How to write a protocol – Master Neuroscience

1. Title page

Title of the module, title of the practical, student name, tutor name

2. Introduction

Short (1-2 pages) introduction of the topic and the main question asked in the practical course. Create a logical structure that leads to the question and justifies the performed experiments. Come up with the theoretical background. Describe what is known, relevant and necessary to understand the question asked. Leave out unnecessary information. Guide the reader logically to the question. Make sure that the information you give leads the reader to the question. Names and terms that are needed later should be abbreviated here the first time. Examples: Interaural Time Delay (ITD), Superficial Neuromast (SN). At the end of the Introduction the question asked should be phrased explicitly.

3. Material and Methods

Presentation of **all** methods and materials used. This part must be written such that anybody can repeat the experiments.

Organize the Methods part in chapters. The easiest way of doing this is to use a chronological sequence, i.e. write in the same order as you proceeded during an experiment: 1. Animal handling 2. Stimulation 3. Calibration 4. Data recording 5. Data analysis 6. Statistics 7. Histology etc. depending on what you did.

You need to keep a laboratory manual into which you note carefully every single detail, e.g., time of day, measures, volumes, incubation times, weights, temperature, concentrations of drugs applied, concentration of buffers and salts, pH etc.

All stimulus parameters need to be explained, e.g., amplitudes, durations, frequencies used. The use of decibel (dB) requires an appropriate reference (e.g., dB re: 1 µPa). Similarly, all parameters analysed need to be explained and it must be defined how they were determined, e.g., response rate in AP/s, mean and standard deviation etc. Please be extremely precise with every description.

Figures are very helpful in the Methods section and mandatory in the protocol. For instance, a schematic diagram of the experimental set-up, a wiring diagram of the stimulation and recording equipment, calibration curves, tables explaining the experimental protocol or experimental groups.
Important note: even though it sounds awkward, often the Methods part is written in passive.
Example: „The animal was treated ...“ or: „The data were analysed ...“

4. Results

Present **all** results! Do not leave anything out without proper reason. Give the number of all elements (animals, slices, cells, gels etc.) encountered, even if it was only once or for a short time. Give the number of analysed elements, the number of elements responding to your stimulus, the number of elements that did not, and so on. Be complete. Do not ever lie or come up with false numbers or faked data.

Note: To give these numbers you need to have a comprehensive laboratory manual into which you note every single detail (e.g., single cell nr. 5 recorded at depth 200 µm from 2 p.m. – 2:30 p.m., recorded responses to amplitudes 10, 10, and 30, data in file nr. 1, 2 and 3).

In the Results section, the original data must be reduced in an appropriate manner and the way of **data reduction needs to be intrinsically clear** from the data presentation. For example, show one or two original recordings, then the raster plots and peri stimulus time histograms and then derived values like spike rate or vector strength, and finally data from all cells in one graph.

The results must reproduce the findings. As a general rule one can say that whenever you present something new, you have to show a new figure.

The Results need to structure in appropriate chapters, going along with the Methods section. This also helps you with the data reduction. For instance, first describe the general properties of the recorded neurons, like spontaneous rates and temporal response patterns, then the responses under certain condition, e.g., to different amplitudes or frequencies.

Data need to be shown in appropriate figures and/or tables. These are supposed to be simple, well arranged and therefore easy to grasp. Important: watch for redundancies. Do not show things twice. What you show in a figure does not need to be shown again in a table and vice versa. Use the most appropriate and most intuitive way of showing your data. In most cases a figure is better than a long table.

Very important: The Results consists of text **and** figures. Do not write text alone. Do not ever present figures without text. This means: there is a text body in which the reader is guided through the results and, wherever needed, there is a reference to the respective figure. To write a good Results section, make a plan or an outline of the results structure, then generate the figure and only then write the text "around" the figure. When you write text, tell the reader about the results and refer to a figure. Do not tell the reader what is plotted. Tell them the **result**. To refer to a figure, you need to give the figure number in parentheses. Example: „All cells recorded in the Optic tectum responded to the visual stimulus with an increase in discharge rate (Fig. 1)." Bad example: „The data from the Optic Tectum are shown in Figure 1."

In the Results, only findings are presented. It is written without any evaluation, interpretation or speculation. These things belong into the Discussion. This means that your data must be described quantitatively. Do not use terms like „large", „small", „circa", „somewhat smaller", „sometimes", etc. Use your measures. For example: „The data sample consisted of 25 units. The average firing rate was 10 spikes/s." If some data sets were different (bigger, smaller) than others, then you have to perform statistical analysis with appropriate tests. Report p-values in these cases.

Always write in past tense. You present data that you **obtained** in your experiments. Use of present tense generalizes your data.

5. Discussion

At the beginning of the discussion, the main results may be briefly summarized again but there is no obligation to do so. In the following, the data should be discussed under various perspectives. Often people write a discussion about the mistakes that they made, the problems with the experimental set-up etc. Even though this is important, it gives the impression that you did not plan your

experiments properly because then you would have avoided these mistakes. A good discussion goes beyond this aspect and asks questions like: Why are the results the way they are? How can the data be explained? What do the data mean? What conclusions can be drawn from the data? In any case, the results must be integrated into the existing context. This might be textbook knowledge or scientific literature. Compare your results with previous data obtained in the same or in a different context, within the same or another species, in the same or other brain structures, or with different methods. In order to be able to integrate the results into textbook knowledge or literature, one must of course have read and understood them beforehand. As in the Introduction, literature must be cited in the Discussion just the same way. The Discussion is the place where the results are put into a larger framework or concept. What do the results actually mean, for instance with respect to the sensory system studied, to possible processing mechanisms, to the ecology and behaviour of the studied species? Here is space for speculations, hypotheses, predictions and suggestions for follow-up studies based on one's own results.

6. References

In this chapter, you must give a complete list of **all** references used in your protocol. Please follow the guidelines for the Journal of Comparative Physiology A that you find easily in the internet.

Examples:
1. Original articles:
Bleckmann H, Mogdans J (1999) Peripheral lateral line responses to amplitude-modulated sinusoidal wave stimuli. J Comp Physiol A 185: 173-180 2. Book chapters and reviews:
Bleckmann H, Tittel G, Blübaum-Gronau E (1989) The lateral line system of surface-feeding fish: anatomy, physiology and behavior. In: Coombs S et al. (eds) The Mechanosensory Lateral Line. Neurobiology and Evolution. Springer, Berlin Heidelberg New York, pp. 501-526

Important: A citation that was made in the text must be listed in the reference list **and** everything that is listed must be referenced in the text. Always cross check text and reference list.

7. Figures

Figures must be clear even without the text body. Figures should be simple, clear and well arranged. Graphs must have a proper scaling and labelling of the axes that must not be too small (at least 2mm in the print). Too many subfigures (A, B, C, D etc.) should be avoided if possible.
Each figure must be referred to at the appropriate location in the text so that the relation between written text and figure is clear. Each figure must have a figure legend (figure caption).
Figures must be numbered consecutively from the first (Fig. 1) to the last (Fig. X) independent of where in the text it will occur. Do not use different types of numberings, for instance Fig. 1 for a figure and Photo 1 for a photograph. A photograph is also a figure. Tables are treated the same way as figures, i.e., they are also numbered consecutively from Tab 1 to Tab X.

8. Figure legends

The figures should be included in the text body of the protocol. The figure legend must be given below the respective figure and must have a letter size that is smaller than the size for the main text body. Each legend starts with the number of the figure (e.g., Figure 1) according to the numbering in the text. Then follows a sentence that should read like a title of the figure similar to the title of a book chapter that tells the reader what this figure is about. Then follows an explanation of what is actually shown in the figure and an explanation of symbols (e.g., o, Δ, Ω, etc.), bars, lines etc.

Examples of figures with legends

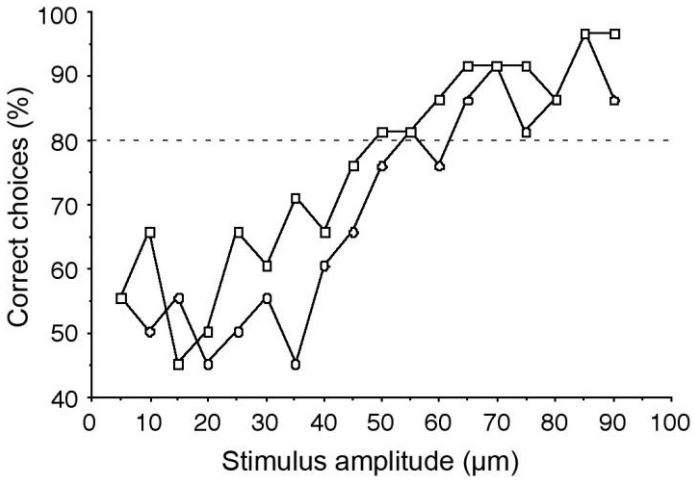

Fig. 1: Detection of a sinusoidally vibrating sphere by goldfish. Percent correct responses from two individuals (□, □) are plotted as function of stimulus amplitude. The horizontal dotted line represents the learning threshold.

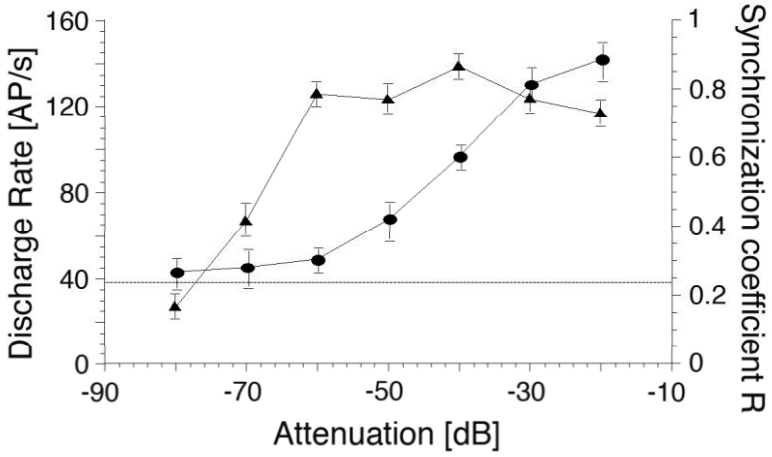

Fig. 2: Level response function and phase coupling of a primary lateral line afferent nerve fibre in the posterior lateral line nerve. Discharge rates (circles, left Y-axis) and synchronisation coefficients R (triangles, right Y-axis) are plotted as function of relative stimulus amplitude of a sinusoidally vibrating

sphere. An attenuation of 0 dB corresponds to 4.2 mm sphere vibration. Stimulus frequency 50 Hz, duration 1 s. The horizontal line represents the mean ongoing discharge rate.

Note again: The figure legend tells the reader what is shown in the figure. It does not describe the result. This is done in the Result section. For the two figures above, the Result section may be written like this: “Percent correct choices increased with increasing stimulus amplitude (Fig. 1)” and “Discharge rate and the degree of phase-coupling increased with increasing stimulus amplitude (Fig. 2)”.

9. Appendix

Place for details that would otherwise go beyond the scope of the text. For instance protocols for histology, listings of original data, contents from files, source codes of programs etc.

10. Language

The protocols in the MSc program must be written in English.

Always use past tense. You write about something that **was** done, that **was** discovered. This is especially important in the Methods and Results. Present tense would render your data generally valid. But they are only valid for **your** investigations under **your** experimental conditions. Present tense may be used when writing about existing facts. Example: „Hair cells are the primary receptor cells in the mammalian inner ear.“

11. Letter type

Use a clear letter type like Arial or Helvetica that is easy to read. Use at least 1.5 lines distance between lines for comments. Leave sufficient margin on both sides. Subtitles should be separated from the text with a space between lines.