A Parker Hannifin White Paper

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# PICOSPRITZER® III

## *Pressure Systems for Ejection of Picoliter Volumes in Cell Research*


## Contents



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## OPERATING MANUAL

### I. INTRODUCTION

Please read this entire set of instructions before attempting to use this instrument for intracellular or extracellular ejections. These instructions cover all currently produced systems (single channel, two channel, and vacuum loading models).

The Picospritzer III is a self-contained, rack-mountable system which supplies repeatable pressure pulses. Volumes dispensed are linear with time and pressure. The system can be initiated three ways: front panel push button external stimulator (5 volt), or remote push button/foot pedal. The Picospritzer III pressure system was designed for rapid and reproducible ejections of picoliter to nanoliter volumes used in conjunction with intracellular or extracellular studies while avoiding the inherent desensitization of nerve cells which accompanies Iontophoretic methodology.

Intracellular applications range from femtoliter ejections of RNA into small (50m diameter) cells to nanoliter ejections into oocytes. Extracellular applications range from picoliter applications of dilute neuroactive substances onto neurons during intracellular recording to the presentation of chemical stimuli to whole animals.

The system comes complete with high speed valve(s) and the necessary tubing assemblies (less pipette and holder). It is designed to fit a standard 19" relay rack. The Picospritzer III comes fully adjusted and ready to use. Set-up entails the following steps:

1. Position the control unit in the desired location. Position the remote valve box within two feet of the experimental site (VELCRO® is supplied for mounting) and within 6 feet of the control unit.

2. Connect the black ¼ inch pressure tubing to the side port on the front panel regulator and to a source of clean dry compressed gas (air, nitrogen, $CO_2$, or argon) at a maximum of 150 PSI (10 bar). DO NOT USE OXYGEN OR COMBUSTABLE GASES. The fitting on the regulator is a quick-connect type. The end of the tubing should be cut cleanly and inserted fully into the opening in the fitting. An o-ring seals around the outside of the tube. To remove the tubing from the fitting you must first vent the pressure from the line then press the ring on the end of the fitting (around the tubing) toward the body of the fitting while pulling on the tubing.

3. Use the 6-foot log 1/8-inch tubing assembly to connect the front panel regulator to the remote valve box. The connection at the regulator is also a quick-connect type while that to the remote valve box is threaded (1/4-28 male). There should be a ferrule pressed in to this end of the tubing.

4. The 3-foot long 1/16-inch tubing assembly is used to connect the remote valve box to a pipette holder (optional). Both ends of this assembly have ¼-28 nuts and ferrules pre-assembled to it.

**PLEASE NOTE:** An important detail in arranging the location of the solenoid in relation to the pipette holder is to maintain a loose coil in the interconnecting small bore (1/16 inch) Teflon® tubing to absorb or dampen the pressure pulse, thus avoiding movement of the pressure pipette. DO NOT stretch the tubing out tight between the valve housing and the pipette holder.

## II. SPECIFICATIONS

**Useful Pressure Range:**
10-100 PSI, self-bleeding pressure regulator (lower pressure ranges available).

**Maximum Inlet Pressure:**
150 PSI
(Do not use oxygen or combustible gas.)

**Pulse Durations:**
2 to 999 ms, 1 ms intervals, 0.1 to 99.9 seconds, 0.1 to 99.9 minutes

**Pulse Initiation:**
Pushbutton on panel, external stimulator, or remote pushbutton/footswitch

**Time Mark:**
5-volt TTL from Channel One

**Power Requirements:**
Universal 90-250 V.A.C.
50-60 Hz.

Unit is equipped with a universal power supply which matches available line voltage ranging from 90 to 250 V.A.C.

## III. CONTROLS AND USE

The unit is activated when the power switch is in the ON position and the red pilot light is on. The unit must be connected to a source of clean, dry compressed gas at a pressure at least as high as that desired for ejections. Clockwise rotation of the knurled knob on the right of the rack mount panel will increase the pressure (indicated on adjacent gauge) available for injection purposes. This unit contains a self-bleeding regulator so that the pressure may be reduced by simply rotating the pressure control knob counter-clockwise.

The system is rated to operate up to 100 PSI (6.7 bar). One can be assured that all the connections are gas-tight by rotating the knob on the front panel so that the meter indicates approximately 80 PSI of pressure and then shutting off the main source of pressure to the panel. If a gradual reduction of pressure is observed, it indicates that there is a leak somewhere between the remote valve housing and the main pressure source. The site of this leak may be found by carefully listening for a hissing sound or by application of a dilute soap solution (or "snoop") and watching for the formation of bubbles at various connections. It is essential to have leak proof connections throughout the system to assure reproducible results and to avoid unnecessary loss of gases.

Volume ejected is a linear function of both pulse duration and pressure (see references 1, 3). The pulse duration has a greater dynamic range with more accurate and reproducible settings. Thus, pulse duration will be changed frequently during the course of the experiment while pressure settings will be changed relatively little on a daily basis.

### DURATION SETTING

The pulse duration is indicated by the setting on the three digit thumbwheel switch and the extended range switch (see Extended Range). It may be initiated by pressing the adjacent button on the front panel by remote push button, or by an external input signal. The circuit has a "debounce" control which restricts the action of the push button so that only one pulse is initiated per button press, even if the button is continuously held down. It also restricts the interval between repetitive pulses initiated by the push button to approximately 200 milliseconds. The "PULSE" indicator will light during the course of the pulse.

### EXTENDED RANGE

The basic Picospritzer is furnished with extended range timing controlled by a three-position toggle switch located at the right side of the "Duration" thumbwheel. The top position (labeled "MSEC"), selects timing in the milliseconds range (2 to 999 milliseconds). In the center position (labeled "SEC"), the range is from 0.1 to 99.9 seconds (not the decimal). In the bottom position (labeled "MIN"), the range is from 0.1 to 99.9 minutes.

### INDICATORS

The Picospritzer III contains four LED indicators. "ON" is the power indicator. The "PULSE" indicator shows that the timer is active; the channel 1 and channel 2 indicators show when each channel is active.

### INPUT TRIGGER

The internal timer now has a separate BNC jack to allow it to be triggered from an external source. A low to high (+5 volts)

transition on this BNC will trigger the internal timer just as if the manual pushbutton had been pressed. If a pulse train is applied to this jack, care should be taken to ensure that the duration setting is less than the period of the pulse. Otherwise pulses will be skipped.

**REMOTE PUSH BUTTON**
A jack on the front panel of the Picospritzer is provided for attachment of an optional remote push button or foot pedal for initiating a pulse. This convenience permits the investigator to be some distance from the rack mount panel and to view an ejection through a microscope while initiating the ejection. When using a remote push button, the button has the same function as the panel push button. Optional hand or foot activated switches are available. Refer to Picospritzer Accessories list for additional information.

**EXTERNAL INPUT**
For additional flexibility in experimental use, the Picospritzer III has a front panel BNC jack for each channel which permits independent activation of the two channels from external sources. The selector toggle switch above each jack determines the source of the control signal for that channel. In the "EXTERNAL" position, it operates by energizing the valve whenever the input signal is high (+5 V.D.C.) and de-energizes it when the signal returns to ground. This allows for pulse durations longer than the capacity of the internal timer. In the "TIMER" position, the channel is connected to the internal timer and will activate whenever that timer is triggered (either manually or externally).

**SECOND CHANNEL**
The Picospritzer III is equipped with a separate BNC jack and selector toggle for each of the two channels. This allows 2 external signals to be used to operate the 2 channels independently when the selector toggles are set to the "EXTERNAL" position. Placing a selector toggle to the "TIMER" position connects that channel to the internal timer for operation whenever the timer is triggered (either manually or externally). To prevent a channel from triggering, place the selector switch in the "EXTERNAL" position and do not make a connection to the BNC for that channel.

All Picospritzer III's are designed with a second-channel capability. See Replacements/Spare Parts list to order the remote valve box and tubing for a second channel upgrade.

**MARKER**
The time mark provides a convenient indication of the duration of the pulse with respect to a biological signal much like that of the "artifact" associated with iontophoresis. It is a 5-volt TTL signal controlled by channel one. The time mark not only provides a useful indication of the duration of the pulse, but it may also serve as a sync-out signal for triggering the sweep of an oscilloscope, etc.

## HOLDERS FOR PRESSURE PIPETTES

Standard and recording pipette holders are available for pipettes with diameters of 1.0 mm to 2.0 mm in 0.2 mm increments.

For prices and dimensions, see the Picospritzer Accessories list.

***No internal adjustment of the Picospritzer should be undertaken by the user without consulting the manufacturer. Detailed instruction will be provided if necessary.***

## IV. BACKGROUND

In 1977, Dr. R.E. McCaman and associates provided a complete description (1) of a pressure ejection system that utilized a high speed valve. This valve continues to be the heart of the pressure system offering very precise control of ejection volumes (in the picoliter range) and ejection times (in the millisecond range).

Furthermore, these investigators described a series of holders that permitted ejection through micropipettes with sufficiently small tips that could be used for simultaneous intracellular recordings during ejections.

These systems have been used for intracellular as well as extracellular ejections. In listing advantages of the pressure system, these investigators emphasize that the linear relationship between ejection volume and either duration of the pulse or of the applied pressure permits a rapid, convenient and reliable calibration of each pipette (1, 3), unlike that for electrophoretic techniques (7-9).

Pressure ejection seems an ideal approach to delivering uncharged substances such as peptides (4, 6), steroids (4), and enzymes (2, 5). The solutions used for pressure ejections are usually several orders of magnitude more dilute than those used for electrophoretic ejection (1, 3), thus avoiding receptor desensitization commonly experienced with iontophoresis. The fact that the ejection efficiency of the pneumatic systems is not influenced by solute concentration nor by net charge, makes them ideal for intracellular injections of radiolabeled or tracer substances (13-15).

Thus, pressure systems have been used for intracellular injection of radiolabeled precursors or neurotransmitters (10, 11) and

[H3] –sugars as precursors of glycoproteins (12) in order to study neuron-specific transmitter biosynthesis, axonal transport and cellular topography. The reproducible and quantifiable ejections obtained with pressure systems make them ideal for neuropharmacological studies of agonist and drug interactions with membrane receptors (1, 3, 4).

As you find additional uses for your Picospritzer, please send us a reprint for addition to our reference section so that others may benefit from your experience.

N.B.; H3=radioactivity (tritium) label substance.

REFERENCES

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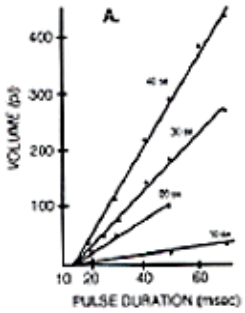
**FIGURE 1**
**DROPLET CALIBRATION CHART**

| (4/3) 4.2 x r3 DIAM. | RADIUS | PICOLITER VOL(F1) |
|---|---|---|
| 10 $\mu$m | 5 | .52 |
| 20 | 10 | 4.2 |
| 30 | 15 | 14.2 |
| 40 | 20 | 33.6 |
| 50 | 25 | 65.6 |
| 60 | 30 | 113 |
| 65 | 32.5 | 144 |
| 70 | 35 | 180 |
| 75 | 37.5 | 221 |
| 80 | 40 | 269 |
| 90 | 45 | 383 |
| 100 | 50 | 525 |
| 110 | 55 | 699 |
| 120 | 60 | 907 |
| 125 | 62.5 | 1025 |
| 140 | 70 | 1441 |
| 150 | 75 | 1772 |
| 160 | 80 | 2150 |
| 170 | 85 | 2579 |
| 175 | 87.5 | 2814 |
| 180 | 90 | 3062 |
| 200 | 100 | 4200 |
| 225 | 112.5 | 5900 |
| 250 | 125 | 8203 |
| 275 | 137.5 | 11037 |
| 300 | 150 | 14175 |
| 325 | 162.5 | 18022 |
| 350 | 175 | 22509 |
| 375 | 187.5 | 27685 |
| 400 | 200 | 33600 |
| 450 | 225 | 47840 |
| 500 | 250 | 65625 |
| 600 | 306 | 113,000 |
| 625 | 312.5 | 128,000 |
| 750 | 375 | 221,480 |

Calibration chart provided courtesy of Dr. Joyce K. Ono, Department of Biological Science, California State University

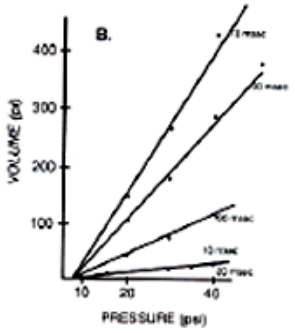
**FIGURE 2**
**CHARACTERISTICS OF THE PRESSURE EJECTION SYSTEM**
The diameter of the droplet ejected in air was measured with an ocular micrometer in a dissecting microscope. The volume was calculated for droplets formed by varying pressure or pulse duration parameters. The following graphs demonstrate that each pipette can be calibrated by varying these two major determinants of the volume ejected.

**FIGURE 2A: LINEARITY OF VOLUME EJECTED WITH VAYRING PULSE DURATION AT CONSTANT PRESSURE**



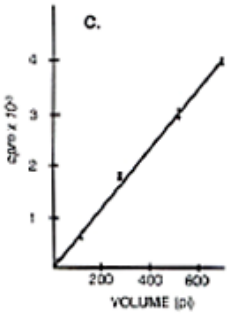

The X-intercept (15 MSEC) represents the mechanical lag time of the particular solenoid valve.

**FIGURE 2B: LINEARITY OF VOLUME EJECTED WITH VARYING PRESSURE AT CONSTANT PULSE, DURATION**




The X-intercept (7.5PSI) represents the minimum pressure necessary for ejection and is a characteristic of a particular pipette.

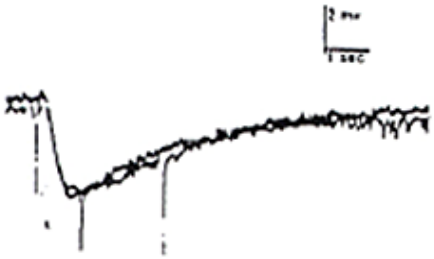
**FIGURE 2C: LINEARITY OF THE AMOUNT OF AN H3 STANDARD WITH VARIOUS EJECTED VOLUMES**




The points of this graph are highly correlated (R=.97) with the independently determined specific activity (6.5 x 106 CPM/mL) of the radioactive solution.

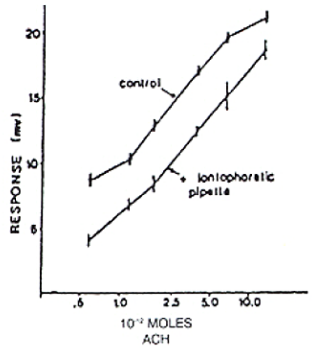
**FIGURE 3**
**COMPARISONS OF RESPONSES OF *APLYSIA CALIFORNICA* NEURONS TO IONTOPHORETIC AND PRESURE APPLICATION OF COMPOUNDS**

**FIGURE 3A:**



Superimposed traces of *Aplysia* buccal neuron responses to ACh delivered by an iontophoretic pulse (1µ amp, 80 MSEC) and a pressure pulse (40 PSI, 60 MSEC, 60 µm diameter droplets of 10-3 M ACh). The amplitude and polarity of the pressure artifacts (negative square pulse in this case) can be manipulated.

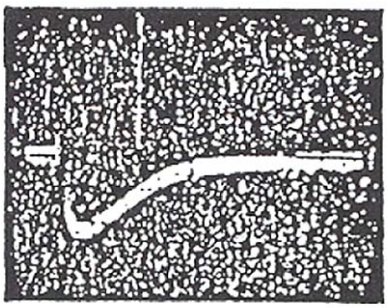
**FIGURE 3B**:




Comparisons of responses to pressure ejected acetylcholine (ACh) in Aplysia neuron in the absence (control) and presence of an iontophoretic pipette (70 MEGOHMS) containing 1 M ACh. The dose-responsive curve is shifted to the right because of desensitization from ACh leaking out of iontophoretic pipette. Each point is the mean response $\pm$ standard error of the mean.

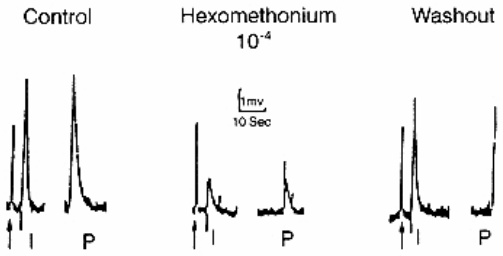
Problems of desensitization can be circumvented with a pressure pipette since the pipette is usually filled with agonists in the concentrations of $10^{-6}$ to $10^{-3}$ M in contrast to iontophoretic pipettes. Braking current is not necessary for the pressure pipette, thus avoiding inconsistent ejection of compounds.

**FIGURE 3C:**



Reproducibility of 12 consecutive responses to acetylcholine (ACh) delivered by a pressure pulse to an *Aplysia* (Sea Hare) neuron. A $10^{-4}$M solution of ACh was ejected by 22 PSI for 5 MSEC to produce a droplet estimated to be 10 PL (=1 Femtomole of ACh).
Calibration: 2 mV, .02 Sec.

**FIGURE 3D:**




Comparison of response to iontophoretic (I) and pressure (P) applied ACh in a drug study. Both responses are equally antagonized by hexamenthonium, even though the ACh in the pressure pipette is ejected in a droplet of normal artificial seawater. Similar results are obtained during substitution experiments. The arrows in the figure point to an input resistance test pulse.