Responsible Conduct of Research Short Course:

The Responsibility of Being Transparent: Science Communication, Sharing, and Publishing

Friday, October 4, 2024, 1-5:30 p.m. CDT, McCormick Place Convention Center Room S103

Agenda:

1:00-1:20pm	Welcome & Overview of course (Lique Coolen) Pre-Survey
1:20-2:10pm	Topic 1: Responsible Conduct of Authorship (Mike Lehman & Christophe Bernard)
2:10-2:20pm	Break
2:20-3:10pm	Topic 2: Responsible Conduct of Peer Review (Christophe & Mike)
3:10-3:20pm	Break
3:20-4:10pm	Topic 3: Open Data Sharing, Rigor, and Reproducibility (Gundula Bosch)
4:10-4:20pm	Break
4:20-5:10pm	Topic 4: Presenting Science to Lay Audiences (Tori Espensen)
5:10-5:30pm	Conclusion & Summary (Lique) Post-survey

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Neuronline Resource: Tricks of the Trade: How to Peer Review a Manuscript

Case 1: Authorship, Transfer of a Project, and Scientific Disagreement

Dr. Cooper had a four-year postdoctoral fellowship in an NIH neuroscience laboratory headed by Dr. Jiang before leaving the NIH for a tenure-track research position at a university. Dr. Cooper published several first-author papers that supported a hypothesis (H1) concerning the role of the immune system in the formation of amyloid-β (Aβ) plaques in Alzheimer's disease in transgenic mice. Dr. Cooper came up with the idea for H1 while in graduate school and joined Dr. Jiang's lab as a postdoctoral fellow with the goal of testing and refining H1. Toward the end of the fellowship, Dr. Cooper began working on a project to determine whether blocking interleukin-10 causes the immune system to remove amyloid-β (Aβ) plaques from the brain. Dr. Cooper developed a protocol for the project and gathered some preliminary data that resulted in their selection for a tenure-track position at the end of the 3rd year of the fellowship. Before leaving, Dr. Cooper and Dr. Jiang agreed, by email, that Dr. Cooper would continue working on the project as an NIH Special Volunteer, would have access to NIH data, and would be the first author of a paper reporting the project's results. Dr. Jiang assigned the project to Dr. Rivas, another postdoctoral fellow. After having difficulty replicating Dr. Cooper's preliminary data, Dr. Rivas consulted with Dr. Jiang, but not Dr. Cooper, and made substantial changes to the protocol. Following these changes, the experiments proceeded smoothly. After completing data collection and analysis, Dr. Rivas wrote the first draft of a manuscript, which listed Dr. Rivas as the first author, Dr. Cooper as second author, and Dr. Jiang as last and corresponding author, with several other coauthors. Dr. Jiang sent the manuscript to Dr. Cooper, who read it carefully and became very upset because 1) Dr. Cooper is listed as second author and not first; 2) Dr. Cooper disagrees with the interpretations of the data, which undermine support for H1 and lend support to a different hypothesis proposed by Dr. Rivas; and 3) Dr. Cooper disagrees with changes to the protocol made by Dr. Rivas without consultation with Dr. Cooper and believes these may have impacted the findings.

1. **Should Dr. Rivas have consulted with Dr. Cooper before making changes to the protocol?**
2. **Who should be first author of this paper? Should Drs. Cooper and Rivas be co-first authors? What factors would you consider in making this decision?**
3. **Does Dr. Jiang's promise to name Dr. Cooper as first author carry any weight?**
4. **Should Dr. Jiang have talked to Dr. Cooper before naming Dr. Rivas as first author? Should Dr. Jiang have done anything else? Who should be listed as co-authors on a paper?**
5. **Do you have any concerns about Dr. Jiang's mentoring of Dr. Cooper? Could Dr. Jiang have done a better job of mentoring Dr. Cooper? How?**
6. **What should Dr. Cooper do to remedy a disagreement with Dr. Jiang about being placed as second, not first author on the paper?**
7. **How should the team go about resolving the dispute about interpreting the data? If they cannot resolve this issue, would it be ethical to publish the paper without naming Dr.**

Acknowledgements: NIH

Using AI to Write a Manuscript (Case #2)

Dr. Blue is principal investigator at the NIH who specializes in cancer genotyping. A prestigious review journal has asked Dr. Blue to write an article reviewing the current state of the field. Dr. Blue is very busy with clinical, research, and administrative responsibilities, so they ask Dr. Green, a postdoctoral fellow working in the lab, to write the review. Without telling Dr. Blue, Dr. Green uses an artificial intelligence (AI) tool to summarize the literature on this topic and generate references. Dr. Blue reads the review and congratulates Dr. Green on a job well done. They submit the solicited review to the journal. The article lists Drs. Blue and Green as authors but does not acknowledge the use of the AI in preparing the article. Two months after publication, an anonymous critique of the article, appearing in a post-publication peer review blog, claims that two of the citations in the article are fake. The editors of the review journal inform Dr. Blue about this and ask them to submit a correction. Dr. Blue meets with Dr. Green about the issue and asks how the problem occurred. Dr. Green admits to using an AI tool to help write the article and says the tool must have made the mistakes. Dr. Blue is furious at Dr. Green for using this tool without consulting with them first. They both carefully examine the references and verify that the two references mentioned by the critic are indeed fake. They also discover that three additional references are inaccurate, three are irrelevant, and two sentences in the article are copied word-for-word from another article without quotation marks or attribution.

Questions for Case #2 discussion (with facilitator notes)

1. When Dr. Blue and Dr. Green submit their correction to the journal, should they also address the inaccurate and irrelevant references and the copied sentences and acknowledge the use of the AI tool?

2. Should they explain how the problem occurred, i.e., that the AI tool made the mistakes?

3. Should they retract the article?

4. Did they commit research misconduct, i.e., plagiarism?

5. What are the responsibilities of authors when using AI tools to review the literature?

[End of case study #2]

Acknowledgements: NIH

Case Study 3: Who is an Author?

Susan Jacobs, a Ph.D. student from a small university, sets up, as part of finishing her dissertation, a six-month internship at a prestigious larger institution in order to learn a new molecular-biological technique. Ms. Jacobs contacted the laboratory leader, Dr. Marvin Frank, a world-renowned scientist, in the hope of developing new skills for her research and also to foster a relationship with Dr. Frank, who is well connected in her field of biochemistry.

When Ms. Jacobs comes to Dr. Frank's laboratory, she is greeted warmly as a member of the team. Dr. Frank, the graduate students, the postdoctoral fellows, and the technicians include Ms. Jacobs in the weekly laboratory meetings, in which everyone participates in a free exchange of ideas about the ongoing projects in the laboratory, and which last for hours. In the meetings, Ms. Jacobs finds some of the ideas helpful but others less so, and gives her point of view concerning the ongoing projects. In addition, she meets weekly, one on one, with Dr. Frank, who provides significant scientific advice and one or two recommendations, which advance her work and move her in a slightly different direction. She discusses the results of her research with her mentor, Dr. Melissa Seabrook, back at her home college, by weekly e-mails and occasional phone calls, interactions that also push ahead the project she started in Dr. Seabrook's lab three years ago.

Ms. Jacobs makes great progress during the six months she spends in Dr. Frank's laboratory, and she writes a paper reflecting some important findings. Ms. Jacobs puts herself down as first author, Dr. Frank as second author, and Dr. Seabrook as last author on the paper. At the end of the paper, she gives an acknowledgment to a technician who showed her several techniques and worked with her on a few experiments.

Ms. Jacobs based her listing of authors on her understanding of the guidelines put forth by the International Committee of Medical Journal Editors (ICMJE), which say that an author is someone who has made significant contributions to the conception and design, or to the acquisition of data, or to the analysis and interpretation of data; was involved in drafting the article or revising it critically for important intellectual content; and provided final approval of the version to be published. The guidelines, which are followed by approximately 500 medical journals, say that all three criteria must be met for authorship. Ms. Jacobs would like to send her manuscript to a journal that follows ICMJE guidelines as soon as possible, because of what she feels is the importance of her results.

Ms. Jacobs gives Dr. Frank and Dr. Seabrook a draft of her manuscript for review on a Friday, hoping for feedback by Monday. Dr. Seabrook sends her comments by e-mail to Ms. Jacobs. Dr. Frank sends his comments back to Ms. Jacobs and changes the authorship listing to include Ms. Jacobs, the technician, two postdocs in his lab, two graduate students in the lab, himself, and Dr. Seabrook. Dr. Frank also gives a copy of the draft to all the members of his laboratory for discussion at the next meeting.

Ms. Jacobs is shocked that Dr. Frank added the other laboratory members to the draft, explaining to him the ICMJE guidelines and maintaining that the major intellectual and physical work in preparing the paper was done by her and by Dr. Seabrook and Dr. Frank. Dr. Frank is equally surprised by Ms. Jacobs's feelings, responding that he and Ms. Jacobs benefited from the input of all the other lab members. Dr. Frank adds that a graduate student in the laboratory, Lisa Bain, is writing a short paper that is based on some very exciting preliminary findings, and that Ms. Jacobs would be included in the list of authors. Dr. Frank says that the results of Ms. Bain's research would need further elaboration in the laboratory and that a second paper using the same data and additional studies would be more comprehensive, and that Ms. Jacobs would be included on the second one, too.

Dr. Frank insists to Ms. Jacobs that the contributions of all the laboratory members were sufficient to satisfy the ICJME guidelines for both papers, adding that the idea of a scientist acting as an independent entity is an outdated concept and that those who work around a scientist contribute significantly, helping him or her to function.

Ms. Jacobs tells Dr. Frank that she does not want to be included on Ms. Bain's paper, feeling that she did not contribute adequately. Dr. Seabrook, who follows ICMJE guidelines but was intimidated by Dr. Frank's stature, advises Ms. Jacobs not to rock the boat, to use Dr. Frank's revisions and some of the changes suggested during the laboratory review and to submit the paper to the journal with the authorship he suggested.

Questions:

1: Why should Ms. Jacobs and Dr. Frank have discussed the laboratory□s approach to authorship issues when she started working in his laboratory?

2: Why is the order of authorship and the listing of authors important in a research paper?

3: What is the difference between an acknowledgment and a listing as an author?

4: Although many journals subscribe to the guidelines of the International Committee of Medical Journal Editors, many do not, and many researchers do not follow the practices that it recommends. What tends to happen, and how are ICMJE standards being challenged?

5: Who among the authors takes responsibility for submitting the paper to a journal and following up with the editor and peer-review revisions?

6: What are some potential problems with Dr. Frank’s submitting a paper on preliminary findings and not performing sufficient corroboratory experiments?

7: What kind of problems may arise if the same data is used in multiple papers in the research literature?

8: What might happen if someone is listed as an author on a paper for which he or she did not do any work?

9: What might have been done to resolve Ms. Jacobs's ethical dilemma with Dr. Frank about the authors on the paper?

Acknowledgements:

RCR case study from produced by the Columbia Center for New Media Teaching & Learning (CCNMTL) in collaboration with the Columbia University Office for Responsible Conduct of Research. This Responsible Authorship and Peer Review module in the Responsible Conduct of Research series was authored by Robin Eisner, Daniel Vasgird, and Ellen Hyman-Browne. This case was adapted, with permission, from:
"When in Rome: Conventions in Assignment of Authorship"
Research Ethics: Cases and Commentaries
Volume 2, Section 1, Authorship.
Brian Schrag, ed. Association for Practical and Professional Ethics
Bloomington, Indiana, February, 1998
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Activity D
Authorship Expectations Case Study

Purpose This activity helps you learn to apply authorship criteria in a real life scenario. After completing this activity, you will be able to determine who should be listed as an author on your papers and justify those choices using standard criteria.

Procedure Work in small groups to discuss the following scenario. Be ready to share your group's ideas with the rest of the class.

DISCUSS

Read the following scenario and write down your answers to the questions, then discuss with your group. Try to apply what you have learned about best practices for publication ethics.

Case Study: Why not me?

Dr. Mac started her lab 4 years ago. Her lab is active and growing. Right now, there are three graduate students in the lab (Sarah, 4th yr.; Raj, 3rd yr.; and Jess, 1st yr.), as well as a research technician, Norman, and two part-time undergraduate students, April and Becky. Dr. Mac encourages everyone to work together on their projects with the theory that if one does well, everyone benefits.

Raj's research project is going well. He has started to prepare the results for publication and has almost finished the first draft of his manuscript. Dr. Mac asks him to present the outline of his manuscript at the next lab meeting to discuss how best to complete the manuscript for publication.

At the next lab meeting, Raj presents to the group the title "Sugar water increases body mass of Wnt10b mice" and "Raj Nice and Henrietta Mac" as the authors of his draft manuscript. Several lab members provide immediate feedback.

Sarah: "Why am I not on the authorship list? I taught you everything you know! And the cell culture data in the paper were done by me, not you. I NEED to be an author on this paper!"

Jess: "Yeah, I mean I fed the mice the sugar water every day for 6 months. You said that if I helped you, I would be an author."

Sarah: "And what about Norman? He did all of the assays. You just analyzed the results."

Norman: "The assays were routine work. Raj, I'm glad your study went so well."

April: "I didn't necessarily think that I would be an author, but I did help you every afternoon for the past year and a half. I even did parallel studies to rule out some of your experimental candidates. Does that qualify for authorship?"

1. **Should Raj revise his authorship list? Why or why not?**

2. **If so, what do you suggest and why? (USE the worksheet)**

3. **How could Raj have avoided this tense situation?**

WRITE

REMEMBER: **Note ideas that you want to add to your My Authorship Checklist.**

Activity E
Major Revision Case Study

Purpose This activity helps you learn to apply authorship criteria in a real life scenario. After completing this activity, you will be able to adapt an authorship plan as the participants in your work change over time. You will be able to identify the stakeholders' perspectives and recognize the value of authorship to each (PI, student, postdoc, technician, etc.).

Procedure Work in small groups to discuss the following scenario. Be ready to share your group's ideas with the rest of the class.

DISCUSS

Read the scenario and write down your answers to the questions, then discuss with your group. Try to apply what you have learned about best practices for publication ethics.

Case Study: Major Revision

Raj submits his paper entitled "Sugar water increases body mass of Wnt10b mice" by "Raj Nice, Sarah Roswell, April Smith, and Henrietta Mac" to *AJP-Endocrinology and Metabolism*. The reviews come back: "MAJOR REVISION." The reviewers note that he needs to do more mouse and cell culture experiments to rule out some alternative interpretations of the data.

Raj had not planned to do any more work on this paper. In fact, he is scheduled to defend his thesis in just 2 weeks and start a postdoc in 4 weeks. Dr. Mac suggests that he ask Jess to perform the experiments that the reviewers have suggested. Raj agrees with Dr. Mac, and he asks Jess to finish up the paper. Dr. Mac even promises to add Jess as an author.

Six months later, Dr. Mac meets with Jess to discuss the revised paper. Dr. Mac notices that Jess's name is now listed as second author and asks her to explain the order.

Jess replies: "I have been working on these revisions all day for 6 months. Raj and Sarah have both left the lab and have not been much help besides reviewing the revised manuscript. I performed the requested experiments, revised the manuscript, and even re-did some of Raj's experiments to confirm the results with the new reagents. I deserve to be second author, possibly even first author considering that the paper would not be published without my effort."

1. **Do you agree with Jess? Why or why not?**

2. **Should Raj remain as first author? Why or why not?**

3. **Should Jess be included as an author at all? Why or why not?**

4. **What should Dr. Mac do to determine how best to revise the authorship list?**

5. **What should be the final order of the authorship list? Should anyone be acknowledged?**

6. **What should Dr. Mac do to avoid these situations in her lab in the future?**

WRITE

REMEMBER: Note ideas that you want to add to your My Authorship Checklist.

eNeuro

The Good Reviewer's Guide to the Publishing Galaxy

After having spent months/years doing experiments and analyses, we are finally ready to tell our story to our fellow scientists. However, before knowledge is transmitted to others, we must pass under the yoke of the review process. In many instances, it is painful, stressful, and even, sometimes, humiliating. The "best" review I ever received was from one of the two *you-know-who* journals. Verbatim, the full review was "It is incredible if it is true." I cherish it as a souvenir and use it as a perfect example of what must *not* be done. Quality in peer review is this year's topic for Peer Review Week.

What is a good-quality review? The answer is surprisingly easy: a good review is a helpful and useful one. When early career scientists come to see me when they have a paper to review, sometimes for the first time, I tell them that the only thing they have to do is to check whether the interpretations/conclusions are supported by the presented data and analyses. If there are some issues, they must try to help the authors provide a better case without asking them to do unnecessary experiments.

Our review process at *eNeuro* is based on these basic principles. The reviewing editors pay great attention to what is transmitted to authors: reviews must be factual, not emotional, and should include improvement suggestions (if necessary). If the reviewers agree that more experiments are needed and that experiments will require more than two months' work, the paper is automatically rejected (with the possibility to resubmit). This procedure allows researchers to really ponder which additional experiments are truly necessary. Finally, reviewers and the reviewing editor must reach a consensus on what comments will be transmitted to authors. Therefore, the authors receive a one-voice factual report. This provides a clear directive toward the path to publication and eliminates the need for authors to try to interpret the priorities of separate reviewers. Sometimes, generating one consensus review requires several exchanges and discussion between the reviewers and the reviewing editor, dialogue is the key to success. We know that the system works as, since the launch of *eNeuro* in 2014, I can count on two hands the number of appeals I have received. Even if one may be unhappy after rejection, the decision is accepted because the facts and reasons are provided. I am 100% convinced that this type of reviewing (pioneered by *eLife*) is today's best solution to the concerns raised by traditional peer review. It is easy to implement, but it takes more time per manuscript. For obvious reasons, it works best if the reviewing editor is an active scientist. We also know that *eNeuro*'s system works based on the positive comments we receive from authors (included those with rejected papers) and reviewers regarding the quality of our peer review process. Before hopefully becoming the norm, mentalities must change.

The best way forward is to teach the young generations the fundamentals of a good-quality review. Unfortunately, there are few teaching courses provided by research institutions on how to review a paper. The Society for Neuroscience offers a mentorship program (https://www.jneurosci.org/content/sfn-reviewer-mentor-program) to train graduate students, postdocs, or established researchers to write good and helpful reviews; trainees are then invited to become reviewers at *eNeuro*. When you are a reviewing editor, you may think that you are taking a risk when selecting a non-seasoned reviewer. But so far, reviewing editors who have used trainees from the program have been enthusiastic regarding the quality of their reviews. I believe that this will induce a virtuous circle. The happier authors become with the review process, the better their own reviews will be, making even more authors happy, etc. I am not overly optimistic; it is working at *eNeuro*. We have the opportunity to shape the future of the publication field. Let us seize it.

And for those who have read the Guide, you know that the final answer is to be found on page 42.

Christophe Bernard
Editor-in-Chief
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Brief Communication

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Brain clearance is reduced during sleep and anesthesia

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It has been suggested that the function of sleep is to actively clear metabolites and toxins from the brain. Enhanced clearance is also said to occur during anesthesia. Here, we measure clearance and movement of fluorescent molecules in the brains of male mice and show that movement is, in fact, independent of sleep and wake or anesthesia. Moreover, we show that brain clearance is markedly reduced, not increased, during sleep and anesthesia.

Sleep is a state of vulnerable inactivity. Because of the risks that this vulnerability entails, most researchers assume that sleep must confer some essential benefit[1–3]. However, what this is remains a mystery. One suggestion is that sleep clears the brain of metabolites and toxins using the 'glymphatic' system, a process that cannot operate efficiently during the waking state[3,4]. This attractive idea has important implications. For example, diminished toxin clearance brought about by chronically poor sleep might exacerbate, if not cause, Alzheimer disease[5,6].

How metabolites and toxins are cleared from the brain is unresolved. Disputes surround both the anatomical pathways[7–9] and the mechanisms of clearance[7,10,11]. The glymphatic hypothesis contends that bulk flow of fluid, rather than just diffusion, actively clears solutes from the brain parenchyma during non-rapid-eye-movement (NREM) sleep[3]. This flow is proposed to be driven by hydrostatic pressure gradients established by arterial pulsations[12]. Anesthetics at sedative doses, which induce states resembling deep NREM sleep[2,13], were also reported to increase clearance[3,14,15]. However, whether sleep does enhance clearance by increased bulk flow is unresolved, with findings both supporting[3,4,12,14–16] and challenging[10,11,17–19] the idea. Here, we directly measure clearance and fluid movement in the brains of mice during different vigilance states (awake, sleeping or sedated).

We first determined the diffusion coefficient (D) of a fluorescent dye (fluorescein isothiocyanate, FITC-dextran) in brains of mice (Fig. 1a). We injected 4 kDa FITC-dextran into the caudate putamen (CPu) and then monitored the fluorescence arriving in the frontal cortex. The first series of experiments involved waiting for steady state and then bleaching the dye in a small volume of tissue in the neocortex and determining D from the rate that unbleached dye moved into the bleached region, a technique pioneered by others[20,21].

We validated our methodology by measuring the diffusion of FITC–dextrans of various molecular weights in agarose 'brain phantom' gels, modified to approximate the light-scattering and optical-absorption properties of brain tissue[22] and found (Extended Data Fig. 1) that the distribution of light intensity was well approximated by a hemispherical Gaussian distribution. Immediately following 30 s of bleaching, we recorded the recovery of the fluorescence as unbleached dye moved into the bleached volume. Figure 1b shows a typical recording for 4 kDa FITC-dextran (blue trace). There was excellent agreement between these data and the time course predicted using equations (4) and (5) (Methods and Extended Data Fig. 2).

Using this method, our measured diffusion coefficients were in good agreement with literature values in aqueous solutions[23,24] and their mass dependence (inset to Fig. 1b). Our diffusion coefficients also agreed well (Fig. 1c) with values obtained using a direct method (Extended Data Fig. 3) that did not involve photobleaching.

We then measured D in vivo using 4 kDa FITC-dextran, which after injection into the CPu, could be detected in the frontal cortex, where its fluorescence peaked at about 6–7 h postinjection, then slowly declined at ~6% per hour (Extended Data Fig. 4a). During the slowly declining phase, approximating to steady state, the recovery from bleaching

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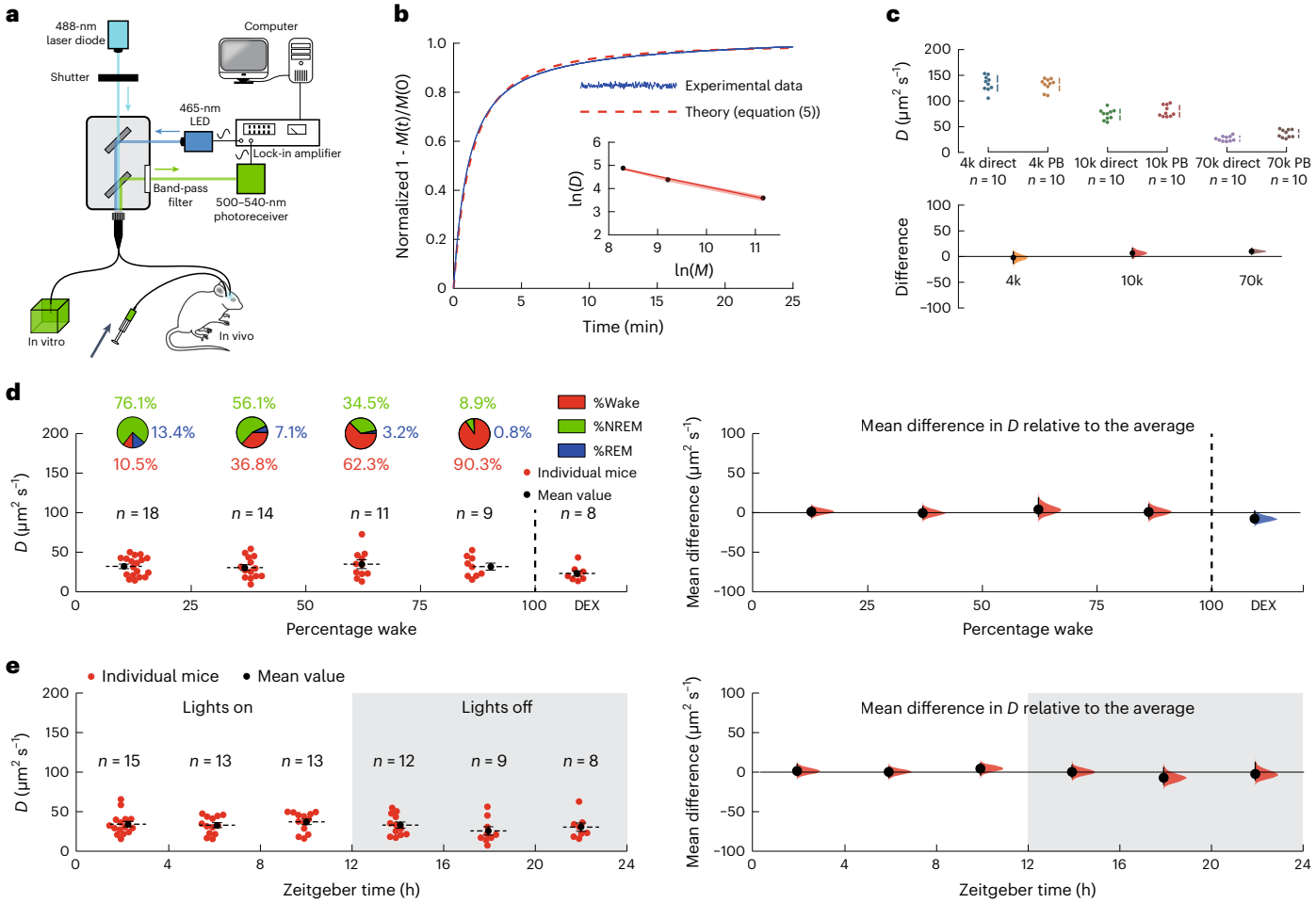

Fig. 1 | Changes in local diffusion with vigilance states. a, The experimental setup. Light from a 488-nm laser diode was passed through a 200-µm optical fiber into either an agarose gel brain phantom in vitro or the frontal cortex of a mouse in vivo. For the in vitro experiments, the agarose gel contained 4 kDa FITC-dextran while, for the in vivo experiments, the brain had been injected with 4 kDa FITC-dextran some hours earlier. **b**, A typical recording of photobleaching in an agarose gel brain phantom, fitted by least-squares to equation (5), to give (for this example) a value of $D = 136\ \mu m^2\ s^{-1}$. The inset shows that the diffusion coefficient follows a power law, with $D \propto M^{-0.44}$. The red shading in the inset shows the s.e.m. **c**, A comparison between the diffusion coefficients determined directly (direct) (Methods and Extended Data Fig. 3) and those determined using the photobleaching method (PB) was not significantly different (two-way ANOVA $P = 0.10$). Top, the individual data points. Bottom, the differences in the diffusion coefficients determined using the two methods. The agreement between the methods was excellent at 4 kDa FITC-dextran and this was used for the in vivo measurements. **d**, Left, the diffusion coefficients of 4 kDa FITC-dextran as a function of the percentage of wake (state) during the hour the diffusion coefficient was being measured (the distribution of vigilance states is shown in the pie charts above). Each point represents the average of typically four measurements for an individual mouse and the number of mice, *n*, is shown above. The last group of data on the right-hand side were recorded during dexmedetomidine (DEX) sedation. Right, the mean differences relative to the average diffusion coefficient across all vigilance states. A one-way ANOVA gave $F(4,55) = 0.90$; $P = 0.47$. (A difference of ~35% in *D* would have been detected.) **e**, Left, the diffusion coefficients as a function of zeitgeber time. Right, the mean differences relative to the average diffusion coefficient recorded over the circadian cycle. A one-way ANOVA gave $F(5,64) = 0.88$; $P = 0.50$. In **c**–**e**, the vertical solid lines show the 95% confidence intervals; the shaded areas show the distributions of likelihood. In **d** and **e**, the horizontal solid and dashed lines show the s.e.m. and the mean, respectively.

was recorded (and baseline corrected) (Methods). The spread of light in a brain using a brain slice (Methods) confirmed that the distribution was also well approximated by a hemispherical Gaussian distribution (Extended Data Fig. 4b). As with the gel experiments described above, the fluorescence recovery agreed well with the theoretical predictions (Extended Data Fig. 4c) and we derived values for the effective tissue *D* from the time courses, while also determining the vigilance states (Extended Data Fig. 4d).

We observed no significant change in the diffusion coefficient of 4 kDa FITC-dextran with either vigilance state or dexmedetomidine (200 µg kg^{-1}; intraperitoneal (i.p.) sedation (Fig. 1d) or during the day–night cycle (Fig. 1e)). The mean value for *D* across all vigilance states was $32.1 \pm 1.9\ \mu m^2\ s^{-1}$ ($n = 52$; mean ± s.e.m.), which corresponds, using equation (3), to a tortuosity of ~2.5 (having corrected the aqueous *D* to 37 °C using the Stokes–Einstein equation[25]). This is consistent with values reported for rodent neocortex[25] and suggests that the movement of 4 kDa FITC-dextran in the cortex is predominantly by diffusion, a conclusion previously reached by others[11,18,19]. Notably, these results show that diffusion kinetics do not change during sleep or anesthesia. From separate in vitro measurements (Extended Data Fig. 5), we estimate that we could have detected a change in bulk flow between vigilance states of >0.5 µm s^{-1} but our results cannot rule out changes in pairwise flows in opposite directions over small distances in the surrounding tissue, which might have averaged out, so that brain clearance might, nonetheless, have changed. We therefore extended our experiments to measure brain clearance itself during different vigilance states.

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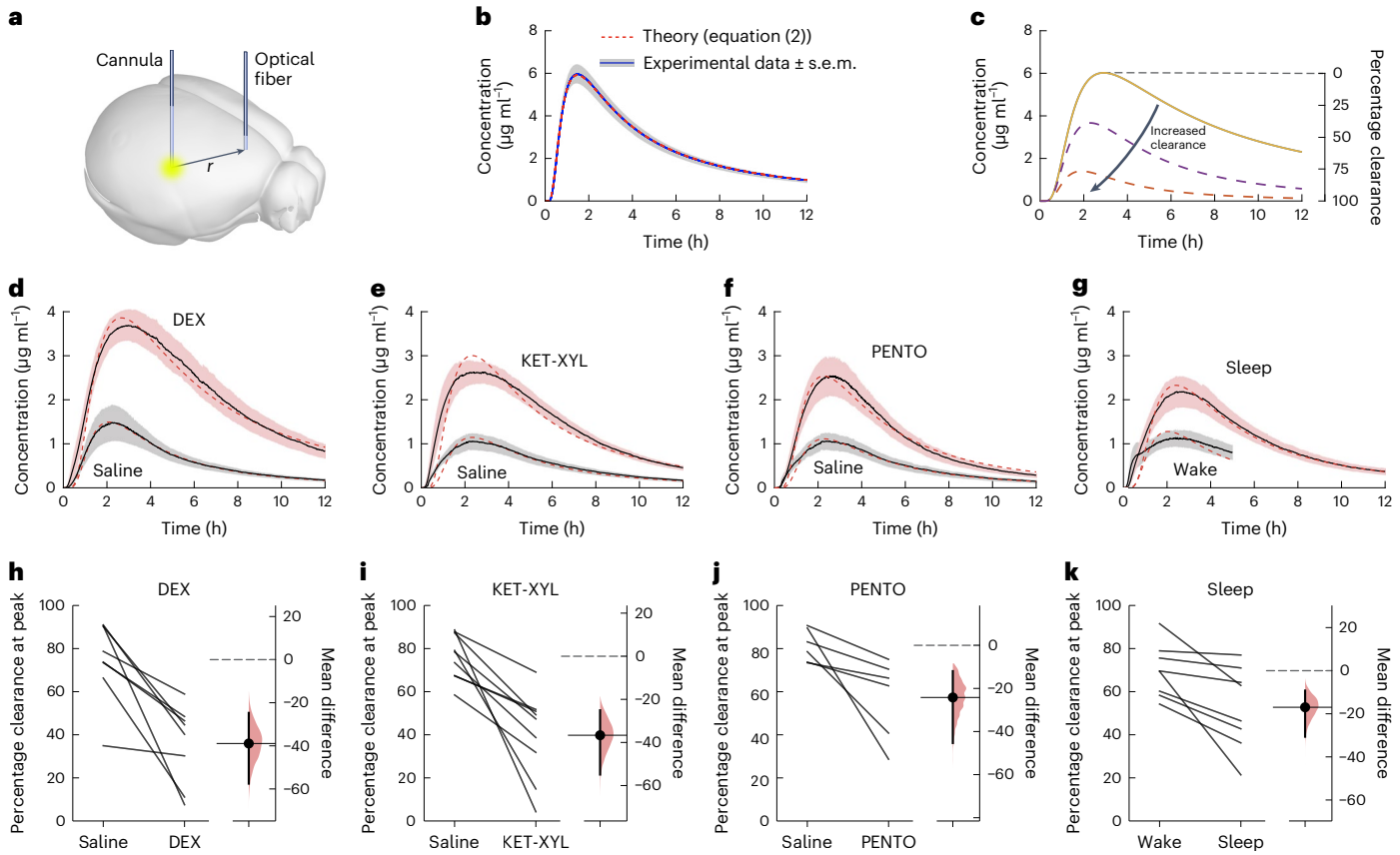

Fig. 2 | Photometry data show that brain clearance is reduced by sleep and anesthesia. a, A fluorescent dye (AF488) was injected into the CPu and the fluorescence monitored over time in the frontal cortex. **b**, The spread of the dye could be accurately predicted by equation (2) in an agarose gel with a diffusion coefficient of 295 $\mu m^2 s^{-1}$, where there was zero clearance. The error envelope represents the s.e.m. **c**, If brain clearance of the dye is assumed to increase with time as described by equation (9), then the concentration in the frontal cortex is predicted to follow the time course given by equation (8) and is shown by the dashed lines. Knowing the concentration that should have arrived at the cortex had there been no clearance (solid line), the percentage clearance can be calculated at any time. **d**–**g**, Observed concentration curves recorded following either saline injection or DEX anesthesia (**d**), KET-XYL anesthesia (**e**), PENTO anesthesia (**f**) and during the waking state or during sleep (**g**). The observed concentrations were significantly lower (two-way ANOVA with Bonferroni–Holm multiple comparisons correction) in the waking state compared to DEX ($P < 10^{-6}$), ketamine-xylazine (KET-XYL) ($P < 10^{-6}$) or pentobarbital (PENTO) ($P < 10^{-6}$) anesthesia or during sleep ($P < 10^{-6}$). The error envelopes represent the s.e.m. **h**–**k**, Peak clearance observed following either saline injection or DEX anesthesia (**h**), KET-XYL anesthesia (**i**), PENTO anesthesia (**j**) and during the waking state or during sleep (**k**). For both anesthesia and sleep, the percentage of brain clearance was significantly reduced (two-tailed paired *t*-test): DEX ($P = 0.0029$), KET-XYL ($P = 0.0015$) or PENTO ($P = 0.037$) anesthesia or during sleep ($P = 0.016$). The vertical bars represent 95% confidence intervals about the mean (horizontal solid lines) and the shaded areas are the distributions of likelihood.

The approach we took to measuring brain clearance used the same experimental setup as shown in Fig. 1a. However, it has recently been shown[16] that a small dye which moves freely in the parenchyma can be used to accurately quantify brain clearance (Fig. 2a). This would also allow a complete time course to be recorded in the cortex as the dye spread throughout the brain. We used AF488 (~570 Da) and first showed that the spread in a gel, with no clearance possible, could be accounted for by equation (2), the spread from a Gaussian source. Figure 2b, shows that equation (2) fitted the experimental data essentially perfectly, with an aqueous diffusion coefficient of 295 $\mu m^2 s^{-1}$. In the absence of clearance and, if r (the distance between where dye is injected and where it is recorded) is constant, then the timing of the peak is determined only by the diffusion coefficient (Extended Data Fig. 6). If clearance occurs, the height of the peak would be reduced (Fig. 2c and equation (8)).

We then repeated these experiments in mice which had been injected (i.p.) with either saline or an anesthetic (Fig. 2d–f). A comparison was also made between the sleeping and waking states (Fig. 2g). For the saline controls, the peak concentrations were much lower than that predicted by equation (2) but could be accounted for accurately by assuming clearance had occurred, as described by equations (8) and (9). There was excellent agreement between the photometry data and equation (8), with the discrepancies at small times possibly being due to dye finding its way across the brain via the ventricles[16]. At the peak concentration (~2–3 h) the clearance was 70–80% with saline-injected controls, indicating that the normal mechanisms of brain clearance had not been disrupted. Notably, in the presence of anesthetics, this clearance was substantially reduced. This was true for dexmedetomidine (Fig. 2d,h), ketamine-xylazine (Fig. 2e,i) and pentobarbital (Fig. 2f,j). Reduced clearance was also observed in mice that were sleeping, compared with mice that were kept awake (Fig. 2g,k and Extended Data Fig. 7). By contrast, the diffusion coefficients, reflecting the rate of spread in the brain parenchyma and the time to reach the peak in the photometry data (Fig. 2d–g), did not change significantly during sleep or anesthesia (Extended Data Table 1). If these diffusion coefficients reflect pure diffusion, then they would correspond to a tortuosity of ~1.4. We cannot rule out that spread might be enhanced by local fluid movement without bulk flow; however, these do not change with vigilance state. We also measured the EEG power spectra (Extended Data Fig. 8a–d) and found a weak negative correlation between peak clearance and delta (0.5–4 Hz) power (Extended Data Fig. 8e), implying that the deeper the sleep, the lower the clearance.

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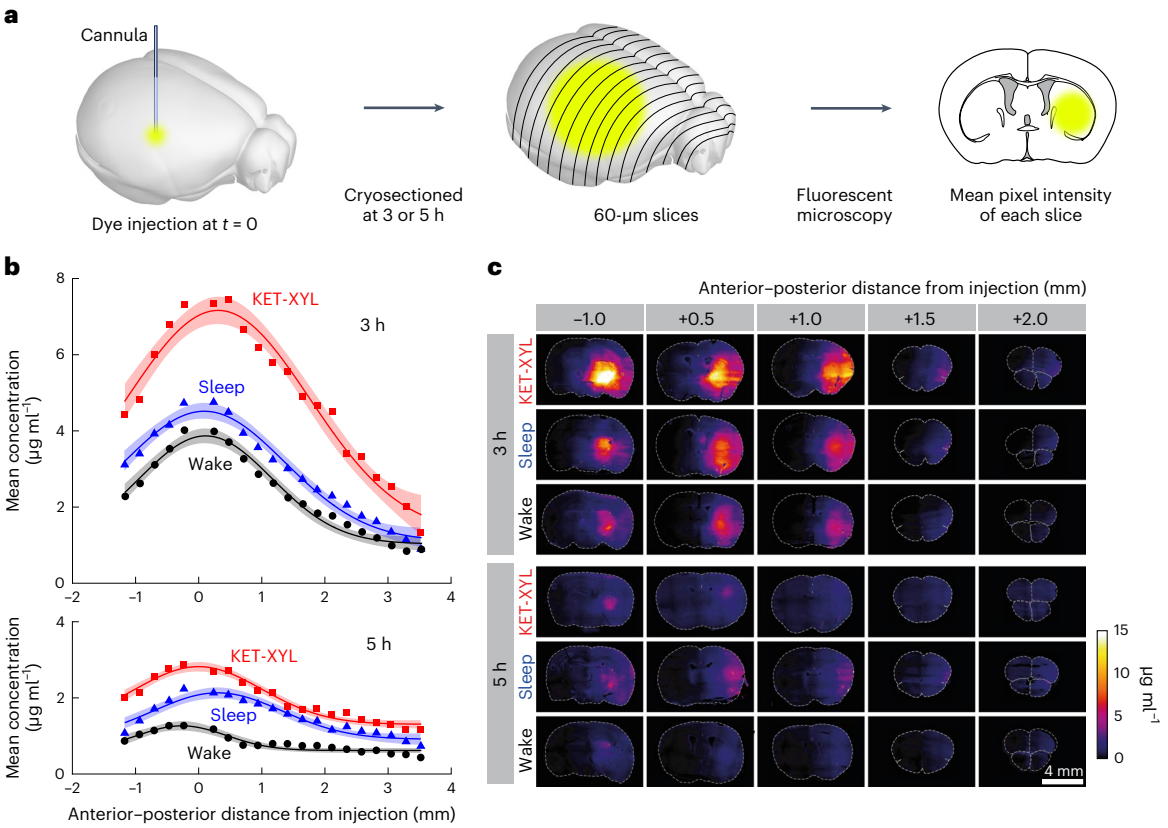

Fig. 3 | Histology data confirm that brain clearance is reduced by sleep and anesthesia. a, At either 3 or 5 h following injection of AF488 into the CPu, the brain was frozen and cryosectioned at 60 µm. The average fluorescent intensity across each slice was obtained by fluorescent microscopy; then the mean intensities across groups of four slices were averaged. **b**, The mean fluorescence intensity was converted to a concentration using the calibration data in Supplementary Fig. 1 plotted against the anterior–posterior distance from the point of injection for wake (black), sleep (blue) and KET-XYL (red) anesthesia. Top, the data after 3 h. Bottom, the data after 5 h. The lines are Gaussian fits to the data and the error envelopes show the 95% confidence intervals. At both 3 and 5 h, the concentrations during KET-XYL ($P < 10^{-6}$ at 3 h; $P < 10^{-6}$ at 5 h) and sleep ($P = 0.0016$ at 3 h; $P < 10^{-4}$ at 5 h) were significantly larger than wake (two-way ANOVA with Bonferroni–Holm multiple comparisons correction). **c**, Representative images of the brain slices across the brain (anterior–posterior distance from the site of AF488 injection) at both 3 h (top three rows) and 5 h (bottom three rows). Each row represents data for the three vigilance states (wake, sleep and KET-XYL anesthesia). The color scale on the right shows the concentrations, determined using the calibration data in Supplementary Fig. 1.

Histology experiments (Fig. 3) confirmed the photometry results. At both 3 h (Fig. 3b, top) and 5 h (Fig. 3b, bottom) after dye injection, the concentration of dye was higher during sleep and ketamine-xylazine anesthesia. As expected, (equation (8)), the spread was Gaussian (fitted curves in Fig. 3b), with characteristic widths roughly in line with those predicted using the diffusion coefficients derived from the photometry experiments. These data show that redistribution of the AF488 dye is essentially by diffusion alone and confirm that sleep and ketamine-xylazine anesthesia inhibit clearance. Representative brain sections are shown in Fig. 3c at 3 h (top) and 5 h (bottom).

Our experiments show that brain clearance is reduced during sleep and anesthesia, the opposite conclusion of ref. 3. Those authors observed that fluorescent dyes injected into the cerebrospinal fluid (CSF) via the cisterna magna penetrated further into the cortex during sleep and anesthesia. They interpreted this as showing that molecular movement into the cortex must be faster during these states. However, the concentration of dye in any brain region will always be the difference between its rate of arrival and its rate of departure and so increased dye penetration in sleep and anesthesia can be equally well explained by a reduced rate of clearance rather than an increased rate of entry. Indeed, almost all the experiments that have been interpreted as showing that sleep or anesthesia change brain clearance have involved introducing markers into the CSF, which then move into the brain parenchyma[14,26–30]. Under these circumstances, entry, exit and redistribution of the marker are all occurring simultaneously, greatly confounding any quantification of clearance.

Our data in Figs. 2 and 3 show that, averaged across the brain, clearance is reduced by both sleep and anesthesia. Although clearance might vary with anatomical location, the extent of this variation appears small (Extended Data Fig. 9). Moreover, the inhibition of clearance by ketamine-xylazine is highly significant independent of location. These data are for a small dye that can freely move in extracellular space. Molecules of larger molecular weights may behave differently. Exactly how anesthetics and sleep inhibit brain clearance is unclear, although it is notable that CSF outflow from the brain is markedly reduced by anesthetics[30]. Whatever the mechanism, however, our results challenge the idea that the core function of sleep is to clear toxins from the brain.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41593-024-01638-y.

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Methods

Theoretical basis of three-dimensional photobleaching method

We assume that, following bright illumination, the bleached fluorescent dye is distributed over a hemispherical volume with a concentration, $Q(s)$, that falls off as a Gaussian distribution (see main text and Extended Data Figs. 1b and 4b for experimental confirmation):

$$Q(s) = Q(0)\exp\left(-\frac{s^2}{2\sigma^2}\right)(s \geq 0) \quad (1)$$

where $Q(0)$ is the maximum tissue concentration of the bleached dye at the origin of the hemisphere, s is the radial distance from the center of the distribution and σ is the standard deviation of the Gaussian distribution. Then, following bleaching, the concentration $C(r,t)$ of bleached dye as a function of time, t, and distance, r, from the center of the hemisphere can be shown to be:

$$C(r,t) = C(0,0)\left\{\left[1+\frac{2Dt}{\sigma^2}\right]^{-\frac{3}{2}}\left[\exp\left(\frac{-r^2}{4Dt+2\sigma^2}\right)\right]\right\}, \quad (2)$$

where D is the effective diffusion coefficient governing movement through the tissue. (This result was originally obtained[31] for the case of a spherical 'volume source' in the atmosphere and the subsequent diffusion of material from the source.) The effective diffusion coefficient, D, through the tissue is related to the aqueous diffusion coefficient, D_{aq}, by

$$D = D_{aq}/\lambda^2 \quad (3)$$

where the dimensionless parameter λ is the empirical tortuosity, which accounts for the resistance to diffusion and increased path length which a membrane-impermeable dye encounters when diffusing through the tortuous extracellular space[32].

The fluorescent signal $I(t)$ which is recorded at any time t after bleaching is due to unbleached dye diffusing back into the bleached volume. If we assume the volume being recorded from is a hemispherical volume of radius R and that $I(0)$ is the signal recorded immediately after bleaching (at $t = 0$) and $I(\infty)$ is the signal recorded when equilibrium has been re-established (which is also the signal recorded immediately before bleaching), then $M(t)$, the number of moles of bleached dye in the hemispherical volume at a time t, is related to the observed fluorescent intensities by:

$$M(t) = M(0)\left[\frac{I(\infty)-I(t)}{I(\infty)-I(0)}\right] \quad (4)$$

where $M(0)$ is the number of moles of bleached dye in the hemisphere immediately following bleaching.

The total number of moles $M(t)$ of fluorescent dye in a hemisphere of radius R, is given by equation (2) multiplied by the area of a hemisphere ($2\pi r^2$), integrated from $0 \rightarrow R$, which leads to (Extended Data Fig. 2):

$$M(t) = \frac{2\pi C(0,0)\sigma^3}{\sqrt{(2Dt+\sigma^2)}}\left\{\sqrt{\frac{\pi(2Dt+\sigma^2)}{2}}\,\mathrm{erf}\left(\frac{R}{\sqrt{(4Dt+2\sigma^2)}}\right) - R\exp\left[-\frac{R^2}{(4Dt+2\sigma^2)}\right]\right\}. \quad (5)$$

Hence, as the ratio $M(t)/M(0)$ can be determined experimentally (using equation (4)), D can be derived using equation (5), provided σ and R are known. If we assume that the distance that light penetrates into the tissue to initiate bleaching will be comparable to the distance light penetrates to record the fluorescence as dye diffuses back into the bleached volume, then we can set $R = \sigma$. In fact, while the time course of $M(t)$ is sensitive to values of D and σ, it is insensitive to values of R (Extended Data Fig. 2), so this assumption has little impact on the derived value of D.

In the presence of fluid flow with a velocity v, the integral of equation (2) to give $M(t)$ becomes:

$$M(t) = 2\pi C(0,0)\left[1+\frac{2Dt}{\sigma^2}\right]^{-\frac{3}{2}} e^{-\frac{v^2t^2}{4Dt+2\sigma^2}}\int_0^R r^2\exp\left[\frac{-(r^2+2rvt)}{4Dt+2\sigma^2}\right]dr. \quad (6)$$

The integral cannot be solved analytically but can be evaluated numerically (Extended Data Fig. 5).

In vitro photobleaching protocol

The experimental setup is shown in Fig. 1a. Light from a 488-nm laser diode (Doric Lenses) was passed through a 200-µm optical fiber (Doric Lenses) into an agarose gel brain phantom (see 'Preparation of agarose gel brain phantoms') containing FITC-dextran (25 mg ml^{-1}; Merck Life Science UK). The power at the tip of the optical fiber was measured to be 1.3 mW. Following a 30-s period of photobleaching at 20 °C, controlled by an electronic shutter triggered once every hour, the recovery of fluorescence was recorded using an LED for excitation (465-nm wavelength) and a photoreceiver (New Focus) with a 500–540-nm-wavelength Mini Cube filter) (Doric Lenses). The signal was amplified by a lock-in amplifier (Stanford Research Systems), operating at 125 Hz and stored on a computer. All photometry data were recorded with the software Doric Neuroscience Studio (v.5.4.1.23, Doric Lenses).

In vivo photobleaching protocol

An identical setup was used for the in vivo experiments but with the 200-µm optical fiber being implanted into the frontal cortex of a male C57BL/6J mouse with coordinates: medial–lateral (ML) −1.00 mm, anterior–posterior (AP) 2.22 mm, dorsal–ventral (DV) −2.00 mm and a guide cannula being implanted in the CPu (coordinates: ML −2.55 mm, AP −0.58 mm, DV −3.00 mm) for injection of the 4 kDa FITC-dextran. At the start of the experiment, 4 kDa FITC-dextran was injected into the CPu (25 mg ml^{-1} in saline; 0.1 µl min^{-1} over 100 min), with injections being made (with different animals) throughout the 24-h cycle. The dye took about 2 h to be measurable in the frontal cortex, where it reached a peak about 6–7 h after injection (Extended Data Fig. 4a). Thereafter, there was a slow decline in baseline intensity (~6% per hour), which was corrected for by fitting the baseline to a least-squares cubic spline curve. After ~6 h, the recovery of fluorescence following photobleaching was recorded every hour for up to 24 h.

Measurement of the distribution of bleached dye in agarose gels and the brain

The experimental setup used to measure the distribution of bleached dye from the optical fiber in both agarose gels and the brain is shown in Extended Data Fig. 1a. A brain slice (800 µm) or sheet (800 µm) of an agarose gel brain phantom (see 'Preparation of agarose gel brain phantoms') containing FITC-dextran was sandwiched between two 500-µm blocks of clear agarose (0.5% w/v). (The purpose of the blocks of clear agarose was to eliminate internal reflection at the gel–air interfaces which would have existed in their absence, potentially artefactually increasing the spread of light, particularly along the axial direction of the fiber.) An optical fiber (diameter 200 µm) was inserted into the central gel or brain slice and an image taken of the light distribution of a 488-nm laser diode at an intensity which avoided complete bleaching at the center of the distribution. The image was digitized and fit to a hemispherical Gaussian distribution (Extended Data Fig. 1b). To account for the small spread of the dye during the 30-s bleaching, equation (2) was integrated over 30 s and this distribution was fit to a Gaussian. This small correction never exceeded 8% (Extended Data Fig. 1c).

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Preparation of agarose gel brain phantoms

Brain phantom gels, to mimic the optical scattering and absorbance of brain tissue, were composed[22] of 1% agarose (Sigma-Aldrich A9539) in phosphate-buffered saline (10 mM phosphate buffer, 2.7 mM KCl and 137 mM NaCl, pH 7.4; Sigma-Aldrich P4417) with 8% dried skimmed milk powder (Sigma-Aldrich 70166) and 0.1% Indian ink (Winsor and Newton 1010754). For validation of the method, 0.3 mg ml^{-1} of FITC-dextran (molecular weights 4, 10 and 70 kDa) (Sigma-Aldrich 46944, FD10S and 46945, respectively) was added to the brain phantom gel.

Direct measurement of diffusion coefficients in agarose gel brain phantoms

Accurate values of the diffusion coefficients of the FITC-dextran molecules were determined by measuring the efflux of the fluorescent dye from a sheet of agarose gel of known thickness L. If, at $t = 0$, a molecule has a uniform concentration of C_0 in a membrane of thickness L and if the membrane is bounded on one side (at $x = 0$) by an impermeable barrier, then as the molecule diffuses out of the membrane across the boundary $x = L$, the concentration across the membrane as a function of time is given by[33]:

$$C(x,t) = \frac{4C_0}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1}\exp\left(-\frac{D(2n+1)^2\pi^2 t}{4L^2}\right)\cos\frac{(2n+1)\pi x}{2L} \quad (7)$$

Because of the cosine term, for values of x that are small compared to L (~20% or less), $C(x,t)$ is very insensitive to x. Consequently, if the concentration can be measured close to the impermeable barrier (that is, close to $x = 0$), then the time course provides an accurate measurement of D, provided only that L is known.

We constructed 1-mm sheets of 1% agarose gel brain phantoms containing a chosen molecular weight of FITC-dextran (concentration 25 mg ml^{-1}), bounded on one side by a glass slide and the other being exposed to a stirred solution of phosphate-buffered saline at a constant temperature (20 °C) containing the same concentrations of milk solids (8%) and India ink (0.1%). A 200-µm optical fiber was inserted immediately adjacent to the impermeable glass slide (so that $x/L = 0.1$) (Extended Data Fig. 3).

Protocol for measuring brain clearance

For the experiments used to measure brain clearance, a similar experimental arrangement to that described above for bleaching was used (Fig. 1a), with the same coordinates for the CPu injection and cortical recording. In these experiments, however, we injected a much smaller volume of dye (0.5 µl at 5 mg ml^{-1} over 10 min) into the CPu and used a smaller dye (AF488) to speed up the dye movement and allow a complete time course to be recorded. After injection, the cannula was capped and the fluorescent intensity recorded in the cortex over several hours. We assumed that the dye spread according to equation (8) (see Fig. 2 for experimental verification and also Extended Data Fig. 6) but where σ is now the characteristic width of the initial Gaussian distribution of dye, rather than the width of the bleached dye, as was the case for the bleaching experiments. To account for the loss of dye due to brain clearance, the equation was multiplied by a term $(1 - \frac{t}{t+\tau})$, where τ is the half time for clearance, giving:

$$C'(r,t) = C(0,0)\left(1-\frac{t}{t+\tau}\right)\left\{\left[1+\frac{2Dt}{\sigma^2}\right]^{-\frac{3}{2}}\left[\exp\left(\frac{-r^2}{4Dt+2\sigma^2}\right)\right]\right\}, \quad (8)$$

where $C'(r,t)$ is the concentration when clearance is present. The percentage clearance can be calculated from the ratio of the concentrations given by equations (2) and (8):

$$\text{Clearance}\,(\%) = \left[1-\frac{C'(r,t)}{C(r,t)}\right]\times 100 = \frac{t}{t+\tau}\times 100 \quad (9)$$

In many cases, the distance r between the optical fiber and the cannula could be measured postmortem but, when this was not available, the calculated distance (3.335 mm) between the two sets of coordinates was used. The average of the measured distances was 3.368 ± 0.064 mm (mean ± s.e.m.; $n = 15$).

For the anesthesia experiments, mice were injected with either an anesthetic (see 'Anesthesia') or saline, 1 week apart and in random order. For the sleep experiments, mice were sleep deprived for 5 h and then allowed to sleep (Extended Data Fig. 7). Recordings were made either during the wake period (for 5 h) or during the recovery sleep period, starting at the first sleep episode. These recordings were made on the same animal, 1 week apart and again in random order.

Calibration of fluorescent intensity

The observed fluorescent intensity was converted to concentration using the data shown in Supplementary Fig. 1. For both the bleaching experiments and clearance experiments, there were linear relationships between fluorescent intensity and dye concentration. For the bleaching experiments, this was confirmed by measuring fluorescent intensity in solution as a function of concentration of 4 kDa FITC-dextran (Supplementary Fig. 1). The solution was that used to prepare the brain phantom gels (see 'Preparation of agarose gel brain phantoms'). For the clearance experiments, fluorescence was measured either from solutions or from brain slices which had been incubated in different concentrations of dye (Supplementary Fig. 1) and imaged as described below for the histology experiments (Fig. 3).

Mice

All experiments were performed in accordance with the UK Home Office Animal Procedures Act (1986) and all procedures were approved by the Imperial College Ethical Review Committee. Mice used in the experiments were adult male C57/BL6 mice (3–7 months old). Mice were maintained on a 12 h:12 h, light:dark cycle at constant temperature (20 °C) and humidity (50%) with ad libitum food and water. All measurements were made on mice in their home cage.

Stereotaxic surgery

Mice were anesthetized with 2% isoflurane in oxygen by inhalation and received buprenorphine injection (0.1 mg kg^{-1} subcutaneous (s.c.)) and carprofen (5 mg kg^{-1} s.c.) and placed in a stereotaxic frame (Angle Two, Leica Microsystems) on a heat mat (ThermoStar Homeothermic Monitoring System, RDW Life Science) at 36.5 °C. Mice were implanted with two miniature screw electrodes (+1.5 mm Bregma, +1.5 mm midline; −2.0 mm Bregma, +1.5 mm midline—reference electrode) with two EMG wires (AS634, Cooner Wire). The EMG electrodes were inserted between the neck musculature. A multipin plug for an EEG–EMG device (see 'EEG/EMG recording and sleep scoring') was affixed to the skull with Orthodontic Resin power and Orthodontic resin liquid (TOC Dental). Mice were also implanted with a 200 µm optical fiber (Doric Lenses) in the frontal cortex (coordinates: ML −1.00 mm, AP 2.22 mm, DV −2.00 mm) and a guide cannula for delivering the FITC-dextran or AF488 into the CPu (coordinates: ML −2.55 mm, AP −0.58 mm, DV −3.00 mm). Mice were allowed to recover from surgery for at least 1 week before any experiments were performed.

Anesthesia

For the experiments during anesthesia, mice were anesthetized (i.p.) with 200 µg kg^{-1} (60 µg ml^{-1}) dexmedetomidine (Orion Parma), 100 mg kg^{-1} (20 mg ml^{-1}) ketamine (Zeotis) with 20 mg kg^{-1} (4 mg ml^{-1}) xylazine (Dechra) or 50 mg kg^{-1} (10 mg ml^{-1}) pentobarbital (Animalcare), and kept on a heat mat (ThermoStar Homeothermic Monitoring System, RDW Life Science) at 36.5 °C. Control injections were with saline.

EEG/EMG recording and sleep scoring

EEG and EMG signals were recorded using a miniature datalogger attached to the skull[34]. The data were downloaded and waveforms

visualized using MATLAB (MathWorks). The EEG signals were high-pass filtered (0.5 Hz, −3 dB) using a digital filter and the EMG was band-pass filtered between 1 and 50 Hz (−3 dB). Power in the delta (1–4 Hz), theta (5–10 Hz) bands and theta to delta band ratio were calculated, along with the root-mean-square value of the EMG signal (averaged over a bin size of 5 s). All of these data were used to define the vigilance states of Wake, NREM sleep and rapid-eye-movement (REM) sleep, initially by an automatic script using a probability-based algorithm and Gaussian Mixture Model ('Code Availability'). The sensitivity and specificity when compared to experienced human sleep scorers were very high (see below). Nonetheless, after automatic scoring, each vigilance state was then screened and confirmed manually afterwards.

	Scorer 1			Scorer 2		
	Wake	NREM	REM	Wake	NREM	REM
Sensitivity	0.91	0.97	0.91	0.94	0.93	0.95
Specificity	0.98	0.92	0.99	0.96	0.96	0.98

Histology experiments

At a chosen time following dye injection into the CPu, mice were killed and their brain taken by dissection and frozen immediately in liquid pentane on dry ice. The brain was then embedded in OCT embedding matrix (CellPath) and kept frozen. Next, the brain was sliced in 60-µm coronal sections using a cryostat (CryoStar NX70, Thermo Fisher Scientific), then immediately dried and mounted on slides using DPX mountant (06522, Sigma-Aldrich). The coronal sections were imaged with a widefield microscope and Zeiss Zen Pro software (Axio Observer, Carl Zeiss) at a magnification of ×5. The average intensity of each slice was measured using ImageJ and the mean intensity in groups of four along the anterior–posterior distance was calculated. The data, when plotted against the anterior–posterior distance from the site of injection, were fitted to Gaussian curves, with variable width, amplitude, baseline and position.

Quantification and statistical analysis

All quantitative results are quoted as means ± 95% confidence intervals or means ± s.e.m. Normality was confirmed using the Kolmogorov–Smirnov test. Comparisons were made using estimation statistics and one-way or two-way analysis of variance (ANOVA). Confidence intervals and sampling distributions (that is, distributions of likelihood) were calculated using bias-corrected and accelerated bootstrapping[35]. The sampling distributions were calculated using 5,000 bootstrap samples. Data collection and analysis were generally not performed blind to the conditions of the experiments. However, the automatic sleep-scoring algorithm was done blind and the vigilance states then checked manually. No statistical methods were used to predetermine sample sizes but our sample sizes are similar to those reported in previous publications[3,4,12].

Data exclusions

For the diffusion coefficient measurements, bleaching recordings that could not be fitted by the custom curve-fitting algorithm were excluded. For the photometry recordings, poor fits to the theoretical curves were excluded and recordings where one of the paired recordings (saline or anesthetic; or sleep and wake) was not successful. For the histology experiments, brain sections that were substantially damaged were excluded from the quantitative analysis.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All source data for the main figures and Extended Data figures are available on figshare at https://doi.org/10.6084/m9.figshare.25483339 (ref. 36). Source data are provided with this paper.

Code availability

The MATLAB script for automatic sleep scoring is available on figshare at https://doi.org/10.6084/m9.figshare.25483339 (ref. 36).

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Author contributions

N.P.F. and W.W. conceived the study. A.M., T.L., B.H. and M.G. performed the experiments and with N.P.F. analyzed the data. C.J.E. contributed to the theoretical basis of the methodology. R.T.C.W. calculated the EEG power spectra. T.G.C. contributed to developing data-logging equipment. N.P.F. and W.W. wrote the first draft of the paper and all authors contributed to and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41593-024-01638-y.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41593-024-01638-y.

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Peer review information *Nature Neuroscience* thanks Vartan Kurtcuoglu and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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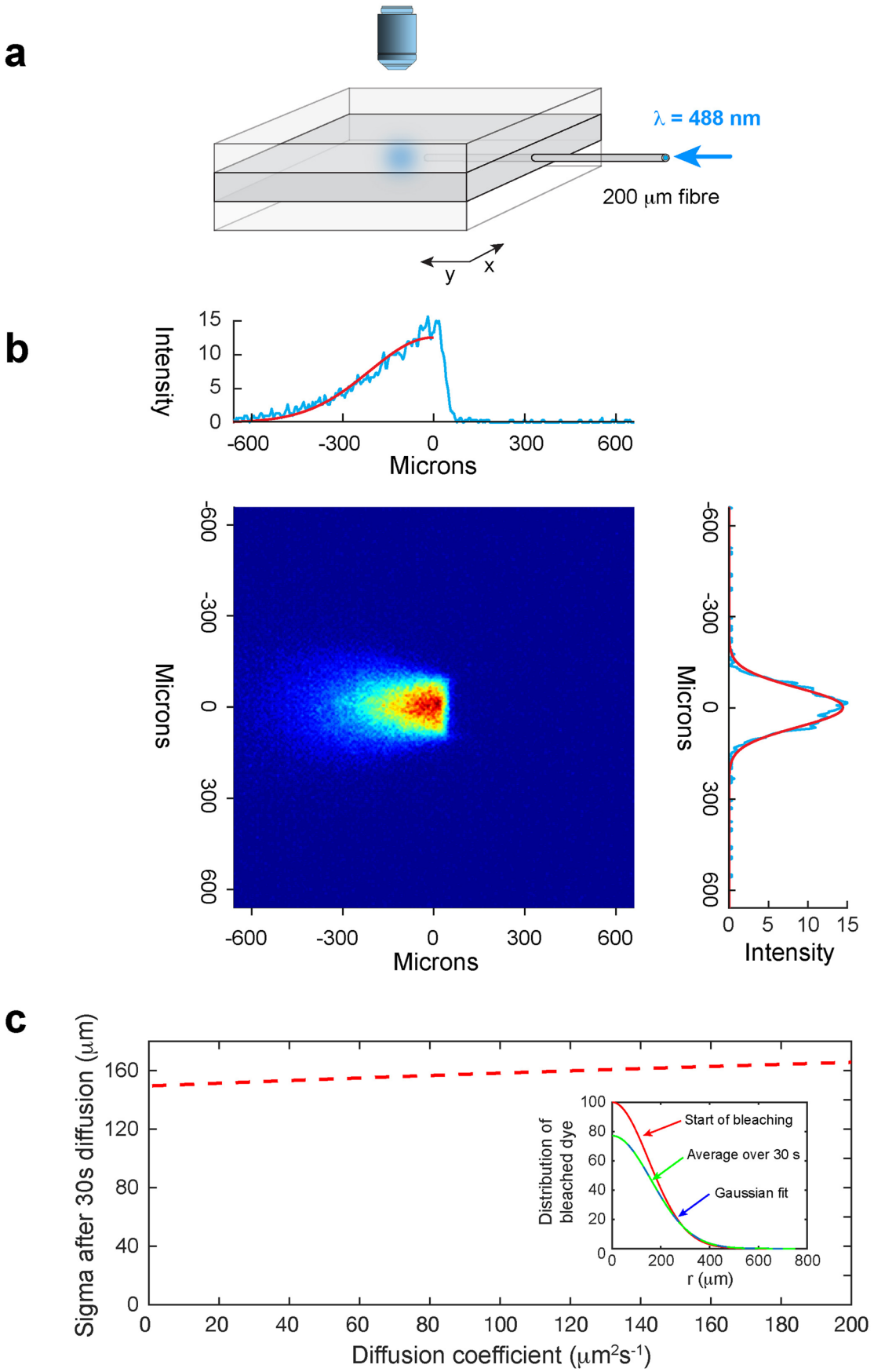

Extended Data Fig. 1 | See next page for caption.

 https://doi.org/10.1038/s41593-024-01638-y

Extended Data Fig. 1 | Measurement of the distribution of bleached dye. a, A thin brain slice or sheet of an agarose gel brain phantom containing FITC-dextran was sandwiched between two blocks of clear agarose (Methods). An optical fiber (core diameter 200 µm) was inserted into the brain slice or central gel and an image taken of the light distribution of a 488 nm-laser diode. **b**, The intensity distribution from a digitized image (blue lines) was then fit to a hemispherical Gaussian distribution (red solid lines). The average value for the standard deviations of the Gaussian fits was $\sigma = 149.5$ µm (CI [140.7, 162.6]; $n = 8$ independent experiments). **c**, There is a small change in this standard deviation due to diffusion during the 30 s of bleaching (red dashed line), which differs for each molecular weight due to the different diffusion coefficients. This was estimated by averaging the dye distribution (equation [2]) over 30 s and then fitting this to a Gaussian. Inset: An example of how the dye distribution changes during bleaching for 4 kDa FITC-dextran, $D = 133.9$ µm^2 s^{-1}. The red curve is the Gaussian distribution at the start of bleaching ($\sigma = 149.5$ µm), the green curve is the average distribution over 30 s, fitted to a Gaussian (blue dashed line) which gives ($\sigma = 161.0$ µm).). The values of σ that were used for the diffusion measurements in agarose gel for 4 kDa, 10 kDa and 70 kDa FITC-dextran were 152.1 µm, (CI [143.3, 165.0]; $n = 8$ independent experiments), 156.2 µm, (CI [147.7, 169.0]; $n = 8$ independent experiments) and 161.0 µm, (CI [152.6, 173.2]; $n = 8$ independent experiments), respectively.

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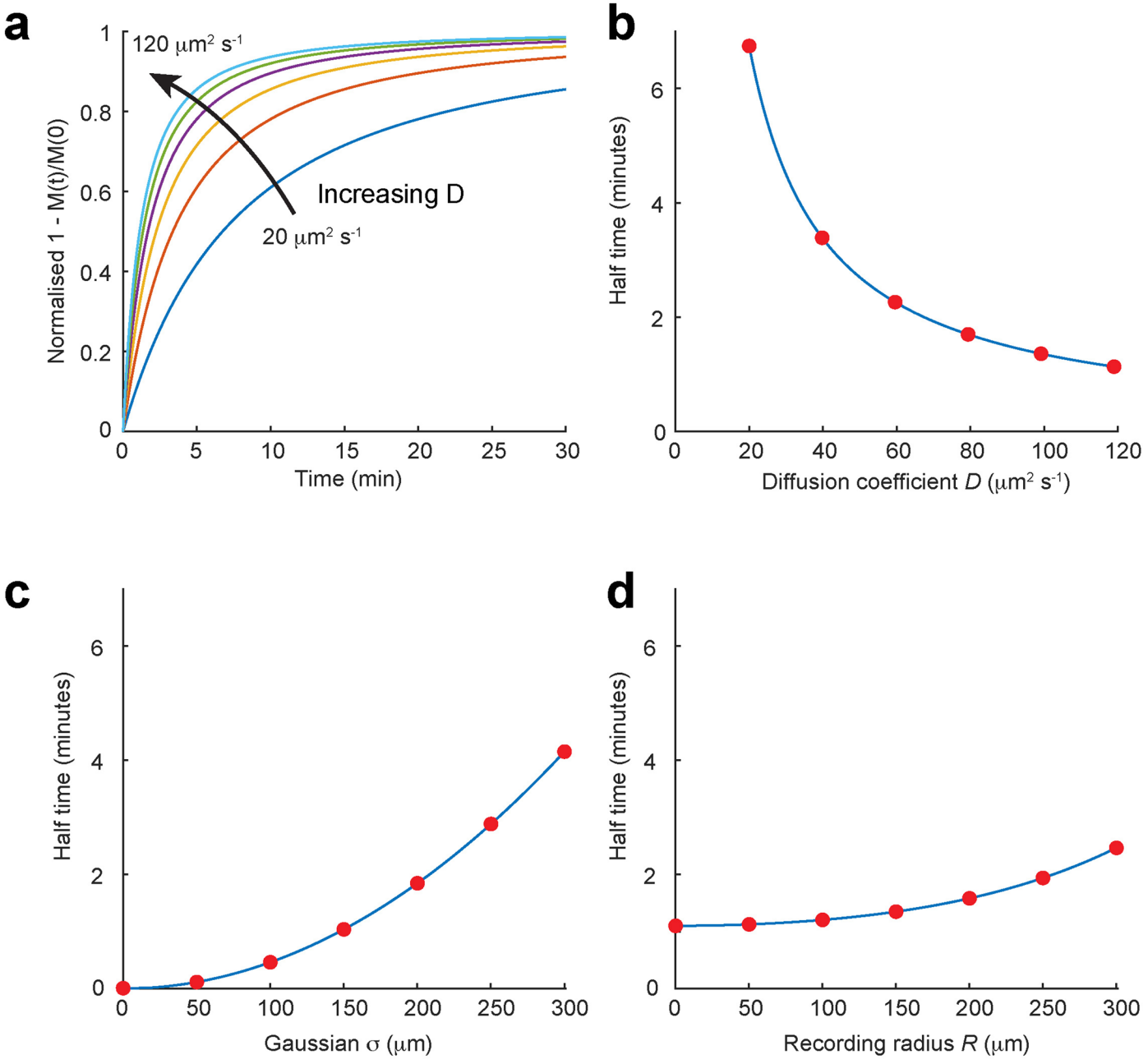

Extended Data Fig. 2 | The time course of *M(t)* (equation [5]) is sensitive to values of *D* and *σ*, but insensitive to values of *R*. **a**, The time course of $M(t)/M(0)$ for values of D from 20–120 μm² s⁻¹. **b**, Corresponding half times of $M(t)/M(0)$ over the same range of D showing that the half times change greatly with D. **c**, Corresponding half times of $M(t)/M(0)$ over the same range of D showing that the half times change greatly with σ. **d**, Corresponding half times of $M(t)/M(0)$ over the same range of D showing that the half times change little with R. **Derivation of equation** [5]. The total number of mols *M(t)* of fluorescent dye in a hemisphere of radius R, is given by equation [2] multiplied by the area of a hemisphere ($2\pi r^2$), integrated from 0→R (because we have assumed that the volume being recorded from is a hemisphere of radius R): $M(t) = C(0,0)[1+\frac{2Dt}{\sigma^2}]^{-\frac{3}{2}}\int_0^R 2\pi r^2 exp[\frac{-r^2}{4Dt+2\sigma^2}]dr$

This can be written as: $M(t) = a\int_0^R r^2 exp[-br^2]dr$, where $a = 2\pi C(0,0)[1+\frac{2Dt}{\sigma^2}]^{-\frac{3}{2}}$ and $b = (4Dt+2\sigma^2)^{-1}$ Integrating by parts gives:

$M(t) = [-\frac{aR}{2b}exp[-bR^2]] + \int_0^R \frac{a}{2b}exp[-br^2]dr$ Using the standard integral:

$\int_0^R exp[-br^2]dr = \sqrt{\frac{\pi}{4b}}erf(\sqrt{b}R)$, we have $M(t) = \frac{a}{2b}\{\sqrt{\frac{\pi}{4b}}erf(\sqrt{b}R) - Rexp[-bR^2]\}$

so, finally, substituting in a and b we have Equation [5]:

$M(t) = \frac{2\pi C(0,0)\sigma^3}{\sqrt{(2Dt+\sigma^2)}}\{\sqrt{\frac{\pi(2Dt+\sigma^2)}{2}}erf(\frac{R}{\sqrt{(4Dt+2\sigma^2)}}) - Rexp[-\frac{R^2}{(4Dt+2\sigma^2)}]\}$.

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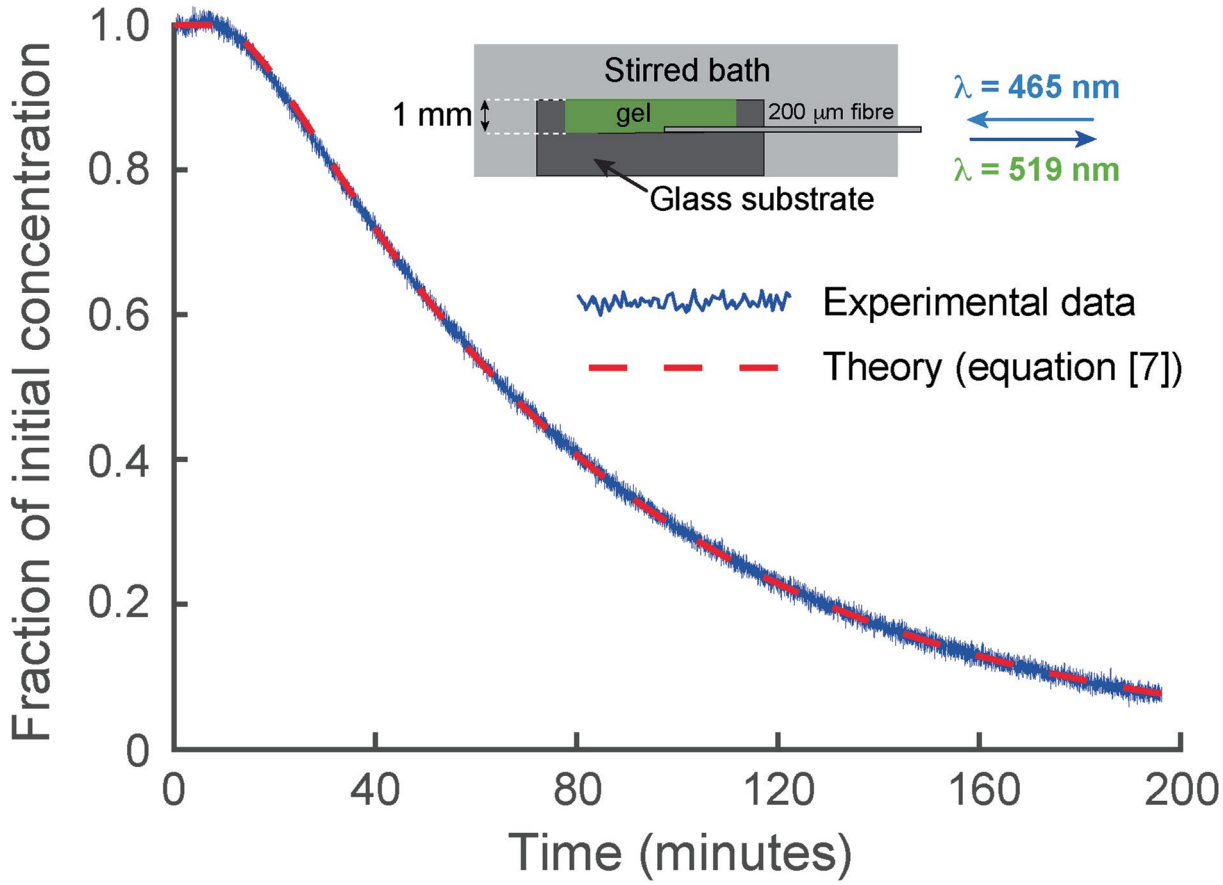

Extended Data Fig. 3 | Direct measurement of diffusion coefficients. The diffusion coefficients of the FITC–dextrans in the brain phantom agarose gel were determined directly by measuring the time course of diffusion of FITC-dextran from a 1-mm thick sheet of gel, into an effectively infinite stirred water bath containing all the components of the brain phantom (except the agarose and FITC-dextran). By recording the reduction in the fluorescent signal close to the impermeable glass surface on which the gel was set, as a function of time, the diffusion coefficient could be directly determined using equation [7] (ref. 26). The figure shows data from a typical experiment using 4 kDa FITC-dextran (blue trace) and the red dashed line shows the change predicted by equation [7].

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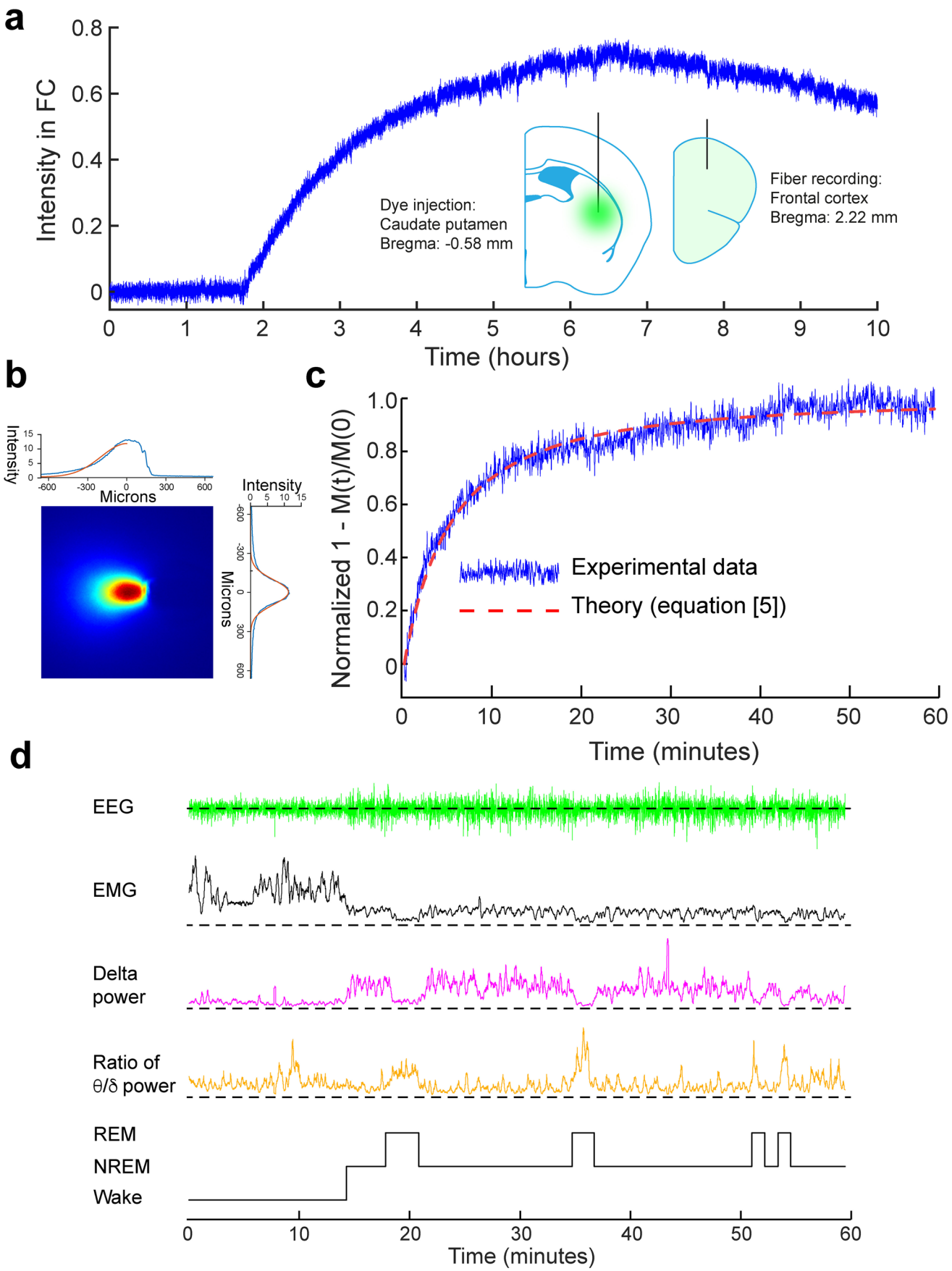

Extended Data Fig. 4 | See next page for caption.

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Extended Data Fig. 4 | Measurement of movement *in vivo* using photobleaching. a, Fluorescent intensity measured in the frontal cortex following injection of 4 kDa FITC-dextran into the CPu (at $t = 0$). After a delay, fluorescent intensity rises to a maximum and then slowly decays. **b**, As with the experiments in gels, the spread of light in the brain had to be established. This was done using brain slices (Methods) and this figure shows a typical image obtained from a brain slice, which provided a measure of the standard deviation of the hemispherical gaussian σ. **c**, A typical recording *in vivo* of the recovery of fluorescence after photobleaching. A value for D was derived from the theoretical fit (red dashed line) to Eq. 5, as described in Methods. **d**, Throughout the experiment, the EEG and EMG signals were recorded and the power in the delta band (1–4 Hz) and theta band (5–10 Hz) derived, so that the vigilance state (WAKE, NREM or REM) could be determined (Methods).

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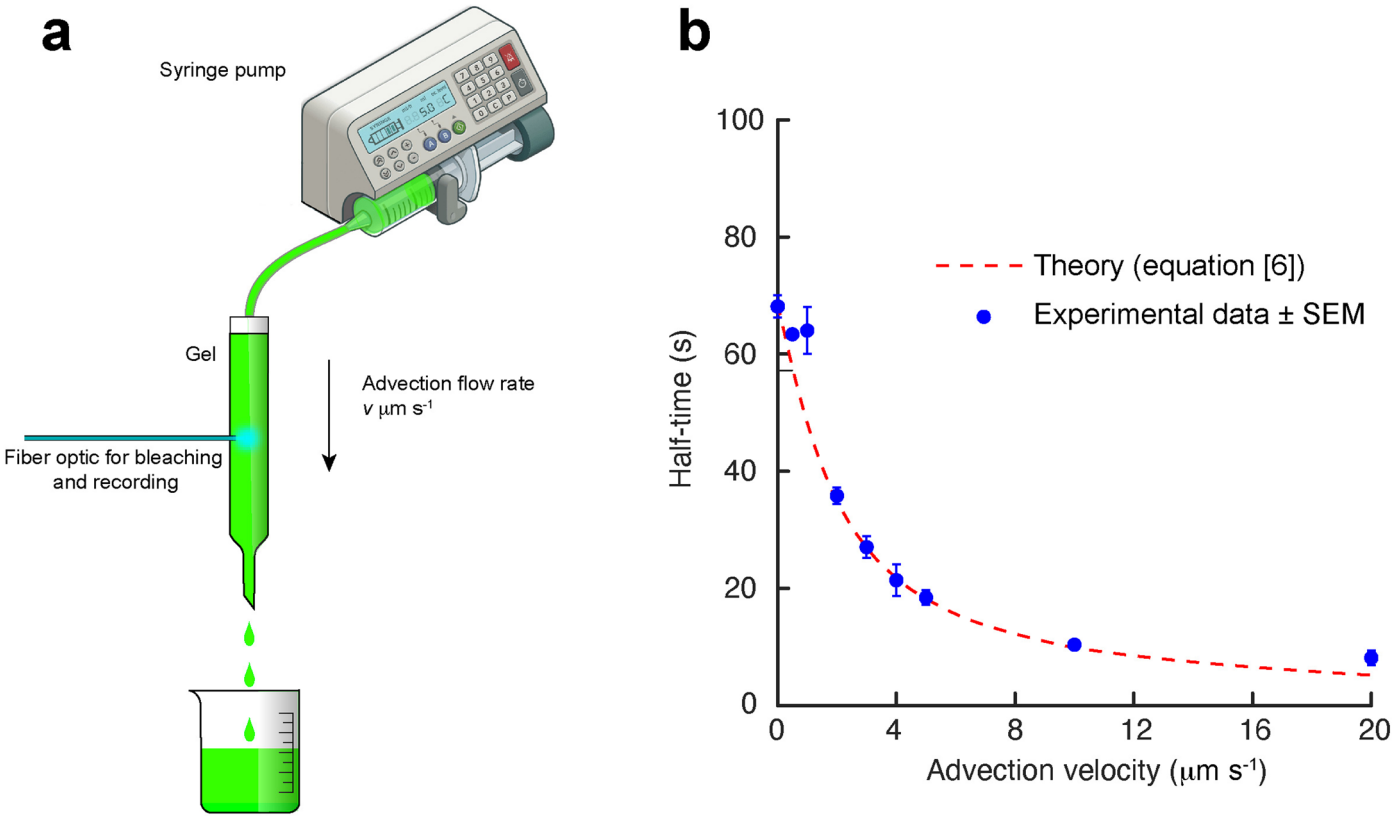

Extended Data Fig. 5 | The effect of advective flow on the time course of recovery of fluorescence after photobleaching. This was assessed in an *in vitro* experiment illustrated in **a**. A solution of 4 kDa FITC-dextran was passed through a gel (Methods) at a constant flow rate and the time course for the recovery of photobleaching recorded using an optical fiber, exactly as used in the experiments described in the text *in vitro* and *in vivo*. **b**, The observed half times were accurately predicted from equation [6] and reduced rapidly with increasing advective velocity. From the precision with which we could record changes in diffusion coefficients *in vivo* (Fig. 1d,e right panels) and their corresponding half times, we estimate that we would have been able to detect a change in advective flow of about 0.5 μm/s, or greater. Where error bars (SEM; $n = 5$ independent experiments) are not shown they were smaller than the size of the symbol.

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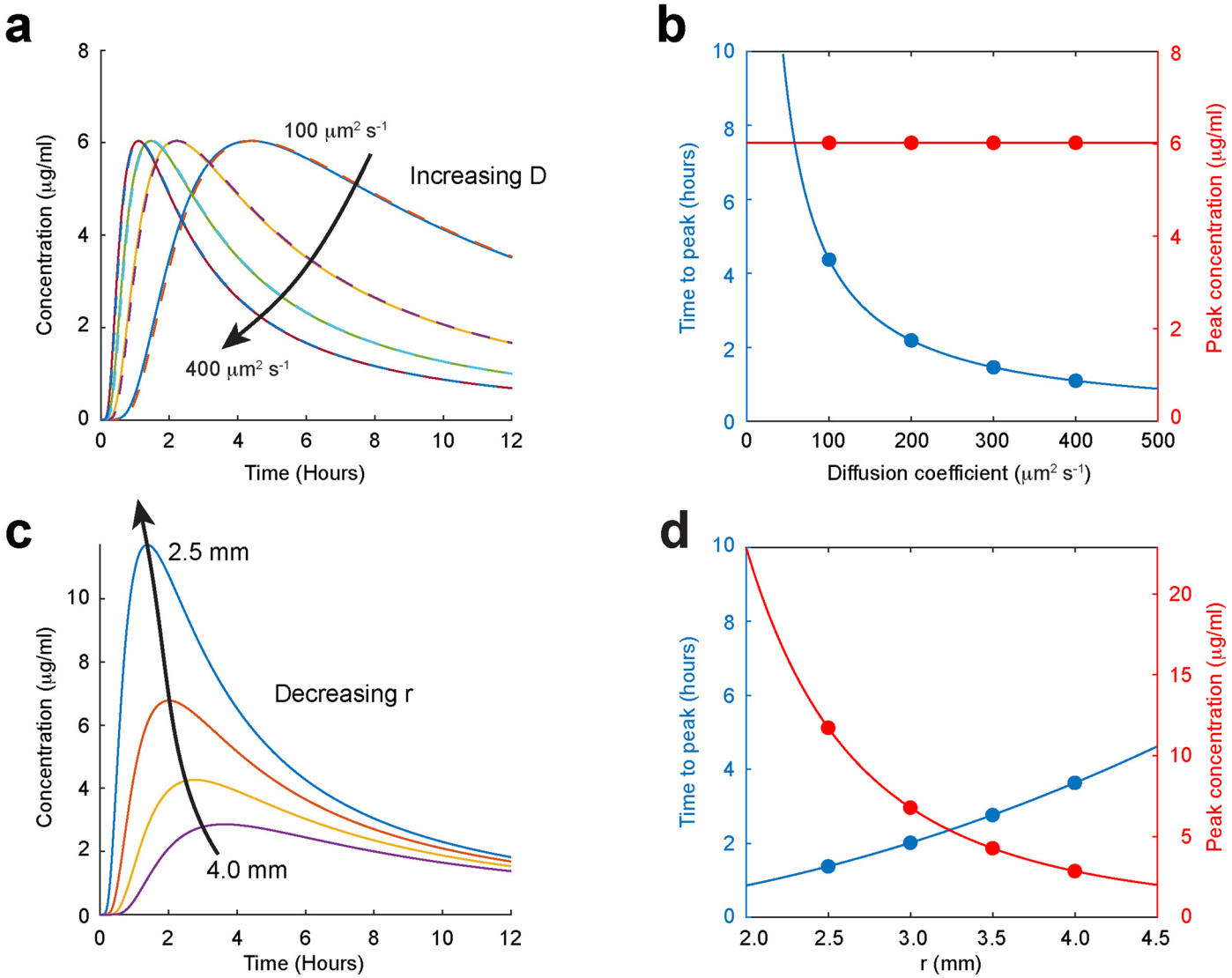

Extended Data Fig. 6 | The time course of C(r, t). a, **b** According to equation [2], the concentration at a fixed distance, *r*, from a Gaussian source (solid lines) reaches a peak with time that depends only on the diffusion coefficient *D*, while the peak concentration does not change. Almost identical concentrations are predicted if the source is a sphere, rather than a Gaussian, containing the same number of moles (dashed lines). (The equation for the concentration as a function of time from a spherical source has been solved by Crank[26].) **c**,**d**, The peak concentration with time for a fixed diffusion coefficient, decreases with increasing distance *r*, with relatively small changes in the time to peak.

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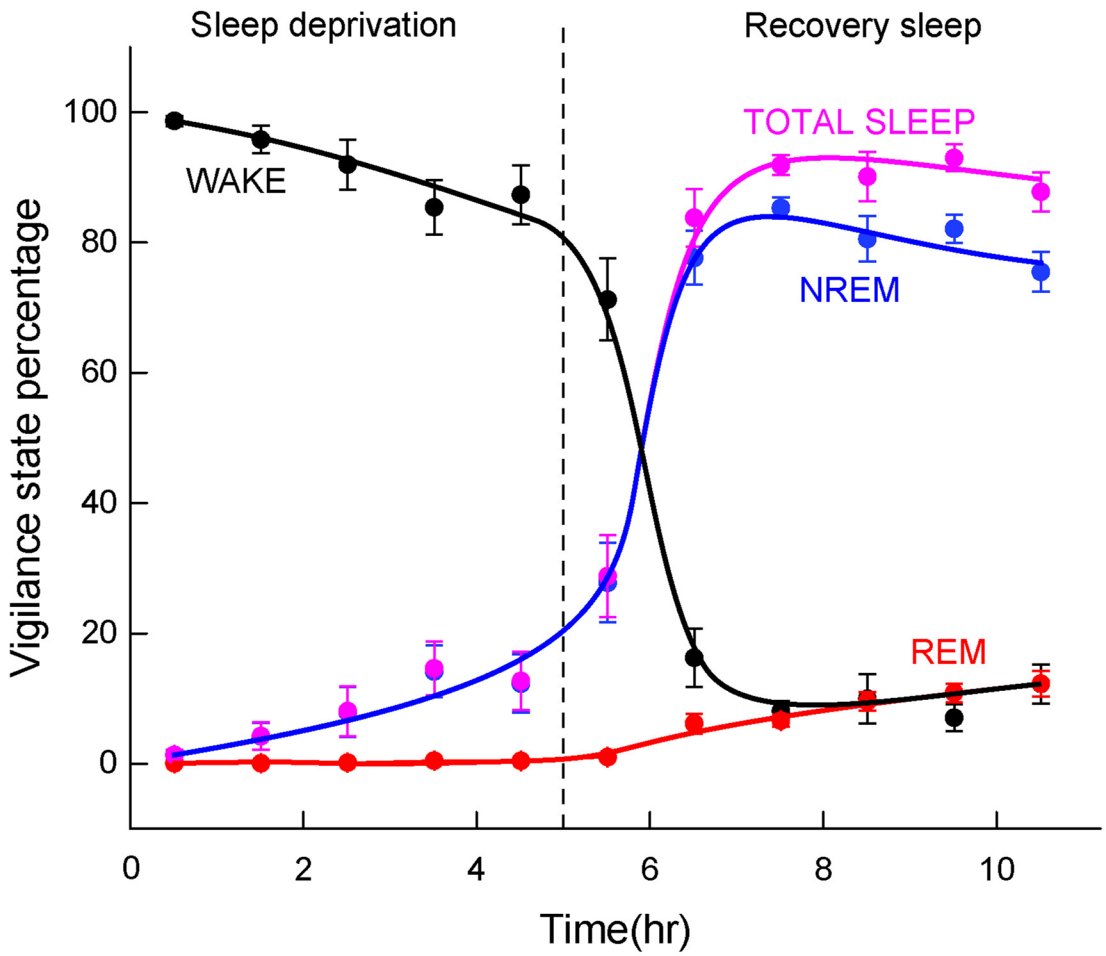

Extended Data Fig. 7 | Vigilance-state percentages for the sleep photometry experiments. During the sleep experiments, mice were first sleep deprived by placing novel objects in their cage and after 5 hours were then allowed to sleep. The photometry measurements during the WAKE state were carried out during the five hours of sleep deprivation, where the WAKE state occurred, on average, 92% of the time (8% NREM and 0% REM). The photometry measurements during the SLEEP state were carried out after the first sleep episode following sleep deprivation. During the first five hours the vigilance state percentages were: WAKE 9.3% (n = 11 mice), NREM 80.8% (n = 13 mice), REM 9.9% (n = 13 mice), TOTAL SLEEP 90.7% (n = 13 mice). Sleep scoring of vigilance states was carried out as described in Methods. The errors bars represent SEMs.

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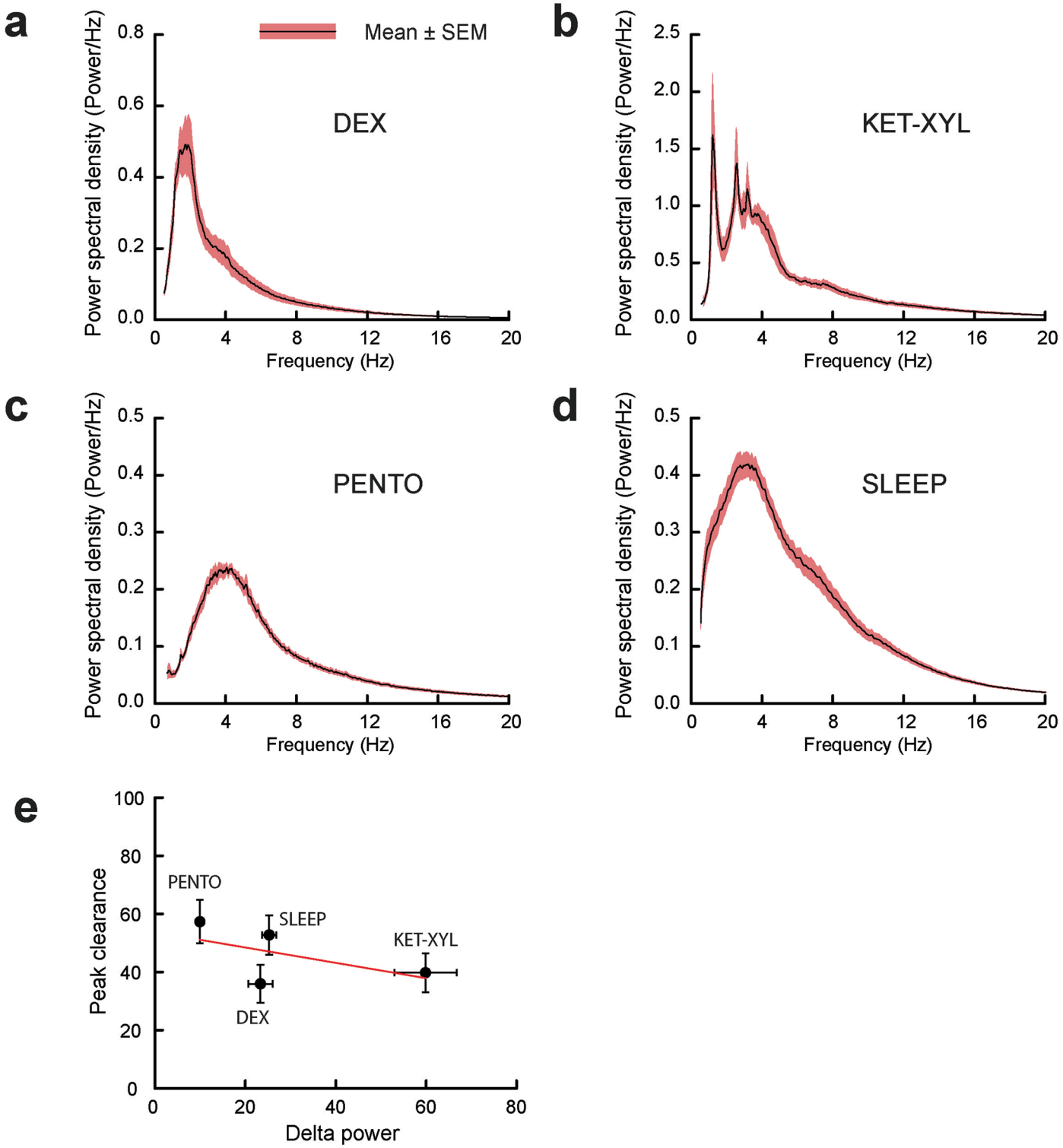

Extended Data Fig. 8 | Power spectra during anesthesia and correlation with peak clearance. Power spectral density plots during anesthesia were calculated for the three anesthetics **a-c** and **d**, during recovery sleep. For the anesthetics, the power spectra were carried out using the EEG recorded during the first 2 hours of anesthesia (excluding the first ten minutes following injection). For sleep, the power spectra were calculated during 2 hours of recovery sleep, which included some time in WAKE (9.3%) and REM (9.9%) states. **e**, There was a weak negative correlation (Pearson's correlation coefficient −0.58) between delta (0.5–4.0 Hz) power and peak clearance (see Fig. 2d–g and Extended Data Table 1). PENTO (n = 10 mice), DEX (n = 9 mice), SLEEP (n = 9 mice) and KET/XYL (n = 9 mice). The errors bars represent SEMs and where they are not shown they were smaller than the size of the symbol.

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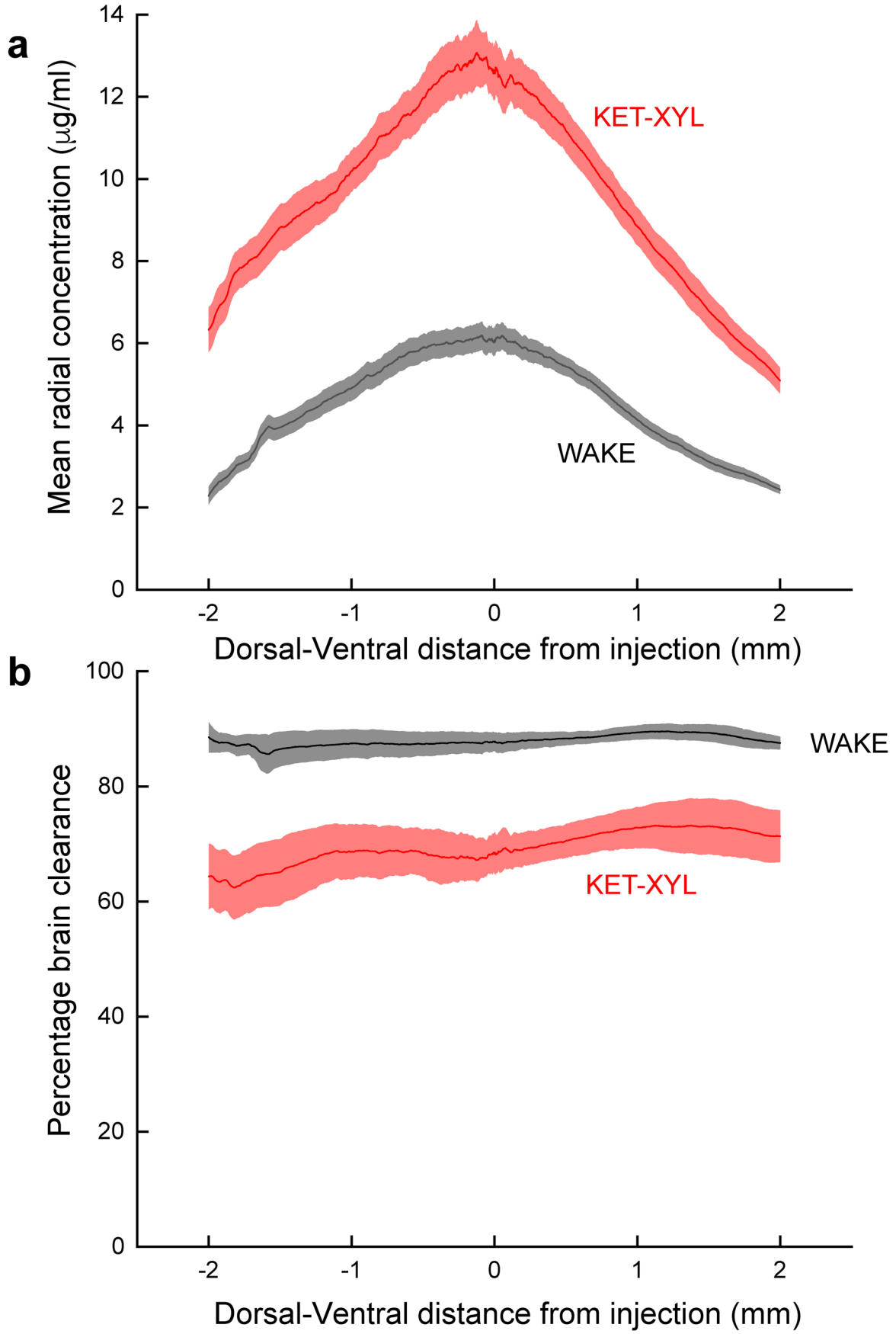

Extended Data Fig. 9 | See next page for caption.

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Extended Data Fig. 9 | Brain clearance is uniform across the brain. The concentration of AF488 dye 3 hours after injection into the CPu was measured at an anterior-posterior coordinate 1 mm from the site of injection. **a**, The concentration of dye was then calculated as a function of radial distance from the peak concentration in both the dorsal and ventral directions. As predicted by equation [8], this results in a Gaussian curve. **b**, Using these data (predicted by equation [8]) together with equation [2], the percentage clearance can be calculated in the dorsal and ventral directions. Two-way ANOVA shows that there is no significant change in brain clearance across the brain ($p = 0.99$) for both WAKE animals and those anesthetized by ketamine-xylazine. In contrast, the inhibition of clearance by ketamine-xylazine is highly significant ($p < 10^{-6}$). For both panels the means are for $n = 3$ animals and the error envelope shows the SEMs.

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Extended Data Table 1 | Summary of percentage clearances and diffusion coefficients

Dexmedetomidine (200 μg/kg)				
	Saline injection (mean ± SEM)	DEX injection (mean ± SEM)	*n*	P (Paired *t*-test)
Percent clearance at peak	74.9 ± 6.6	36.0 ± 6.5	8	0.0029
Diffusion coefficient	137.3 ± 12.0	138.2 ± 9.7	8	0.94

Ketamine (100 mg/kg) - Xylazine (20 mg/kg)				
	Saline injection (mean ± SEM)	KET-XYL injection (mean ± SEM)	*n*	P (Paired *t*-test)
Percent clearance at peak	76.4 ± 3.5	39.8 ± 6.7	9	0.0015
Diffusion coefficient	162.7 ± 14.6	200.5 ± 31.3	9	0.12

Pentobarbital (50 mg/kg)				
	Saline injection (mean ± SEM)	PENTO injection (mean ± SEM)	*n*	P (Paired *t*-test)
Percent clearance at peak	81.5 ± 3.1	57.3 ± 7.5	6	0.037
Diffusion coefficient	127.4 ± 11.6	139.3 ± 9.3	6	0.39

Sleep				
	WAKE (mean ± SEM)	SLEEP (mean ± SEM)	*n*	P (Paired *t*-test)
Percent clearance at peak	69.7 ± 4.3	52.8 ± 6.8	8	0.016
Diffusion coefficient	225.8 ± 55.4	169.0 ± 17.5	8	0.23

Summary of percentage clearances at the peak photometry signal (see Fig. 2h-k) and diffusion coefficients for the different vigilance states as means±SEMs. The statistical test used was a two-tailed paired *t*-test.

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☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
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☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
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☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	EEG/EMG data was collected with a customized portable recording device (Hsieh, B. et al. 2019). Photometry data was collected with Doric Lenses photometry system (Doric Lenses, Quebec Canada) and recored with the software Doric Neuroscience Studio (version 5.4.1.23, Doric Lenses, Quebec Canada). Histology data was collected with a widefield microscope Zeiss Axio Observer 3 with Zeiss Zen Pro software (version 3.8, Carl Zeiss NY U.S.)
Data analysis	Automatic sleep scoring based on EEG/EMG and photo-recovery curve fitting were carried out simultaneously with a customized script performed in software Matlab (The MathWorks Inc, Natick, Massachusetts, USA, version R2024a, 24.1). Vigilance states were checked manually. The script and related documentation is included in the Code and Software Submission. Statistical calculations were made using the online resource https://www.estimationstats.com/#/ (Ho, J. et al. 2019), Matlab (The MathWorks Inc, Natick, Massachusetts, USA, version R2024a, 24.1 or OriginPro (OriginLab Corporation, Massachusetts, USA, version 9.8.0.200). Figures were prepared with Adobe Illustrator (version 26.5.2). Mean pixel intensity for histological data and preparation of representative images were performed with FIJI ImageJ (version 1.54f).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data are available from the corresponding authors on request.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N.A.
Reporting on race, ethnicity, or other socially relevant groupings	N.A.
Population characteristics	N.A.
Recruitment	N.A.
Ethics oversight	N.A.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications (Iliff et al.,2012 PMID: 22896675; Xie et al., 2013 PMID: 24136970; Mestre et al., 2018 PMID: 30451853).
Data exclusions	For the diffusion coefficient measurements, bleaching recordings that could not be fitted by the custom curve-fitting algorithm were excluded. For the photometry recordings, poor fits to the theoretical curves were excluded, and recordings where one of the paired recordings (either saline or anesthetic, or sleep and wake) was not successful. For the histology experiments, brain sections that were significantly damaged were excluded from the quantitative analysis.
Replication	The main method of photo-bleaching and recovery has been validated independently in vitro with FITC-dextran at different molecular weights. In vivo photo-bleaching and recovery experiment were carried out in a replication of 24 animals. Each animal were recorded during multiple experiments each contains multiple bleach/recovery successions. For in vivo photometry experiments, at least 6 animals were tested for each anesthetics and vigilant states. Multiple measurements were made for each experimental conditions. Histology images shows in Figures were repeated in at least three mice. All above mentioned attempts at replication were successful.
Randomization	Selection of animals from the stock cohort were randomized. In vivo photo-bleaching and recovery experiments were started at random time of the day. For the anesthesia experiments, mice were injected with either an anesthetic or saline in random order. For the sleep experiments, recordings were made on the same animal, one week apart, in random order.
Blinding	Data collection and analysis were generally not performed blind to the conditions of the experiments. However, the automatic sleep-scoring algorithm was done blind, and the vigilance states then checked manually.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Return to Title Page

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	C57BL6j (Jackson laboratory), male, aged between 3-7 months
Wild animals	No wild animals were used in the research
Reporting on sex	Metabolite clearance and the function of sleep are not considered as a sex dimorphism according to the current literature. Therefore only male mice were used in this research. We do not expect that the results we are reporting are sex-dependent.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All experiments were performed in accordance with the United Kingdom Animal Scientific Procedures Act 1986 under personal and project licenses granted by the United Kingdom Home Office. Ethical approval was provided by the Ethical Review Panel at the Imperial College London

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	N.A.
Novel plant genotypes	N.A.
Authentication	N.A.

What are Data?

Raw data, processed data, secondary data, survey data

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if hrs <=40 :
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    print(total_pay)
else :
    ot_pay = ((hrs - 40) * (1.5 * rph))
    base_pay = 40 * rph
    total_pay = base_pay + ot_pay
    print(total_pay)
```

Data analysis/visualization scripts

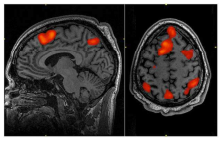
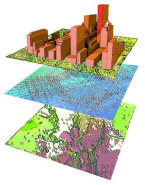


Image data, audio/video recordings, photos

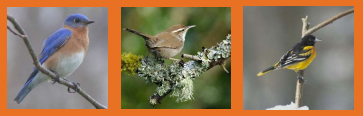


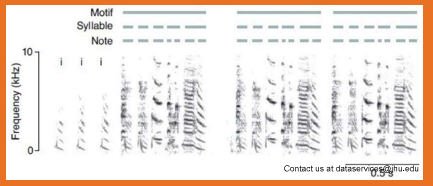
Geospatial data

Scenario: Birdsong Project

Research lab and PI:
You are an assistant professor at the Dept. of Biology, Johns Hopkins University.

Grant:
One of your grant proposals has just been funded by National Science Foundation!! This is a 3-year project to study the birdsong variation for several songbird species in Eastern United States.





Scenario: Birdsong Project

Lab members:
Graduate students: Two students will use part of this project for their dissertations.
Lab technician: A lab technician will help with data collection and analysis.
Undergraduate students: They will help with data analysis.

Collaborators:

University of Delaware
University of Maine
Smithsonian Institution
State University of New York
University of North Carolina, Chapel Hill

COLLABORATION

Scenario: Birdsong Project

Data collection:
Cornell Lab of Ornithology: the Cornell Guide to Bird Sounds
Citizen scientists: record birdsongs by a smartphone and submit it to the lab's website
Lab members and collaborators: Recordings collected by the lab and collaborators in the field

Data analysis:
Cornell Lab of Ornithology: Raven Pro software to process birdsongs
Lab members: Write custom Python scripts to further analyze song parameters and do statistical analysis

Data Examples

#	Datasets (in workflow order)	Format(s)	~size	Share
1	*Audio recordings of birdsong by citizen scientists and collaborators*	.wav	10 GB	Yes (Macaulay Library, Cornell Lab of Ornithology)
2	*The Cornell Guide to Bird Sounds: United States and Canada*	.wav	2.2 GB	No (proprietary data, but can be purchased from here)
3	*Raven Pro for visualizing and analyzing birdsongs*	.exe	107 MB	No (proprietary software, can be purchased and downloaded here)
4	*Python scripts for song classification and statistical analysis*	.py	10 MB	Yes (Johns Hopkins Research Data Repository)
5	*Sound parameters*	.csv	100 MB	Yes (Johns Hopkins Research Data Repository)

Funder's Role: Data Access

Federal

Private

NIH Policy for Data Management and Sharing

NIH DMSP: takes effect January 25, 2023

- All data-producing research funded by NIH require a Data Management and Sharing Plan
- How data will be managed, which data will be shared
- Plans should address privacy of human participants
- Data management and sharing cost
- Scientific Data Sharing by NIH: https://sharing.nih.gov/

Data Services provides **consultations** and **workshops** for writing your Data Management and Sharing plan

Goals of Data Stewardship (Data Management)

Facilitate data sharing at the end of a project

Make your data Findable, Accessible, Interoperable, and Reusable (FAIR)

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How to Make Data FAIR?

Findable
- Descriptive keywords
- Persistent Identifier (DOI)

Accessible
- Easy to retrieve by machines and humans
- Data in a repository

Interoperable
- Open formats
- Consistent vocabulary

Re-Usable
- Clear reuse licenses
- Good documentation

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St. Lawrence Global Observatory (2018) *FAIR Principles*. Retrieved 20190918 on https://oesl.ca/en/fair-principles

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Best Practices for Data Management

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Plan Early

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Data Management Plan (DMP)

- A document describing how research data will be **gathered, stored, documented and shared**
- Many funders ask for DMPs to go with proposals
  - Some PIs were asked to revise grant proposals due to weak DMPs
- It is still a good practice to write a DMP, even if your funder does not ask for it

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Essential Components of DMPs

Data Acquisition, Storage and Security

- What are you producing?
- Assessing risk of disclosing personal & health identifiers
- Preparing data to minimize risk for internal use
- Finding secure storage and collaboration platforms

Data Organization and Documentation

- Standardize protocols for file naming, organization, documentation (focused on collaboration)

Data Sharing

- What data will be shared, how, and under what conditions?

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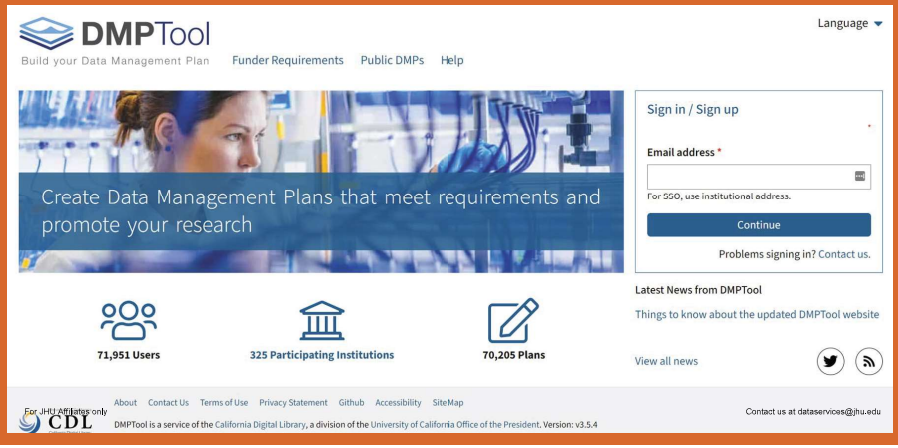
DMPTool

https://dmptool.org/



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Organize and Document

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We hope you cannot relate to this…

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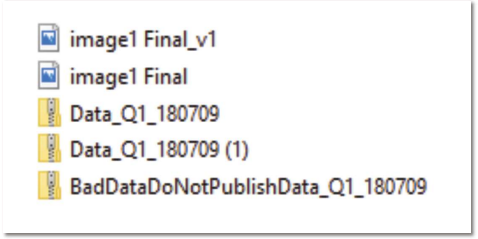
What's in a Name?

Can you tell what these are without opening them?



Use *filenaming* to
organize and document research files

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Quick Tips for File Naming

File naming tip	Poor names	Good names
Be specific	Image1 UseThisOne_v.2 Data	**StemCell_SMA** **WillowCreek_SpList_2012** **PerceptionExp_Subj1**

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Quick Tips for File Naming

File naming tip	Poor names	Good names
Be specific	Image1 UseThisOne_v.2 Data	**StemCell_SMA** **WillowCreek_SpList_2012** **PerceptionExp_Subj1**
Be consistent	Data_v1 ResearchData_v2 Results_v3	**Azaleas_Stem** **Azaleas_Pollen** **Azaleas_Petal**
Use certain characters (Stick with letters, numbers, -, _ and avoid spaces and special characters)	Perception Exp: Survey Rhododendron[Plot1] StemCell.SMA.15A*	**Perception_Exp_Subj1** **RhododendronPlot1** **StemCell-SMA-15A**
Use Standard Date/Time Format (YYYYMMDD hh:mm:ss)	April_10_2018 04102018	**20180410** **2018-04-10**

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Folder, Variable and Other Names

The same file naming tips can also be applied to:

Folder names

Variable names

	A	B
1	Weekly_Demand	Price_per_Gallon
2	85	3.89
3	95	2.78
4	105	3.01
5	115	3.23
6	125	1.68
7		
8		

Function names

```
function compare(a, b)
{
    return a == b;
}

var are_equals = compare(3, 5);
```

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A Tip for A Research Group

Create a File Naming Convention document for your lab group so everyone can follow the same naming convention

Example file name:

Recording team | Date: YYYYMMDD

Ssialis_JHU_BaltimoreMD_20210504_rec01.wav

Bird species | Location | Recording No.

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Resources: File Naming

- Using File Naming to Organize Research Files by Data Services
- Use a batch renaming software
  - On Windows 10
  - On Mac
  - Other software: Rename-It!, Bulk Rename Utility
- Best practices for file naming by Stanford Libraries: tips, software and case studies

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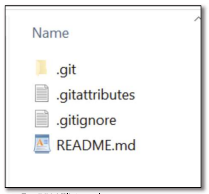
Version Control: Use a Version Control Software

git | CVS | mercurial | SUBVERSION



Pro: Can track changes from multiple users; can return to any previous version
Con: Learning curve can be steep

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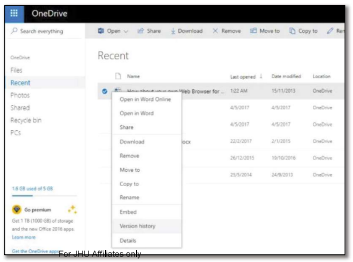
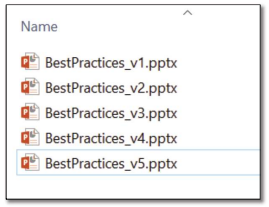
Version Control: Cloud Storage with Version History

OneDrive | Dropbox | OSF | Google Drive



Pro: Updates to new versions automatically
Con: It depends on the service provider to decide how many versions they keep and what constitutes a new version

Version Control: Use Filenames to Show Versions



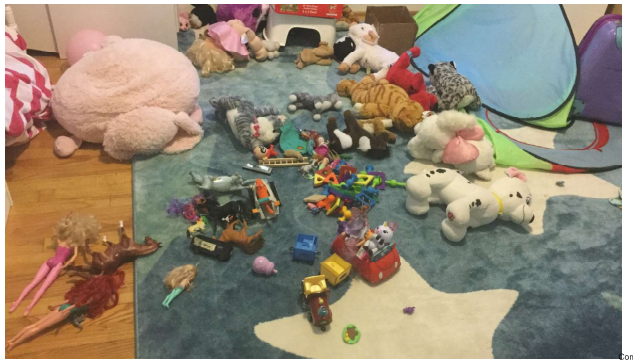
Pro: Don't have to be very computer-savvy to use it

Con: It's not automatic so you may overwrite previous versions, can get unruly

Documentation

What is it?	• Data that provides **descriptive information** • Also known as **metadata**
What gets documented?	• Data, procedures, code, variables and values, derived data, restrictions on use of data, etc.
Why should I do it?	• Enables other to search for, trust, and **reuse** your data • Critical for research **reproducibility**

Can You Please Organize This Mess??



Good Organization

But Organization Is Not Enough; Need Documentation

How much detail should I add to my documentation?

Enough for your future self or collaborators

Birdsong Project: Documentation

You just came back from attending North American Ornithological Conference. A researcher from University of California, Davis was very interested in your research and would like to collaborate with you. She asked if you can share your preliminary data with her. So you sent her your data in a spreadsheet.

Data in the next slide were modified from this dataset: Kleyn, Tristan; da Cruz Kaizer, Mariane; Figueiredo Passos, Luiza (2020), Acoustic and temporal birdsong measurements, Dryad, Dataset, https://doi.org/10.5061/dryad.n02v6wwv3

Species	LF	HF	Dur 90%	BW 90%	Notes	State
B. culicivorus	3100.8	8930.2	0.80	3117.20	8	MD
C. falcularius		4555.3		60	3	
C. gujanensis	2	3409.4		60	8	
C. mer		1489.8		70	35	NY
H. poicilotis	2825.2	4805.8	1.80	1195.30	8	NY
H. poicilotis	2843.5	4773.9	1.60		8	VA
L. albicollis	5357.1	6551	1.50	937.50	8	PA
L. albicollis	5282.3	6574.2	1.90	1007.80	10	NH
M. leachii		5	2.00	750.00	3	RI
P. fasciatus	1785.	2575.6	1.50	679.70	3	MA

What does this missing value mean?

What is the unit for each variable?

What does each variable mean?

Why there are numbers in the Notes column?

Is this value correct?

Am I looking at the correct sheet?

Original | DATA | Metadata

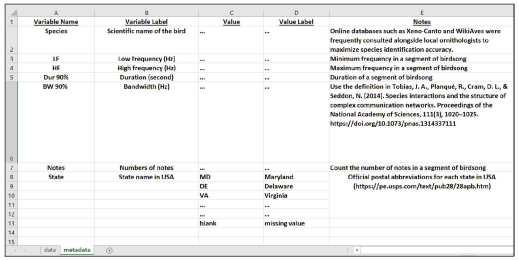
Example Documentation: Codebook

	A	B	C	D	E
1	**Variable Name**	**Variable Label**	**Value**	**Value Label**	**Notes**
2	Species	Scientific name of the bird	...	...	Online databases such as Xeno-Canto and WikiAves were frequently consulted alongside local ornithologists to maximize species identification accuracy.
3	LF	Low frequency (Hz)	...	...	Minimum frequency in a segment of birdsong
4	HF	High frequency (Hz)	...	...	Maximum frequency in a segment of birdsong
5	Dur 90%	Duration (second)	...	...	Duration of a segment of birdsong
6	BW 90%	Bandwidth (Hz)	...	...	Use the definition in Tobias, J. A., Planqué, R., Cram, D. L., & Seddon, N. (2014). Species interactions and the structure of complex communication networks. Proceedings of the National Academy of Sciences, 111(3), 1020–1025. https://doi.org/10.1073/pnas.1314337111
7	Notes	Numbers of notes	...	...	Count the number of notes in a segment of birdsong
8	State	State name in USA	MD	Maryland	Official postal abbreviations for each state in USA (https://pe.usps.com/text/pub28/28apb.htm)
9			DE	Delaware	
10			VA	Virginia	
11			...	...	
12			...	...	
13			blank	missing value	
14					
15					

Separate Metadata Sheet

data | metadata

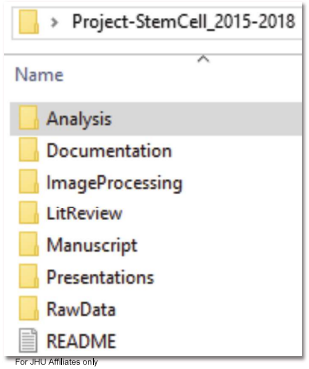
File Level Documentation



Examples

- Codebook to define values in a spreadsheet
- README explaining how to run a code file

Project Level Documentation



Examples

- Author's name, PI's name, file location, etc.
- Permanent identifier, such as DOI
- Written description of a dataset, such as a README for a project

Birdsong Project: Project Level Documentation

Project: Birdsong variation in Eastern United States songbird species
Funder and grant number: NSF Grant # BIO-12345678
PIs: Dr. Robin Cardinal, Johns Hopkins University
Dates: August 2021 to August 2024
Name and location of key files:
Code – https://github.com/birdsong-variation and published in Johns Hopkins Research Data Repository (doi: 10.7281/T10Z715B)
Protocol – published Nature Protocols (https://doi.org/10.1038/s0587-245-01)
Data – birdsongVariation.zip published in Johns Hopkins Research Data Repository (doi: 10.7281/T10Z715B)
Codebook – In same zip file as data
File naming convention:
Dates recorded as YYYYMMDD
All files should start with species name (scientific name), whether it is raw or processed, and date
Use standard abbreviations for variable names

Resource: ReadMe file template and best practices by Cornell University

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A Tip for Documenting Data

Use standards in your research fields when available or develop your own standards!

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Documentation: Metadata Standards

Why do we need standards?

- **Transparency**: Facilitate comprehension and trust of research
- **Reuse**: Make it easier to combine disparate data sets
- **Community standards**: Some communities have developed their own documentation standards

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What are YOUR standards?

- Metadata Standards Directory Working Group
  http://rd-alliance.github.io/metadata-directory/
- NIH Common Data Elements
  https://www.nlm.nih.gov/cde/
- FAIRsharing metadata standards
  https://fairsharing.org/standards/

RDAP

NIH CDE Repository

FAIRsharing.org
standards, databases, policies

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Resources: Documentation

To learn more, watch Self-paced **Online Trainings** by JHU Data Services:

Planning for Software Reproducibility and Reuse

- Best practices for creating understandable, reusable, and citable software and scripts.
- Intellectual property considerations
- Long-term accessibility of code.

Documenting Your Research Data

- Introduction to Documentation
- Metadata and Metadata Standards
- Tabular Data
- Code
- Medical data
- Geospatial data

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Data Storage, Backups and Collaboration

Things to Consider For Storage

Size

Collaboration

Copies

Security

Versions

Simple Security Tips

- Use **Pulse Secure VPN** when not on campus
- **Encrypt** your laptop, tablet, external hard drive, etc. or encrypt particular folders/files
- Password **** **Change passwords** frequently or use a password manager
- **Don't** send unencrypted sensitive information via email
- **Don't** look at sensitive data in public place (e.g., Starbucks)

Collaboration: Tips During a Project

Always Practice Good Data Management

- Share your data management plans with collaborators
- Track versions, retain multiple copies of data, and document research
- Use same terminology and acronyms across data and documents

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Sharing

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At the End of a Project

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Tips for Preserving Data

Select	• Determine **what to preserve** for future use • Example: data behind a figure in a publication
Migrate	• Preserve data in **"open" formats** if possible • Ex. Instead of xlsx format, convert to csv
Document	• Retain SOPs, exp. design, codebook, etc. • **Include basic information** on set of files such as author(s), title, grant, dates, etc.
Copy	• Make sure you have **multiple copies** • Check copies periodically

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Find a Data Repository

1. Determine if your grant requires **a particular repository**
2. If one is not required, you can choose
   a. Disciplinary repository
   b. Generalist repository

National Institute of Mental Health Data Archive (**NDA**)

NIH

- A list of NIH-supported data repositories
- Most accept data from NIH-funded projects (and others), with some exceptions

https://www.nlm.nih.gov/NIHbmic/nih_data_sharing_repositories.html

re3data.org
REGISTRY OF RESEARCH DATA REPOSITORIES

- A registry of research data repositories
- Use re3data.org to search a data repository appropriate to host your data

https://www.re3data.org/

Example Data Repositories

ICPSR — a partner in social science research

- A data repository for research in the social, behavioral sciences and public health data
- With the options of open or restricted data repositories

https://www.icpsr.umich.edu/icpsrweb/ICPSR/

- Controlled-access repository, clinical focus
- Anonymized data
- Patient consent allows for general research and commercial use
- Free for datasets under 500 GB

Vivli — CENTER FOR GLOBAL CLINICAL RESEARCH DATA

https://vivli.org/

USAID

- For USAID-funded projects

https://data.usaid.gov/

Data Sharing and Human Participants

In general, if you have human participants, you should use a **controlled-access repository** UNLESS

1) your consent language will allow for public sharing,
2) you are able to de-identify your data, and
3) the risk of disclosure is minimal. Always contact your IRB if you are not certain or have concerns.

Plan for data sharing prior to beginning your study!

Consent Language

Work with **your IRB team** to develop a consent form for data sharing. In addition to thinking about open versus controlled-access repository

- JHU SOM informed consent template
- NIH's Informed Consent for Secondary Research with Data and Biospecimens

Birdsong Project: Deposit to Open-Access JHRDR

#	Datasets (in workflow order)	Format(s)	~size	Share
1	*Audio recordings of birdsong by citizen scientists and collaborators*	.wav	10 GB	Yes (Macaulay Library, Cornell Lab of Ornithology)
2	*The Cornell Guide to Bird Sounds: United States and Canada*	.wav	2.2 GB	No (proprietary data, but can be purchased from here)
3	*Raven Pro for visualizing and analyzing birdsongs*	.exe	107 MB	No (proprietary software, can be purchased and downloaded here)
4	*Python scripts for song classification and statistical analysis*	.py	10 MB	Yes (Johns Hopkins Research Data Repository)
5	*Sound parameters*	.csv	100 MB	Yes (Johns Hopkins Research Data Repository)

Birdsong Project: Deposit to JHRDR

#	Datasets (in workflow order)	Format(s)	~size	Share
4	*Python scripts for song classification and statistical analysis*	.py	10 MB	Yes (Johns Hopkins Research Data Repository)
5	*Sound parameters*	.csv	100 MB	Yes (Johns Hopkins Research Data Repository)

Additional info/files that we would want

A **ReadMe** file to provide instruction for how to use your code and data
- Manifest of key files/folders and a short description of them
- Python version and how python files work

A **data dictionary** for the sound parameters

Summary

PLAN & DESIGN

Plan Early:

Write a Data Management Plan

COLLECT & CAPTURE

Organize & Document:
- Use filename to organize files
- Provide good documentation

MANAGE, STORE, PRESERVE

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Contact Dept IT:
- Storage
- Data backup
- Security

SHARE & PUBLISH

Share:
- Find a data repository
- Get a DOI
- De-identify PHI/PII data