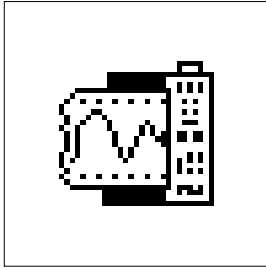


# Pharmacology Experiments Manual



# Introduction

This manual provides practical descriptions of a range of pharmacological experiments on isolated animal tissues. It includes notes on the dissection and set-up, as well as suggestions for hardware and software settings, and methods of data analysis in ADInstruments' Chart software. All the experiments are suitable for undergraduate laboratory classes, although some are more advanced than others. The manual could provide a basis for a course of undergraduate laboratory experiments, or a useful source of ideas for supplementary experiments in an existing course, but is not a complete student laboratory manual as such.

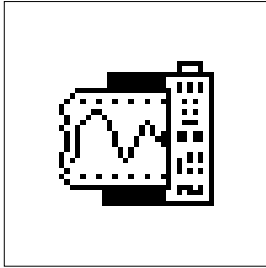
An earlier manual (the *Physiology Experiments Manual*, also available from ADInstruments) described physiological experiments on human subjects. It contained detailed step-by-step instructions for student laboratory classes. We have chosen not to follow that approach in the present manual. Step-by-step instructions are likely to be inappropriate, because each pharmacological laboratory tends to have its own designs of organ bath and stimulus electrodes, and its own methods for attaching tissues to transducers.

Instead, this booklet is intended for academic staff, senior students, and technicians, who are assumed to have experience in dissecting and mounting tissues for pharmacological experiments, and know how to use, or are interested in, the PowerLab system.

In preparing this manual we have referred frequently to two books that are unfortunately now out of print: *Pharmacological Experiments on Isolated Preparations*, and *Textbook of in vitro Practical Pharmacology* (see References). These books give details of a wide range of additional experiments.

## General Requirements

It is expected that you have a reasonably well-equipped pharmacological laboratory, with organ baths, stimulus electrodes, tissue holders, and a PowerLab system. Likewise, it is expected that you have well-established methods for experiments in vitro. A workshop of some sort is vital for experimentation: the tissue holders described in the experiments were all custom-made in a workshop rather than obtained commercially. This ensured that they were suited to the particular organ baths and procedures for which they were designed.



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## **Introduction**

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## **1 Mammalian Diaphragm**

Stimulation of the phrenic nerve causes twitch responses in rat diaphragm muscle. The effects of drugs on this fast response are examined, along with reversal of those effects.

## **2 Chick Biventer Cervicis**

The chick biventer cervicis muscle has both fast and slow muscle fibres. Stimulation of the attached tendon nerve causes twitch responses. The effects of drugs on fast and slow muscle fibres are examined.

## **3 Toad Rectus Muscle**

Toad rectus muscle shows only slow contractile responses, since it does not contain fast muscle fibres. The effects of drugs on the slow muscle fibres present are examined.

## **4 Vascular Smooth Muscle**

Vascular smooth muscle, such as arterial rings, can be used to show not only responses to classical autonomic drugs, but also the more recently discovered role of the endothelium in modulating vascular responses.

## **5 Perfused Rat Hindquarters**

The isolated hindquarters preparation allows examination of drug effects on resistance vessels. Responses to a range of vasoactive drugs can be clearly seen.

## **6 Guinea Pig Atria**

The spontaneously beating atria from the heart of a small animal can be used to show a variety of responses to drugs that affect the frequency and strength of muscle contraction.

## **7 Rabbit Heart**

The isolated perfused rabbit heart (Langendorff preparation) provides a way of studying drug effects on ventricular muscle.

## **8 Guinea Pig Ileum**

The classic guinea pig ileum preparation has many uses in undergraduate student laboratory classes, ranging from dose-response studies to demonstration of selective antagonism.

## **9 Rabbit Jejunum**

The isolated innervated rabbit jejunum (Finkelman preparation) provides a way of studying drug effects on sympathetic nerve function.

## **10 Mammalian Uterus**

The isolated uterus of a rat or guinea pig provides a good example of a tissue containing inhibitory  $\beta_2$ -adrenoreceptors. Stimulatory agonists such as oxytocin can also be studied in this preparation.

## **11 Rat Vas Deferens**

The rat vas deferens is rich in sympathetic innervation, and is useful in showing the effects of  $\alpha$ -adrenoreceptor agonists and antagonists, and a range of drugs that presynaptically inhibit neurotransmission.

## **Plates**

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## Physiological Saline

Laboratories have their own standard solutions, and these can be used instead of the particular ones mentioned here. A Krebs–Henseleit-type solution is suitable for all the mammalian experiments. An appropriate composition (g/L in distilled water) is:

NaCl	KCl	MgSO <sub>4</sub> ·7H <sub>2</sub> O	KH <sub>2</sub> PO <sub>4</sub>	NaHCO <sub>3</sub>	Glucose	CaCl <sub>2</sub>
6.92	0.35	0.29	0.16	2.1	2.1	0.28

Ideally the solution should be made fresh each day. Some laboratories find it possible to keep solutions in a refrigerator for a day or two, especially if the glucose is initially omitted, and added just before use. Calcium should be added as a solution to prevent precipitation (visible as cloudiness). Mammalian physiological saline should be bubbled with 'carbogen' gas (95% O<sub>2</sub>, 5% CO<sub>2</sub>).

A suitable frog-Ringer or toad-Ringer solution (g/L in distilled water) is:

NaCl	KCl	NaHCO <sub>3</sub>	CaCl <sub>2</sub>
6.5	0.14	0.16	0.16

## Drug Exposure

Concentrations of drugs in most of the experiments are given as final molar concentrations in the bath. For reproducible results in dose–response experiments, a standardised cycle of drug exposure is required. For example, successive agonist doses may be given every five minutes, with 45 s exposure, followed by one or two washes. It is also important to allow time for equilibration after the tissue is first set up in physiological saline. Typically, 20–30 minutes of equilibration suffices; during this time the bath should be washed out once or twice.

## Organ Bath

A water-jacketed organ bath of internal volume 25–50 mL is required. Wash-out should preferably be by overflow, since washing out by drainage disturbs the mechanical arrangements and tends to dislodge nerves from their stimulating electrodes. Many laboratories use a custom design. Those without access to glass-blowing facilities could purchase an organ bath such as the ones available from ADInstruments. Single-bath (LE11) and multiple-bath (LE01) versions are available.

## Stimulator and Electrodes

Some experiments require an external stimulator to activate nerves in the preparation. ADInstruments does not supply equipment suitable for these experiments. In trial experiments we used a Grass model SD9, but almost any commercial stimulator can be used, provided it is capable of a wide range of output currents or voltages, and can deliver single stimuli at frequencies down to 0.1 or 0.2 Hz. It is not necessary for the outputs to be

electrically isolated from ground. Chart can control an external stimulator using the PowerLab's analog outputs, but this was not done for these experiments. For further details of nerve stimulation, see the application note AN326 'Principles of Nerve Stimulation', available free from the web site [www.ADIstruments.com](http://www.ADIstruments.com) or from your ADInstruments distributor.

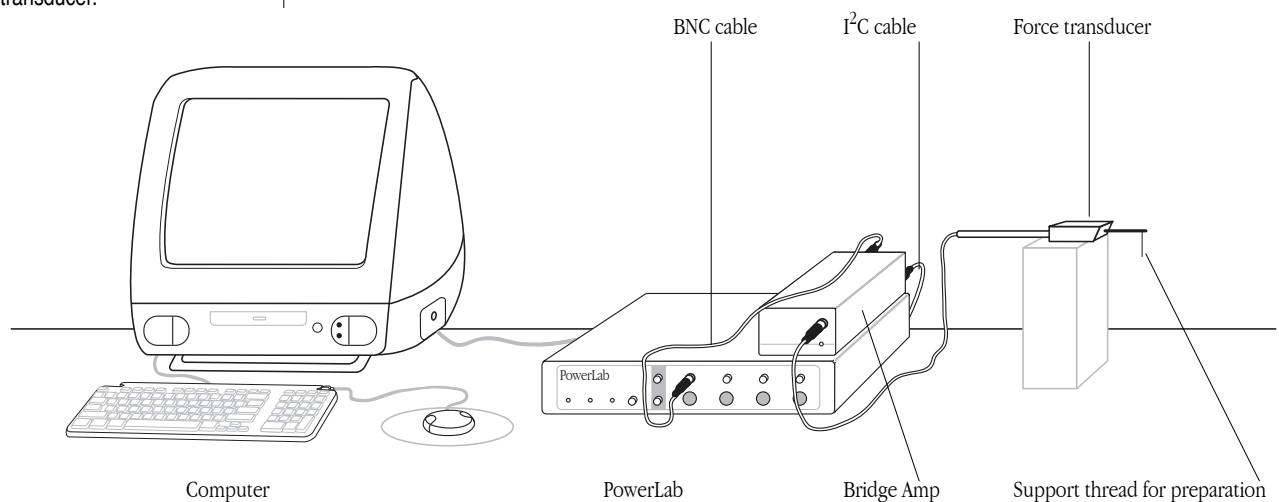
## PowerLabs and Transducers

The experiments in this manual involve recording on one or two channels only, at modest sampling rates (200 or 400 samples/s maximum). Any model of PowerLab (or MacLab) is therefore suitable. All experiments use a force transducer except for Experiment 5 (Perfused Rat Hindquarters), which uses a pressure transducer (suitable models include the ADInstruments MLT1050 or MLT0380 transducers).

The force transducer should be capable of recording forces in the range 0.1–20 gram weight (about 1–200 mN; g wt is discussed later). A suitable low-cost ADInstruments transducer is the MLT050. Another transducer, with better specifications, is the MLT001. Many other commercial transducers may be used, such as the Grass FT03. The transducer connects to the PowerLab with a bridge front-end amplifier (the ML110 Bridge Amp, the ML118 Quad Bridge Amp, or the ML119 Octal Bridge Amp). The maximum bandwidth of the Quad and Octal bridge amplifiers is 100 Hz, but this is sufficient for monitoring smooth muscle contractions, and even skeletal muscle twitches in pharmacological experiments. The single Bridge Amp has a higher bandwidth. (In the future, low-cost Pod connectors may be available that do not require a Bridge Amp for most purposes.) The PowerLab/415 may be of use, although it cannot perform Experiment 5 — it is a four-channel PowerLab with built-in bridge amplifiers that have the same specifications as the Quad Bridge Amp.

Details of connections are provided in the hardware manuals. Figure 1 shows a set-up with a single Bridge Amp connected to Channel 1 on a PowerLab. The force transducer connects to the Bridge Amp. It is itself fixed to a retort or stand, and usually a thread is attached from it to the preparation.

**Figure 1**  
Connections to the force transducer.



## Chart

All experiments require an up-to-date version of ADInstruments' Chart software, either Chart for Macintosh v3.6 or Chart for Windows v3.4. Recent (but not early) versions of Chart can be updated to the latest ones with free updating software from the web site, [www.ADIstruments.com](http://www.ADIstruments.com). The settings are given for the Macintosh version: some minor adjustments may be necessary for the Windows version. Chart for Windows will not manage some of the lowest suggested sampling rates, so if you use it, you should sample at a higher rate and compress the view more to compensate, so that a similar waveform is seen. The 'Cycle Variables' Chart extension is referred to in a couple of experiments, but its use is not mandatory. Chart extensions, Cycle Variables among them, should be available with the next release of Chart for Windows.

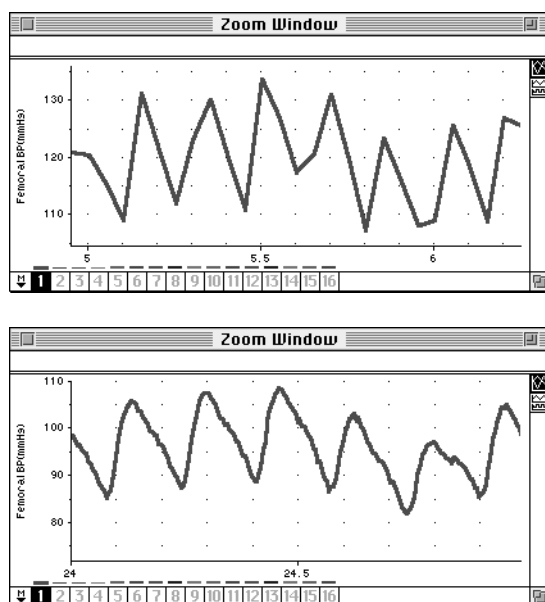
Some general guidelines on recording and settings that apply to all experiments are given below. Specific settings are indicated as appropriate in individual experiments. The manuals for the software products describe their features in detail.

### General Recording Techniques

The sampling rate must be set fast enough to record waveform peaks accurately. As a rough guide there should be at least 10–20 samples for each peak. The outcome from setting the sampling rate too low is illustrated in Figure 2. Setting the sampling rate higher than necessary has no ill effect on the fidelity of recording. It does however, inflate the size of a saved disk file, and may slow things down if you are scrolling through or analysing the data on an older computer.

**Figure 2**

Rat blood pressure sampled at 20/s (top) and 200/s (bottom). This rapidly changing signal is recorded with poor accuracy at 20 samples per second.



Equally important is the range setting (which determines the amplification of the signal). A range should be chosen such that the recorded signal does not go out of range, even with the maximum changes of biological activity.

An out-of-range signal is clipped to the range value, causing severe loss of information.

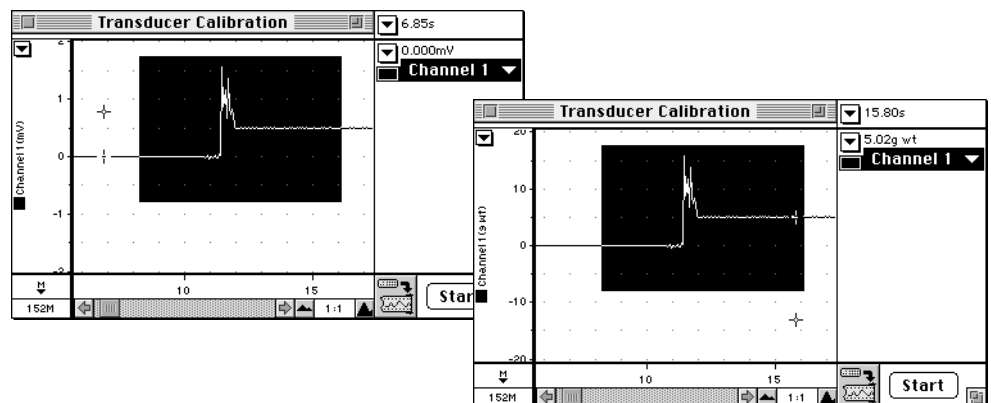
There is a good introduction to data acquisition provided in the documentation for Chart, Scope, and Scope for Windows (from v3.6), and Chart for Windows (from 3.4). This information on general recording techniques is also available in one of the Application Notes (AN022 'Basics of Data Acquisition') that can be downloaded from the ADInstruments web site or obtained from your ADInstruments distributor.

### Transducer Calibration

Three related tasks should be performed at the start of each day's experiments: zeroing the Bridge Amplifier, setting the Bridge Amplifier's Range, and calibrating the transducer. The displayed range in the Chart window and the Bridge Amplifier dialog box is affected by the drag scale settings as well as by the range itself. To avoid confusion, it is best to set the drag scale to its full bipolar range, by double clicking once or twice as necessary in the vertical axis region. This ensures that the whole of the working voltage range is displayed.

To calibrate a force transducer, record data for a few seconds while known forces are applied. Commonly, small known weights are attached by a cotton loop to the transducer. Units conversion is then applied to calibrate the transducer, letting one read off directly the force measured by the transducer rather than the base volts or millivolts. This method has the advantage that the recorded calibration data can be saved along with each day's experiment files. Figure 3 shows a typical calibration, before and after: the initial recording is of a known weight attached to the transducer. The selection includes data both before and after the weight was applied (the spikes are temporary oscillations while the weight was added).

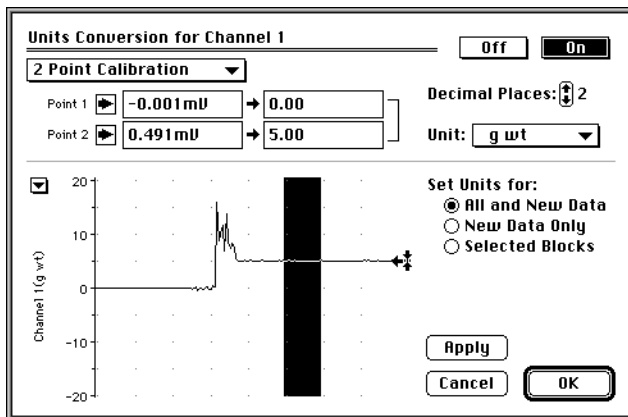
**Figure 3**  
Chart recordings showing the attachment of a 5 g weight to the transducer, before (left) and after (right) units conversion.



The selected data are transferred to the Units Conversion dialog box (Figure 4), where values are entered for the known weights and the conversion applied.

**Figure 4**

The Units Conversion dialog box, showing the selected data from Figure 3.

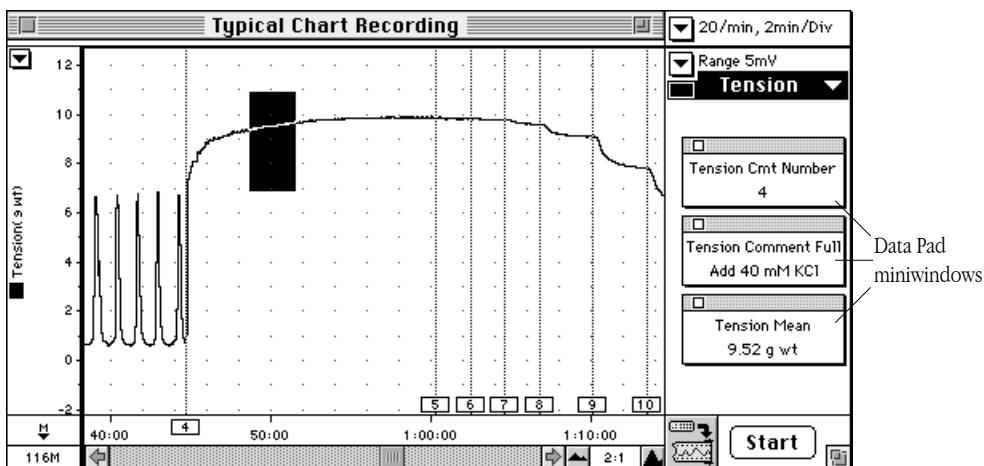


### Comments and Miniwindows

The best way of marking dose times and dose sizes in a Chart file is to enter a Chart comment by pressing the Return or Enter key as the drug is added. Comments and measurements of various sorts can be read off as required from Data Pad miniwindows, which can be set up wherever convenient on the screen (over the channel control area is a good place). Figure 5 shows a series of comments in a file, with Data Pad miniwindows at the right (see the Chart user's guide for details) showing the comment number and the text of the comment to the left of the data selection.

**Figure 5**

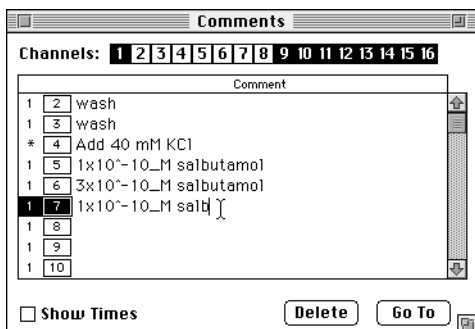
The Chart window with comments indicating addition of drugs and so on.



If the drug additions are being made at short intervals, there may not be enough time to enter full details in the comment text. In such cases text can be added or edited later, in the Comments window (Figure 6).

**Figure 6**

Chart's Comments window, in which the dose list is being updated by editing the comment text.



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On the Macintosh, Chart's Notebook window can be used for general description of the experiment; on Windows, a Data Pad column can be used for this general description.

## Terms and Units

The terms describing the software and hardware are the standard ones used in the manuals. Sampling rates may at times be abbreviated from 200 samples per second to 200/s, and so on. The terms dose-response, concentration-effect, and variants on them are interchangeable.

Molar concentrations of drugs described using prefixes (mM,  $\mu$ M) or in powers of ten ( $10^{-3}$  M,  $10^{-6}$  M) depends on the laboratory's practice. A mix of units is used in this manual. Many pharmacology laboratories take the unit of force to be gram weight (g wt, usually but wrongly abbreviated to g), as used in this manual. Calibration can equally easily be made in the SI unit of force, the newton (N), given that 1 N equals about 102 g wt. As a handy mnemonic, the newton is the weight of a medium-sized apple.

## Alternatives

Specific animal tissues and drug doses are cited in the experiments. The isolated tissues were chosen because they have been traditionally used or they demonstrate some concept particularly well. In some cases, alternatives are suggested in the Further Work sections. Where they are not, a standard search of the literature should indicate what alternatives are suitable, the appropriate dose ranges of various agonists, and so on.

## Acknowledgements

We are grateful to Margot Story, Wayne Hodgson, and other staff and graduate students of the Department of Pharmacology, Monash University, for carrying out a series of isolated organ bath experiments from which we have drawn in the production of this manual. In particular, it is a pleasure to acknowledge the skilled technical assistance of Margaret Wastell, and the expertise of the Monash University Medical Illustrations Unit who contributed the photographs.

## References

Staff of the Department of Pharmacology, University of Edinburgh, *Pharmacological Experiments on Isolated Preparations*, second edition (E. & S. Livingstone, Edinburgh, 1970).

I. Kitchen, *Textbook of in vitro Practical Pharmacology* (Blackwell Scientific Publications, Oxford, 1984).

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This document was, as far as possible, accurate at the time of printing. Changes may have been made to the software and hardware it describes since then, though: ADInstruments reserves the right to alter specifications as required. Late-breaking information may be supplied separately.

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