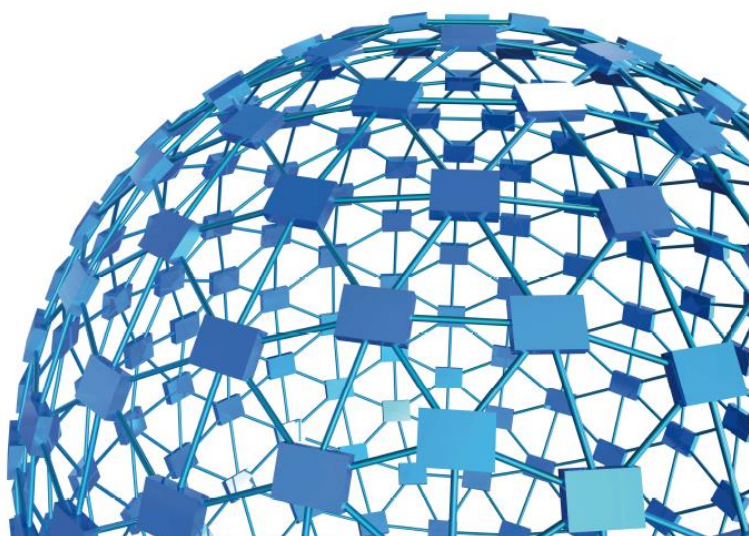




instruction manual

**Fear Conditioning Systems-NG
Series 46001**



UGO BASILE S.R.L.

sales@ugobasile.com / service@ugobasile.com www.ugobasile.com

UGO BASILE S.R.L.

Via G. Di Vittorio, 2
21036 GEMONIO, VA, ITALY
Phone: +39 0332 744574

sales@ugobasile.com / service@ugobasile.com
www.ugobasile.com

instruction manual

Fear Conditioning Systems Series 46001

Serial No.

SAFETY CONSIDERATIONS

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.

New Fear Conditioning System

Series 46001

General

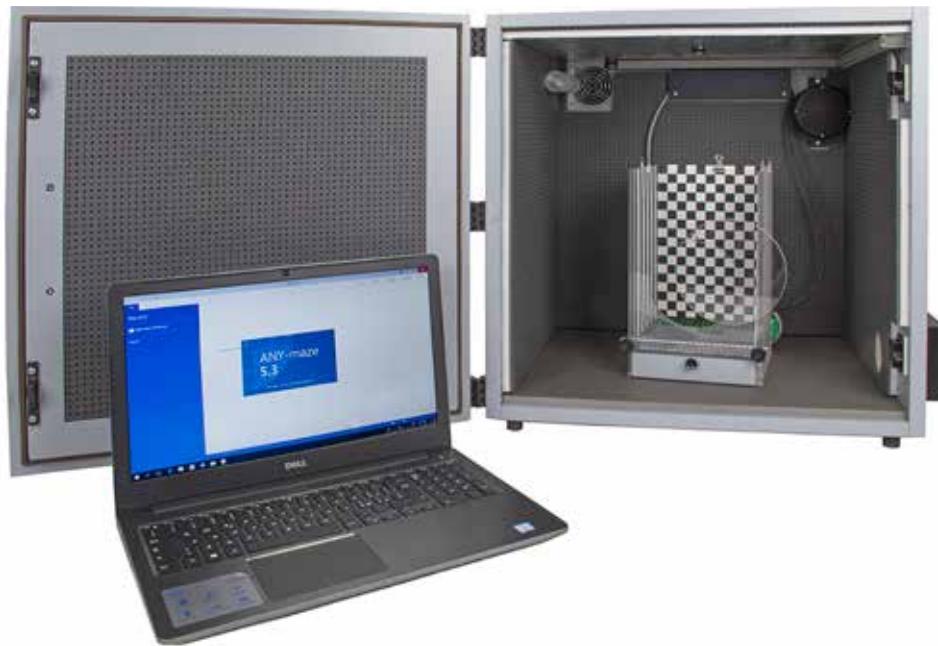
The **UGO BASILE ANY-maze controlled Fear Conditioning system** automates the two most common fear conditioning paradigms: **Contextual Fear Conditioning** and **Cued Fear Conditioning**. The detection of Freezing is automated and based on video analysis.

A typical **FC-System** consists of:

- an FC-Unit, encompassing a Sound-Attenuating Box, with ventilating fan, a dual (visible/I.R.) light, a speaker & a USB-camera. Each FC-Unit has an individual controller on-board
- an FC-cage, for mouse or rat, (see ordering information for available models), with electrified floor & context kit (3 floors and 3 sets of patterned walls)
- ANY-maze software (from version 6.0, FC or full license)

Multiple systems, with virtually no limit, are easily assembled by multiplying the number of FC-Units and FC-cages, with no additional multiplexer or interface required.

ANY-maze controls the shocker, the sound generator and the light (I.R. and visible), and automatically detects the animal **freezing**, reporting information such as: total freezing time, number and duration of freezing episodes, latency times between stimuli and freezing events.



"I have been using your fear conditioning setup pretty heavily in the last months and I am really happy..."

Dr. Alexandra Klein, Max Planck Institute

Main Features

- **AUTOMATIC** detection of **FREEZING** also in **total darkness**
- Specific versions for rats or mice
- Select the most suitable cage: two sizes, available with optional features, such as specific versions for tethered animals, etc.

NEW to 2.1 version

- Extremely simple to set-up: just plug a USB cable into a single USB port on the FC-Unit
- Multiple Cage Set-up, with virtually no limit in number
- ANY-maze controls all phases of the test, managing the experimental parameters, detecting the animal response and analyzing the experimental data.

CHECK-LIST

Series 46001 Fear Conditioning Systems

CLIENTE / CUSTOMER _____

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

UB code	CAT.No.	DESCRIPTION	40001	46001-2	46001-3	46001-4	other
	46001	FC-Unit, including	1	2	3	4	
	46000-596	Sound-Attenuating Cubicle					
	46000-165	Speaker (assembled in the Cubicle)					
	46000-325	Dual Light (assembled in the Cubicle)					
	46000-105	Controller on-board & hub (assembled on the Cubicle)					
	47400-035	USB-Camera, with 2m USB cable					
E-AU 078		Power Supply for Controller 46000-105					
	52010-323	USB cable with connect. A/B 1.8mt	1	2	3	4	
E-WP 008		Power Cord	1	2	3	4	
E-AU 041	41500-302	Instruction manual of USB flash drive (E-AU 041)	1	1	1	1	
ANIMAL CAGE/s							
	46002	Rat Cage					
	46003	Mouse Cage					
	46004	Mouse Cage XL					
	46002-D03	Rat Cage (for tethered animals)					
	46003-D03	Mouse Cage (for tethered animals)					
	46004-D03	Mouse Cage XL (for tethered animals)					

DATE ____ / ____ / ____ Serial No. _____ PREPARATO DA / PACKED BY _____

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**

FROM: Name _____ Company/Institution _____

DATE _____ REF. _____

NOTE :

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Fear Conditioning Units - ANYmaze

Series 4 6 0 0 1

1 GENERAL

1.1 Rationale for the technique

Fear Conditioning is a quick and reliable method to assess memory in rodents.

When rodents are exposed to fearful stimuli, they respond, among the others, with immobility behavior, also called “freezing”. All different Fear Conditioning procedures imply the measurement of the freezing response (*i.e.* the fraction of time spent immobile).

A rodent can learn to fear a previous neutral stimulus if that has been associated with an instinctively aversive one (such as an electric shock), or, in other terms, after having been conditioned.

After the conditioning has occurred, and the two stimuli have become associated, the animal is presented the neutral stimulus and, as a consequence, it will **freeze**¹. The time spent freezing is indicative of undergone memory and learning processes, which are at the basis of the association between neutral and aversive stimuli.

Fear Conditioning is a sensitive and quick test, which requires very little training (usually only one trial) to the animals. It is therefore a valuable tool for basic behavior research, as well as for high throughput and drug discovery studies. Moreover, the conditioning established during the Fear Conditioning procedure can be very strong (*i.e.* long-lasting), allowing for long term experiments.

1.2 Principle of Operation of the Fear Conditioning Systems

The Ugo Basile Fear Conditioning systems are designed to run experiments of Pavlovian conditioning on mice or rats.

Single-frequency sounds are delivered as neutral or conditioned, stimulus (CS). Experimental procedures which contemplate the sound as CS are usually referred to as **Cued Fear Conditioning**.

Electric current from the grid floor is delivered as the naturally aversive, or unconditioned stimulus (US).

Tests are run in an animal cage with a grid floor, whose appearance can be easily altered by attaching patterned contexts on the walls and floor. This allows for the investiga-

¹ Freezing is commonly defined as complete immobility, apart from respiratory movements

tion of **Contextual Fear Conditioning**, in which the CS is not the sound but the visual appearance of the cage (*i.e.* the context) itself. In this case the animal associates the electric shock to the environment where it was received.

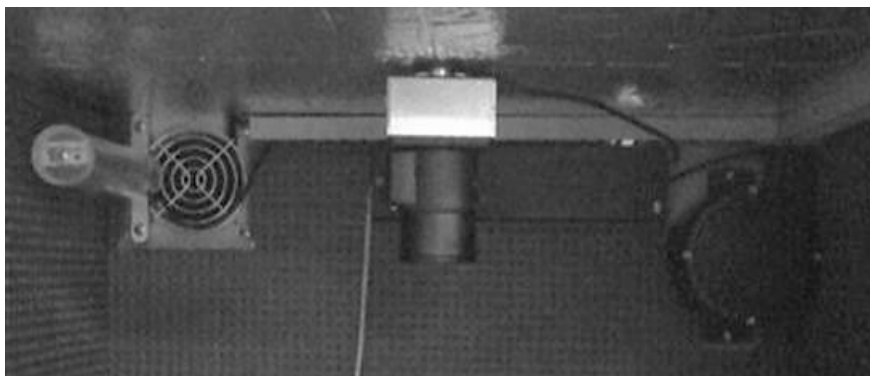
The detection of Freezing is automated and based on video analysis. The shock, light and sound parameters are controlled by software (USB) or manually, via the new Electronic Unit, based on touchscreen technology.

The context kit provided with each cage alter the animal cage appearance, allowing for both Contextual and Cued procedures to be run with the same system.

2 SYSTEM CONFIGURATION

The new FC-Unit 46001 encompasses all that you need to set-up a Fear Conditioning test:

- a Sound-Attenuating Chamber **46000-596**, see *paragraph 3.1*
- a noiseless fan for ventilation
- a dual (visible and I.R.) LED light **46000-325**
- a loudspeaker **46000-165**
- a controller on-board **46000-105**
- a USB camera **47400-035**, see *paragraph 3.3*



The system is completed by adding

- one or more animal cages, see *paragraph 3.4*
- ANY-maze software, see *paragraph 3.5*

It is extremely simple to set-up the new FC-Unit: just plug a USB cable into a single USB port on the FC-Unit.

2.1 Multiple Systems

Multiple Cage Set-up, with virtually no limit in number, are easily assembled by multiplying the number of FC-Units and FC-cages, with no additional multiplexer or interface required.

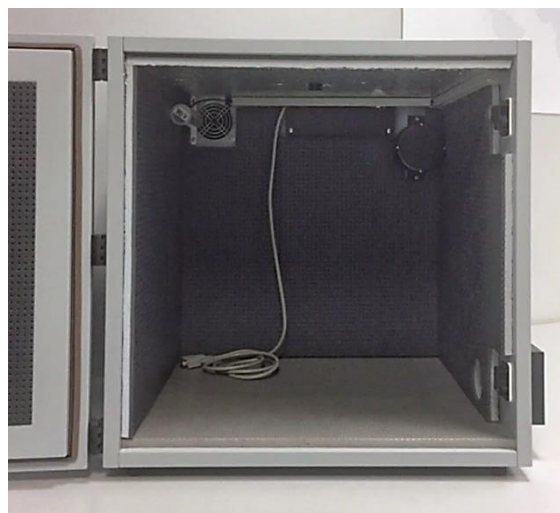
3 SYSTEM COMPONENTS

3.1 Isolation Cubicle

The Sound-Attenuating Cubicle **46000-596** is dimensioned 50(w)x(40)d)x50(h)cm, inside dimensions 48.5(w)x38.5(d)x48.5(h)cm).

It incorporates a noiseless ventilation fan, For air intake and exhaust, and a fixture to hold the USB-camera.

High-tech insulating material & door seal guarantee high-level sound-proof efficacy, a feature particularly useful when testing FC in multiple cage systems.



3.2 FC Controller On-Board

Each FC-Unit has an individual controller **46000-105** on-board.

It is the interface which receives instructions from ANY-maze and manages all the FC parameters, shock, light, sound.

See also paragraph 5.8-Connections.



3.3 USB-Camera

The 47400-035 USB-camera is sensitive to IR light, which allows for freezing detection even in total darkness.

Wide angle lenses and IR filters are included, as well as the necessary USB cable.



3.4 Animal Cages with Electrified Grid Floor

Ugo Basile Fear Conditioning Cages with Electrified Grid Floor are available in three models, with the following dimensions:

- **46002** Rat Cage
I.D.: 25.5x25.5x36(h)cm, external: 29x33x41(h)cm
- **46003** Mouse Cage,
I.D.: 17x17x25(h)cm, external: 24x20x30(h)cm
- **46004** Mouse XL Cage
I.D.: 25.5x25.5x36(h)cm, external: 29x33x41(h)cm

The mouse electrified grid is an array of 2mm diam. bars, spaced 8mm apart. In the rat cage bars have a diameter of 3mm and are spaced 12mm apart.



A set of removable contexts is provided to alter the colour and texture of the box walls and floor.

Each animal cage includes 3 striped walls, 3 checkered, 3 grey walls and 3 plastic floors (white, black, grey).

Custom contexts are available on request.



3.4.1 Optional FC-Cage for Tethered Animals

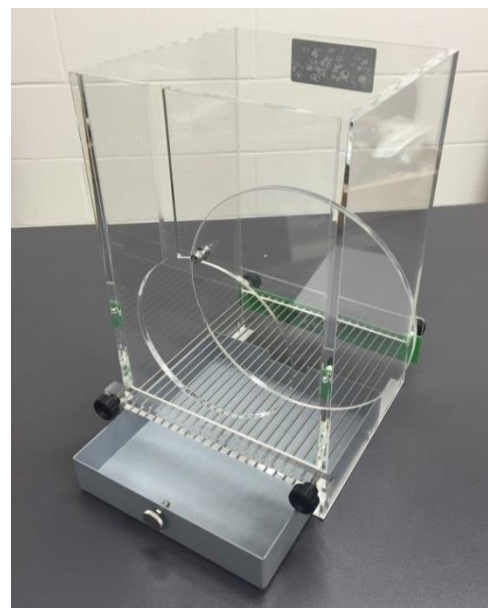
Optional models for tethered animals are available:

- **46002-D02** Rat Cage
- **46003-D03** Mouse Cage
- **46004-D03** Mouse XL Cage

With the same dimensions and grids as the standard models.

These cages are provided with a slot, for an easy insertion of the tethered animal into the cage, and transparent walls, for an unobstructed lateral camera view.

These special cages are particularly useful for optogenetics studies.



3.5 ANY-maze Software

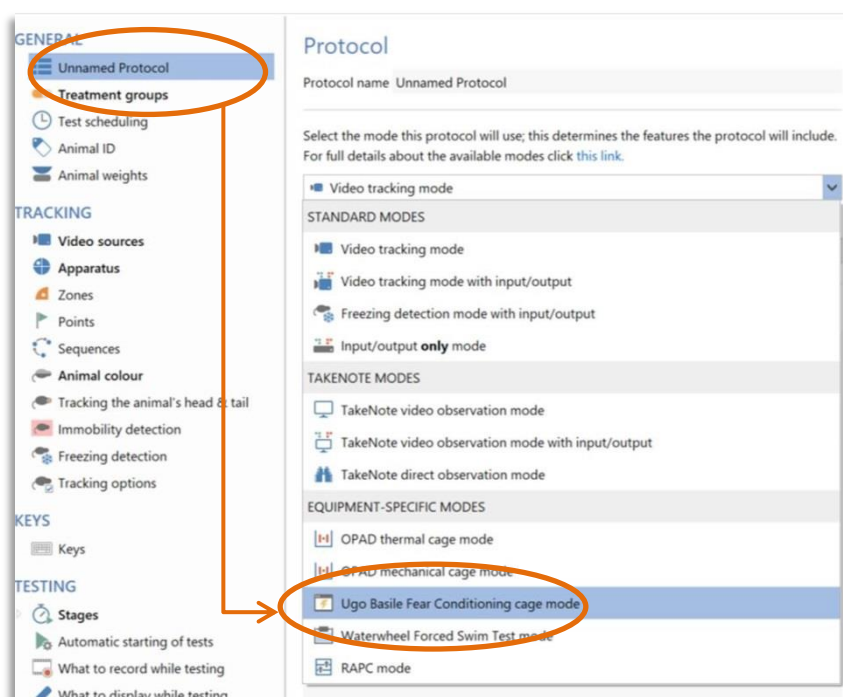
ANY-maze is the brain of our new FC-system.

It controls all phases of the test, managing the experimental parameters, detecting the animal response and analyzing the experimental data



An **Ugo Basile Fear Conditioning mode** is provided in the Protocol list to facilitate the set-up:

by asking some questions about the way you want to set up the cages, **ANY-maze creates lots of the protocol items for you!**



During the testing phase, ANY-maze detects freezing, collects experimental data, and analyzes the results, reporting information such as: total freezing time, number and duration of freezing episodes, latency times between stimuli and freezing.

ANY-maze is available in the following versions:

- **60000-FC** a specific version dedicated to Fear Conditioning Test
- **60000** Full Licence, a versatile tracking software, which also includes the dedicated FC version.

For instructions on ANYmaze, please refer to the **HELP section** online.

NOTE: to manage the new FC-Units, ANYmaze version 6 or higher are required. Upgrades are available. Ask for details

4 SYSTEM SPECS.

Warranty	
Warranty	Ugo Basile Fear Conditioning Systems are covered by a 24-month warranty.
Setting	
Shock	constant current
Shock Intensity	from 0.1 to 3.0 mA, in 0.1 mA steps
Shock Duration	duration: from 0.5 to 99 seconds
Visible Light	variable from 0 to 100%
I.R. Light	variable from 0 to 100%
Sound	100Hz-18KHz, 0-100% (100%=100dB \pm 3dB) and white noise 0-100% (100%=77dB \pm 3dB)
Physical	
Power	80V/240Vac, 50/60 Hz
Dimensions	Cubicle 55(d)x60(w)x57(h)cm Mouse Cage 24(w)x20(d)x30(h)cm, ID 17x17x25(h)cm Rat Cage 33(w)x29(d)x41(h)cm, ID 25.5x25.5x36(h)cm
Shipping Dimensions	82x71x57cm for each unit
Weight	35Kg, for each unit
Shipping Weight	42Kg, approx. for each unit

4.1 PC Requirements



ANY-maze will run on almost any Windows based computer: ANY-maze runs under all versions of Windows from XP to Windows 10.

If you're only planning to track a single animal then there aren't really any special requirements and almost any computer built in the last five years should work fine. However, if you want to track in multiple set-up simultaneously then you may need a more powerful machine.

	Minimum specification	Recommended specification	Power system
Windows version	XP service pack 3	Windows 10	Windows 10
Processor	Pentium Dual-core	Core i3	Core i7
RAM	1GB	4GB	8GB
Hard drive	1GB available	500GB	1TB
Display	1366 x 768 24-bit colour	1600 x 900 32-bit colour	1920 x 1080 32-bit colour
USB ports	1 available	2 available	4 available
Expansion	Not applicable	Not applicable	PCI express ports

If you're planning to track in multiple apparatus, or track for long periods (> 12 hours), or include large numbers (> 500) of animals in experiments then we recommend you read the whitepaper *Choosing a computer for ANY-maze* which discusses various technical considerations that you may need to take into account.

4.2 Intended Use

The 46000-Series fear Conditioning Systems are intended for investigation on laboratory animals only.

4.3 Intended Environment

Storage Conditions:

- Temperature : 0°C to 40°C
- Humidity: 0 to 70%

Operating Conditions:

- Temperature : 10°C to 30°C
- Humidity: 0 to 60%

5 INSTALLATION

5.1 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.

5.2 Notes on the Instruction Manual

The Instruction Manual included in the package (on the USB flash drive) is necessary for the correct installation and operation of the instrument.

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

Free of charge copies of the instruction manual are available upon request: download them from our web page, or contact our service department (see paragraph 7.4- Customer Support) specifying the series number of your instrument.

5.3 ANY-maze Software



Make sure you install ANY-maze on your PC **first** and then connect FC system.

See <http://www.anymaze.co.uk/anymaze-downloads.htm>

5.4 Before Applying Power

The instrument is powered by a single external power supply, no manual voltage selection is required because instrument automatically adapts to line voltage.

5.4.1 Mains Cord

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

5.5 General Safety Instructions

The following guidelines must be followed to ensure safe operation.

- ! **DO NOT** attempt to open or perform any service work
- ! **DO NOT** connect up human subjects



5.5.1 Additional Safety Consideration

- a. Place your system on a steady flat surface.
- b. Do not obstruct a comfortable access to the power module.
- c. Use original accessories and spare parts only, see paragraph 8.
- d. Immediately disconnect and replace damaged mains cord.
- e. Do not operate in hazardous environments or outside prescribed environmental limitations (i.e. +10C° / +40C°, 95% max. relative humidity, non-condensing)
- f. Do not spray any liquid on the connectors and on the geared motor.

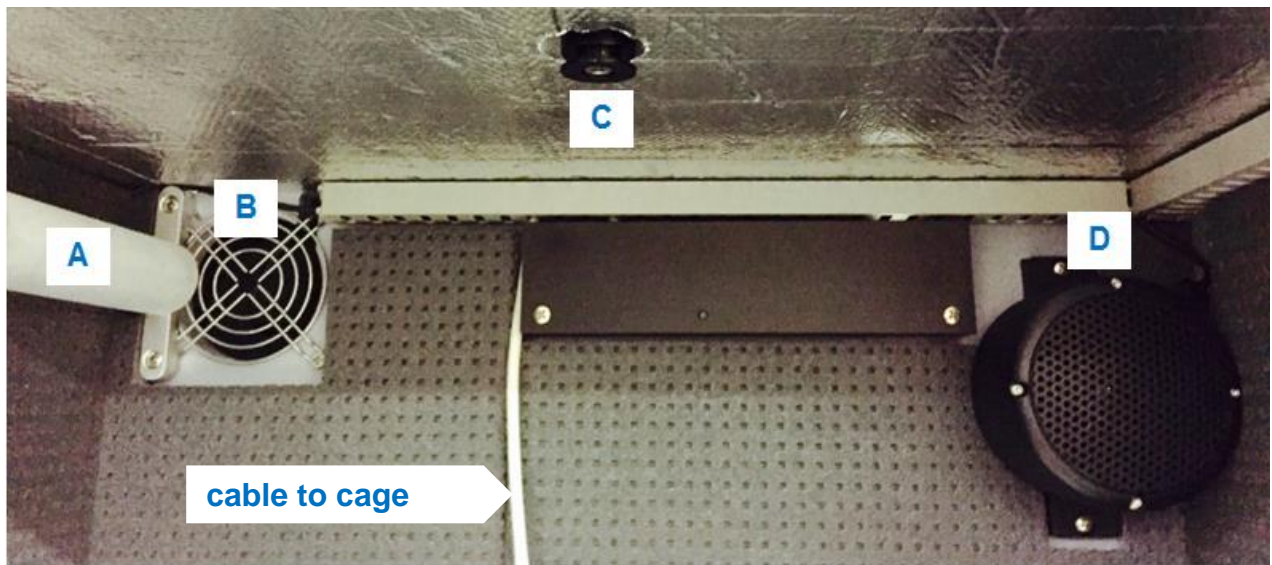
UGO BASILE DOES NOT ACCEPT ANY RESPONSIBILITY FOR PROBLEMS OR HARM CAUSED TO THINGS OR PERSONS, ARISING FROM:

- 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

see also paragraph 7-MAINTENANCE.

5.6 Setting Up the FC Unit/s

The Fear Conditioning Unit 46001 is delivered assembled and tested, the visible/I.R. light, the fan are in fact already assembled inside the Cubicle. Here's a view of the cubicle inside, where you can see, from left to right the dual light **A**, the fan **B**, the camera fixture **C**, and the speaker **D**:



Assemble the USB-camera on its mounting block, gliding on the clamp fixed to the cubicle ceiling.

5.7 Cage

Position the cage inside the cubicle.

Connect the D-SUB cable you find inside the cubicle, to the matching connector located on the back wall of the cage. See picture.

You will notice the LED on the left of the socket connector; the LED blinking which indicates the current is correctly delivered to the floor bars.

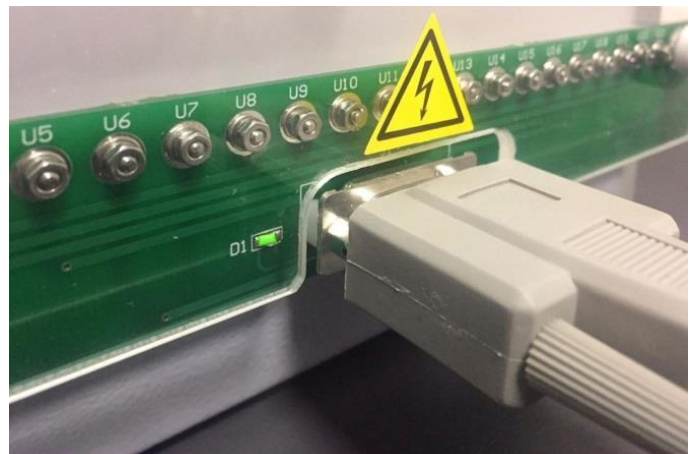


Figure 1 "Cage Connection"

5.8 Connections

On the back of the isolation cubicle, you will find, neatly assembled, the controller on board 4600 **E**, and the connection panel **F**, which encompasses the following connectors:

- **Connector 1** (grey): shock
- **Connector 2** (red): light
- **Connector 3** (black): sound
- **Connector 4** (blue): fan and door-switch

Possible mismatching is prevented by color coding

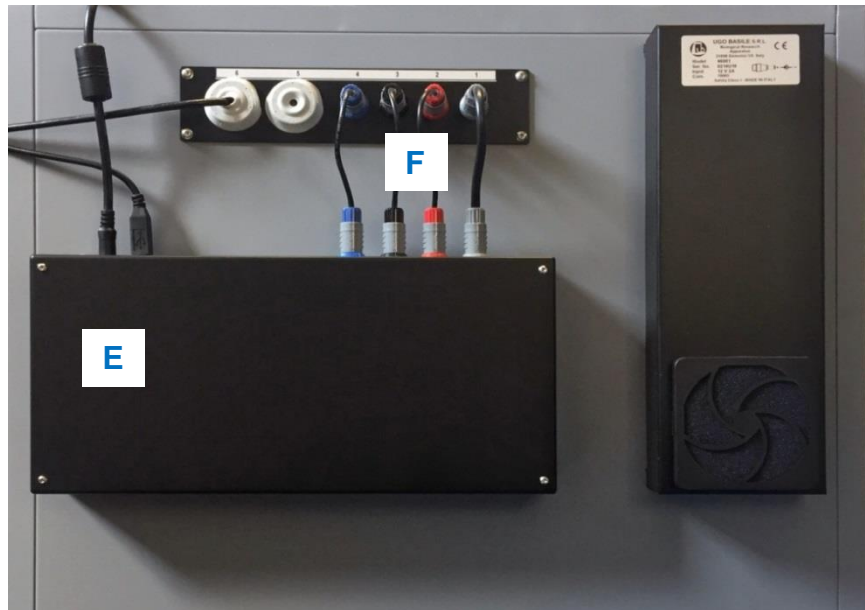


Figure 2 “Cubicle Back Panel”

In addition, on top of the controller on board, you will see, from left to right:

- Power switch
- Cable to power supply
- USB cable

As well as the 4 connectors to cage, with the color code described in previous paragraph.



The socket labelled “**I/O box**” enables the connection of our 4+4 channel TTL (in&out) I/O Box 46000-150, while the “BUS A-B” connector is not used.

5.8.1 Hub/USB-Camera Connections

The hub is part of the connection arrangement, as it gathers the connections to the controller on board, the USB-camera, and to the PC.

Make sure the connection scheme follows the labels on the hub, see picture:



Figure 3 “Hub Labelling”

6 CONTROLS, DETECTION AND DATA COLLECTION

All controls, including stimulus timing, freezing detection and data collection are all managed by ANYmaze (from version 6.0, FC or full license).

ANY-maze controls the shocker, the sound generator and the light (I.R. and visible), and automatically detects the animal **freezing**, reporting information such as: total freezing time, number and duration of freezing episodes, latency times between stimuli and freezing.

An **Ugo Basile Fear Conditioning mode** is provided in the **Protocol** list to facilitate the set-up: by asking some questions about the way you want to set up the cages, **ANY-maze creates lots of the protocol items for you!**

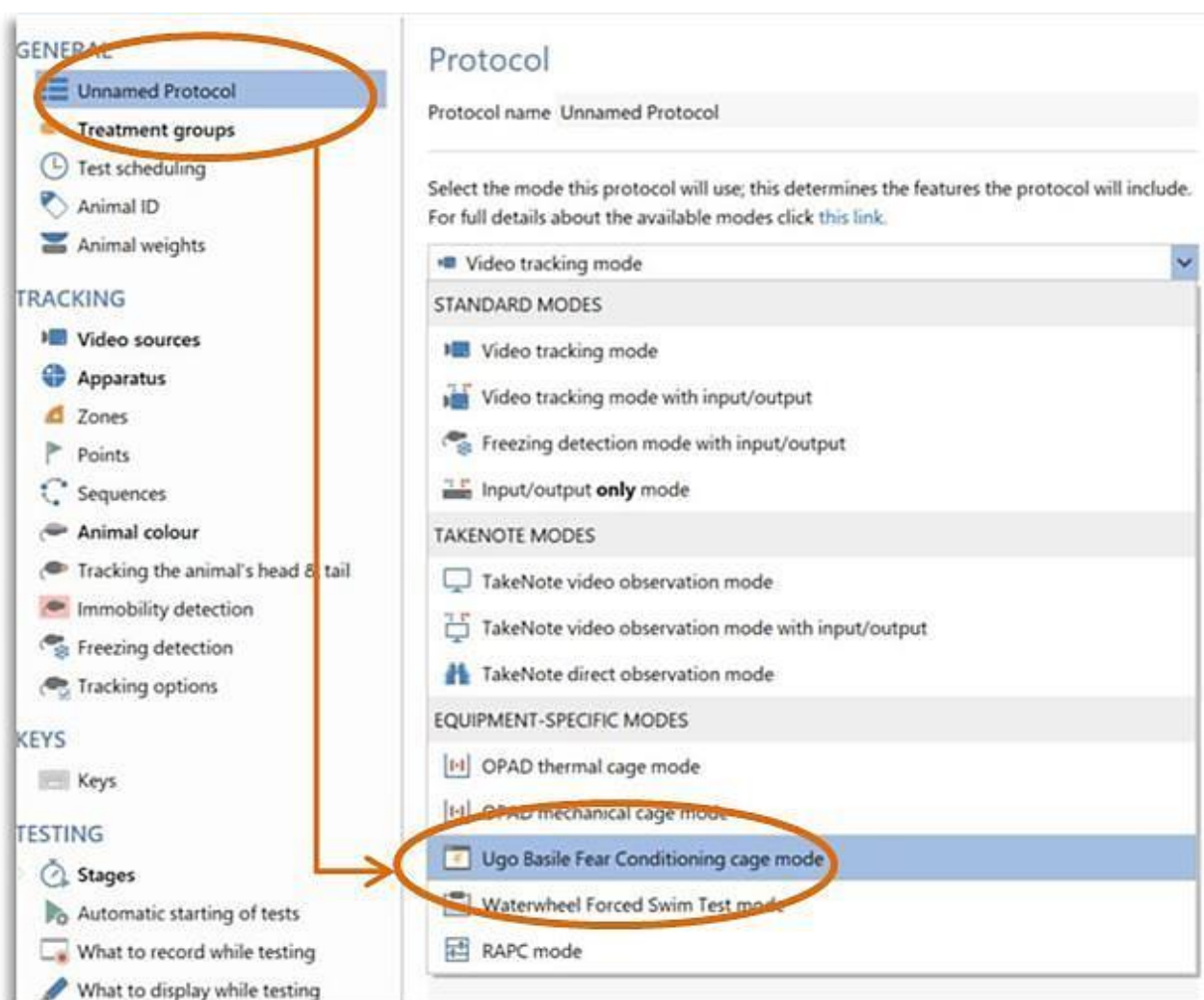


Figure 4 “Ugo Basile FC Protocol on ANY-maze”



Please refer to ANY-maze extensive HELP section for complete instructions.

6.1.1 Update SW

Upload the software update received from Ugo Basile on a USB flash drive or downloaded from our web page. Insert the flash drive on one of the USB ports available on the side panel of the controller on-board. Additional instructions are provided with the update.



Wait at least 15 seconds, then press the UPDATE SW icon. Press OK to continue with the update.



An error message will appear if the file is not available on the USB key or if the name is not correct.

7 MAINTENANCE

Ugo Basile Fear Conditioning Systems are covered by a 24-month warranty. Service, if necessary, has to be done by authorized UB personnel only; servicing by anyone other than an authorized service facility will void the warranty. If a problem occurs, contact your representative, see paragraph 7.4-Customer Support.



UNPLUG THE MAINS CORD BEFORE CARRYING OUT ANY MAINTENANCE JOB!

7.1 Cleaning and Sterilization

The Fear Conditioning hardware may be wiped down with a dry, lint-free cloth. Do not use chemicals or abrasive elements.

Under no circumstances allow moisture to penetrate the instrument. Unplug power cord from AC outlet before cleaning.

When cleaning the FC cage, avoid the use of organic agents, which will impair the transparent walls.

7.2 Disposal

Send to a recycling center equipped to handle electronics.

7.3 Abnormal conditions

Operate the instrument only as intended by the manufacturer. If you suspect the FC protection has been impaired, disconnect the power cord and secure the instrument against any unintended operation.

The protection is likely to be impaired if, for example, the instrument shows visible damage or has been subjected to severe transport stress. Proper use of instrument depends on careful reading of all instruction and labels.

7.4 Customer Support

For any further information you may desire concerning the use and/or maintenance of the Fear Conditioning Systems series 46000, please do not hesitate to contact our **service department** (or our local distributor) either directly or via the support page on our web site www.ugobasile.com.

Before sending any instrument to our factory for repair, please contact our logistics department to obtain a return authorization (RMA) and shipping/packing instructions.

FC systems are delivered in a pliable wooden crate, to be stocked for future use: in case it is necessary to return the system for repair/control, whenever possible, make sure to use the original packing. We may not be held responsible for damages during transport due to poor packing:



UGO BASILE s.r.l.

Via G. Di Vittorio 2
21036 GEMONIO – Varese, ITALY



Phone : +39 0332 744574



service@ugobasile.com
logistics@ugobasile.com
sales@ugobasile.com

8 ORDERING INFORMATION

8.1 FC-Units and Bundles

46001	Fear-Conditioning Unit, including Isolation Cubicle 46000-596 (inside dimensions 48.5(w)x38.5(d)x48.5(h)cm), dual visible/I.R. light 46000-325, speaker 46000-165, controller on-board 46000-105, camera 47400-035, cables and power supply.
46001-2	Bundle of two FC-Units 46001
46001-3	Bundle of three FC-Units 46001
46001-4	Bundle of four FC-Units 46001

complete the system according to your experimental needs, choosing one or more cages among the available ones, and the suitable ANY-maze license (FC or full license):

8.2 Animal FC-Cages

46002	Rat Cage for Fear Conditioning, with electrified grid floor, 25x25x35(h) cm
46003	Mouse Cage for Fear Conditioning, with electrified grid floor, 17x17x25(h) cm
46004	Mouse Cage XL for Fear Conditioning, with electrified grid floor (mouse bars), 25x25x35(h)cm

8.3 ANY-maze

60000-FC	Software ANY-maze for fear conditioning (freezing detection)
60000-FC	Software ANY-maze , full license
60050	ANY-maze 1-year after sales assistance. For update of older versions of ANY-maze



You own a previous version of our FC-System? No worries: you can upgrade it to the new version, and/or add extra units.
Ask for details!

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10 CERTIFICATIONS

CE Compliant, UL and cUL Listed

10.1 CE Declarations of conformity

The system meets requirements of EMC Directive 2004/108/EEC for Electromagnetic Compatibility and Low Voltage Directive 2006/95/EEC for product safety and 2011/65/UE on the restriction of use of hazardous substances in electrical and electronic equipment.

10.2 EMC Directive

- EN61326-1:2006
- EMC requirement for electrical equipment for measurement, control and laboratory use.

10.3 Electromagnetic emission

- EN 55011/A2:2002, Radiated and conducted emission (Class A)
- EN 61000-3-2/A2:2005 Harmonic Current Emission
- EN 61000-3-3/A2:2005 Voltage fluctuation and flickers

10.4 Electromagnetic Immunity

- EN 61000-4-2:2001 Electrostatic Discharge (2kV contact, 2kV air, 2kV Vert/Hor coupling planes)
- EN 61000-4-3:2006 RF Radiated electromagnetic field (3V/m, 80-1000MHz,;3V/m, 14000MHz – 2GHz)
- EN 61000-4-4:2004 Electrical fast transient (1kV on PSU Line, 0,5kV on IO Signal data and control lines)

- EN 61000-4-5:2006 Surges (1kV Main Lines, L-N,L-PE,N-PE)
- EN 61000-4-6:2007 RF Conducted Electromagnetic field (3Vrms, 0.15MHz – 80MHz)
- EN 61000-4-11:2004 Mains Dips and interruptions.

10.5 Low Voltage directive

- EN 61010-1:2001 Safety requirements for electrical equipment for measurement, control and laboratory use.

10.6 UL and cUL certifications

- UL Standard UL 61010-1 2nd edition
- Canadian Standard: CSA-C22.2 No. 60101-1-04

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Notes

Handwriting practice lines consisting of 25 horizontal dashed lines.



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CE CONFORMITY STATEMENT

Manufacturer **UGO BASILE srl**
Address Via G. di Vittorio, 2 – 21036 Gemonio, VA, ITALY
Phone n. +39 0332 744574
Fax n. +39 0332 745488

We hereby declare that

Instrument. **FEAR CONDITIONING SYSTEM - SERIES 46000**
Catalog number **Rat series: 46102, 46152, 46202, 46252, 46402, 46452**
Mice series: 46103, 46153, 46203, 46253, 46403, 46453

*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