes ☐ No ☐ Unknown

If yes, describe: _____

18. Was there evidence of overlay? (overlay is an obstruction of the nose or mouth, or compression of the neck or chest as a result of a person rolling on top of or against an infant) ☐ Yes ☐ No ☐ Unknown

If yes, describe: _____

19. Was the infant breathing when found? ☐ Yes ☐ No ☐ Unknown

If no, did anyone witness the infant stop breathing? ☐ Yes ☐ No ☐ Unknown

20. Describe the infant's appearance when found. (*indicate all that apply*)

Appearance	Present?	Describe and specify location
Discoloration around face, nose, or mouth	☐ Yes ☐ No ☐ Unknown	
Secretions or fluids (<i>e.g., foam, froth, or urine</i>)	☐ Yes ☐ No ☐ Unknown	
Skin discoloration (<i>e.g., livor mortis, pale areas, darkness, or color changes</i>)	☐ Yes ☐ No ☐ Unknown	
Pressure marks (<i>e.g., pale areas, or blanching</i>)	☐ Yes ☐ No ☐ Unknown	
Rash or petechiae (<i>e.g., small, red blood spots on skin, membrane, or eyes</i>)	☐ Yes ☐ No ☐ Unknown	
Marks on body (<i>e.g., scratches or bruises</i>)	☐ Yes ☐ No ☐ Unknown	
Other: _____	☐ Yes ☐ No ☐ Unknown	

21. What did the infant feel like when found? (*check all that apply*)

- ☐ Sweaty ☐ Warm to touch ☐ Cool to touch ☐ Limp/flexible ☐ Rigid/stiff ☐ Unknown
☐ Other, specify: _____

22. Did EMS respond? ☐ Yes ☐ No ☐ Unknown

If yes, was the infant transported? ☐ Yes ☐ No ☐ Unknown

23. Was resuscitation attempted? ☐ Yes ☐ No ☐ Unknown

If yes: By whom? (*e.g., EMS, bystander, or parent*) _____

Date: (*mm/dd/yyyy*) _____ Time: _____

Type of compression? (*check all that apply*)

- ☐ Two finger ☐ One hand ☐ Two hands

Was rescue breathing done? ☐ Yes ☐ No ☐ Unknown

The following questions refer to the caregiver(s) at the time of death.

24. Has the caregiver ever had a child under their care die suddenly and unexpectedly?

☐ Yes ☐ No ☐ Unknown

If yes, explain: (*include familial relationship of child and infant, and cause of death*) _____

25. Were the infant and caregiver in the *same room* at the time of the incident, but not sharing the same sleep surface?

☐ Yes ☐ No ☐ Unknown ☐ N/A - sharing a sleep surface

26. Was the infant's caregiver using any of the following during the incident? (*indicate all that apply*)

Substance	Caregiver used?	Frequency
Over the counter medications	☐ Yes ☐ No ☐ Unknown	
Prescription medications	☐ Yes ☐ No ☐ Unknown	
Opioids	☐ Yes ☐ No ☐ Unknown	
Tobacco, specify: (<i>e.g., cigarettes or e-cigarettes</i>)	☐ Yes ☐ No ☐ Unknown	
Alcohol	☐ Yes ☐ No ☐ Unknown	
Herbal remedies	☐ Yes ☐ No ☐ Unknown	
Other, specify: _____	☐ Yes ☐ No ☐ Unknown	

Was the infant's caregiver asked to consent to blood or urine for drug/alcohol testing? ☐ Yes ☐ No ☐ Unknown

If yes, what were the results? _____

INVESTIGATION SUMMARY

1. Arrival dates and times.

Person(s) involved	Hospital	Incident scene
Infant		N/A
Law enforcement		
Death investigator		

2. Agencies conducting an investigation? (check all that apply) ☐ Child protective services
☐ Death investigator from medical examiner or coroner office ☐ Law enforcement, specify: _____
☐ Other, specify: _____

3. Indicate when the form was completed. Date: (mm/dd/yyyy) _____ Time: _____

4. If more than one person was interviewed, does the information provided differ? ☐ Yes ☐ No ☐ N/A
 If yes, detail any differences or inconsistencies of relevant information. (e.g., placed on sofa or last known alive on chair)

5. Indicate the task(s) performed. (check all that apply) ☐ Additional scene(s) (forms attached) conducted ☐ Photos or video taken
☐ Materials collected or evidence logged ☐ Next of kin notified ☐ 911 tape obtained ☐ EMS run sheet or report obtained
☐ Witness(es)/caregiver(s) interviewed

6. Was the family offered grief counseling services? ☐ Yes ☐ No ☐ Unknown

7. Was a doll scene reenactment performed? ☐ Yes ☐ No ☐ Unknown

If no, why? _____

If yes: How was it documented? (check all that apply) ☐ Photographed ☐ Videoed ☐ Other, specify: _____

Where was it performed? ☐ Incident scene ☐ Hospital ☐ Other, specify: _____

Indicate when the doll reenactment was performed. Date performed: (mm/dd/yyyy) _____ Time performed: _____

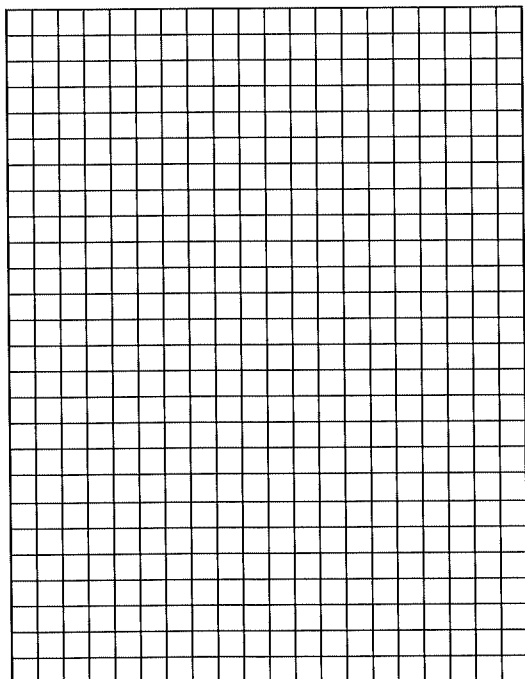
Were photos provided to the pathologist? ☐ Yes ☐ No ☐ Unknown

Do the scenarios given during the doll reenactment(s) match what was seen during the preliminary investigation?

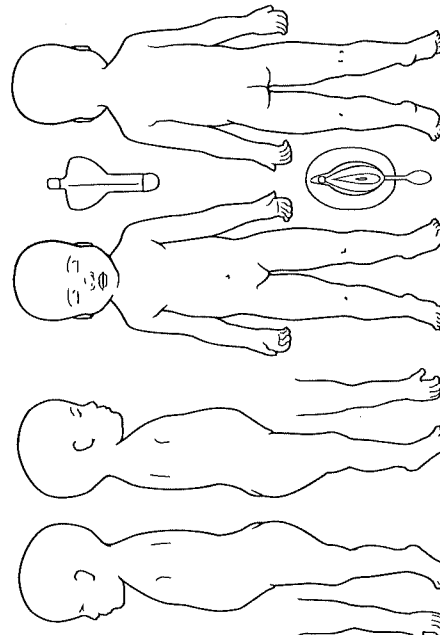
☐ Yes ☐ No ☐ N/A

INVESTIGATION DIAGRAMS

1. Scene diagram (illustrate the infant's sleep environment)



2. Body diagram (note visible injuries, livor mortis, or rigor mortis)



3. Scene and doll reenactment photos (include with form)

SUMMARY FOR PATHOLOGIST

1. Investigator information. Name: _____ Agency: _____
 Phone: _____ Email address: _____
2. Indicate when the investigation took place. Date: *(mm/dd/yyyy)* _____ Time: _____
3. Indicate when the infant was pronounced dead. Date: *(mm/dd/yyyy)* _____ Time: _____
4. Indicate when it is estimated the infant died. Date: *(mm/dd/yyyy)* _____ Time: _____
5. Location of death: *(e.g., home or hospital)* _____
6. Data sources consulted to complete this form. *(check all that apply)* ☐ Infant medical records ☐ Birth records ☐ Prenatal records
☐ Witness interview ☐ Photos/videos from caregivers demonstrating injuries, developmental milestone, or medical concerns
☐ Other, specify: _____

7. Indicate whether preliminary investigation suggests any of the following. *(indicate all that apply)*

Sleeping Environment	Yes	No
Asphyxia <i>(e.g., evidence of overlying, wedging, choking, nose or mouth obstruction, re-breathing, neck or chest compression, or immersion in water)</i>	☐	☐
Sharing of sleep surface with adults, children, or pets	☐	☐
Change in sleep condition <i>(e.g., unaccustomed stomach sleep position, location, or sleep surface)</i>	☐	☐
Hyperthermia or hypothermia <i>(e.g., excessive wrapping, blankets, clothing, or hot or cold environments)</i>	☐	☐
Environmental hazards <i>(e.g., carbon monoxide, noxious gases, chemicals, drugs, or devices)</i>	☐	☐
Unsafe sleep condition <i>(e.g., non-supine, couch, adult bed, stuffed toys, pillows, or soft bedding)</i>	☐	☐

Infant History	Yes	No
Diet <i>(e.g., solids introduced)</i>	☐	☐
Recent hospitalization	☐	☐
Previous medical diagnosis	☐	☐
History of acute life threatening events <i>(e.g., apnea, seizures, or difficulty breathing)</i>	☐	☐
History of medical care without diagnosis	☐	☐
Recent fall or other injury	☐	☐
History of religious, cultural or alternative remedies	☐	☐
Cause of death due to natural causes other than SIDS <i>(e.g., birth defects or complications of preterm birth)</i>	☐	☐

Family Information	Yes	No
Prior sibling deaths	☐	☐
Sudden or unexpected death before the age of 50 or heart disease <i>(e.g., cardiomyopathy, Marfan or Brugada syndrome, long or short QT syndrome, catecholaminergic polymorphic ventricular tachycardia)</i> among the infant's blood relatives <i>(e.g., siblings, parents, grandparents, aunts, uncles, or first cousins)</i>	☐	☐
Previous encounters with police or social service agencies	☐	☐
Request for tissue or organ donation	☐	☐
Objection to autopsy	☐	☐

Exam	Yes	No
Preterminal resuscitative treatment	☐	☐
Signs of trauma or injury, poisoning, or intoxication	☐	☐

Other	Yes	No
Suspicious circumstances	☐	☐
Other alerts for pathologist's attention	☐	☐

If yes to any of the above, explain in detail: (description of circumstances)

8. Medical examiner or pathologist information.

Name: _____

Agency: _____

Phone: _____ Fax: _____ Email address: _____

Visit <https://www.cdc.gov/sids/SUIDRF.htm> for Additional Investigative Scene Forms of Body Diagram, EMS Interview, Hospital Interview, Immunization Record, Infant Exposure History, Informant Contact, Law Enforcement Interview, Materials Collection Log, Non Professional Responder Interview, Parental Information, Primary Residence Investigation, and Scene Diagram.

4. SDT FOR HISTOLOGY SLIDES

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IN THE SUPERIOR COURT OF THE STATE OF WASHINGTON
IN AND FOR THE COUNTY OF KING

STATE OF WASHINGTON,)
)
Plaintiff,) CAUSE NO. _____
Vs.) SUBPOENA
(client name))
)
Defendant.)

GREETINGS: King County Medical Examiner's Office
908 Jefferson St.
Seattle, WA 98104

YOU ARE HEREBY COMMANDED to provide to ASSOCIATED COUNSEL FOR
THE ACCUSED, a public defense law firm:
Biological slides related to the examination of (child name and D.O.B.) in KCMEO
(autopsy case number)

YOU ARE FURTHER COMMANDED to provide the aforementioned materials on or
before the (date), by calling for pick up or mailing to the offices of (defense law firm
address) , ATTN: (name, address, email, phone number of person who picks it up)

DONE IN OPEN COURT this _____ day of _____ 202__.

Judge
(attorney name and signature) _____

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RULE CR 45 SUBPOENAS

(c) Protection of Persons Subject to Subpoenas.

(1) A party or an attorney responsible for the issuance and service of a subpoena shall take reasonable steps to avoid imposing undue burden or expense on a person subject to that subpoena. The court shall enforce this duty and impose upon the party or attorney in breach of this duty an appropriate sanction, which may include, but is not limited to, lost earnings and a reasonable attorney's fee.

(2)(A) A person commanded to produce and permit inspection and copying of designated books, papers, documents or tangible things, or inspection of premises need not appear in person at the place of production or inspection unless commanded to appear for deposition, hearing or trial.

(B) Subject to subsection (d)(2) of this rule, a person commanded to produce and permit inspection and copying may, within 14 days after service of the subpoena or before the time specified for compliance if such time is less than 14 days after service, serve upon the party or attorney designated in the subpoena written objection to inspection or copying of any or all of the designated materials or of the premises. If objection is made, the party serving the subpoena shall not be entitled to inspect and copy the materials or inspect the premises except pursuant to an order of the court by which the subpoena was issued. If objection has been made, the party serving the subpoena may, upon notice to the person commanded to produce and all other parties, move at any time for an order to compel the production. Such an order to compel production shall protect any person who is not a party or an officer of a party from significant expense resulting from the inspection and copying commanded.

(3)(A) On timely motion, the court by which a subpoena was issued shall quash or modify the subpoena if it:

(i) fails to allow reasonable time for compliance;

(ii) fails to comply with RCW 5.56.010 or subsection (e)(2) of this rule;

(iii) requires disclosure of privileged or other protected matter and no exception or waiver applies; or

1 (iv) subjects a person to undue burden, provided that the court may condition
2 denial of the motion upon a requirement that the subpoenaing party advance the
reasonable cost of producing the books, papers, documents, or tangible things.

3 (B) If a subpoena

4 (i) requires disclosure of a trade secret or other confidential research,
development, or commercial information, or

5 (ii) requires disclosure of an unretained expert's opinion or
6 information not describing specific events or occurrences in dispute and
7 resulting from the expert's study made not at the request of any party, the
8 court may, to protect a person subject to or affected by the subpoena, quash
9 or modify the subpoena or, if the party in whose behalf the subpoena is
issued shows a substantial need for the testimony or
material that cannot be otherwise met without undue hardship and assures
that the person to whom the subpoena is addressed will be reasonably
compensated, the court may order appearance or production only upon
specified conditions.

10 (d) Duties in Responding to Subpoena.

11 (1) A person responding to a subpoena to produce documents shall produce
12 them as they are kept in the usual course of business or shall organize and
label them to correspond with the categories in the demand.

13 (2)(A) When information subject to a subpoena is withheld on a claim that it is
14 privileged or subject to protection as trial preparation materials, the claim shall
be made expressly and shall be supported by a description of the nature of the
documents, communications, or things not produced that is sufficient to enable
15 the demanding party to contest the claim.

16 (B) If information produced in response to a subpoena is subject to a claim of
17 privilege or of protection as trial-preparation material, the person making the
claim may notify any party that received the information of the claim and the
18 basis for it. After being notified, a party must promptly return, sequester, or
destroy the specified information and any copies it has; must not use or
disclose the information until the claim is resolved; must take reasonable steps
19 to retrieve the information if the party disclosed it before being notified; and may
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1 promptly present the information in camera to the court for a determination of
2 the claim. The person responding to the subpoena must preserve the
3 information until the claim is resolved.
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DECLARATION OF SERVICE:

I declare under penalty of perjury under the laws of the State of Washington
that the foregoing information contained in the proof of service is true and
correct.

Executed on this _____ day of _____, 202 in the City of
Seattle.

(defense agency address)

DECLARATION

I, (investigator or name of person serving SDT) , hereby declare under
penalty of perjury under the laws
of the State of Washington that the original copy of this subpoena was
served on the above named witness on the _____ day of _____, 2020.
Service of this subpoena was made in the following manner:

5. SDT FOR NEWBORN SCREENING
VICTIM NOTIFICATION
DECLARATION FOR JUDGE



**Department of
PUBLIC DEFENSE**

*Upholding the Constitution,
one client at a time.*

ACA Division—Kent Office
420 West Harrison, Suite 201
Kent, WA 98032
(206) 477-8996 Fax: (253) 520-6635
TTY Relay 711

(date)

Washington State Newborn Screening Program
1610 NE 150th Street
PO Box 55729
Shoreline, WA 98155
Care of: John Thompson, Lab Director

(next of kin name and various addresses you found)

Re: *State of Washington v. (case name and number)*

Dear Washington State Newborn Screening Program / (name of victim's next of kin)

I represent (client) . He is currently charged with (charge). (names of prosecutors) are the assigned deputy prosecutors (phone number of both prosecutors) respectively. The alleged victim is (full name of child and date of birth). We are seeking blood splotch information that is currently held by the Washington State Newborn Screening Program which is relevant to the defense in our case.

Pursuant to Criminal Rule 4.8, I am giving you notice that on or about (at least two weeks out per court rule) I will be asking the court to sign one subpoena for the blood splotch described above. I have enclosed a copy of the subpoena for your review.

If you have any questions, please contact me at (attorney name, paralegal name, email and phone numbers)

Sincerely,

(signature, name, work address)

CC: (prosecutors' names , Deputy Prosecution Attorneys)



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IN THE SUPERIOR COURT OF THE STATE OF WASHINGTON
IN AND FOR THE COUNTY OF KING

STATE OF WASHINGTON,	)	
	)	
Plaintiff,	)	CAUSE NO. (number)
Vs.	)	
	)	SUBPOENA DUCES TECUM
(case name)	)	
	)	
Defendant.	)	

GREETINGS: Washington State Newborn Screening Program
1610 NE 150th Street
PO Box 55729
Shoreline, WA 98155
Care of: John Thompson, lab director

YOU ARE HEREBY COMMANDED to provide to (name of law firm) a public
defense law firm:

Blood splotch taken at birth of (name of child and date of birth) and held in the
custody of the Washington State Newborn Screening Program. The requested material
is to be provided to (name of lab recommended by defense expert) referencing (defense
medical expert's name) , defense medical examiner, (name of child and d.o.b.) and
KCMEQ case number (case file number on autopsy). Defense Counsel (name of
attorney (s)) can coordinate as needed.

YOU ARE FURTHER COMMANDED to provide the aforementioned materials and/or
work with defense counsel to send the materials out on or before the (date decided above),

1 by calling for pick up or mailing to the offices of (Full defense firm name, address, attorney
2 names, emails and phone numbers).

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5 DONE IN OPEN COURT this _____ day of _____ 2021.

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7 _____
8 Judge

9 /s)(attorney signature, name, bar, attorney for client)
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RULE CR 45 SUBPOENAS

(c) Protection of Persons Subject to Subpoenas.

(1) A party or an attorney responsible for the issuance and service of a subpoena shall take reasonable steps to avoid imposing undue burden or expense on a person subject to that subpoena. The court shall enforce this duty and impose upon the party or attorney in breach of this duty an appropriate sanction, which may include, but is not limited to, lost earnings and a reasonable attorney's fee.

(2)(A) A person commanded to produce and permit inspection and copying of designated books, papers, documents or tangible things, or inspection of premises need not appear in person at the place of production or inspection unless commanded to appear for deposition, hearing or trial.

(B) Subject to subsection (d)(2) of this rule, a person commanded to produce and permit inspection and copying may, within 14 days after service of the subpoena or before the time specified for compliance if such time is less than 14 days after service, serve upon the party or attorney designated in the subpoena written objection to inspection or copying of any or all of the designated materials or of the premises. If objection is made, the party serving the subpoena shall not be entitled to inspect and copy the materials or inspect the premises except pursuant to an order of the court by which the subpoena was issued. If objection has been made, the party serving the subpoena may, upon notice to the person commanded to produce and all other parties, move at any time for an order to compel the production. Such an order to compel production shall protect any person who is not a party or an officer of a party from significant expense resulting from the inspection and copying commanded.

(3)(A) On timely motion, the court by which a subpoena was issued shall quash or modify the subpoena if it:

(i) fails to allow reasonable time for compliance;

(ii) fails to comply with RCW 5.56.010 or subsection (e)(2) of this rule;

(iii) requires disclosure of privileged or other protected matter and no exception or waiver applies; or

1 (iv) subjects a person to undue burden, provided that the court may condition
2 denial of the motion upon a requirement that the subpoenaing party advance the
reasonable cost of producing the books, papers, documents, or tangible things.

3 (B) If a subpoena

4 (i) requires disclosure of a trade secret or other confidential research,
development, or commercial information, or

5 (ii) requires disclosure of an unretained expert's opinion or
6 information not describing specific events or occurrences in dispute and
7 resulting from the expert's study made not at the request of any party, the
8 court may, to protect a person subject to or affected by the subpoena, quash
9 or modify the subpoena or, if the party in whose behalf the subpoena is
issued shows a substantial need for the testimony or
material that cannot be otherwise met without undue hardship and assures
that the person to whom the subpoena is addressed will be reasonably
compensated, the court may order appearance or production only upon
specified conditions.

10 (d) Duties in Responding to Subpoena.

11 (1) A person responding to a subpoena to produce documents shall produce
12 them as they are kept in the usual course of business or shall organize and
label them to correspond with the categories in the demand.

13 (2)(A) When information subject to a subpoena is withheld on a claim that it is
14 privileged or subject to protection as trial preparation materials, the claim shall
be made expressly and shall be supported by a description of the nature of the
documents, communications, or things not produced that is sufficient to enable
15 the demanding party to contest the claim.

16 (B) If information produced in response to a subpoena is subject to a claim of
privilege or of protection as trial-preparation material, the person making the
17 claim may notify any party that received the information of the claim and the
basis for it. After being notified, a party must promptly return, sequester, or
18 destroy the specified information and any copies it has; must not use or
disclose the information until the claim is resolved; must take reasonable steps
to retrieve the information if the party disclosed it before being notified; and may
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1 promptly present the information in camera to the court for a determination of
2 the claim. The person responding to the subpoena must preserve the
3 information until the claim is resolved.
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DECLARATION OF SERVICE:

I declare under penalty of perjury under the laws of the State of Washington that the foregoing information contained in the proof of service is true and correct.

Executed on this _____ day of _____, 2021 in the City of _____.

(office address and name)

DECLARATION

I, _____, hereby declare under penalty of perjury under the laws

of the State of Washington that the original copy of this subpoena was served on the above named witness on the _____ day of _____, 2021.

Service of this subpoena was made in the following manner:

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7 IN THE SUPERIOR COURT OF THE STATE OF WASHINGTON IN AND FOR THE COUNTY OF
8 KING
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10 STATE OF WASHINGTON) Cause No.: (number)
11)
12 Plaintiff,) DECLARATION
13)
14 vs.)
15 (case name))
16)
17 Defendant)
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I, (attorney) declare as follows:

I am a licensed attorney in the state of Washington and a staff attorney at (office name) . I am over the age of 18 and I am competent to testify to the matters set forth herein.

1. I am one of the attorneys for (client). He is charged with (charge)

2. I will seek a subpoena for records from the Washington State Newborn Screening Program for blood splotch materials belonging to (name and date of birth of child in full) , the alleged victim, taken during his birth. This information is relevant to the defense in this case.

1 3. Pursuant to Criminal Rule 4.8, I wrote a letter to the Estate of
2 (child's initials) , providing notice of my intent to seek this
3 subpoena on (2 weeks out per rule) through the King County
4 Prosecutor's Office and at the most recent addresses that could be
5 found through the Department of Licensing and CLEAR - a database
6 defense has access to. The letter is attached. I also sent this
7 declaration and the letter to the King County Prosecutor's office on
8 (date sent).

9 4. By conveying these materials on (date of sent to victim's family) I
10 believe enough time has been provided for the Estate (child's
11 initial) to respond by the selected requested signature date of
12 (signature date).

13 5. Because no objection has been noted, Defense is seeking signature of
14 the attached Subpoena.

15
16 I hereby certify under penalty of perjury under the laws of the State of
17 Washington that the foregoing is true and correct.

18
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20 DATED THIS (date) in Seattle, WA.

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22
23 (attorney name and signature)
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**6. SDT FOR BLOOD SPLOTCH
VICTIM NOTIFICATION
DECLARATION FOR JUDGE**



**Department of
PUBLIC DEFENSE**

*Upholding the Constitution,
one client at a time.*

ACA Division—Kent Office
420 West Harrison, Suite 201
Kent, WA 98032
(206) 477-8996 Fax: (253) 520-6635
TTY Relay 711

(date)

King County Medical Examiner's Office
HMC Box 359792
325 9th Ave
Seattle, WA 98104-2499

(next of kin name and various addresses)

Re: *State of Washington v. (name of client and case number)*

Dear King County Medical Examiner's Office / (name of next of kin)

I represent (client name). He is currently charged with (charge). (prosecutor names) are the assigned deputy prosecutors (their contact phone numbers) respectively. The alleged victim is (child's initials and date of birth). We are seeking blood splotch information that is currently held by the King County Medical Examiner's Office, number (autopsy number) pursuant to an autopsy performed on (initials of the child and date of birth) which is relevant to the defense in our case.

Pursuant to Criminal Rule 4.8, I am giving you notice that on or about (notice period per statute) I will be asking the court to sign one subpoena for the blood splotch described above. I have enclosed a copy of the subpoena for your review.

If you have any questions, please contact me at (email and phone number of attorney and paralegal)

Sincerely,

(signature, address, name of attorney)

CC: (name of prosecutors), Deputy Prosecutors



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IN THE SUPERIOR COURT OF THE STATE OF WASHINGTON
IN AND FOR THE COUNTY OF KING

STATE OF WASHINGTON,	)	
	)	
Plaintiff,	)	CAUSE NO. (number)
Vs.	)	
	)	SUBPOENA DUCES TECUM
(case name)	)	
	)	
Defendant.	)	

GREETINGS: King County Medical Examiner's Office
HMC Box 359792
325 9th Ave
Seattle, WA 98104-2499

YOU ARE HEREBY COMMANDED to provide to (attorney's address):
Blood splotch evidence taken from an autopsy of (initials and date of birth of child)
and held in the custody of the King County Medical Examiner's Office. The requested
material is to be provided to ZRT Laboratory, 8605 SW Creekside Place, Beaverton,
OR 97008 referencing (defense expert), defense medical examiner (full name and date
of birth of child), and KCMEO case number (autopsy number).. Defense Counsel
(attorneys) can coordinate as needed.

YOU ARE FURTHER COMMANDED to provide the aforementioned materials and/or
work with defense counsel to send the materials out on or before the (date of subpoena), by
calling for pick up or mailing to the offices of (address for attorneys, emails, phone
numbers)

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DONE IN OPEN COURT this _____ day of _____ 2021.

Judge

/s/ (signature)
(attorney name and bar number)

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RULE CR 45 SUBPOENAS

(c) Protection of Persons Subject to Subpoenas.

(1) A party or an attorney responsible for the issuance and service of a subpoena shall take reasonable steps to avoid imposing undue burden or expense on a person subject to that subpoena. The court shall enforce this duty and impose upon the party or attorney in breach of this duty an appropriate sanction, which may include, but is not limited to, lost earnings and a reasonable attorney's fee.

(2)(A) A person commanded to produce and permit inspection and copying of designated books, papers, documents or tangible things, or inspection of premises need not appear in person at the place of production or inspection unless commanded to appear for deposition, hearing or trial.

(B) Subject to subsection (d)(2) of this rule, a person commanded to produce and permit inspection and copying may, within 14 days after service of the subpoena or before the time specified for compliance if such time is less than 14 days after service, serve upon the party or attorney designated in the subpoena written objection to inspection or copying of any or all of the designated materials or of the premises. If objection is made, the party serving the subpoena shall not be entitled to inspect and copy the materials or inspect the premises except pursuant to an order of the court by which the subpoena was issued. If objection has been made, the party serving the subpoena may, upon notice to the person commanded to produce and all other parties, move at any time for an order to compel the production. Such an order to compel production shall protect any person who is not a party or an officer of a party from significant expense resulting from the inspection and copying commanded.

(3)(A) On timely motion, the court by which a subpoena was issued shall quash or modify the subpoena if it:

(i) fails to allow reasonable time for compliance;

(ii) fails to comply with RCW 5.56.010 or subsection (e)(2) of this rule;

(iii) requires disclosure of privileged or other protected matter and no exception or waiver applies; or

1 (iv) subjects a person to undue burden, provided that the court may condition
2 denial of the motion upon a requirement that the subpoenaing party advance the
reasonable cost of producing the books, papers, documents, or tangible things.

3 (B) If a subpoena

4 (i) requires disclosure of a trade secret or other confidential research,
development, or commercial information, or

5 (ii) requires disclosure of an unretained expert's opinion or
6 information not describing specific events or occurrences in dispute and
7 resulting from the expert's study made not at the request of any party, the
8 court may, to protect a person subject to or affected by the subpoena, quash
9 or modify the subpoena or, if the party in whose behalf the subpoena is
issued shows a substantial need for the testimony or
material that cannot be otherwise met without undue hardship and assures
that the person to whom the subpoena is addressed will be reasonably
compensated, the court may order appearance or production only upon
specified conditions.

10 (d) Duties in Responding to Subpoena.

11 (1) A person responding to a subpoena to produce documents shall produce
12 them as they are kept in the usual course of business or shall organize and
label them to correspond with the categories in the demand.

13 (2)(A) When information subject to a subpoena is withheld on a claim that it is
14 privileged or subject to protection as trial preparation materials, the claim shall
be made expressly and shall be supported by a description of the nature of the
documents, communications, or things not produced that is sufficient to enable
15 the demanding party to contest the claim.

16 (B) If information produced in response to a subpoena is subject to a claim of
17 privilege or of protection as trial-preparation material, the person making the
claim may notify any party that received the information of the claim and the
18 basis for it. After being notified, a party must promptly return, sequester, or
destroy the specified information and any copies it has; must not use or
disclose the information until the claim is resolved; must take reasonable steps
19 to retrieve the information if the party disclosed it before being notified; and may
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1 promptly present the information in camera to the court for a determination of
2 the claim. The person responding to the subpoena must preserve the
3 information until the claim is resolved.
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DECLARATION OF SERVICE:

I declare under penalty of perjury under the laws of the State of Washington that the foregoing information contained in the proof of service is true and correct.

Executed on this _____ day of _____, 2021 in the City of Seattle.

(attorney's address)

DECLARATION

I, _____, hereby declare under penalty of perjury under the laws

of the State of Washington that the original copy of this subpoena was served on the above named witness on the _____ day of _____, 2021.

Service of this subpoena was made in the following manner:

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7 IN THE SUPERIOR COURT OF THE STATE OF WASHINGTON IN AND FOR THE COUNTY OF
8 KING
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10 STATE OF WASHINGTON) Cause No.: (number)
11)
12 Plaintiff,) DECLARATION
13)
14 vs.)
15 (case name))
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17 Defendant)
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29 I, (attorney), declare as follows:

30 I am a licensed attorney in the state of Washington and (where you
31 work). I am over the age of 18 and I am competent to testify to the matters
32 set forth herein.

33 1. I am one of the attorneys for (client's name). He is charged with
34 (charge).

35 2. I will seek a subpoena for records from the King County Medical
36 Examiner's Office for blood splootch materials belonging to (victim
37 initials and date of birth), the alleged victim, taken during autopsy
38 in this case.

1
2 3. Pursuant to Criminal Rule 4.8, I wrote a letter to the Estate of
3 (child's initials) providing notice of my intent to seek this
4 subpoena on (date you'll get the signature) through the King County
5 Prosecutor's Office and at the most recent addresses that could be
6 found through the Department of Licensing and CLEAR - a database
7 defense has access to. The letter is attached. I also sent this
8 declaration and the letter to the King County Prosecutor's office on
9 (when you sent the letter).

10 4. By conveying these materials on (date you send the letter) I believe
11 enough time has been provided for the Estate MHA to respond by the
12 selected requested signature date of (date for signature 14 plus days
13 out)

14 5. Because no objection has been noted, I am now seeking signature of
15 the attached Subpoena.

16
17 I hereby certify under penalty of perjury under the laws of the State of
18 Washington that the foregoing is true and correct.

19
20
21 DATED THIS (date) in Seattle, WA.

22
23 (attorney signature, name, bar number and name of client)
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7. DEPOSITION TRANSCRIPT SDT

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5 IN THE SUPERIOR COURT OF THE STATE OF WASHINGTON IN AND FOR THE COUNTY OF
6 KING
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8 STATE OF WASHINGTON) Cause No.: (case number)
9)
10) Plaintiff,) SUBPOENA DUCES TECUM
11)
12) vs.)
13) (client name))
14)
15) Defendant)
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Greetings:

(court reporter company, name, address, email)

Requested depositions:

- (name of witness and name of court reporter)

You are hereby ordered to provide complete transcripts in .pdf/.docx file format of the above referenced depositions relating to cause number (number of civil case) to (attorney address) by no later than (reasonable date). Please send transcripts to (attorney email) or via mail to (attorney address) If there are questions about this subpoena please send emails to the above or call (attorney phone)

JUDGE

Presented by:

/s/ (attorney name and signature)

RULE CrR 4.8 SUBPOENAS

a) For Attendance of Witnesses at Hearing or Trial. A subpoena commanding a person to attend and give testimony at a hearing or at trial ("a subpoena for testimony") shall be issued as follows:

(1) Form; Issuance.

(A) A subpoena for testimony shall (i) state the title of the action, the case number, the name of the court in which the action is pending, and, if different, the name of the court from which the subpoena is issued; and (ii) command each person to whom it is directed to attend and give testimony at a specified time and place.

(B) The court in which the action is pending or before which attendance is required may issue a subpoena for testimony under the seal of that court, or the clerk may issue the subpoena for testimony in response to a praecipe. An attorney for a party also may sign and issue a subpoena for testimony.

(C) A command to a person to produce evidence or to permit inspection may be joined with a subpoena for testimony or may be issued separately under section (b) of this rule.

(2) Notice. Notice to each party of the issuance of a subpoena for testimony is not required; provided that, when a subpoena for testimony also commands the person to whom it is directed to produce evidence or to permit inspection of things, the serving party shall give advance notice of such subpoena in the manner described in subsection (b) of this rule.

(3) Service-How Made. A subpoena for testimony may be served by any suitable person over 18 years of age, by giving the witness a copy thereof, or by leaving a copy at the witness's dwelling house or usual place of abode with some person of suitable age and discretion then residing therein. When service is made by any person other than an officer authorized to serve process, proof of service shall be made by affidavit or declaration. A subpoena for testimony may also be served by first-class mail, postage prepaid, together with a waiver of personal service and instructions for returning such waiver to the attorney of record of the party to the action in whose behalf the witness is required to appear. Service by mail shall be deemed complete upon the filing of the returned waiver of personal service, signed in affidavit or declaration form.

(4) When Witness Excused. A witness subpoenaed to attend a hearing or trial is excused from further attendance as soon as the witness has given testimony in chief and has been cross-examined thereon, unless a party moves in open court that the witness remain in attendance and the court so orders. Witness fees will not be allowed any witness after the day on which the witness's testimony is given, except when the witness has in open court been required to remain in further attendance, and when so required the clerk shall note that fact in the minutes.

(b) For Producing Evidence or Permitting Inspection. A subpoena commanding a person to produce and permit inspection and copying of designated documents, tangible things, or premises in the possession, custody, or control of that person ("a subpoena for production") shall be issued as follows:

(1) Form; Issuance.

(A) A subpoena for production shall (i) state the title of the action, the case number, the name of the court in which the action is pending, and, if different, the name of the court from which the subpoena is issued;

1 (ii) command each person to whom it is directed to produce and permit inspection and
2 copying of documentary evidence, tangible things, or premises in the possession,
3 custody, or control of that person at a specified time and place; and (iii) set forth
4 the text of subsection (b)(4) of this rule.

5 (B) The court in which the action is pending or before which attendance is required
6 may issue a subpoena for production under the seal of that court or the clerk may
7 issue the subpoena in response to a praecipe. An attorney for a party also may sign
8 and issue a subpoena for production.

9 (C) A subpoena for production may be joined with a subpoena for testimony, or it may
10 be issued separately, provided that a subpoena to inspect premises may not be combined
11 with other subpoenas issued pursuant to this rule.

12 (2) Notice. Notice to parties of the issuance of a subpoena for production is not
13 required; provided that, whenever a party intends to serve a subpoena for production
14 seeking evidence or inspection of things or premises belonging or pertaining to a
15 defendant who is not the party seeking or issuing the subpoena, then the serving party
16 must give all parties advance notice; and provided that, whenever any party intends to
17 serve a subpoena for production seeking evidence or inspection of things belonging or
18 pertaining to an alleged victim or complaining witness, then the serving party shall
19 provide advance notice to all parties and to the alleged victim or complaining
20 witness; and provided that a subpoena for inspection of premises must be signed by the
21 court and only after good cause is shown and advance notice is provided to all parties
22 and the owner or occupier of the premises.

23 (A) Time and Manner. If advance notice is required under this rule, then no fewer than
24 five days prior to service on the person named in the subpoena for production, notice
25 shall be provided in the manner prescribed by CR 5(b). The parties may agree to
26 shorten the time for advance notice when a subpoena seeks solely evidence or tangible
27 things belonging or pertaining to a defendant. The court may shorten the time for
28 advance notice upon a showing of good cause by a party; provided that, any alleged
victim or complaining witness whose evidence, tangible things, or premises are sought
shall receive notice and an opportunity to be heard on any motion to shorten time.

(B) Court May Excuse Notice. A court on ex parte motion may excuse compliance with the
advance notice requirement upon the serving party's showing of good cause; any such
court order, along with a copy of the subpoena for which notice is excused, shall be
filed under seal pursuant to GR 15.

(3) Service-How Made. A subpoena for production shall be served in the manner
prescribed in CR 5(b); provided that, if the subpoena for production is joined with a
subpoena for testimony, then subsection (a)(3) of this rule shall govern service.

(4) Protection of Persons Subject to Subpoena for Production. On timely motion, the
court may quash or modify a subpoena for production if it (A) fails to allow
reasonable time for compliance; (B) requires disclosure of privileged or other
protected matter and no exception or waiver applies; (C) is unreasonable, oppressive,
or unduly burdensome; or (D) exceeds the scope of discovery otherwise permitted under
the criminal rules. The court may condition denial of a motion to quash or modify
upon the advancement by the party on whose behalf the subpoena for production is
issued of the reasonable cost of producing the books, papers, documents, tangible
things, or premises.

(5) Applicability of Other Notice and Privacy Provisions. The provisions of this rule
do not modify or limit privacy protections and notice requirements provided by court
rule, statute, regulation, or other applicable law.

(c) Contempt. Failure by any person without adequate excuse to obey a subpoena served
upon that person may be deemed a contempt of the court from which the subpoena issued.

CERTIFICATION OF SERVICE

I, _____, hereby certify under penalty of perjury under the laws of the State of Washington that the original copy of this subpoena was served on the above named witness on the ____ day of _____ 2018 at _____ hours. Service of said subpoena was made in the following manner:

____ Personal Accepted by: _____

____ Posted Address: _____

____ Fax Fax Number: _____
Confirmed with Recipient? ____ Yes ____ No

____ Email Email Address: _____

____ Other Delivery Method: _____

SIGNED and DATED this ____ day of _____ (year), in Kent, WA.

(law firm address)

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7 IN THE SUPERIOR COURT OF THE STATE OF WASHINGTON IN AND FOR THE COUNTY OF
8 KING
9

10 STATE OF WASHINGTON) Cause No.: (number)
11)
12 Plaintiff,) DECLARATION
13)
14 vs.)
15 (case name))
16)
17 Defendant)
18)

19 I, (attorney name) , declare as follows:
20
21

22 I am a licensed attorney in the state of Washington and a staff attorney
23 at (where you work). I am over the age of 18 and I am competent to testify
24 to the matters set forth herein.
25

26 1. I am one of the attorneys for (client name) He is charged with
27 (charge).
28

29 2. I will seek a subpoena for records from the King County Medical
30 Examiner's Office for blood splootch materials belonging to (child's
31 initials and D.O.B.) the alleged victim, taken during autopsy in
32 this case.

1
2 3. Pursuant to Criminal Rule 4.8, I wrote a letter to the Estate of
3 (child's initials) providing notice of my intent to seek this
4 subpoena on (at least two weeks out) through the King County
5 Prosecutor's Office and at the most recent addresses that could be
6 found through the Department of Licensing and CLEAR - a database
7 defense has access to. The letter is attached. I also sent this
8 declaration and the letter to the King County Prosecutor's office on
9 (date sent).

10 4. By conveying these materials on (date notification sent to family of
11 victim) I believe enough time has been provided for the Estate
12 (victim initials) to respond by the selected requested signature date
13 of (signature date for judge).

14 5. Because no objection has been noted, I am now seeking signature of
15 the attached Subpoena.
16

17 I hereby certify under penalty of perjury under the laws of the State of
18 Washington that the foregoing is true and correct.
19
20

21 DATED THIS (date) in Seattle, WA.
22

23 / (attorney
24 signature, name, bar and Attorney for XXX
client)
25
26
27
28

8. SDT FOR PATHOLOGIST'S CASENOTES

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7 **IN THE SUPERIOR COURT OF THE STATE OF WASHINGTON**
8 **IN AND FOR THE COUNTY OF KING**

9 STATE OF WASHINGTON,
10 Plaintiff,

11 vs.

12 (client name)

13 Defendant.

)
) Case No: (case number of your case)
)
)

) SUBPOENA DUCES TECUM
)
)

14
15 GREETINGS: King County Medical Examiner's Office
16 HMC Box 359792
17 325 9th Avenue
18 Seattle, WA 98104-2499
(or your medical examiner's office address and name)

19 YOU ARE HEREBY COMMANDED to provide to (name of your law firm or public defender office):
20

21 All "case notes maintained by King County Medical Examiner's Office number (autopsy number), including
22 But not limited to notes, logs, recordings – be they audio visual – emails, letters, communications, and
23 anything at all pertaining to meetings, including but not limited to doctors from (any other hospital doctors
24 involved in the discussions with ME and prosecutors, often SCAN doctors or those who recently treated
25 child), relating to a death investigation and autopsy of (initials and date of birth of child). This subpoena is
26 not seeing internal King County Prosecuting Attorney Office files (or your prosecutor's office) files – only
27 files and records held in the custody of the King County Medical Examiner's Office.
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YOU ARE FURTHER COMMANDED to provide the aforementioned materials on or before the (date you
picked at least 2 weeks out), by calling for pick up or mailing to the offices of (your office name), ATTN:
(attorney name, email, fax, phone number).

DONE IN OPEN COURT this _____,

Judge

Attorney name and bar number

RULE CR 45 SUBPOENAS

(c) Protection of Persons Subject to Subpoenas.

(1) A party or an attorney responsible for the issuance and service of a subpoena shall take reasonable steps to avoid imposing undue burden or expense on a person subject to that subpoena. The court shall enforce this duty and impose upon the party or attorney in breach of this duty an appropriate sanction, which may include, but is not limited to, lost earnings and a reasonable attorney's fee.

(2)(A) A person commanded to produce and permit inspection and copying of designated books, papers, documents or tangible things, or inspection of premises need not appear in person at the place of production or inspection unless commanded to appear for deposition, hearing or trial.

(B) Subject to subsection (d)(2) of this rule, a person commanded to produce and permit inspection and copying may, within 14 days after service of the subpoena or before the time specified for compliance if such time is less than 14 days after service, serve upon the party or attorney designated in the subpoena written objection to inspection or copying of any or all of the designated materials or of the premises. If objection is made, the party serving the subpoena shall not be entitled to inspect and copy the materials or inspect the premises except pursuant to an order of the court by which the subpoena was issued. If objection has been made, the party serving the subpoena may, upon notice to the person commanded to produce and all other parties, move at any time for an order to compel the production. Such an order to compel production shall protect any person who is not a party or an officer of a party from significant expense resulting from the inspection and copying commanded.

(3)(A) On timely motion, the court by which a subpoena was issued shall quash or modify the subpoena if it:

(i) fails to allow reasonable time for compliance;

(ii) fails to comply with RCW 5.56.010 or subsection (e)(2) of this rule;

(iii) requires disclosure of privileged or other protected matter and no exception or waiver applies; or

(iv) subjects a person to undue burden, provided that the court may condition denial of the motion upon a requirement that the subpoenaing party advance the reasonable cost of producing the books, papers, documents, or tangible things.

(B) If a subpoena

(i) requires disclosure of a trade secret or other confidential research, development, or commercial information, or

(ii) requires disclosure of an unretained expert's opinion or information not describing specific events or occurrences in dispute and resulting from the expert's study made not at the request of any party, the court may, to protect a person subject to or affected by the subpoena, quash or modify the subpoena or, if the party in whose behalf the subpoena is issued shows a substantial need for the testimony or

1 material that cannot be otherwise met without undue hardship and assures that the person
2 to whom the subpoena is addressed will be reasonably compensated, the court may order
appearance or production only upon specified conditions.

3 (d) Duties in Responding to Subpoena.

4 (1) A person responding to a subpoena to produce documents shall produce them as they
5 are kept in the usual course of business or shall organize and label them to correspond with
the categories in the demand.

6 (2)(A) When information subject to a subpoena is withheld on a claim that it is privileged or
7 subject to protection as trial preparation materials, the claim shall be made expressly and
8 shall be supported by a description of the nature of the documents, communications, or
things not produced that is sufficient to enable the demanding party to contest the claim.

9 (B) If information produced in response to a subpoena is subject to a claim of privilege or of
10 protection as trial-preparation material, the person making the claim may notify any party
that received the information of the claim and the basis for it. After being notified, a party
11 must promptly return, sequester, or destroy the specified information and any copies it has;
must not use or disclose the information until the claim is resolved; must take reasonable
12 steps to retrieve the information if the party disclosed it before being notified; and may
promptly present the information in camera to the court for a determination of the claim. The
13 person responding to the subpoena must preserve the information until the claim is
14 resolved.

CERTIFICATE OF SERVICE

I, (PERSON WHO SERVED), hereby certify under penalty of perjury under the laws of the State of Washington that the original copy of this Subpoena was served on the above named witness on the (DATE AND TIME OF SERVICE). Service of said document was made in the following manner:

Personal

Accepted by:

☒ X Posted

Address:

King County Medical Examiner

HMC Box 359792, 325 9th Ave

Seattle, WA 98104

Fax

Fax Number:

Confirmed with Recipient? ☐ Yes ☐ No

Email

Email Address:

Other

Delivery Method:

Signed and Dated this (date) in (location),

Signature, name and address of person serving

CHECKLISTS

Anna Samuel

MEDICAL EVIDENCE FROM STATE

CHECKLIST 1

1. Autopsy
2. SUIDI –CDC forms
3. Histology- Harborview
4. Neuropathology -UW
5. Virology/bacterial testing-PH
6. Radiology – full body scan-UW
7. Dexis imaging
8. SCAN records
9. Toxicology
10. Oral and Anal swabs –cytology and DNA
11. Neck dissection
12. Photos of autopsy/histology
13. Forms filled out by ME
14. Draft and final death certificate
15. Emails between ME and others
16. Case notes - SDT
17. Evidence shared with ME

OTHER DISCOVERY



CHECKLIST 2

1. Civil case discovery
2. Depositions – under oath
3. Research, papers published
4. Presentations by doctors about this subject/case
5. Meetings between prosecutors, doctors and detectives
6. Emails
7. SCAN doctors
8. DCYS or CPS records – subpoena with notice
9. Children's hospital – only SW and nurses are employees
10. Others are private doctors
11. Which hospitals are connected to each other
12. Follow the money
13. How are they paid? Grants, salaries, special projects?

TIMELINE AND FAMILY

CHECKLIST 3

1. Calendars for each year of life
2. important dates/birthdays/events
3. Last 72 hours of child in detail
4. Persons present last 72 hours
5. Any video/phone calls
6. Messenger/text/social media
7. Client
8. Each parent
9. Surrogate parent
10. Grandparents
11. Caregivers
12. Friends

MEDICAL EVIDENCE FROM PARENTS



CHECKLIST 4

1. Prenatal
2. Birth
3. Well baby checks
4. Vaccination
5. Birth defects
6. CPS/school records
7. Medication/Over the counter meds
8. Symptoms
9. Motrin/Tylenol
10. Hospital visits
11. Other illness/injury

ABOUT THE CHILD

CHECKLIST 5

1. Developmental stage
2. Walking, talking
3. Potty training – details/who trains
4. Bathing, dressing
5. Any medically identified concerns
6. Communication, talking, words
7. Discipline – who does it, details
8. Photos/videos over time
9. Favorite food, drink
10. Toy/games
11. Books
12. TV shows
13. Clothes
14. Friends
15. Sleeping patterns
16. Day's schedule in detail