Facultad de Ciencias, Enero 2019/Laboratorio de Neufisiología, Farmacología y Conducta. Investigador: Marco Atzori

El siguiente documento contiene normas de conducta para el uso de las instalaciones y del equipo en el LNFyC.

Es de fundamental importancia conocer y respetar tales reglas para optimizar el uso del equipo y para minimizar el riesgo de daños físico al usuario y al equipo utilizado.

Por favor, antes que cerrar el laboratorio de electrofisiología, después de cada sesión experimental es necesario verificar la siguiente checklist:

- **Verificar que los tanques de gas (oxígeno y CO2) estén cerrados**
- **Verificar que todas las salidas de oxigeno utilizadas por el usuario estén cerradas (salidas del setup, salida de las rebanadas, otras salidas utilizadas**
- **Limpiar todos los recipientes usados**
- **Tirar las rebanadas sobrantes y limpiar los vasos de precipitado de la incubación**
- **Verificar que TODOS los aparatos eléctricos estén apagados. La permanencia de aparatos eléctricos sin apagar es causa de malfuncionamiento y a veces de desarreglo permanente de tales aparatos.**
- **Quitar los capilares no usados de los headstages (electrodos de registro) como de los holders de estimulación, especialmente si se rompen**
- **Lavar el objetivo con ácido (0.05-0.10 M HCl) y luego con agua destilada.**
- **Lavar los ductos de la perfusión como las jeringas para las drogas con ácido (0.05-0.10 M HCl) y luego con agua destilada.**
- **Reportar el malfuncionamiento y el desarreglo de cualquier aparato que tenga problemas.**

Todo el personal de laboratorio que trabaje con animales tiene que tomar la vacuna contra el TETANO. La vacuna es ofrecida gratis en varias instalaciones.

Al igual que cada avance en los respectivos proyectos son logros individuales como de todo el laboratorio, de la misma manera es de suma importancia que cada usuario se haga responsable del uso del equipo, y deje el equipo en condiciones impecables. Cada falta de un usuario que deja algo sin funcionar o en mal estado es una pérdida de tiempo para el usuario que le sigue en el calendario. Por respeto a sus propios compañeros, por favor, limpien y dejen en orden el sistema y todo lo que usan del laboratorio.

En caso de dudas sobre el funcionamiento del equipo, o de no saber procedimientos a utilizarse, por favor, siempre consultarse con el investigador, o preguntar a los compañeros del laboratorio que ya conozcan tales procedimientos.

Es responsablilidad de cada uno de los miembros del laboratorio que desarrollen proyectos registrar los datos que adquieran, de tal manera que DEBEN hacer por lo menos 3 copias de respaldo de sus datos y estos deben estar actualizados por lo menos cada semana para evitar pérdida de datos ya que pueda impactar de manera negativa en su proyecto.

Abajo vienen unas reglas de seguridad (en inglés) que es buena norma respetar, y también unos tips para avanzar más rápidamente con las mediciones de patch-clamp. Les tomara' entre 30 y 60 minutos leer las instrucciones y normas abajo. Les pido por favor, que se tomen su tiempo para leerlas, porque así se podrán ahorrar mucho más tiempo en la ejecución de sus proyectos. Con gusto les traduciré el contenido si es necesario. Enfóquense especialmente en la segunda parte, la parte específica para el laboratorio.

Site Specific Safety and Training Rules

The Laboratory Safety Standard further requires that the employee's supervisor provide training, which covers the specific topics described in the checklist below. This training must be provided at the time of the student or employee's initial assignment, on a refresher basis and upon updating procedures. A current copy of this signed form must be on file for each laboratory student or worker with Environmental Health & Safety.

Requirements for Site-Specific Training: Each Principal Investigator/Laboratory Supervisor should conduct laboratory-specific training. This checklist may be used to assist employers with the laboratory-specific training requirements. Students and employees operating in the laboratory are required to 1) review the following material safety information, 2) know materials and equipment stored or dealt with in the laboratory, procedures for related experimental protocols, and 3) take of necessary precautions to avoid any risks associated with operating such materials, protocols and equipment:

- □ **Safety Handbooks, Material Safety Data Sheet (MSDS) , Laboratory-Specific Standard Operating Procedures**
  - □ Review contents of Chemical Safety Handbook
  - □ Review procedures for how to access MSDS
  - □ Review laboratory-specific procedures, equipment in use
- □ **Chemical Inventory**
  - □ Review chemicals used and define hazards
  - □ Safe handling, locations for storage and segregation of hazardous chemicals, container labeling
- □ **Compressed Gas Cylinders / Liquid Nitrogen**
  - □ Inspection, storage, labeling, and handling of cylinders
- □ **Emergency Information**
  - □ Review location of fire extinguishers, fire alarm pull stations, safety showers, eyewash stations, first aid supplies
  - □ Review Laboratory-Specific Evacuation Plan – alarm activation, building evacuation & re-entry procedures, equipment shutdown procedures, and special consideration for non-fire hazards (poisons, corrosives, irritants, radioactive materials, biological hazards)
- □ **Chemical Waste Management Procedures**
  - □ Selection of Containers, Labeling, Secondary Containment
  - □ Laboratory's Storage Location for Hazardous Waste
  - □ How to Request a Chemical Pick-Up
- □ **Chemical Purchasing/Procurement**
  - □ Review UTHSCSA Procurement Card Policy

- ☐ Laboratory's Procedures for Ordering Chemicals
- ☐ **Annual Laboratory Safety Evaluations**
  - ☐ Review Results of Most Recent Lab Safety Evaluations and Corrective Actions
- ☐ **Laboratory Hygiene & Personal Protective Equipment**
  - ☐ Personal Protective Equipment (PPE) Requirements for the Lab
  - ☐ Laboratory-Specific Housekeeping Procedures
- ☐ **Environmental Monitoring & Safety Equipment**
  - ☐ Review Use of Specific Chemicals That Would Warrant Chemical Exposure Monitoring
  - ☐ How to Request Monitoring from Environmental Health & Safety
  - ☐ Review Laboratory Directional Airflow, Chemical Fume Hoods, Biological Safety Cabinets
- ☐ **Medical Surveillance**
  - ☐ Review Laboratory-Specific Requirements - Hepatitis B Virus Vaccine, TB Skin Test, Baseline Serum Sampling, Respirator Use & Fit Test
- ☐ **Chemical Spills and Emergency Response**
  - ☐ Review Laboratory-Specific Spill Response, Decontamination, Procedure for Reporting Injuries & Illnesses
  - ☐ Review Recent Spills/Accidents/Injuries and Discuss Prevention Measures

The information contained in this Safety Module is meant to minimize the risk associated with operating in the laboratory. Non compliance with Safety Rules will be reported and enforced according with federal and state laws and by-laws. Although compliance with the rules described here is required, it is by no means warrantee of safety. Please, be always alert and use common sense when using any laboratory equipment, materials, and procedures. Refer to senior laboratory personnel, or to the principal investigator (Dr. Atzori, personal tel. 444 1739 256), whenever you have any doubt concerning how to operate in the laboratory.

Site specific recommendations

Laboratory of Neurophysiology, Pharmacology and Behavior, LNPB

This is a list of rules, recommendations and suggestions for the activity in the Laboratory of Cell and Synaptic Physiology. Whether you are a visiting professor, a post-doctoral fellow, a graduate or undergraduate student or just a visitor collaborating with the laboratory, you are probably extremely busy. Still, it is important that you take your time to read all the safety related paragraphs, and all the other paragraphs relevant for your projects, and return one signed copy to the PI. I would recommend that you to read the document throughout.

Activity

The laboratory works on cellular and synaptic properties of cortical and subcortical circuits with focus on the neocortex. We use rodent animal models with a combination of electrophysiological techniques (patch-clamp on brain slices and in-vivo recording), neuroanatomy and behavior. Your contribution is important to the laboratory and, for the best yield out of your activity, it is important to observe a number of precautions. Most of the things indicated are just common sense, some other are meant to direct newcomers in the laboratory activity. All suggestions are given for avoiding actual laboratory problems previously occurred elsewhere.

General

Listening to radio or music is allowed if all the persons inside the lab explicitly agree. Loud radio or music is not allowed in any way. The space in the lab is limited, so that people will have to work in strict physical contact with each other: please, take care of your personal hygiene. Please, limit the use of cell phones to the minimum possible, and in case of long calls, quit the lab space: your colleagues need not to witness your calls. Food and drinks are generally forbidden outside the student room, particularly when doing any experimental activity. Web-surfing and use of personal e-mail and is allowed, but should be limited to the minimal indispensable. It is absolutely forbidden to download music files and access adult web sites or use any UTD resources for illicit purposes. Please keep clean and ordered the working spaces. Thanks for helping to keep a healthy working environment!

Rules for Specific activities

General activity, safety, and procedures

The laboratory conducts experimental research on the role of neurotransmitters in the modulation of brain activity. Currently, a main line of our activity is the identification of cortical circuit activity is reversibly changed by the presence of neuromodulators. The effects of endogenous and exogenous chemicals are tested with behavioral and electrophysiological techniques in vitro and in-vivo. Main focus of the lab are the effects of Cholinergics, Monoamines and Cytokines.

The laboratory conducts experiments using multiple techniques and expertise. The nature of the investigation is such that many people may happen to interact, whether for collaborating on a common project, or for using shared equipment, tools, or just the being in the same facility for an any length of time. If you are reading this sheet you are probably a:

- Guest PI
- Post-doctoral fellow
- PhD, doctoral student
- Master student
- Undergraduate student
- Technician

Regardless of your role in the lab, and of the duration of your stay, please, read and respect the following rules:

General

- Have a clear scientific plan for your activity (it could be from simply prepare a project using the informatics resources, or to start and finish a series of targeted experiments for publication)
- Clean after yourself: you want to find an ordered environment. The only way to keep the environment tidy is if everyone cleans and reset all the used tools, and equipment (including glassware and capillars for electrode backfilling) clean and usable. In some cases not cleaning provokes rapid deterioration of equipment causing major monetary and time losses.
- Care about personal hygiene: you are working close to many other colleagues for many hours, remember that the nose is connected to the amygdala, a main emotional center. Refrain from talking loudly, it just irritates whoever is not interested, plus, some in-vivo experiments consist in the presentation of auditory stimuli: loud noises or voices can interfere with the experiment
- Most experiments in the lab require mechanical stability: do not touch recording setups by any means unless you are conducting the experiment. Do not produce unnecessary mechanical vibrations (no door or objects slamming)

- If lights are turned off there is a reason (typically for the use of light sensitive drugs: ask before turning lights on.
- If you are using oxygen or other gases, make sure that there are no leakages and that you close the supply before leaving.
- Turn off all electrical devices (including PCs) when you are done with the experiment.
- Actively participate to weekly lab meeting for discussing project advancements. It is the only opportunity happening on a regular basis for discussing also how to fix project-related problems.

Safety: General Guidelines

Safety in the lab is the number-one priority for students, technicians, and researchers. To ensure laboratory safety, we have developed the set of regulations contained in this document. Every prospective lab member receives a copies of this document before joining the lab and is required to read and understand its contents. By signing and returning the last page, you affirm that you have read and understood this safety document. This document is to be kept in your notebook for reference.

Proper laboratory technique is essential to the education of successful scientist. Your success will depend on your attitude and conduct. An interest in your work, an understanding of its purpose and a clear interpretation of your results are essential to your success. The laboratory is a safe place to experiment if you are careful. You must assume responsibility not only for your own safety, but also other lab (team) members.

- All precautions need to be taken to avoid to injure, or otherwise induce short- or long-term physical damage to yourselves or anybody else, inside or outside the lab. If you have any doubt about the risks involved in using any laboratory substance or procedure, ask the PI.
- Whenever you use reagents, salts, or drugs, be aware of what are the dangers associated with the substance used. Some substances have potential for chemical damage to you and/or the environment, some other are biological toxins. **Use gloves** before using any potentially dangerous substance. **Dispose of the left over as required by the specific rules for the substance**. If you have any doubt, please ask the PI or other members of the lab who are acquainted with the use of the substance.
- **Sharp and needles need to be collected in a special container**. Do not leave them around after use, and make sure that even during use their presence is clearly identifiable, to avoid any possible risk of injury.
- **All substances need to be stored in their appropriate location** (safe boxes, 4C refrigerator, -20C refrigerator, -80C freezer, dessicator, or chemical storage shelves, trying to keep the alphabetical order).
- **Animals must be used according to UTD and state rules.** All animal users need to present proof of a tetanus shot to the UASLP and watch the animal training movies.

Animal anesthesia, sacrifice, must be performed using appropriate spaces and tools.

Make sure that you:

Conduct yourself in a responsible manner at all times in the laboratory.

Plan your project in detail before you start experiments. Compute required amounts of reagents before you begin and familiarize yourself with the Material Safety Data Sheet (MSDS) for each reagent you plan to

use. Follow all written and verbal instructions (from senior lab personnel) carefully. If you do not understand the directions or part of a protocol, ask the lab member in charge before proceeding.

If there are steps that require unusual safety precautions, avoid conducting these at times when you are alone in the lab.

Do not eat food, drink beverages, except in the dedicated areas, or chew gum in the laboratory. Do not use laboratory glass- or plasticware as containers for food or beverages. Also, do not leave empty beverage cans, bottles, food wrappers, or food-service items in the lab.

Safety glasses or goggles are required in the laboratory; lab coats are recommended and aprons are occasionally required for some protocols (e.g., handling hot acids). Appropriate hand coverings (e.g., latex or insulated gloves) should be worn in accordance with standard procedures. Avoid wearing expensive clothing to the lab and keep exposed skin to a minimum.

Observe good housekeeping practices. Work areas should be kept clean and tidy at all times. Bring only your protocol instructions, worksheets, and/or reports to the lab bench. Other materials (books, purses, backpacks, etc.) should be left on desks or writing areas.

Know the locations and operating procedures of all safety equipment including first aid kits, eyewash stations, safety showers, spill kits, fire extinguishers, and fire blanket. Know where the fire alarm and lab exits are located.

Be alert and proceed with caution at all times in the laboratory. Use of iPods or similar personal audio devices is strongly discouraged. Notify senior research personnel immediately of any unsafe conditions.

Dispose of all waste materials properly. Solid chemicals, electrophoresis gels, metals, matches, filter paper, and other insoluble materials must be disposed of in proper waste containers, not in the sink.

Be familiar with appropriate disposal procedures for hazardous reagents. Check the label of a waste container twice before adding your chemical waste to the container. Update chemical waste logs.

Razor blades, hypodermic needles, and other sharp objects must be disposed of in approved “sharps” containers. Cracked or broken glass should be placed in the special container for “Broken Glass.”

Labels and equipment instructions must be read carefully before use. Set up and use the prescribed apparatus as directed in the laboratory instructions provided by senior lab personnel.

Keep hands away from your face, eyes, mouth, and body while using chemicals. Wash your hands with soap and water after working at the bench. Clean all work surfaces and equipment at the end of your experiment.

Experiments must be personally monitored at all times. You will be assigned a laboratory station at which to work. Do not distract others or interfere with their laboratory experiments.

Know what to do if there is a fire drill while working in the laboratory. In the event of fire, close reagent containers, turn off gas valves, electrical equipment, and fume hoods, if instructed.

If you spill acid, strong alkali, or any other corrosive chemical on your skin or clothes immediately wash the area with large amounts of water (remember that small amounts of water may be worse that no water at all). After decontaminating yourself and your clothing, bring the incident to the attention of senior personnel. Use a spill kit to decontaminate floor or counter-top areas.

Pooling water is a common hazard (e.g., freezer or refrigerator condensate). Mop up any standing water on the floor of the lab or in the vicinity of the lab.

If you are the last person in the lab at the end of the day, ensure that: a) gas outlet valves are shut off; b) water faucets are off; c) your desk and bench top are clean; d) unused equipment and light is off.

Safety: Specific Rules

The laboratory hosts a number of potentially dangerous substances and pieces of equipment. Following a few precautions will avoid any foreseeable problems:

Chemicals: again, always use gloves when handling any chemical substance, salt, toxin or enzyme. Never ingest, contact with skin or eyes or breathe any of the chemicals in the laboratory. Most substances are not dangerous but some might be highly toxic or carcinogenic. Many drugs have not been fully tested and might be toxic in unexpected ways, especially if contacted directly or by inhalation.

Instruments: always handle with care sharp objects and blades. Never be in a rush when handling sharp objects, blades or glassware. Electrical equipment should always be kept far from moist or water. Report any problems to your supervisor. In case of emergency for intoxication, wound, or electrical shock, call 9-1-1, or bring the injured person to the Emergency Room of the closest hospital (Hospital Lomas, Palmira 600, 78218 San Luis, S.L.P., u Hospital del Parque, Avenida Chapultepec).

Personal Safety, Accidents, and Injuries

Do not divulge personal information (home addresses, personal phone numbers, etc.) for any member of the group. Also, do not disclose a group member's whereabouts to any unfamiliar persons. Clear any lab visitors with the PI. Suspicious individuals (loiterers, etc.) should be reported to UTD police immediately. If you are working alone in the lab after hours, close and lock the front door. Keep a cell phone or the lab phone handy to call UTD police in an emergency. Report any accident (spill, breakage, etc.) or injury (cut, burn, etc.) to senior lab personnel immediately, no matter how trivial it may appear.

If you or any lab member are hurt, immediately alert senior research personnel. Everyone should turn off burners and prepare to help if needed. Reagents in the eye(s) should be treated by washing with running water at an eyewash station for at least 20 minutes. Notify senior lab personnel immediately.

Handling Laboratory Chemicals

All chemicals in the laboratory are to be considered dangerous. Do not touch or taste any reagent. Intentional exposure to chemical fumes is discouraged unless advised by senior lab personnel. The proper technique for investigating chemical fumes (when instructed to do so by senior lab personnel) is to gently fan the air above the reagent toward your nostrils. Breathe normally. Double-check the label on chemical bottles before removing any of the contents. Tap out dry reagents onto weighing boats or weighing paper. Use a clean spatula to remove and discard excess reagent. Measure liquid reagents using a graduated cylinder (usually TD type). Never return unused chemicals to their original containers. Avoid inserting spatulas and pipets into stock reagent bottles. Follow proper procedures when using toploading and analytical balances (consult the respective manuals). Clean the balance of any chemical residue after use. If you are not familiar with balance maintenance procedures, see senior lab personnel for instructions before using the balances. Never use mouth suction to fill a pipet. Use a pipette bulb or pipet filler. Acids must be handled with extreme care. ALWAYS ADD ACID SLOWLY TO WATER, with slow stirring and swirling, being aware of the heat produced. Extreme heat generated by acid dissociation can cause solutions to boil, (e.g., with sulfuric acid.). Handle flammable hazardous liquids over a pan to contain spills. Never dispense flammable liquids anywhere near an open flame or source of heat.

Never take chemicals or other materials from the laboratory area except using approved transport methods. Exercise care when transferring acids and other chemicals from one part of the laboratory to another. Grip bottles securely or use an approved carrier.

Handling Glassware and Equipment

Inserting and removing glass tubing from rubber stoppers can be dangerous. Always lubricate glassware (tubing, thistle tubes, thermometers, etc.) before attempting insertion into a stopper. Protect your hands with towels or cotton gloves when inserting glass tubing into, or removing it from, a rubber stopper. If a piece of glassware becomes "frozen" in a stopper, take it to senior lab personnel for removal. When removing an electrical plug from its socket, grasp the plug, not the electrical cord. Hands must be completely dry before touching an electrical switch, plug, or outlet. Examine glassware before each use. Never use diry, chipped, or cracked glassware. Do not immerse hot glassware in cold water; it may shatter (even Pyrex). Report damaged electrical equipment immediately. Look for things such as frayed cords, exposed wires, and loose connections. Do not use damaged electrical equipment.

If you do not understand how to use a piece of equipment, ask senior lab personnel for help.

HANDLING FLAMES AND HEAT SOURCES

SHOULD THE BUNSEN BURNER GO OUT, IMMEDIATELY TURN OFF THE GAS AT THE GAS OUTLET VALVE. If you wish to turn off the burner, do so by turning off the gas at the gas outlet valve first, then close the needle valve and barrel. Never reach over an exposed flame. Never leave a lit burner unattended. Never leave anything that is being heated or is visibly reacting unattended. Always turn the burner or hot plate off when not in use. You will be instructed in the proper method of heating and boiling liquids in laboratory glassware. Do not point the open end of a test tube being heated at yourself or anyone else.
Heated metals, glass, and ceramics remain very hot for a long time. They should be set aside to cool on a trivet and then picked up with caution. Use tongs or heat-protective gloves if necessary. Determine if an object is hot by bringing the back of your hand close to it prior to grasping it.

Locate the closest laboratory shower, in case you need to use it after ignition of clothes, chemical spill over any part of your body, or any other safety need.

Planning experiments

The vast majority of difficulties can be avoided through careful planning of an experiment. Always consider all necessary controls before starting experiments. Without proper controls, the data obtained may be of no use, because they are impossible to interpret. If you have any doubts, questions or problems do not hesitate to ask senior laboratory personnel. It will help you to save time and sometimes it will help the lab to conserve other resources (reagents, glass- and plasticware, and other consumables).

Training

Whether you are an experienced research or an undergraduate student or anything in between, you will are most likely carrying out a collaboration, and you have to interact strongly with a number of people. The laboratory is NOT a democracy: there is a hierarchy to follow in making decisions. Bearing this in mind, it is essential that all voices are heard before making important decisions, and we want to always maximize the well being of everyone, using simple behavioral rules, respecting everyone's activity.
If you are a student, you will probably have to learn one or more techniques from a person more senior than you in the lab. S**eniority does NOT involve age or academic rank, but only experience matured in the specific activity carried out** (for example a PhD or master student might have to learn and use a technique from an undergraduate student, this brings that particular undergraduate above him/her in the lab hierarchy. Teaching is an obligation associated with knowledge. The person in the upper hierarchical level is literally giving away his/her time, which could have been used to do another experiment, finishing a project, or for other academic or personal use. The person who learns owes to his/her teacher, and for this reason, whenever sharing resources, the teacher needs to be consulted prior to using sharing resources (animals, electrophysiology setups, slicers, or any other shared laboratory space or tool). The hierarchy relationship student-teacher is not absolute: the use of different techniques or expertise might occasionally involve an inversion of the parts (an electrophysiologist might teach his discipline to an anatomist and viceversa).
Because of the continuous need to exchange information (and not because you all are good persons) it is of paramount importance that everyone respects hierarchies, and everyone who is teaching them.

Collaborations and authorships

It is very rare that scientific project in biology are carried out by one person only. For this reason is important to understand a series of authorship rules. Scientific authorship does not obey simple

mathematic rules and varies from field to field. It is currently tacitly accepted that all authors of a publication should be acquainted with the results and experimental details of the study, but only the first, and the corresponding author (which is usually the last) receive 100% of credit, unless otherwise specified. This rule ensures that every project has a main responsible who is in charge of the scientific execution (experiment planning analysis and realization, but is not necessarily involved in administrative and financial handling of the laboratory), and another person (typically the PI) who is in charge of the initiation of the project and of the administrative, legal, and financial aspects of the study. Other authors typically contribute with additional techniques (experimental or analytical), and/or with substantial intellectual contribution (suggest important experiments and or data interpretation). Authorship is the most important recognition of the work done, and it depends on many factors, some of them objective, some others more difficult to measure. For this reason authorship is the largest cause of serious friction between laboratory members. In order to avoid unnecessary distastes it is important to have a main responsible for every specific project, who will be the first author of the associated final manuscript. A person who is starting his/her activity be assigned one mentor/teacher and will be acknowledged in the work as co-author. Only when the person/student will be fully independent he/she will be eventually assigned a new project of which he/she will possibly be responsible.

Different objectives for lab members.

While the current research system has numerous limitations, we all have to accept to work with the constraints of the current model. This implies the acceptance of different levels of involvement of lab members in the laboratory activity: some undergraduate students only want to receive a short exposure to research for complying with the course requirements, some others want to learn a technique for professional development, pre-med student want to increase the probability to access med school by having an authorship in a publication, colleagues investigators have an interest in finishing a project and publishing the results for furthering their career. It is therefore natural that each person's scientific retribution will vary according to their commitment and capability. Please, understand this well, whatever the expectations from your work in the lab (if you are a PhD student working for your dissertation do not expect that every undergraduate is a genius devoting all his free time to carry out your crazy experiment, and if you are the undergraduate spending just a couple of afternoons on a project that does not advance do not expect to get much recognition of your work).

In spite of all the limitations, the laboratory intends to offer a service of quality to everyone willing to focus on an interesting project in our specific field, and likewise willing to dedicate his/her time and mental energy to it. The more time, dedication, and care you put in your project, the more you increase the probability of starting and completing a significant piece of work. I would estimate the typical payoff for a project in about 6-months to 2 year time for completing one significant project for a team of 2-5 people, from the start of the project to the production of a submittable manuscript. Large variations are typically due to partial time and lags in project continuation following interruption. In case of controversy, it is in everyone's interest that any issue is recognized and discussed among the interested persons, before the PI is involved. In no agreement is reached, the PI will make a decision on the issue.

Recommendations for patch-clampers

For those who are starting to use patch clamp electrophysiology, or even for those who use it routinely, I write here a check list that I would hope it is useful to fix bugs during working:

Symptom: no cells alive in the preparation

Possible reasons:

- Mistake in the preparation of the slice

- Extracellular solution wrong

Solution:

- Make sure that brain has not been compressed by skull bone or scissors or any surgical tool during removal from skull
- Make sure brain did not dry while gluing it to chamber
- Check for wrong vibrations of cutting chamber (separate recording from surrounding table)
- Avoid ice blocks contact with brain
- Be gentle with slice removal and transport to incubation/perfusion chambers
- Check pH and osmolarity of external solution or just re-do it.

Symptom:

Cells look good but sealing is difficult:

Possible cause

It could still be any of the above, in addition, it could be

- Flow of external solution too slow or too fast
- Intracellular solution is bad (pH, osmolarity, or just composition)
- Wrong electrodes shape and/or resistance
- Mechanical vibration of electrode during sealing
- The volume between external tubing and electrode leaks air (pressure loss) (check pressure)

Electrical noise in the recording

- Ground electrode defective (check soldering between pellet and wire, re-do if necessary)
- External devices not properly grounded (ground them properly)
- Chlorinate conducting silver wire, ground pellet, silver wire to perfusion inlet and outlet
- Screen electrical stimulation cable(s) to the recording chamber
- Make sure that signal power supply come from clean (orange) line and PC and monitor from the other line

There are sometimes unforeseeable causes inducing hassles. Once a laboratory had dead slices until all the otherwise new oxygen tubings were substituted. It turned out that the oxygen was reacting with a chemical in the plastic line which was intoxicating and killing the slices in the incubation chamber. This is just for keeping you alert and aware of unforeseeable events that can hurdle you progress.

I have limited if any experience in behavior and in vivo recording. I would appreciate if the personnel working with these techniques would make a similar list of possible glitches that can simplify debugging.

Behavior

Behavioral setups can have problems with: Hardware, Software, Miscellaneous

In vivo recording: there can be problems with: Sound generation, Preparation (problems in anesthesia, surgery), Electrical noise, Electrical grounding, (sources of noise)

Please, all patch-clamper, behavioral, and in-vivo recording laboratory people are invited to get me an up-dated list adding possible common or rare causes of mistakes and bugs in the experiments.

Other techniques are also occasionally performed in the lab (immunohistochemistry, anatomy, specific in-vivo protocols like deafness-induction, foot-shock, intraventricular injections. Please, refer to your instructor(s) or discuss it with the PI for gathering specific details on how to best carry out your experiments.