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SUPERFUSION SYSTEM (Raiteri's Method)

Cat. No. 1 4 9 0 0



INSTRUCTION MANUAL

UGO BASILE

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Via G. Borghi 43

21025 COMERIO - Varese, ITALY

INSTRUCTION MANUAL

SUPERFUSION SYSTEM

Cat. No. 1 4 9 0 0

Series No. _____ Mfg. date _____

THIS INSTRUMENT IS WIRED FOR

- | | | |
|--------------------------|-------------------|-----------|
| <input type="checkbox"/> | 115 Volts – 60 Hz | OPERATION |
| <input type="checkbox"/> | 115 Volts – 50 Hz | |
| <input type="checkbox"/> | 230 Volts – 50 Hz | |
| <input type="checkbox"/> | 230 Volts – 60 Hz | |

SAFETY CONSIDERATION

ALTHOUGH THIS INSTRUMENT HAS BEEN DESIGNED WITH INTERNATIONAL SAFETY STANDARD, THIS MANUAL CONTAINS INFORMATION, CAUTIONS AND WARNINGS WHICH MUST BE FOLLOWED TO ENSURE SAFE OPERATION AND TO RETAIN THE INSTRUMENT IN SAFE CONDITIONS.

SERVICE AND ADJUSTMENTS SHOULD BE CARRIED OUT BY QUALIFIED PERSONNEL, AUTHORIZED BY UGO BASILE ORGANIZATION.

ANY ADJUSTMENT, MAINTENANCE AND REPAIR OF THE OPENED INSTRUMENT UNDER VOLTAGE SHOULD BE AVOIDED AS MUCH AS POSSIBLE AND, WHEN INEVITABLE, SHOULD BE CARRIED OUT BY A SKILLED PERSON WHO IS AWARE OF THE HAZARD INVOLVED.

CAPACITORS INSIDE THE INSTRUMENT MAY STILL BE CHARGED EVEN IF THE INSTRUMENT HAS BEEN DISCONNECTED FROM ITS SOURCE OF SUPPLY.

Instruction Manual dated July 2001
Revision 0



Superfusion System

Cat. No. 14900

General

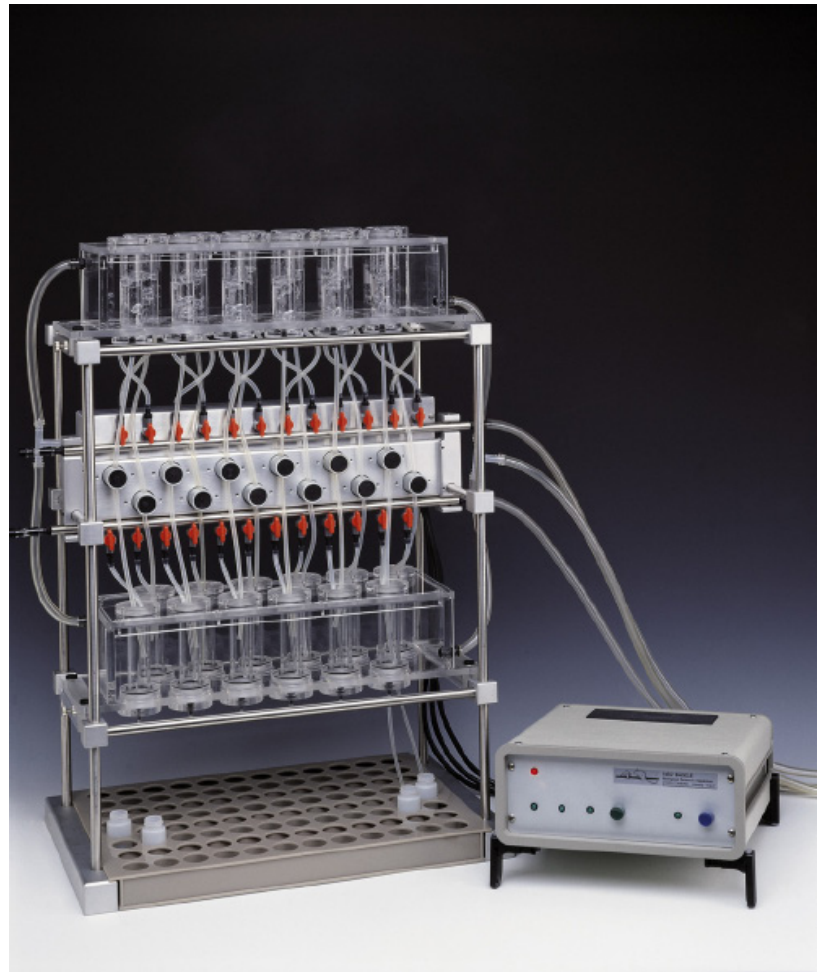
Neurotransmitter release is the major step of neurotransmission. Abnormalities in neurotransmitter release have been proposed to be involved in many pathological conditions.

Therefore, understanding the physiological mechanisms of transmitter release and how the process can be modified by pathological states is essential to develop therapeutically useful pharmacological agents.

UGO BASILE 14900 Superfusion System has been especially designed to perform release studies from synaptosomes, although brain slices can be employed as well.

On the other hand, presynaptic nerve terminals are the sites where release specifically occurs; therefore superfusion of synaptosomes is best suited to explore presynaptic events.

Superfused synaptosomes are the preparation of choice to study release-regulating presynaptic receptors and to explore the intimate mechanisms of neurotransmitter release.



RAITERI'S METHOD

Synaptosomes Release Studies

Main Features

- Specifically designed to perform release studies from synaptosomes
- Brain slices can be employed as well
- More than 300 full papers using superfused synaptosomes have been published



UGO BASILE S.R.L.
BIOLOGICAL RESEARCH
APPARATUS

CHECK-LIST

CAT. 14900 SUPERFUSION SYSTEM

CLIENTE / CUSTOMER _____

No. Ordine / Order No. _____ Data / Date ____ / ____ / ____

UB code	CAT.No.	✓	Q.t y	DESCRIPTION		DESCRIZIONE	
	14900-302		1	INSTRUCTION MANUAL		MANUALE DI ISTRUZIONE	
E-WP 008			1	MAINS CABLE	EUROPA	CAVO RETE	EUROPA
E-WP 008-1		U.S.A.			U.S.A.		
E.WP 008-2		U.K			U.K		
	14900-001		1	ELECTRONIC UNIT		UNITA'ELETTRONICA	
	14900-002		1	SUPERFUSION BATH		GRUPPO BAGNO	
	14900-004		1	SUCTION PUMP		POMPA DI DRENAGGIO	
M-LM 133	14900-301		1	DUST COVER		COPERTINA	
M-CM 340-F	14900-338		2	FILTERS		CONFEZIONE FILTRI	
M-CM 336-F	14900-328		100	PHIALS		FIALE	
CU2			1	HEX-WRENCH 2		CHIAVE A BRUGOLA 2	
CU3			1	HEX-WRENCH 3		CHIAVE A BRUGOLA 3	
CU4			1	HEX-WRENCH 4		CHIAVE A BRUGOLA 4	
	14900-325		1	PHIAL RACK		RACK FORATO	
	14900-326		1	DRAIN PAN		VASCHETTA RACCOLTA	
E-FT 014			2	FUSES FOR 115V (T 2.5 mA)		FUSIBILI PER 115 V (T 2.5 mA)	
E-FT 010-1			2	FUSES FOR 230 V (T 1.25 mA)		FUSIBILI PER 230 V (T 1.25 mA)	
			Verificare che cambio tensione e fusibili inseriti nel blocco alimentazione corrispon- dano ai dati di targa				
OPTIONAL							
	14900-010		1	MASTERFLEX PERISTALTIC PUMP			
	14900-003		1	WATER CIRCULATOR/HEATER			

DATE / /	PREPARATO DA / PACKED BY
Set for	<input type="checkbox"/> 115V 50 Hz <input type="checkbox"/> 230V 50 Hz <input type="checkbox"/> 115V 60 Hz <input type="checkbox"/> 230V 60 Hz
Serial Number	14900-001 14900-002 1490-004
IMPORTANT/IMPORTANTE:	
Check the shipment for completeness immediately after receipt: should you find any discrepancy, please fill in the following part and transmit it to our fax No. +39 0332 745488	
Al ricevimento della merce controllate che la spedizione sia completa: in caso di discrepanza, completate il formulario di seguito riportato ed inviatelo al nostro fax No. 0332 745488	
FROM: Name	Company/Institution
DATE	REF.
NOTES	
MOD.04 REV 0	



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SUPERFUSION SYSTEM (RAITERI'S METHOD)

Cat. 1 4 9 0 0

1 GENERAL

Neurotransmitter release is the major step of neurotransmission. Abnormalities in neurotransmitter release have been proposed to be involved in many pathological conditions.

Therefore, understanding the physiological mechanisms of transmitter release and how the process can be modified by pathological states is essential to develop therapeutically useful pharmacological agents.

UGO BASILE **14900 Superfusion System** has been especially designed to perform release studies from synaptosomes, although brain slices can be employed as well.

On the other hand, presynaptic nerve terminals are the sites where release specifically occurs; therefore superfusion of synaptosomes is best suited to explore presynaptic events. Superfused synaptosomes are the preparation of choice to study release-regulating presynaptic receptors and to explore the intimate mechanisms of neurotransmitter release.

UGO BASILE 14900 Superfusion System is a semi-automated version of that originally developed in Raiteri's laboratory, where about 200 papers have been published exploiting the technique, see paragraph 6-BIBLIOGRAPHY.

We have developed this Superfusion System in order to make commercially available an instrument in which the original design of the superfusion chambers has remained intact.

1.1 Instrument Description

The 14900 Superfusion System consists of:-

- 1) a Superfusion Bath Cat. 14900-002
- 2) an Electronic Unit Cat. 14900-001
- 3) a Suction/Draining Pump Cat. 14900-004

An optional (not supplied with standard package) Water Circulator/Heater and a multi-channel Peristaltic Pump are necessary complements to this system and are to be purchased separately.

See paragraph 5.1-Optional.



1.1.1 Superfusion Bath

The Superfusion Bath 14900-002 consists of 12 open superfusion chambers, working in parallel, combined with 12 upper reservoirs. Both reservoir and chamber sets are lodged into appropriate temperature-controlled baths.

Pre-warmed oxygenated media of the desired composition can be delivered simultaneously from the reservoirs to the superfusion chambers.

Synaptosomes deposit as very thin layers on microporous filters placed on suitable glass-filter supports.

Synaptosome or slice superfusion is provided by a multi-channel peristaltic pump; superfusate samples may be directly collected into scintillation vials.

1.1.2 Electronic Unit

The Electronic Unit 14900-001 is lodged into a resilient cabinet provided with splash-proof side slots for ventilation and tilting front feet.

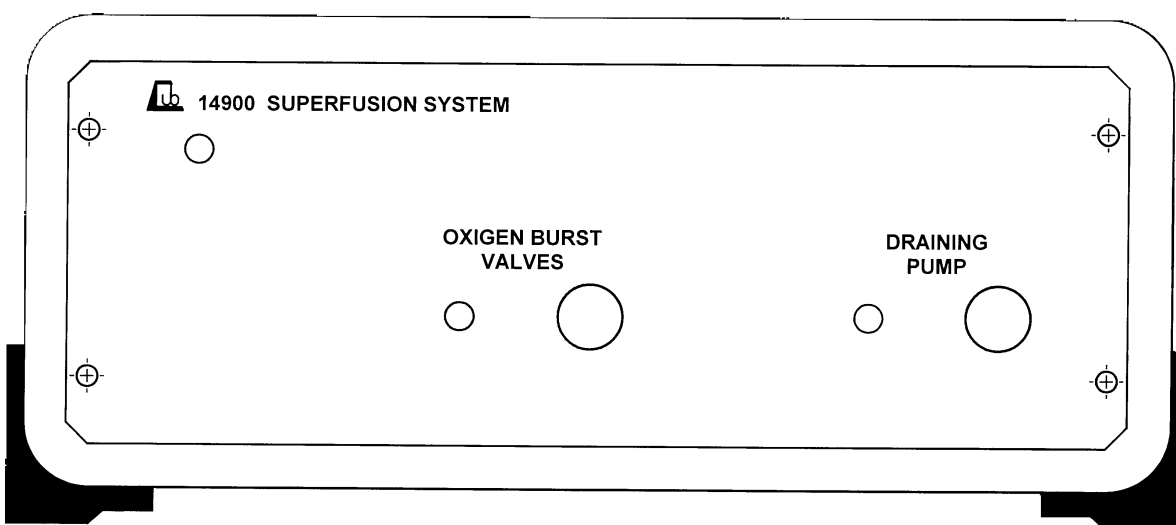


Figure 1, "Front Panel "

The cabinet **front panel** (see Figure 1) of anodized aluminium feature extremely durable engraved markings, indicating:-

OXYGEN BURST VALVES : The green switch which opens the valves must be held depressed until the liquid from the upper chambers has completely been drained into the lower chambers.

See also 3-OPERATION, 3rd and 4th paragraphs.

DRAINING PUMP : this blue push-button activates the draining (or suction) pump for the duration of 4 seconds. A green LED, when lit, indicates that the pump is working. See also paragraph 3-OPERATION.



POWER : this red LED indicates when the power is ON.

The **back panel** (see Figure 2) embodies, beside the POWER MODULE the following socket connectors:-

SUCTION PUMP : to plug the suction/draining pump, see paragraph 3-OPERATION.

OXYGEN : to plug the cable of the oxygen-burst valves located on the bath assembly, see paragraph 3-OPERATION.

ELECTROVALVES : to branch the electrovalve block located on the bath assembly. A second connector is provided to plug-in a second bath assembly (optional), see paragraph 3-OPERATION.

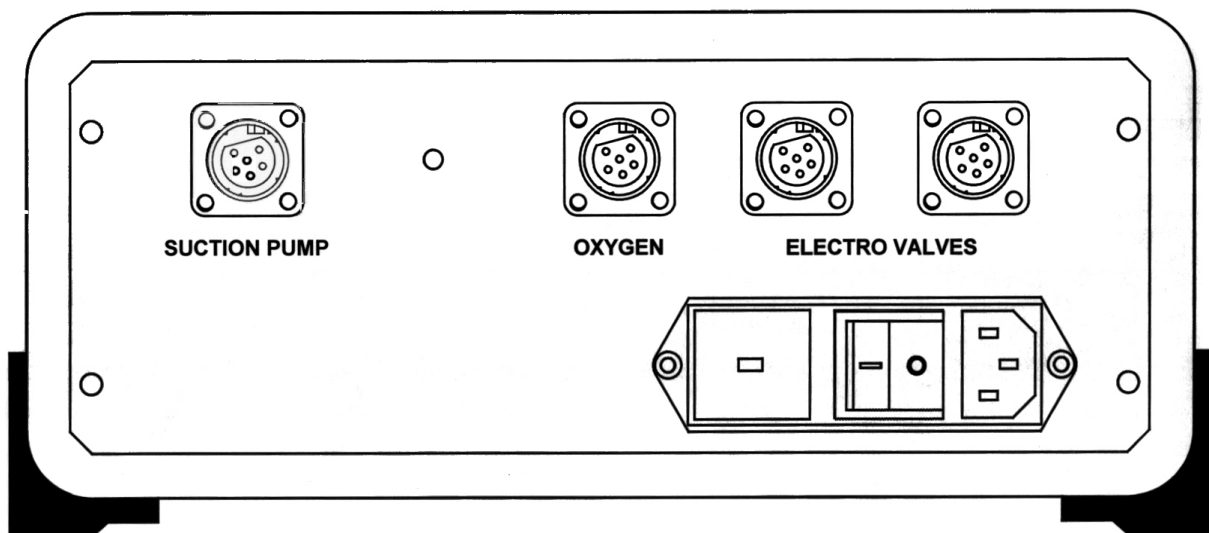


Figure 2, "Back Panel "

1.1.3 Suction Pump

This is a self-priming 24V DC electric pump, provided with strainer, whose purpose is the rapid drain of the superfusion chambers via the draining manifold.

The pump is directly fed by the electronic unit

2 INSTALLATION

2.1 How to Handle the Instrument

The Superfusion Bath is a relatively bulky and heavy and it is recommended that two people lift it.

Once the set-up is assembled, do not attempt to lift or move the combined unit.



2.2 Unpacking & Preliminary Check

Check the contents of the shipment for completeness, packing list to hand, and visually inspect the instrument as soon you take it out of the packaging. Use the **Check List** supplied.

If the instrument is damaged or, after having tested it, fails to meet rated performances, notify the carrier and our company immediately.



Protect the environment!

Dispose of packaging properly, according to existing and applicable waste management rules and regulations.

2.3 Notes on the Instruction Manual

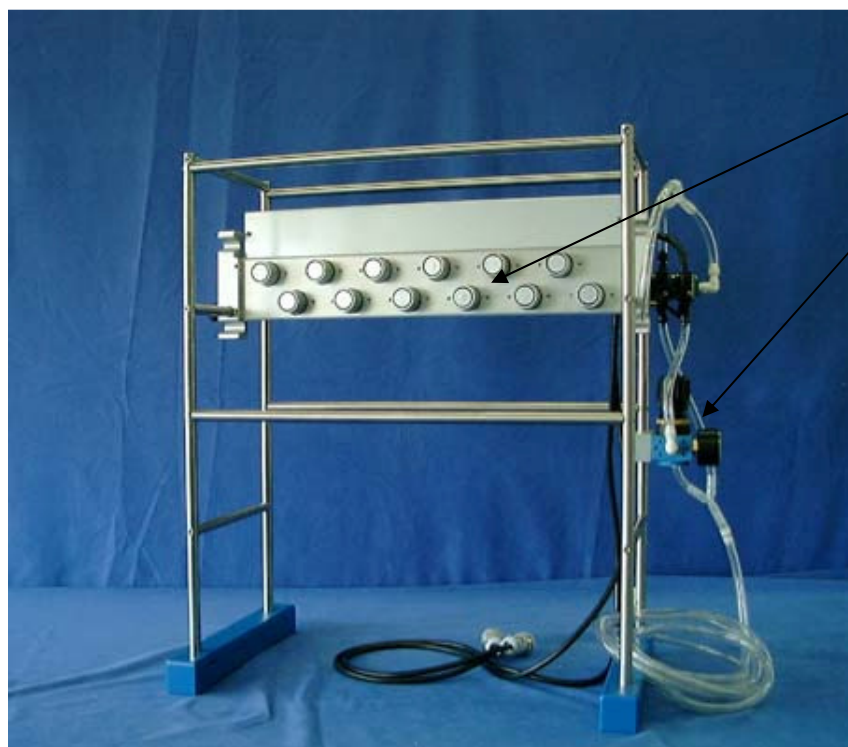
The 14900Instruction Manual included in the Electronic Unit package is necessary for the correct installation and operation of the instrument.

We recommend keeping the manual in good conditions, ready to be consulted by the qualified personnel who use the instrument.

Free of charge copies of the instruction manual are available upon request: please contact our service department (see paragraph 4.4-Customer Support) specifying the series number of your instrument.

2.4 Assembling the Instrument

Assemble the instrument on a stable and reasonably flat bench or table surface, following the scheme suggested by the pictures 3 to 10 for a correct assembly of the whole system.



The Main frame includes:

the **electro-valve block**

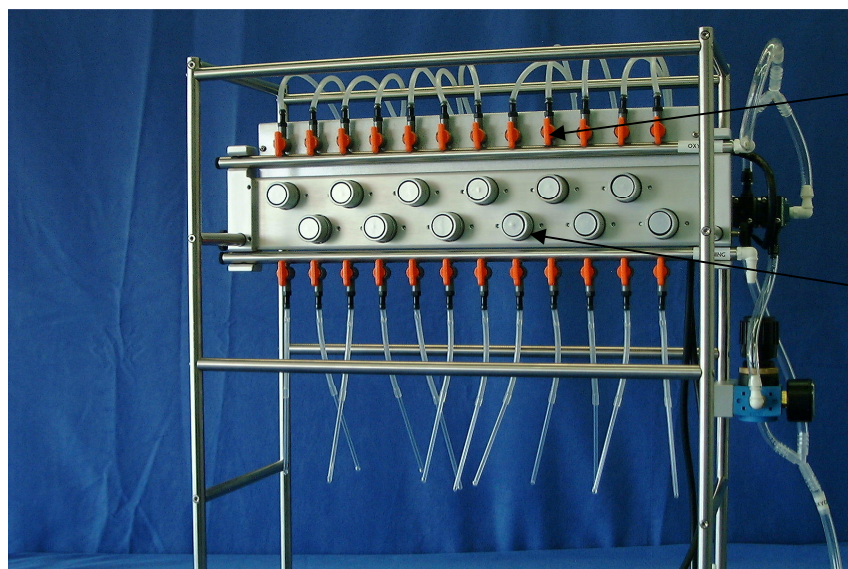
and

the **pressure gauge**.

This block is the basic unit and is shipped assembled as shown in picture Figure 3 “Main Frame”.

Figure 3 “Main Frame”

Assemble the Oxygenation and Draining Manifolds, see Figure 4 “Manifolds”. Both manifolds must be locked with the 3mm hex. (Allen) wrench provided.

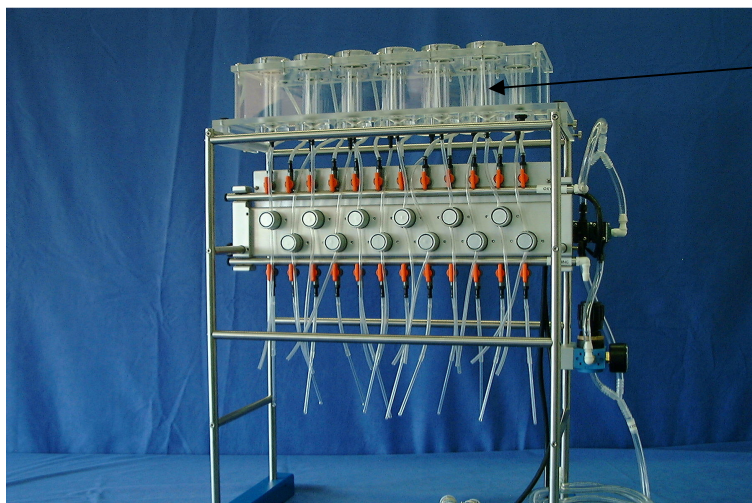


Oxygenation Manifold

Draining Manifold

Figure 4 “Manifolds”

Assemble the Upper Chamber Bath and lock it by the knobs, mounted on the bath itself. See Figure 5 “Upper Chamber Bath”.



Upper Chamber Bath

Figure 5 “Upper Chamber Bath”

Assemble the Lower Chamber Bath proceeding as for the Upper Bath.

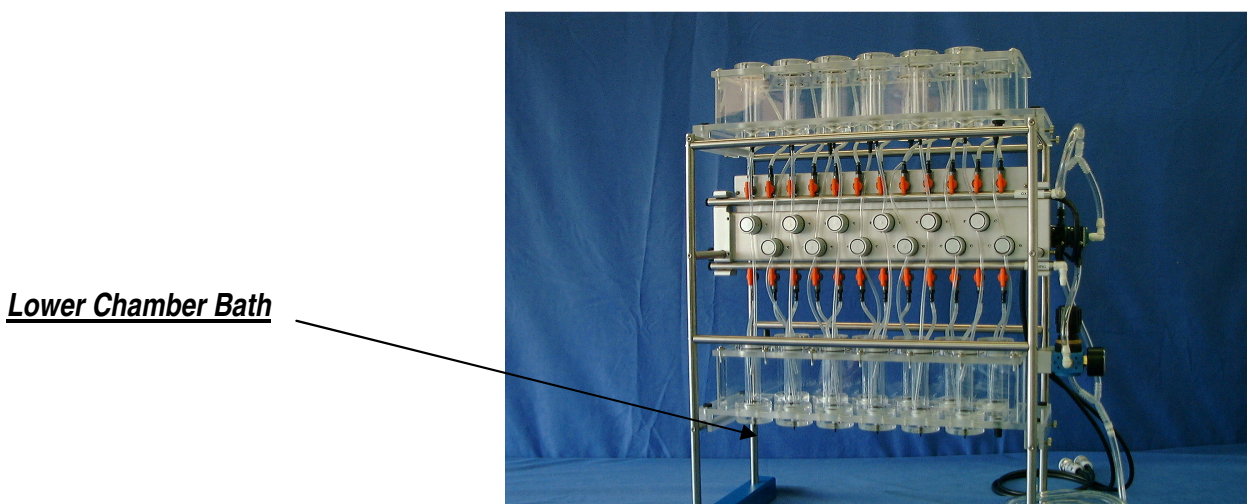


Figure 6 “Lower Chamber Bath”

Now consider the Suction Pump:

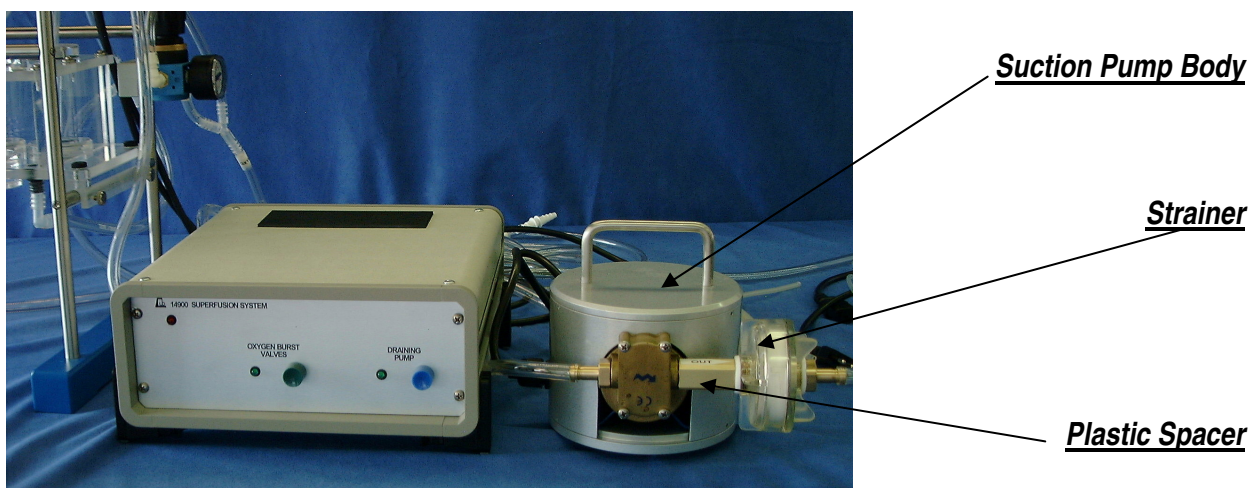


Figure 7 “Suction Pump”



First lock the plastic spacer on the pump body, then position the strainer as shown by Figure 7 “Suction Pump”.

2.5 Connections

Connect the Tygon tubes as indicated in Figure 8 “Piping”.

Carefully fasten all the Tygon tubes by the plastic Tie-Wraps provided.



Figure 8 “Piping”

The upper and lower baths must be connected in parallel and fed by the Water Circulator/Heater (optional).

Connect the circulator output to the lower water outlet of both lower and upper baths by the “T” junction provided.

The output tubes of these baths are led to the circulator by another “T” junction. In this way, the bubble-purging operation becomes easier.

The Oxygenation “Y” tube is mounted on the main frame and connected to the pressure gauge of the O₂ manifold on one arm of the “Y” and to the O₂ Electrovalve on the other arm. Link the “Y” stem to an Oxygen cylinder, adjusted for a pressure of about 1 or 2 bars.

The pressure must be held quite low, just to have a moderate air flow bubbling into the chambers.

Connect the suction pump to the draining manifold (see Figure 4 “Manifolds”), paying attention to the vacuum direction, as shown by an arrow marked on the pump itself.

Each chamber of the upper bath is provided with a small oxygenation tube; connect it to the corresponding output of the O₂ manifold.

Connect a tube to the bottom outlet of each chamber in the upper bath; the tube goes throughout its electrovalve and drops into the corresponding chamber on the lower bath.



Connect the bottom outlet of the lower chambers to a Multi-channel Peristaltic Pump (optional), in order to collect the perfusate.

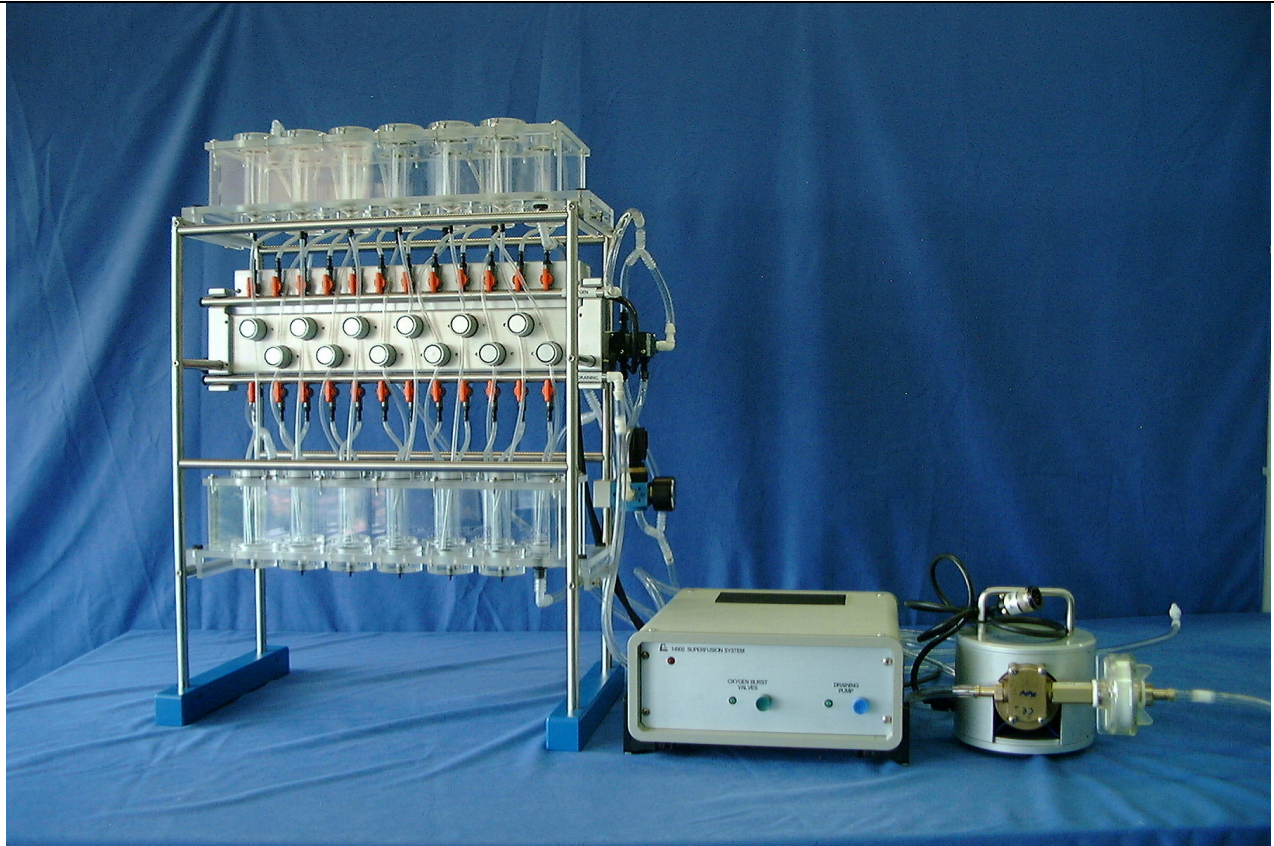


Figure 9 “Assembled Instrument”

The Suction Pump and the Valve Block must be electrically linked to the back panel of the Electronic unit, via the corresponding connectors, see also paragraph 2-Electronic Unit. A second connector marked “electrovalves” is provided in case the system is doubled to 24 chambers.

2.6 Before Applying Power

Take a look at the Power Module, on the right of the Electronic Unit back-panel, which encompasses – from left to right - the inlet connection of the mains cord, the mains switch and the fuse holder/voltage selector.

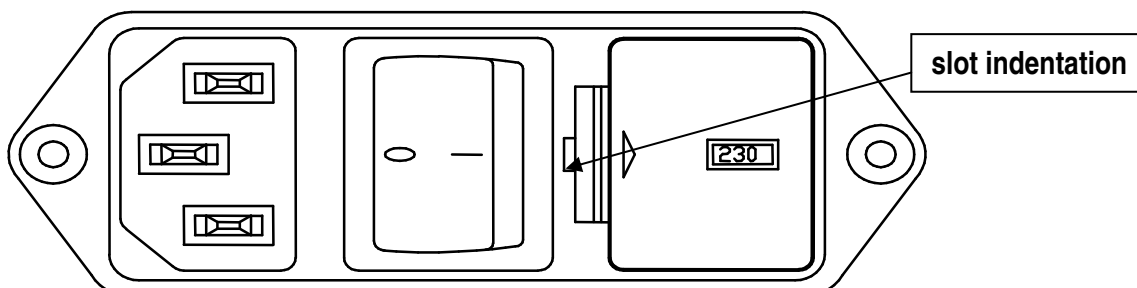


Figure 10 “Power Module”



2.6.1 Mains Switch

This two-pole toggle switch, which complies with international safety standards, provides a visual cue, meaning:-

- **OFF** when the “O” side is depressed
- **ON** when the “I” side is depressed

2.6.2 Fuse Holder & Voltage Selector

The fuse holder comprises two fuses, one on the live, and the other on the neutral. For operation at 220-230 Volts, we recommend 1.25 A timed fuses (type T1.25). Use 2.5 A fuses (type T2.5) for operation at 115 Volts. To replace the fuses, see paragraph 4.1-Electrical.

The fuse holder also embodies the Voltage Selector. Make sure that the flag indicates the correct voltage (i.e., the voltage of your mains). To change the selected voltage, see paragraph 4.1.

2.6.3 Mains Cord

It is a standard cable, Cat. # E-WP008. Make sure your power outtake is provided with a reliable ground connection, see also 2.7 & 2.8.

2.7 Connection to the Mains

Connect the mains cord of the Electronic Unit to a power outtake, **provided with a reliable earth connection**, protected by a differential earth-leakage switch (CGFI) that breaks at the threshold of 0.03A, within a max. acceptable delay of 1s. The maximum breaking compliance required by the CGFI is 10kA at rated current is 6A.

2.8 Additional Safety Consideration



UGO BASILE DOES NOT ACCEPT ANY RESPONSIBILITY FOR PROBLEMS OR HARM CAUSED TO THINGS OR PERSONS ARISING FROM:

- ◆ INCORRECT ELECTRICAL SUPPLY;
- ◆ INCORRECT INSTALLATION PROCEDURE;
- ◆ INCORRECT OR IMPROPER USE OR, IN ANY CASE, NOT IN ACCORDANCE WITH THE PURPOSE FOR WHICH THE INSTRUMENT HAS BEEN DESIGNED AND THE WARNINGS STATED IN THE INSTRUCTION MANUAL SUPPLIED WITH THE INSTRUMENT;
- ◆ REPLACEMENT OF ORIGINAL COMPONENTS, ACCESSORIES OR PARTS WITH OTHERS NOT APPROVED BY THE MANUFACTURER;
- ◆ SERVICING CARRIED OUT BY UNAUTHORIZED PERSONNEL.

- a. Place your Superfusion Bath Assembly on a steady flat surface (e.g. your experimentation table).



- b. Do not obstruct free and comfortable access to the power module.
- c. Use original accessories and spare parts only, see also paragraph 5-ORDERING INFORMATION.
- d. Immediately disconnect and replace a damaged mains cable.
- e. Do not operate in hazardous environments or outside prescribed environmental limitations (i.e. $+10^{\circ}\text{C}$ / $+40^{\circ}\text{C}$, 95% relative humidity, non-condensing), see also paragraph 5.2-Specifications.
- f. Do not spray any liquid on the connectors; see also paragraph 4-MAINTENANCE.

3 OPERATION

Switch on the electronic unit. The two push-button switches located on the electronic-unit front-panel activate the valves and the pump respectively. The green LED, located near each buttons, show when the valves are open and the pump is working. See also paragraph 1.1.2-Electronic Unit.

The blue switch activates the suction pump for approximately 4 seconds; this time is usually sufficient for carrying out a complete draining.

The green switch which opens the valves **must be held depressed** until the liquid from the upper chambers has completely been drained into the lower chambers.

Note that when the green switch is released, the circuit automatically activates the Oxygen Valves for a short period (approx. 4 seconds), to provide a pressure burst to the system in order to eliminate bubbles. After the burst, the pressure resumes the usual operational pressure, which can be adjusted on the pressure-reducing valve of the oxygen-cylinder, in order to attain 0.5-1 bar.

A visual check is the best way to assess the ideal pressure.

At this point the upper chambers can be filled again for further experimental trials.

The core of the system is 0,65 micron **MILLIPORE FILTER** type DA, mounted at the bottom of the lower chambers. Its individual plastic holding-ring is attached to the chamber by 4 small magnets.

It is important each chamber is filled with the same amount of liquid in order to have a constant oxygenation rate and to ensure a complete simultaneous draining of all the chambers.

See paragraph 7-APPENDIX – RELEASE EXPERIMENTS (written by Prof. Raiteri and his team) for a description of the experiment step by step.

A reprint of paper mentioning the 14900 Superfusion System is also attached for reference, see also paragraph 6.2.



4 MAINTENANCE

While any service of the instrument is to be carried out by Ugo Basile personnel or by qualified personnel, authorized by UGO BASILE organization, this section of the instruction manuals describes normal maintenance procedures which can be carried out at the customer's facilities.



UNPLUG THE MAINS CORD BEFORE CARRYING OUT ANY MAINTENANCE JOB!

4.1 Electrical

To inspect and/or replace the fuses, **disconnect the mains cable first!** Insert a miniature screwdriver in the slot indentation, see paragraph 2.6, and snap out the slide which houses the fuses.

For operation at 220-230 Volts, we recommend 1.25 A timed fuses (type T1.25). Use 2.5 mA fuses (type T2.5) for operation at 115 Volts.

Having extracted the fuse slide, the Voltage selector becomes accessible. The same miniature screwdriver will help you to pry out the cross jumper on which the operation voltage is engraved. Place the jumper upside down if you have to shift from 115 to 230V or viceversa.

Snap in the fuse slide: the mechanical “click” ensures that it is locked. Check the voltage flag before applying electrical power.

4.2 Cleaning

Organic solvents should not be used for cleaning the Bath assembly as they may impair the Perspex surface. A mild detergent and cotton wool are recommended.

4.3 Long Inactivity

The instrument does not require any particular maintenance after long inactivity, except cleaning.

4.4 Customer Support

For any further information you may desire concerning the use and/or maintenance of the Dynamic Plantar Aesthesiometer, please do not hesitate to get in touch with our local distributor or with our **service department** at:-



UGO BASILE

Viale G. Borghi 43
21025 COMERIO – Varese, ITALY



Phone : +39 0332 744574



Fax : +39 0332 745488



e-mail : service@ugobasile.com

We recommend you to get in touch with our service department **before sending any instrument to our factory for repair.**

5 ORDERING INFORMATION

14900 SUPERFUSION SYSTEM, standard package, including:-

14900-001 Electronic Unit

14900-002 Superfusion Bath Complete Assembly

14900-004 Suction Pump

14900-302 Instruction Manual

E-WP008 Mains Cable

Set of 2 fuses for either 115 VAC or 230 VAC mains

5.1 Optional

14900-003 Water Circulator/Heater

14900-005 Masterflex Multi-Channel Peristaltic Pump

5.2 Specifications

Power Requirement : 115 or 230 V, 50/60 Hz, 100 W max.

Operating Temperature : 15° to 30° C

Sound Level : < 70 dB (A)

Dimensions

- electronic unit : cm 38 (w) x 30 (d) x 13 (h)

- assembled bath : cm 46 (w) x 28 (d) x 60 (h)

Total Weight : 34.00 Kg



Shipping Weight : 45.00 Kg approx.
Packing Dimensions : No. 1 carton box 80x60x44 cm
No. 1 carton box 66x50x63 cm

6 BIBLIOGRAPHY

6.1 Method Paper

- M. Raiteri, F. Angelini, G. Levi: "A simple apparatus for studying the release of neurotransmitters from synaptosomes" Eur. J. Pharmacol. 25: 411-414, 1974

6.2 Paper Mentioning 14900

- L Raiteri and M. Raiteri: "Synaptosomes Still Viable after 25 Years of Superfusion" Neurochem. Res. Vol. 25: 463-468, 1996.

THE ABOVE REPRINT IS ATTACHED FOR REFERENCE

6.3 Additional Paper

- A. Pittaluga et al.: "Human brain N-methyl-D-aspartate receptors regulating noradrenaline release are positively modulated by HIV-1 coat protein gp120" AIDS 10: 463-468, 1996.
- M. Di Luca et al. "Increased presynaptic protein kinase C activity and glutamate release in rats with a prenatally induced hippocampal lesion" Eur. J. Neurosci. 9: 472-479, 1997.
- M.V. Clos et al. "D2 dopamine receptors and modulation of spontaneous acetylcholine (ACh) release from rat striatal synaptosomes" Br. J. Pharmacol. 122: 286-290, 1997.
- D. Crespi et al. "Carrier-dependent and Ca²⁺-dependent 5-HT and dopamine release induced by (+)amphetamine, 3,4-methylenedioxymethamphetamine, p-chloroamphetamine and (+)fenfluramine" Br. J. Pharmacol. 121: 1735-1743, 1997.
- E. Schlicker et al. "Effects of selective h5-HT_{1B} (SB-216641) and h5-HT_{1D} (BRL-15572) receptor ligands on guinea-pig and human 5-HT auto- and heteroreceptors" Naunyn-Schmiedeberg. Arch. Pharmacol. 356: 321-327, 1997.
- G. Maura et al. "Glutamate release in human cerebral cortex and its modulation by 5-hydroxytryptamine acting at h 5-HT_{1D} receptors" Br. J. Pharmacol. 123: 45-50, 1998.
- R. Sala et al. "Nerve growth factor and brain-derived neurotrophic factor increase neurotransmitter release in the rat visual cortex" Eur. J. Neurosci. 10: 2185-2191, 1998.
- M. L'Hirondel et al. "Lack of autoreceptor-mediated inhibitory control of dopamine release in striatal synaptosomes of D2 receptor-deficient mice" Brain Research 792: 253-262, 1998.



In addition, more than 300 full papers using superfused synaptosomes have been published



7 APPENDIX – RELEASE EXPERIMENTS

7.1 Preparation of Physiological Solution

1.5 l of standard medium and 0.25 l of stimulation medium are normally sufficient for a typical experiment.

7.1.1 Standard Medium

Two 20 x stock solutions (sol. A and sol. B) are prepared as follows:

Solution A composition (20x)

Salt	final concentration (mM)	g/l (20 x)	MW
NaCl	125	146.1	58.44
KCl	3	4.473	74.56
MgSO ₄ *7H ₂ O	1.2	5.916	246.48
CaCl ₂ *2H ₂ O	1.2	3.528	147.02

Solution B composition (20x)

Salt	final concentration (mM)	g/l (20 x)	MW
NaHCO ₃	22	36.96	84.01
NaH ₂ PO ₄ *H ₂ O	1	2.760	137.99

Sol. A and sol. B are mixed and diluted to the final salt concentration. The correct procedure consists of transferring the needed volume of sol. A in a cylinder and to dilute it with some water, then to add sol. B to reach the final volume.

Note: *never add concentrated A and B solutions together: precipitation of Ca⁺⁺ or Mg⁺⁺ hydroxides may occur.*

The medium prepared as described above is supplemented with 10 mM glucose (1.8 g/l).

The solution is aerated with a mixture of 95% O₂ and 5% CO₂ (carbogen) for about 30 min, which keep the final pH to 7.2-7.4.



Note: this time interval (30 min) is required to equilibrate the O_2 concentration in solution.

7.1.2 Stimulation Medium

Stimulation of release from synaptosomes can be obtained by adding appropriate substances (4-AP, veratrine, ecc) to the standard medium or by using a high potassium depolarising solution. The latter is prepared as to the standard solution using an appropriate "high K^+ " sol. A that should be mixed to the standard sol. B as described above.

Example

Composition of the 15 mM K^+ solution (20 x)

Salt*		g/l (20 x)
NaCl	113 mM	132.1
KCl	15 mM	22.37

* the amount of $MgSO_4 \cdot 7H_2O$ and $CaCl_2 \cdot 2H_2O$ remains unchanged.

7.2 Superfusion Apparatus Preparation

The superfusion apparatus can be prepared during aeration of the solutions:

- the thermostat provided with external circulation is switched on to reach and maintain the temperature of the reservoirs and of the superfusion chambers at 37°C;
- the microporous filters, on which synaptosomes will be stratified, are lodged on the fritted glass holders and fitted to the bottom of each superfusion chamber;

Note: we normally use Millipore filters 0.65 μm (cat. n. DAWP02500) but a number of microporous filters have been proven to work as well (e.g. other Millipore filters, Whatman GF-B filters, etc.)

- when all the holders are positioned, the superfusion chambers will be filled with few ml of water that will be eliminated just before starting superfusion. This enables the leakage due to one or more misplaced filter to be detected;
- two ml of standard medium is placed into each reservoir and aerated with carbogen.

During labelling of synaptosomes (see below) the following procedures are suggested:

- drain the water contained in the superfusion chambers first by vacuum suction, then via the peristaltic pump through filters for a complete elimination;



- b. allow the two ml of warm and aerated medium contained in the reservoirs to descend into the superfusion chambers;
- c. fill the reservoirs with 7 ml of standard medium, which will be used during the first period of superfusion (see below).

Note: *filling of the reservoirs in advance enables the media to warm up and aerate sufficiently before operation. Less than 5 min are normally sufficient for 10 ml of medium to reach 37°C.*

7.2.1 About aeration

While media can be continuously aerated without problems, blowing O₂/CO₂ mixture directly into the synaptosomal suspension or in the superfusion chamber is not recommended.

In fact, due to the lipid materials contained in the synaptosomal preparations, the aeration may create problems (foaming and so on). We overcame this problem by aerating again all the media that will be put in contact with synaptosomes at least for 5 min and by replacing these media at least every 10 min. The pH remains constant during this time interval.

7.3 Labelling

Crude or purified synaptosomes, prepared with standard procedures, are resuspended in 5 ml of standard medium, which had been aerated at room temperature, and transferred into a glass scintillation counting vial.

The correct amount of radioactivity is added to the synaptosomal suspension directly into the vial. The vial ambient is insufflated with carbogen for one minute, then the vial is capped and placed in a rotary water bath at 37°C for 15 min.

At the end of the incubation period, the 5 ml of synaptosomal suspension are transferred to a pre-warmed conical flask and diluted with 35 ml of pre-warmed and aerated standard medium (final volume 40 ml). Three ml of synaptosomes are put in each of the 12 superfusion chambers.

7.4 Superfusion

7.4.1 Stratification

Synaptosomal suspension will be stratified in each chamber by aspiration, using the peristaltic pump at a speed of 1-2 ml/min.

- a. Check that stratification proceeds evenly



Note: *if one or more chambers are much slower than the others it is more convenient to cut them off rather than risking damage of synaptosomes in the other chamber (it is very harmful to leave synaptosomes dry!)*

- b. At the end of the stratification, switch off the peristaltic pump and allow the pre-warmed 7 ml medium to descend into the superfusion chambers.

7.4.2 Superfusion

- a. Set the pump to the desired superfusion speed (we suggest 0.5 ml/min) and start superfusion ($t = 0$).
- b. Add another 7 ml of standard medium in the reservoirs (2nd addition).
- c. At $t = 10$ min superfusion, renew the medium in the superfusion chambers with the 7 ml of pre-warmed and aerated medium already put in the reservoirs, without stopping superfusion.
- d. Fill the reservoirs with another 7 ml of fresh medium (3rd addition).
- e. At $t = 20$ min superfusion, renew the medium chambers, without stopping superfusion.
- f. Fill the reservoirs with another 7 ml of fresh medium (4th addition).
- g. At $t = 30$ min, again allow the pre-warmed medium to move downward for a next superfusion period. The following period lasts 8 min; afterwards synaptosomes will be stimulated.
- h. Fill the reservoirs with 10 ml stimulation medium (5th addition).

Note: *the first three 10 min superfusion will balance the system and allow a constant baseline level.*

Note: *the superfusion flow is about 0.5 ml/min; thus, after each 10 min interval some medium will remain in the chamber. This medium can be eliminated "on line" by the vacuum pump by sucking it directly from the inside of the superfusion chamber.*

Note: *during each of the described periods, synaptosomes can be exposed to different drugs added to the superfusion medium, accordingly to the experimental design. In a typical experiment we add receptor agonists concomitantly with the depolarizing stimuli and antagonists, 8-min before the stimulus.*

7.4.3 Stimulation and sample collecting

This type of experiment is designed to study the effects of drugs on the depolarization-evoked transmitter release: a standard 90 s of depolarization pulse is described.



- a. At $t = 36$ min, collection of the superfusate fractions begins according to the following scheme: two 3-min fractions ($t = 36-39$ and $t = 45-48$, basal release) before and after one 6-min fraction ($t = 39-45$; evoked release);

Note: *superfusate fractions are collected into vials that can be directly counted for radioactivity (when radioactive tracers are used)*

- b. At $t = 38$ min, the medium containing the depolarizing stimulus and the drug(s) under study is introduced in the superfusion chamber. About 10 s before this action, the excess of medium left in the chambers can be drained by vacuum suction;

Note: *Due to the length of the tubing from the filter holder to the collecting vial, a lag time, normally longer than 1 minute takes place; this is the reason why the depolarizing medium is introduced 1 min before the change of the collecting vial*

- c. The reservoirs are filled immediately with 10 ml of pre-warmed and aerated repolarizing medium (usually standard medium);

Note: *you have about 45–50 s to fill the reservoirs, before starting the collection of the second fraction. Filling all reservoirs during this time will be easy if you have to hand a dispenser of adequate capacity, containing the pre-warmed and pre-aerated repolarizing medium.*

- d. At $t = 39$ min, collection of the second fraction is started and continued for 6 min;
- e. At $t = 39$ min and 15 s the excess of depolarizing solution (about 9 ml) in each superfusion chamber is collected by vacuum suction;
- f. At $t = 39$ min and 30 s, allow the prewarmed 10 ml of repolarizing medium to descend into the superfusion chambers (stimulus length = 90 s);
- g. At $t = 45$ min, the collection of the third fraction is started and continued for 3 min;
- h. At $t = 48$ min, the peristaltic pump is switched off and the remaining medium eliminated by suction.

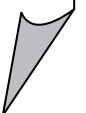
7.4.4 End of the experiment

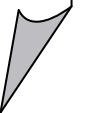
- a. Holders are disconnected from the superfusion chambers and the filters placed into 12 scintillation vials;
- b. Filters are added with a scintillation mixture or extracted for endogenous transmitter determination.
- c. Collected samples and superfused filters are counted for radioactivity or analyzed for their endogenous transmitter content.



N.B.: The apparatus needs to be carefully washed immediately after each experiment. In particular, to avoid ooze formation into the fritted glass we suggest washing with diluted sulphochromic solution (once every 8-10 experiments) followed by prolonged rinsing with distilled water.

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CE CONFORMITY STATEMENT

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We hereby declare that

Instrument. **SUPERFUSION SYSTEM**
Catalog number **14900**

*It is manufactured in compliance with the following European Union Directives
and relevant harmonized standards*

- *2006/95/CE relating to electrical equipment designed for use within certain voltage limits*
- *2004/108/CE relating to electromagnetic compatibility*
- *2011/65/UE on the restriction of the use of certain hazardous substances in electrical and electronic equipment*

Account Manager

Adriano Basile

Nome / Name

April 2014

Date

Firma / Signature