Thermal Gradient Ring User Guide SKU 35530 - Rev. 1.0 - Feb. 2024









SAFETY CONSIDERATIONS

Although this instrument has been designed with international safety standards, it 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 powered instrument 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.



CE CONFORMITY STATEMENT

Manufacturer	UGO BASILE srl	
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	We hereby declare that	
Instrument.	Thermal Gradient Ring	

Catalog number 35550 and 35530

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

- 2006/42/CE on machinery
- 2014/35/UE relating to electrical equipment designed for use within certain voltage limits
- 2014/30/UE relating to electromagnetic compatibility
- 2011/65/UE and 2015/863/UE on the restriction of the use of certain hazardous substances in electrical and electronic equipment

Account Manager

Mauro Uboldi

Nome / Name

Firma / Signature

February 2024

Date

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1. What's in the box

Thermal Gradient Ring is a device that can be used for tethered and non-tethered mice, in the box You will find the necessary parts to set-up the device for both of the experiments.

Thermal Gradient Ring part list				
Q.ty	Description			
1	Thermal Gradient Ring unit			
1	Metallic external maze wall (15 cm high)			
1	Black plastic IR transparent maze inner wall (15 cm high)			
1	Camera stand for tethered mice set-up			
1	Video Camera			
1	IR & Visible illumination assembly			
1	Upper extension for swivel holder with 2 vertical metallic arms and swivel holder horizontal plate.			
1	Gray plastic camera holder bar for the non-tethered mice set-up with 2 knobs			
1	Power supply cable (for Your region)			
1	5 m USB cable for PC connection (USB2.0)			
2	1 m USB cable for Camera connection			
1	USB stick containing: The quality certificate & this guide in PDF format The software ANY-maze Windows installer The video camera Windows driver One ANY-maze sample protocol			

Available Optional parts

High maze walls kit, containing:

- 1 Metallic outer maze wall (24 cm high)
- 1 Black plastic IR transparent maze inner wall (24 cm high)

Thermal conditioned cabinet to hold 2 TGR units for non tethered mice or 1 TGR unit for tethered mice.

2. General

The TGR is a device for THERMAL PREFERENCE PHENOTYPING in mice, according to the method invented by Dr. Katharina Zimmermann from the Erlangen University in Germany.

Previous studies examining thermal behaviours have several limitations. For example, these studies used methods like a two-plate test that does not have high temperature resolution. Meanwhile, the linear thermal gradient method does not preclude the preference of mice to stay in a corner. A stereotypical mouse behaviour is to hide in the corner, which can introduce a bias when mouse behaviours are analysed with cold or hot zones positioned in corners. The Thermal Gradient Ring system was created to overcome these disadvantages. The Thermal Gradient Ring can be less stressful to mice than the two-plate test, and the mice can move freely on the ring rather than remaining in a corner.

2.1. Principle of operation

The Thermal Gradient Ring maze is 15 cm high with a 45 cm and 57 cm inner and outer diameter, respectively. An infrared & visible light video camera placed on the upper side of the apparatus allows monitoring of mouse behaviours in the dark. Part of the inner wall of the apparatus is IR light transparent so that the camera has no blind spots. Cooling and warming devices in the apparatus allow analysis across a range of temperatures from 4 up to 65°C. The apparatus is equipped with a system and a software that records the floor temperature and tracks the animal (ANY-maze software).

The ANY-maze full software version can be used to drive the Ugo Basile TGR (Thermal Gradient Ring) as the TGR limited ANY-maze version which can be used ONLY for the TGR experiment and not for all the other experiment normally available with the ANY-maze full version.

3. Instrument description

3.1. The TGR

The Thermal Gradient Ring 2.0, formerly called TGR can be used with non-tethered and tethered mice, both set-up are included as standard in the box and the necessary parts can be used for preparing the apparatus for one of these set-up.

TGR device, needs ANY-maze software to run, both version, the ANY-maze full version and the TGR ANY-maze limited version can be used to drive the apparatus.

The ANY-maze Windows installer is included into the USB key provided in the TGR package, we advise customer to download the latest version of the software from Internet via the following link: <u>https://www.any-maze.com</u>.

To run the software a Windows PC is required, please refer to the ANY-maze web site for the hardware system requirements in order prepare an appropriate PC machine.

ANY-maze software has an already built-in device set-up, in a way that You can start Your TGR experiment without the tedious need of setting up the connection and create the maze arena.

ANY-maze software is able to read TGR temperature sensors and in this way record tracked animal position and the temperature of the animal position, giving You as a result standard TGR data.

TGR has inside 2 heating/cooling devices, one hot/cold plate at the apparatus left (front view) capable of a temperature range from 4 up to 65 °C and an hot plate at the right capable of a temperature range from ambient temperature up to 65 °C. These 2 devices are thermodynamically and mechanically connected by a thick aluminium ring which create the temperature gradient where the animal stays. One external metallic wall (15 cm high as standard) and a black IR transparent plastic inner wall with the same height, create a circular maze where the mouse is freely moving having no start/end point even no corners.

The front panel let You set the Hot/cold plate and Hot plate temperature.

Depending of the temperature set-up You will use, the apparatus needs time to reach the correct temperature gradient that can be 30 or more minutes. To let You starting the tests in early morning when You arrive at the lab with out waiting for the warm-up time, a timer on the front panel gives You the way to switch the TGR on some time in advance.

An swivel holder is included in the package for general swivels, while we do not know which swivel model You have and want to use, you may need to adapt it at the TGR holder, or let us know in advance the swivel model You plan to use to adapt Your holder.

4. Installation

4.1. Unpacking & preliminary check

Check the contents of the shipment for completeness, "what's in a box" section to hand, and visually inspect the instrument as soon you take it out the packaging.

If the instrument or part of it is/are damaged, immediately inform the shipping agent or carrier and notifying us by email.

Inspect the instrument for damages such as scratches, broken or loose parts. If after having tested it, the instrument fails to meet rated performances, contact our company immediately via email <u>service@ugobasile.com</u>.

Remove the apparatus parts from the package and remove all the packaging materials from the apparatus parts.

Notes on the User Guide

This User Guide can be found on the provided USB Key. We recommend thoroughly to read this manual, as it contains essential informations for the correct installation and operation of the instrument.

Please save the manual in a safe place, ready to be consulted by qualified personnel who use the instrument. Print it only if necessary.

Our User Guide are available as free download on our web site. For any additional information and/or assistance, you are welcome to contact our Service Department <u>service@ugobasile.com</u>, specifying the serial number of the instrument.

4.2. Intended Use

The Ugo Basile TGR is intended for Investigation use on laboratory animal only. DO NOT USE ON HUMANS.

4.3. Additional Safety Consideration

- 1. Use original accessories and spare parts only.
- 2. Immediately disconnect and replace damaged main cord.
- 3. Do not obstruct access to the power module and to the air ventilation inlets
- 4. Do not operate in hazardous environment or outside prescribed environmental limitation.
- 5. Do not spray any liquid on the connectors, display, or on the electronic unit.

Ugo Basile cannot in any way and form be held responsible for damage caused to things and people and warranty will be void, due to:

· 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 component, accessories or parts with others not made by Ugo Basile.
- Servicing carried out by unauthorized personnel.

4.4. Positioning the TGR into the Lab room

TGR apparatus have a total weight of 49.5 kg. and need a flat surface of (no Windows PC included) 100 cm x 70 cm x h 90 cm.

Room temperature is vital for the TGR test; the average temperature the TGR needs is depending of the set-up temperature You choose to use.

You need to calculate the average temperature between the min e max plate temperature setting and this need to be the room temperature.

If room temperature is not equal to the calculated average temperature You risk to not reach the temperature equilibrium on the ring and as a result Your experiment may be not correct.

Average temperature is calculated: (Hot/Cold plate temp. + Hot plate temp.)/2

Hot/Clod plate temp. °C	Hot plate temp. °C	Average temp °C (room temp.
5	30	17.5
15	40	27.5
20	45	32.5

Typical temperature sets are:

You need to have the ability to control the Lab room temperature by an air conditioning system, but also if you can, working in a room temp. of 32.5°C will be quite impossible, despite You come to the room Lab with your swim ware only.

To overcome this matter You may be interested in using the TGR into a temperature controlled cabinet provided by Ugo Basile; our SKU 35580 is a Thermal conditioned cabinet for Thermal Gradient Ring ready to hold 2 TGR for non-tethered mice or 1 unit set-up for tethered mice.

Ask your Ugo Basile representative about this useful device which can ensure You a correct average temperature environment for Your TGR test, while it is able to conditioning the internal cabinet temperature from -10 up to 100°C.

If You need to place the TGR without the cabinet You will need to have a desktop able to support the total apparatus weight (49.5 kg), having a flat and stable surface and the sufficient space.

Place the TGR on the desktop paying attention to not obstruct the ventilation air inlet which are at the left side (hot air coming out) and at the rear side where 2 blowers provide the internal base ventilation.

TGR base has 4 adjustable feet You can regulate to have a correct levelling of the device. (Levelling does not need to be very precise).

4.5. Assembling the essential parts

After placing the TGR base to the right surface You will need to mount the essential parts, which are the two, left and right grey column that holds the illumination assembly, the illuminator assembly itself.

- 1. Remove the 2 circular maze walls from the aluminium ring and place them apart (pay attention they are very fragile).
- 2. Remove by hand the 4 (2 by side) knobs fixing the column at the device base placed in the holes and keep them apart.



3. Find the 2 gray column and place them to the appropriate holes using the 4 knobs to fix them in a stable position.

ATTENTION:

Front device view, the column with the black adhesive line must stay at the left (Hot/Cold plate side)

The column with no mark has to stay at the right (Hot plate side)



- 4. Remove the 4 (2 by side) knobs from the illumination assembly and place the assembly to fit the correct position (cable to the right side) referred to the fixing holes at the top, then screws the 4 knobs to fix it.
- 5. Connect the Illuminator cable red connector to the red matching connector at the device base close to the base at the right column; pay attention to not force it while inserting, there is a right position with a pin, rotate the connector to find the right position for insertion. Cable lays outside the column.

Installation

Now the essential parts are mounted and your TGR should look like this:



4.6. Mounting the TGR for a specific set-up

TGR needs be set-up for the use with tethered or for non-tethered mice, depending of Your requirements You will need to change the set-up of the system. Both configurations share the same video camera but have different parts that needs to be mounted.

4.7. Mounting TGR for the **non-tethered** mice:

1. Find the horizontal bars holding the video camera (which is already mounted on it) like the picture below:



2. Place the horizontal bars at the top of illuminator assembly, paying attention to match the two red marks.



3. Knobs to fix this part are inserted in the holes, remove them, place the bar in position and using the same knobs fix the bar on.

- 4. Using the provided 1 m USB cable connect the camera, passing the cable aside the right column, connect the cable to the connector at the base USB connector which is close to the right column and having a cap that need to be unscrewed to open it.
- 5. Mount in place the two maze walls; the inner black plastic one is hold in position by the grey PVC disk at the centre and the outer wall is kept in place by the side black plastic where the columns stays.



Your set-up should look like this picture:

Pay attention in placing the black plastic inner wall; this wall has a vertical junction that may be seen by the animal inside the maze and may create a experiment bias.

While it is impossible to avoid this junction you need to place it in a zone where animal don't like to stay which is the coolest area.

Identify the junction and rotating the wall placing the junction in front of the left column, the one with the black line on it.

Your TGR set-up for non-tethered mice is now complete.

See "Connecting the Any-maze Windows PC " chapter to connect the Any-maze Windows PC

4.8. Mounting TGR for tethered mice:

Starting from the TGR with the essential parts mounted, here is the way to assembly the TGR for the use of tethered mice.

- 1. If mounted remove the horizontal bar holding the video camera, used for non-tethered mice, removing the 2 knobs.
- 2. If mounted remove the 2 maze walls
- 3. Detach the USB camera cable from the camera De-mount the video camera from the horizontal bar, removing the right side knob paying attention to not lose the washer.



Camera and camera holder has 2 red mark for later re-mounting

- 4. Stock the camera holder bar in a safe place for future use, You do not need it for the Tethered mice.
- Insert the maze walls on the ring starting from the black plastic inner one which need to be rotated with the vertical junction in front of the left column (the one having the line mark.) Then position the metallic gray wall in place (this does not have a specific position).
- 6. Mount the central camera holder coupling the two grey plastic parts matching the 2 red dot marks



7. Insert the central camera holder into the black inner maze wall, from the top and through the illuminator assembly, paying attention in matching the 2 red mark. (this part is very fragile, pay maximum attention)



8. Mount the camera on the central camera holder facing down using the knob and try to place it horizontal. Camera has 2 holes, use the upper one.



- 9. Connect the camera USB cable to the camera connector and to the other cable ends to the USB connector on the base disk.
- 10. Verify that the central camera holder arm with the marked line match the line marked TGR left column. If not, correct Your installation.

11. Mount the disk guide for the animal cable on the top of the central camera holder, just placing it on the top paying attention to match the 2 red marks.



 Mount the 2 grey metallic plates on the top of the each column; you need to loosen (not remove) the 2 knobs of the illuminator assembly, insert the plate (short cut downward) and screw them again. Repeat this procedure for the second plate



- 13. Mount the horizontal swivel holder on the top of the device using the 4 provided (in place) knobs.
- 14. Fix Your preferred swivel at the swivel holder and adjust vertical position as You like.

Now Your setup is ready for the use of tethered mice and look like this picture:



4.9. Connecting the Any-maze Windows PC

Simply connect the 5 mt provided USB cable from the TGR rear connector (close to the power inlet) to a free PC USB port where ANY-maze software is installed.



Installation

5. Operation

5.1. Applying Power

The Power Module (see figure below) is positioned on the back side of the TGR and incorporates, from left to right, the fuse holder, the ON/OFF switch, the power inlet connection.



Power Module

The fuse compartment holds two protection fuses.

Use T5A (6x32) timed fuses for operation at both 115 or 230V, for fuse replacement.

The power cord inlet fits a standard C13 socket.

Make sure your power outlet is provided with a reliable ground connection.

This device is provided by an universal input power system which accept 85-264 VAC, 50-60Hz and absorb a maximum of 400 Watt.

You can now switch on Your TGR.

5.2. Front panel

The TGR front panel provide the following features:

- Visible light intensity
- IR light intensity
- Temperature setting of the Hot/Cold Plate
- · Temperature setting of the Hot Plate
- Power on timer



The visible light intensity knob need to be used to regulate the visible light intensity during the experiment. Usually TGR experiment is conducted in total obscurity, but You may want to record by the video camera the animal behaviour; in this case a little amount of visible light can be applied by this setting.

IR light intensity needs to be regulated viewing the image recorded by the video camera viewed in ANY-maze software, refer to the ANY-maze TGR settings which are included in the on-line software help.

Note that the TGR black inner wall is made of an IR transparent material which

lets You avoid shadows tracking the mouse.

Temperature setting of the Hot/Cold Plate is made by the left controller model TLK33.

Please refer to the attached Ascon Tecnologic instruction manual for the use of this controller.

Temperature setting of the Hot Plate is made by the right controller model K38. Please refer to the attached Ascon Tecnologic instruction manual for the use of this controller.

Power on timer provide You the way to switch the TGR automatically early in morning to let the device warm-up before You came to the Lab finding the device ready to use.

You may want to set the timer at a certain time, please refer to the attached Muller instruction manual.

5.3. ANY-maze Windows software

The Ugo Basile TGR needs ANY-maze software to run the experiment, the software (which is not included and need to be purchased from Ugo Basile) is sold in two versions:

The ANY-maze full version, which is having all the ANY-maze functionality, can be use for a wide variety of experiment in addition to the TGR test.

The TGR limited ANY-maze version, which run ONLY the TGR experiment and is having a cheaper cost.

You need to consider that ANY-maze protection software links the software installation to the PC running the solution and need an activation key to run out of the demonstration mode.

If You need to run TGR and use the same license for other ANY-maze supported experiment (may be in another Lab room or department) You will need to move the ANY-maze PC to the other Lab room, or in order having another set-up buy a second licence of ANY-maze (which cost less than the first)

Usually researcher prefer to have a licence dedicated to TGR test and buy the limited TGR ANY-maze version.

5.4. Installing ANY-maze Windows software

Refer to this link for the ANY-maze system requirement for configuring You PC hardware.

https://www.any-maze.com/equipment/computers/

You will find into the TGR provided USB key the Windows installation kit needed to install the software on Your PC.

You may also want to check for the latest ANY-maze release and download it at this links: https://www.any-maze.com

Click the button "Download ANY-maze for free" to download the latest available version of the software.

Run the installation kit on Your PC to install the software.

Start the software and You will be asked to protect the data by a user name and password, then in order to activate the software You will need to go to the Support tab and select from the left command list "Licensing"

A code will be shown, send this code to <u>sales@ugobasile.com</u> asking for the software activation (if You bought it), we will reply with the activation code You need to insert in the same page.

5.5. Using ANY-maze with the TGR

TGR experiment and TGR connections with the software, are already built into ANY-maze.

You will find in the TGR provided USB key a sample TGR protocol You may want to load into ANY-maze as a starting point.

ANY-maze software has an embedded on-line help; You can access all the informations in the Help Tab or searching in the upper right field inside ANY-maze.

All the needed information to run TGR experiment are well explained there, just search for "TGR"

6. Cleaning

TGR is a set-up designed for single animal test at a time, which drive to a deep cleaning before testing a new animal after testing a previous one.

Deep cleaning implies the cleaning of all the parts the animal may have touched.

Despite any escape, normally the mouse is confined inside the maze during the testing protocol, if the animal did not escape the maze You need to clean:

- Maze walls
- Aluminium ring
- Remove the maze walls and if the tethered animal camera holder is in place, remove it before removing the black inner wall, then remove the metallic wall. Clean the walls with a non aggressive cleaner to remove all the dirty and the animal odours.

Remember the black plastic wall is made by IR Transparent Perspex plastic, do not use aggressive cleaner that obscure the IR transparency of this material.

2. In order cleaning the aluminium ring (floor) you need to remove the 2 column and the 2 black plastic spacers that are in the middle of the column and the aluminium floor.

Clean the aluminium floor and sides with the same cleaner You use for the walls, then clean the 2 black spacers too before remounting them in order remounting the columns

7. Maintenance

No particular maintenance procedures are required for the TGR set-up

UNPLUG THE MAIN CORD BEFORE CARRYING OUT ANY MAINTENANCE JOB

7.1. Electrical

To inspect and/or replace the fuses, disconnect the mains cable first! Insert a miniature screwdriver in the slot indentation and snap out the slide which houses the fuses. Snap in the fuse slide: the mechanical "click" ensures that it is locked.

7.2. Long Inactivity

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

7.3. Customer Support

For any further information you may desire concerning the use and/or maintenance of the TGR, please do not hesitate to contact our service department (or our local distributor) either directly or via our support web form.

Customer Support Contacts

Phone: +39 0332 744574 E-mail: <u>service@ugobasile.com</u> Web form: <u>https://ugobasile.com/support/support-request</u>

Before sending the instrument to our factory for repair, please contact our logistics department to obtain a return authorization number (RMA) and shipping/packing instructions. We may not be held responsible for damages during transport due to poor packing; whenever possible, please use the original packing.

8. Specification

General				
Power Requirement	Universal input 85-264 VAC, 50-60Hz, 400 W max			
Controls	Commands on the heater/cooler front panel			
Pre-set Temperature Read-out	On LCD display on the front panel of the thermal units			
Detection	Via ANY-Maze specific protocol for TGR through integrated USB camera			
Sound emission	68 dB			
Communication interface	Integrated 4 ports USB hub			
Operation				
Temperature Range	Heating Plate: from 20°C to 65°C Heating/cooling Plate: from 4°C to 65°C			
Precision	1°C			
Temperature feedback	By 4 thermocouples monitored in real time by ANY- maze			
Light Intensity (I.R.)	Set by potentiometer with graded scale			
Light Intensity (visible)	Set by potentiometer with graded scale			
Physical				
Internal diameter	45 cm			
Outer diameter	57,5 cm			
Corridor Width	6 cm			
Wall Height standard	15 cm			
Wall Height optional	25 cm			
Dimensions	76 x 60 x 60 (h)cm			
Weight	49.5 kg			
Shipping Weight	69.5 kg			
Shipping Dimension	120x80x70(h)cm (wooden fireproof crate with pallet)			
Warranty				
Warranty	TGR is covered by a 12-month warranty + 12 after product registration on: register.ugobasile.com UB-care12 or UB-care24 months paid warranty extension available.			

9. Bibliografy

Selection of papers mentioning Ugo Basile Thermal Gradient Ring

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F. Touska Z. Winter, A. Mueller, V. Vlachova, J. Larsen and K. Zimmermann, 2016, "<u>Comprehensive thermal preference phenotyping in mice using a novel</u> <u>automated circular gradient assay</u>" Journal Temperature

10. Related Products

Estimates suggest that 20% of adults suffer from pain globally. Chronic pain is the most common cause of long-term disabilities.

Since 1963, Ugo Basile's devices have increasingly acquired a leading role in the field of pain and inflammation preclinical research, becoming precious tools for researchers to achieve their experimental objectives.



37240 - Plethysmometer - Paw Volume & Oedema



37550 - Dynamic Plantar Aesthesiometer - For Automated Mechanical Stimulation and Allodynia



38450 - Electronic Von Frey - e-VF Handheld



37450-275 - Von Frey Filaments manual touch sensitivity kit



37570 - Plantar Test for thermal stimulation - (Hargreaves Apparatus)



37560 - Tail Flick Unit - Thermal stimulation, D'Amour & Smith method





37300 - I.R. Heat-Flux Radiometer for Tail Flick and Plantar Test



38500 - PAM Pressure Application Measurement (for joint pain)



35300 - Hot/Cold Plate NG for screening of thermal hyperalgesia/ allodynia



47885 - Librae Incapacitance Tester (Weight Bearing)



37215 - Analgesy-Meter Randall-Selitto paw-pressure test



31300 - Orofacial Stimulation Test (Fehrenbacher, Henry, Hargreaves method)



35350 - Thermal Place Preference (TPP Test) for Mice & Rats



36103 - Climbing Test - Measures Vertical Activity in Rodents

Related Products

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more than 40,000 citations in the main bibliographic search engines.



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