MVDMLAB MANUAL

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Inspired by similar lab manuals by others. Particularly helpful was the wonderful example from the Aly lab.

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Welcome!

I didn't change my mind, it changed all by itself.

- Luna, Double Feature (1995)

THE VAN DER MEER LAB brings together people who share an interest in how the brain works. We aim to better understand how learning, memory, and decision-making arise from the coordinated activity of neurons. In the pursuit of this goal, we perform brain surgery, design and construct strange mazes, painstakingly build small devices so we can read the minds of rats, solder tiny circuit boards to even tinier wires, write thousands of lines of computer code, collect beautiful data, produce even more beautiful plots, and many other activities.

EACH OF US BRINGS their own particular blend of motivations for engaging in these lab activities, but there are a few common threads: curiosity about how the physical stuff of the brain gives rise to thought and to behavior, a love for animals and computers alike, a desire to help solve some of the big mysteries, and contributing to human knowledge and ultimately a better world.

OUR EFFORTS ARE *collaborative*, not only within the lab, but also with other labs in the department of Psychological & Brain Sciences at Dartmouth. We share equipment, space, and interests with these labs, and collaborate on joint projects and on creating a scientifically exciting and supportive culture where everyone can thrive. We also have joint projects with other labs around the world. We collaborate because the problems we are trying to solve are hard, and because learning from and working with others is one of the great joys of working in science!

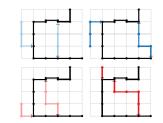


Figure 1: Some strange mazes. (From Emily Irvine's *shortcut* experiment.)

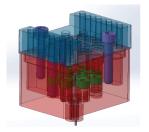


Figure 2: A small device for rat mind-reading (by Andrew Alvarenga).

Working with others brings not only joy, but also expectations. Coding and data analysis can be fun, but are full of pitfalls. Sharing space and expensive equipment with others demands respect. There is satisfaction in getting an experiment to work, but it can be a long slog to get there. Perhaps most importantly of all, the use of animals in research comes with practical as well as moral responsibilities. *This manual is intended to provide guidance on how to navigate these issues, with particular focus on the van der Meer lab.*

IF YOU ARE NEW TO THE LAB, I would like you to read this manual through in its entirety. If you will not be working with animals directly, it may seem like chapter on Animal Management does not apply to you, but it will give you important insights into how the lab operates.

Welcome. Let's do some great science together!

– MvdM

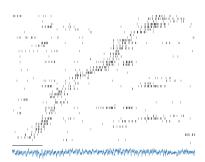


Figure 3: Some beautiful data, recorded from Ro5o's hippocampus (by Alyssa Carey). Vertical tick marks indicate spikes, blue trace shows a local field potential. Scale bar is 1 s.

About this Manual

This document describes the principles that shape how the members of the lab interact and work. Rather than dealing with the nuts and bolts of how to get stuff done in the lab, the manual is about lab philosophy, expectations, and resources. It is an introduction to the lab.

The lab manual is one of several classes (categories) of shared documents in the lab, which are:

- Lab Manual. You are looking at it. It contains information that does
 not change frequently. Only MvdM can change the lab manual,
 but he wants to hear from you if you have thoughts of suggestions!
 Periodically, we will review the manual as a group at lab meeting
 to determine what needs updating.
- Private *GitHub repositories*¹ that contain protocols, i.e. documentation for experimental procedures², and a separate repository for behavioral tasks and associated computer code³. This documentation is on GitHub so that we can say things like, "I used vesion 1.1 of the histology protocol", and so that we can track changes to those protocols as well as the reasons for those changes. If you perform procedures in the lab, you are expected to follow and to contribute to these protocols; see section XXX for a more detailed explanation.
- A *lab wiki*⁴ that contains tutorials, guides, lists of useful links, et cetera. The wiki is a more dynamic, easier to edit resource for content that doesn't rely as much on version control. The wiki currently contains the lab's MATLAB data analysis tutorials.
- We also have a lab *Slack* team⁵ which is used for day to day communication. It hosts shared documents best described as "everything else", something that isn't a protocol, task, or tutorial.

These different venues reflect an overall organization ranging from content that is easy to create and easy to change (low overhead,

- ¹ www.github.com/vandermeerlab. Because these repositories are private, you will not be able to see them unless you are logged in and a member of the vandermeerlab organization.
- ² Examples include electrode plating, histology, drive building, et cetera: mvdmlab-protocols repository
- $^{
 m 3}$ mvdmlab-tasks

4 discovery.dartmouth.edu/~mvdm/wiki

5 mvdmlab.slack.com

quick; Slack, wiki), to content that is moderately stable and takes a bit more effort to change (but easier to track; GitHub), to principles that rarely change (lab manual). For instance, an idea for an experiment may be initially discussed on Slack, lead to a draft protocol that is shared there. Before that protocol is used, it will be pushed to GitHub.

There are a number of other kinds of documents used in the lab that, unlike the above categories, are not typically consulted by multiple lab members. Most important among these are (1) animal records, described in more detail in section XXX, and (2) lab notebooks, described in section YYY.

I assume the lab manual and procedures on GitHub are accurate. This means that you should follow all of the policies and procedures contained in the manual and GitHub. If you notice something that seems to be wrong, please let me know (for the lab manual) or change it yourself (if on GitHub). If there is something in the lab manual or GitHub that you notice people aren't doing, please bring this up at lab meeting, or to me directly. Don't assume this is okay (it's not).

Research Using Animals

We use animals in the lab, based on the conviction that doing so accelerates scientific progess and provides a net benefit to humans, and perhaps to animals, too. There are many examples of scientific discoveries that were directly enabled by resarch in animals⁶. However, these successes do not mean we can experiment freely on any animal we want. The use of animals for research carries a moral, scientific, and legal obligation to only perform experiments where the benefits outweigh the costs, where no suitable alternatives are available, and to care for our animals to the fullest extent possible. These notions are often phrased as the "3 R's" (reduction, replacement, refinement)⁷.

More generally, research in animals demands that we do the best job possible, and maximize the usefulness of the research outcomes.

This means that:

- We strive to design experiments so the outcomes will be as informative as possible.
- We aim to collect the highest quality data possible.
- For the data to be interpretable, records need to be kept.
- We take care of our animals. If animals are comfortable and well cared for, they will perform the behavioral tasks we want, be easy to handle.
- Data is valuable, and should be protected.
- Data should be shared. To facilitate use by others, it needs to be annotated, and stored in a format so that others can use it.

Doing research with animals shapes not only the philosophy of the lab, but also has pervasive consequences for day-to-day work. Later sections of this manual, such as XXX and YYY, will deal in more detail with the nuts and bolts.

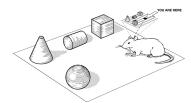


Figure 4: A cartoon rat with some objects. Figure from Dudchenko et al.

⁶ Some good examples include the "Position statement of the Max Planck Society concerning the use of animals in experiments for basic research", https://www.mpg.de/10882259/MPG_Whitepaper.pdf

⁷ Some references on the 3 Rs

In sum, the fact we are working with animals shapes both our lab philosophy and many practical aspects of working in the lab.

All of these components will be discussed on more detail in the relevant sections. This chapter is intended to provide some key definitions, references, and principles.

Animal research at Dartmouth is overseen by the IACUC.

All work with animals (specifically, all procedures) needs to receive prior approval from the IACUC in the form of *protocols*⁸.

Animal care is provided by CCMR.

At a minimum, we are required to log all procedures9.

Apart from the moral obligation to care for our animals and ensure they can contribute to science, we often invest a tremendous amount of time in each animal through behavioral training, construction of chronic implants, surgery, and so on. If any one of these procedures is not performed diligently, then your investment in all the others may come to nothing.

⁸ *Protocol*: document outlining the scientific rationale, aims, and a set of proposed experiments.

⁹ Procedure: Any action or change beyond picking an animal up in the colony room (e.g. for changing cages or weighing) counts as a procedure

Expectations

Everyone

Values: personal and scientific integrity; respect for animals, humans, equipment, data, space. Supportive and collaborative learning atmosphere that fosters personal development, curiosity, exploration, joy of discovery. For this to count, need persistence, resilience, and hard work. This is only possible if you are pursuing something you care about, and take care of yourself and those around you (so they may reciprocate). Be kind. Non-judgmental, recognize that failing often is part of science, changing your opinion is better than having no opinion.

Well-being. Work hard but be kind to yourself and others.

Share your expertise, be respectful of others' time.

Attend weekly lab meetings.

Principal Investigator

Have a vision for where the lab is going, and how to get there.

Provide an environment where great science can be done and people can thrive.

Obtain funding.

Help train you.

Graduate Students

Make an original contribution to knowledge.

Research Technicians

Undergraduates

Onboarding process.

Letters of recommendation.

Funding sources for research and travel.

Code of Conduct

Short version: be nice. Science needs contributions from everyone. There are well-documented barriers to participating that we want to do our best to help eradicate.

Some specific things that work well:

Long version: laid out in Dartmouth policies.

Lab space

Our lab space consists of the following: Include map.

General expectations

In the lab, we assemble precision devices that will be surgically implanted in live animals. We perform behavioral experiments that are sensitive to changes in many different conditions. The amount of time invested in these procedures, and the fact that we are doing this in live animals, demand careful attention. In addition, lab space is subject to general lab safety requirements (link) and IACUC inspections (link).

The principle that guides the use of shared lab space is that any lab member needs to be able to come in, find the items they need, and get things done to a high standard. In addition, the condition in which te lab spaces are kept should reflect pride taken in doing good work – it will be seen and interpreted by others viewing our space. Depending on the specific rooms this can mean somewhat different things, discussed in more detail below, but there are some common principles:

- Clean up after yourself. Use common sense about when to do this. If you're in the middle of something and stepping out to get some lunch, it makes no sense to clear your work area. However, if you've finished what you were working on, definitely clean up. Before going home for the day is usually a good time to at least clean a bit. See the specific room schedules for more specific cleaning requirements.
- Organize where things are stored. Any item in the lab has a home. Some individual items are sufficiently important that they have a dedicated place, such as "final cut scissors" (PICTURE). Some

items don't have a dedicated place but are a member of a more general category, such as "BNC interconnects". If you find an item that does not have an obvious home, check on Slack if anyone has suggestions, and/or create one for it. Creating a home for an item can be as simple as labeling a piece of a shelf somewhere. Make sure you tell everyone about it on Slack, in accordance with the Communication Principle!

- *Track stock levels*. If something is running low, don't just ignore it. Post about it on Slack. Store parts that go with a certain piece equipment with that equipment (for instance, by taping a ziploc baggie to it).
- Label things. Any liquid and food containers MUST be labeled with
 the contents and expiry date, if applicable. When you open a new
 package of something, write on it when you opened it. When a
 new thing comes in, write on it when it was received.
- Treat tools with respect. What may look like a cheap pair of scissors is likely to cost at least \$300. Use tools for their intended purpose, and use the correct tool for the job. For instance, don't use fine scissors to make rough cuts into hard materials. Don't use fine forceps to hold metal parts. 10
- *Be safe.* The general lab safety training covers the basics, but specific spaces have hazards described below. First Aid kits are available in the FAR and in B101.

Experiment rooms

AKA "running rooms"

These are the main spaces that our rats interact with. That has a few consequences:

- If you have a rat out, have the doors closed. Put a sign on the door saying, "Experiment in Progress".
- Don't make changes to the layout of the room while you are running an experiment. Changing the cues and landmarks available can change what strategy animals use to solve a behavioral task.
- Work to keep light levels, sound levels, and odor cues consistent.
- Any surface rats interact with needs to be cleanable. So, no card-board, unsealed wood, and so on.
- Our IACUC protocols require cleaning apparatus at least weekly.

10 Quick guide to forceps: #55 are finest.

Cleanliness and organization is important, but don't let that hold you back in shaping your workspace to enable you do perform your experiments well and efficiently. Tape procedures you are using to the walls, print out the relevant atlas sections, make temporary storage spaces for the tools you use frequently.

Fine Assembly Room (FAR) Surgery and anteroom If there is any single room that is especially important to be clean and organized, this is it! Workshop Beware the Dremel and the belt sander. Wear gloves and goggles. Shared facilities Histology. Vivarium space Our room is... Offices

Digital space

Dropbox, Drive, Calendar

GitHub

Slack

www.slack.com is a software platform for having group conversations in topic-specific channels. The lab Slack is mvdmlab.slack.com.



Wiki Figure 5: Slack.

Mailing list

Computing

Computing plays an increasingly important role in (neuro)science and is central to many aspects of the lab's workflow. The safety of your hard-won data, the integrity and reproducibility of your analyses and results, the ongoing development and sharing of the best protocols and procedures, and the speed with which you can accomplish your goals all depend critically on correct use of the lab's computing resources.

Because many of these are shared (experimental machines) and/or inherently collaborative (lab database, codebase) it is especially important to be aware of the issues below. Experience with some of the more advanced concepts and tools is a highly valued skill in many labs and workplaces; mastery of these will set you apart from many of your peers.

Lab computers

The lab's computers are a mixture of custom builds (by MvdM), designed for a particular role, and general-purpose workstations purchased from Dartmouth Computing Services. The different roles determine the best way to use each machine. The roles are:

- a) single-user machines in offices, one for each grad student/tech/postdoc b) shared machine in B28, for use with NanoZ and high-power scopr
- c) shared machine in B101, for use with the 3D printer d) shared experimental equipment machines: for recording in B18

Single user machines (grad students and postdocs)

One of these will be exclusively yours to use during your tenure in the lab. You are free to install software and change settings to suit your needs as long as any changes (1) do not interfere with the machine's ability to support research, and (2) fall within the Dartmouth Computing guidelines (link).

Single user machines have a boot drive (C:) for the operating system and frequently used software. This is a fast SSD drive with limited space, so be mindful of what you install here. Absolutely do not use this drive for any documents or data.

Save your work and data files on the data drive (D:), because this is automatically backed up using RAID (i.e. there are actually two "mirrored" hard disks which behave as one virtual disk). Run RaidX-pert's check utility periodically to ensure the health of the RAID array.

Shared machines

Unlike the single user machines, when using the shared machine you will need to consider how your actions affect other users. A few guidelines:

- If need to save your work, create a folder with your name on the
 Data drive. Do not leave work or data files on the desktop. If you
 find anything that's not supposed to be there on the desktop or
 anywhere else, put it in the lost-and-found folder on the desktop.
- Do not install any software, or change any software and system settings, without discussing with MvdM first. Do not delete any files, but place them in lost-and-found instead.
- If you log in to web services, make sure you log out when you are done.

Experimental machines

The above considerations for shared machines apply. In addition:

- If you acquire data for your project, use correct renaming and backup procedure when you finish your acquisition session. This is absolutely essential: data is expensive, and you individually and the lab as a whole cannot afford to lose any of it, EVER. Do not leave data in the acquisition folder.
- If you encounter a potential data file or folder that is not yours and seems lost, make an effort to find out whose data it might be.

- NEVER delete anything that looks like it might be data, unless you are absolutely certain that it has been backed up correctly.
- 2. All machines, even the single-user ones, use the mvdmlab account.
- 3. Machines are distributed across physically different locations. To facilitate communication and data sharing, we maintain a list with machine names, MAC and IP addresses, and the network port they are plugged in to. Remember to update this if anything changes.

To move data between machines, we use freeSSHd, Dropbox, and WinVNC.

4. Web services accounts

gmail: mvdmlab@gmail.com dropbox: mvdmlab@gmail.com skype: vandermeerlab (for contacting suppliers and other lab-related business)

PRINTING

Please be considerate when using the printer. Keeping an electronic library of PDFs using an iPad, Dropbox and iAnnotate is a worthwhile investment that will make it much easier to find papers and your comments in the future, and will save a LOT of paper!

The recording computers both have local printers attached for obtaining protocols and weight sheets.

- 6. If you see Windows updates to install on any machine, go ahead and install them. If a computer needs rebooting because of updates and you can do so safely, go ahead.
- 7. Use of University computers (which includes all lab computers) is governed by the Guidelines on Use of Waterloo Computing and Network Resources. These are generally common sense, and not overly long so have a look.

Skills

Growth mindset.

Basic skills: learning about your filesystem, what makes sense to store where

Learn to use the command line.

Learn to code. MATLAB and Python are the most important. In our subfield of neuroscience, MATLAB is still the most used, but Python is gaining ground.

Good coding practices.

Style guide.

Other important software: reference manager, LaTeX if you plan to write a paper or a thesis. Adobe Creative Suite for making figures.

Data management

DATA IS VALUABLE. After taking into account the number of hours that went into training an animal, building a hyperdrive, preparing for and performing surgery, painstakingly turning electrodes; animal housing costs, costs of parts and consumables, and so on, the cost of a single subject worth of data can run into tens of thousands of dollars – and this is without the moral calculation of considering an animal's life. Data therefore needs to be treated with the greatest respect: specifically, it needs to be managed so that it can be maximally useful. The *first requirement* of data management is that data needs to be available – i.e. backed up so that it can never be lost, and accessible by those who need it. Our lab is committed to Open Science, of which public data sharing is one component. Increasingly, journals and funders demand that data is made publicly available. The *second requirement* of data management is that the data needs to be organized such that it can be easily understood and used.

Before discussing how these two requirements – data storage and data organization – are implemented in the lab, it is useful to distinguish between different categories of data.

• Raw data is what gets saved on a data acquisition machine, such as a running room computer connected to a Neuralynx recording system, or a machine connected to a microscope. Depending on the data you collect, a single session may yield many different files, such as behavioral tracking data and neural recording data, or a single file (an image). Raw data is only rarely suitable for analysis beyond a few quick checks. At a minimum, freshly acquired data sets typically must be annotated, and/or the files systematically renamed – for instance, with the ID of the experimental subject and some information about recording locations – so that the analyst can select which files to analyze, and combine results across sessions and subjects. More complex pre-processing steps include spike sorting (the process of assigning spike waveforms

to putative single neurons to obtain their spike times), artefact removal, and many others.

- Promoted data is data that is ready for analysis. Data files need to be organized in a specific folder structure, named according to the naming scheme, and supplied with annotations describing the data. If applicable, various preprocessing steps may have been carried out, for instance spike-sorting of raw voltage traces into spike trains of putative single neurons, filtering of position data to remove artifacts, and definition of trials on a behavioral tasks. PDFs of handwritten notes and procedure logs, and images of histology may also be included. A promoted data set typically does not include the raw data. Promoted data should be sufficiently well described and organized such that a competent reviewer or collaborator can use it.
- InProcess data is data that is being worked on to move it from raw to Promoted.

Data storage

IMMEDIATELY AFTER DATA IS FIRST ACQUIRED, do the following:

- Create and/or rename the new data folder according to the Lab Data Naming Scheme.
- If applicable, compress any files that are large and compressible: typical examples include Neuralynx .nvt (video tracking) files¹¹.
- Upload the data to the *Incoming* folder on the lab server. See section XXX for instructions.
- Move the data out of the location where it is saved, into a folder specific to your subject or experiment.
- Following completion of data collection from that subject or experiment, verify that indeed all data is correct and present on the server, and then delete it from the data acquisition machine.¹²

The lab server uses a redundant data storage system that can tolerate failure of a single hard drive without causing loss of data. The contents of the datavault folder are periodically backed up to "cold" offsite storage. However, it is good practive to also make your own personal backup of your raw and promoted data. One good way of doing this is to store that data on your desktop workstation, or move it to an external HDD, just in case.

¹¹ See section XXX for a quick primer on data compression.

¹² This is an important step, because if there is no space available on a data acquisition machine, we cannot acquire more data!

Data promotion

What exactly is included in a promoted data set depends on the specific experiment, but the main idea is that the data should be organized systematically according to lab conventions, contain annotations in the form of ExpKeys files, contain an overall description of the data set, and include PDFs of relevant notes and histology.

Examples of nice promoted data with description include:

Alyssa's data Jimmie's data

Data use cases

Typical use cases for data include: Your own project. MvdM needs figure for a grant. New student comes in with a different question. A colleague wants to check your results.

Animal care and recordkeeping

Keeping detailed and accurate records on each of our animals is an IACUC requirement (and indirectly a federal issue), as well as scientifically important.

The main principles are that for any animal that is currently alive, there must a binder (or a section in a binder) with that animal that contains an up to date record of all procedures¹³ performed on that animal, as well as records of their weight.

Failure to log a procedure is a serious oversight that, if discovered, could have real consequences, such as creating more work for everyone, or making our IACUC renewals more painful. Don't let it happen to you.

When performing invasive procedures (surgery) and when administering drugs, CCMR requests that these are reported using a special cage card¹⁴. Writing procedures on these cards is *not* sufficient logging: you also need to log the procedure(s) in the animal's binder.

Once you euthanize and animal (or request it to be euthanized), collect all weight and procedure logs, along with any additional information (notes from behavior, for instance), scan them into a single PDF¹⁵ and upload to the data vault.

If you are working with animals, read the Animal Recordkeeping Protocol (link), which provides detailed procedures for the above.

Animal ownership and responsibility

By default, CCMR staff will feed, water, and change cages for all our animals. We pay them a *per diem* fee to do this.

By default, all animals receive ad lib¹⁶ food and water.

¹³ What counts as a procedure: anything beyond what's required to change a cage or weigh an animal. If in doubt, log it anyway.

¹⁴ Picture of procedure cage card.

¹⁵ The departmental copier/scanner is great for this, you can feed it a pile of documents and it can email you a PDF.

¹⁶ Ad libitem is the Latin phrase for, literally, "to your libido", i.e. as much as desired. Incidentally, i.e. is Latin for id est, "it is".

We can also request CCMR to feed rats 18g/day. This is a useful amount that prevents rats from getting obese. To request this, use the relevant cage $card^{17}$.

For any other food/water regimens, you'll need to use the "Experimenter will feed/water" cage card. Doing so implies that the experimenter listed takes ownership¹⁸ of that animal.

When animals first arrive, they are typically group-housed (multiple animals per cage). They are named¹⁹ and start out with communal status. Communal animals do not have an owner yet. They are weighed weekly by the Communal Animal Caretaker²⁰. When communal animals are below 400g in weight, the Caretaker also handles them weekly (about 5 minutes per animal appears to be the sweet spot, although you may want to take ownership of an animal that will be implanted with a hyperdrive early on so that it can be handled more). When communal animals reach 400g, the Caretaker separates them into individual cages so that CCMR can feed them 18g/day.

These procedures are described in more detail in the Animal Recordkeeping Protocol.

¹⁷ This one.

- ¹⁸ As the owner of an animal, you are responsible for logging its weight and all procedures. If the animal is food and/or water-restricted, you are responsible for supplying those things, and logging that you have done so.
- ¹⁹ Rats are named Rxxx, where xxx is a 3-digit counter that it incremented with each new animal. Mice are named Mxxx
- ²⁰ Note that to track weights of grouphoused animals, some kind of mark needs to be used. The lab convention is to write numbers 1, 2, 3 etc. on the base of the tail with a Sharpie. 1 indicates the animal with the lowest number. Mouse tails are too small to write numbers; stripes or rings can be used instead

PBS and Dartmouth

Training required by Dartmouth

General lab safety training

Important people and contact info

PBS staff

CCMR

Dartmouth resources

Dick's House

Graduate School

Postdoc Association

Workshops

Dartmouth design language and files

Beyond the lab

Resources on the internet
Industry vs. academia
Upper Valley

Appendix: Subject email format template

Subject line should read:

Appendix: Yearly evaluation forms

Graduate students

Staff

Bibliography

Eichenbaum, H., Dudchenko, P., Wood, E., Shapiro, M., and Tanila, H. (1999). The hippocampus, memory, and place cells: Is it spatial memory or a memory space?