

SOP: TSE systems PhenoMaster

TSE PhenoMaster

Room: 1.34, ZBMF

Document Version: 2.3

Contact:

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Collaboration:

We will operate the Phenomaster currently without fees in a collaborative manner. We will rehearse the concept, when it is running as routine.

Procedure Checklist

	IMEP	Researcher	Animal caretaker ZBMF
Before the experiment starts			
Contact IMEP via email to register for experiment (at least 3 weeks before you want to start an experiment in the Phenomaster) @ denis.nalbantoglu@uni-ulm.de jan.tuckermann@uni-ulm.de		X	
Approved Tierversuchsantrag (Animal experimentation application)		X	
Set specific date to start the experiment	X	X	X
Plan exact experiment (conditions to be measured, are mice sacrificed afterwards? If mice are about to be returned, get approve from the TFZ. Usually, only mice from the ZBMF can return)	X	X	
Sign access list of room 1.34	X	X	
Fill out room protocols	X		
Perform weight calibrations of Phenomaster devices	X		
Perform Gas calibrations (a day prior to the experiment)	X		
Turn on Phenomaster to let it run warm (a day prior to the experiment)	X		
Leitwarte/ Control pannel is informed about an upcoming Experiment in the Phenomaster	X		
Transfer into the Phenomaster cages			
<i>Note: room 1.34 counts as a satellite room of the mouse housekeeping facility. Therefore, the same hygiene concept applies here as well. read more below.</i>			
All mice need to be weighed prior to the transfer to proper calculate indirect calorimetry	X	X	
Mice are provided ready to pick up in regular cages		X	X
Phenomaster cages are filled with bedding		X	X
Depending on the timescale of the experiment, extra food, water and bedding is provided		X	X
Mice are transported to the Phenomaster room 1.34		X	
Mice are transferred into Phenomaster cages	X	X	
Food and Water from the mice cages are transferred into respective reservoirs in the Phenomaster cages	X	X	

	IMEP	Researcher	TFZ
Operating the Phenomaster (Temp, humidity, light intervals, measurement parameters)	X (operates the software)	X (Check whether all parameters are satisfactory)	
Mice are checked twice a day. <i>If any abnormalities are detected, TFZ and the researchers will be notified to discuss further proceedings</i>	X	X	
Note: Mice must first get accustomed to new environment for at least two days before the measurements can be considered reliable. The IMEP will still perform measurements during this time for in-house quality control			
Start and progress of the measurements			
Data Acquisition (Measurements can last up to 10 consecutive days)	X (checked by)		
During experimental run, mice are checked twice a day. <i>If any abnormalities are detected, TFZ and the researchers will be notified to discuss further proceedings</i>	X	X	
Invasive intervention (injections, weighing the animals in the middle of the experiment, ...)		X	
End of measurements			
Experiment is terminated	X		
Mice are weighed	X	X	
If mice are sacrificed:			
Providing transport boxes			X
Sacrificing mice and organ taking		X	
If mice need to return to regular housing			
Providing new cages with bedding, food, water, enrichment			X
Transfer mice into new cages	X	X	
Return to TFZ		X	
Exporting data files	X		
Analysis and Aid	X (aid)	X (analysis)	
Clean up			
Cleaning the Phenomaster cages			X
Cleaning the Phenomaster	X		
Cleaning food and water reservoirs	X		
Discarding old bedding	X		X
Informing Leitwarte/ control pannel about the end of the experiment	X		

Hygiene concept

To ensure safety for mice and researcher, a certain hygiene protocol needs to be in practice:

- Upon entering the locker rooms, disinfect your hands and put on shoe covers
- In the locker room, put on a body cover, hair (and beard) cover, face mask and gloves
- Upon entering the clean floor 01.38, put on a second layer of shoe covers

- You can transfer between the mouse rooms and 1.34 without changing your protective clothes
- You are not allowed to walk past the washing area 01.35
- Mice are allowed to be housed in the Phenomaster if they come from: DRK, N26, M25, ZBMF room 1.39 & 1.40. If your mice originate from another place, reach out to check the hygiene status and consult TFZ.

Phenomaster operating list

The TSE Phenomaster houses 12 metabolic cages that enables the measurement of mice drinking/ feeding behaviour, movement, activity and indirect calorimetry in real time.

Hardware

The Phenomaster is made up of the following components with respective functions.

1. Phenomaster cabinet
 - Three displays give information about **temperature, humidity and light intensity**
2. Cages
 - 12 cages to use + 1 “reference cage” (tube at the back)
 - Right hook: food reservoir
 - Left hook: water reservoir
 - Front: treadmill
 - *Note 1: do not add extra (enrichment) equipment if not necessary, as this can interfere with the movement sensors*
 - *Note 2: Do not put in too much saw dust, as this can interfere with the movement sensors*
 - *Note 3: The Software will tell you if something interferes with the movement sensors*
 - The cages are placed on 3 shelves that can be pulled out of the cabinet. Only pull out one of the shelves at a time
 - Temperature sensor: Every cage has its own temperature sensor. The temperature measured in the cages can be slightly off to the set temperature of the cabinet. This is due to activity of the mouse in the cage
 - Air sensor: transparent tube at the back of the cages draw air out of the cage. Slits on the lid of the cage draw fresh air into the cages.
 - air pulled from the cage will be transported to the O₂/Co₂ sensor to measure its content. The System takes roughly 3 min to measure o₂ and co₂ concentration per cage and it can only measure one cage at a time.
 - With 12 cages and 1 reference cage, the system needs 33 minutes for one rotation, thus o₂/Co₂ conc. Can be measured every 39 min per cage
 - Tubes should be replaced every 1-2 years depending on usage frequency
 - Treadmill: measure physical activity of the mice in rotation/min or meter/sec
 - Cage controller: black box at the end of each cage. Communicate all measurement of the cage to the software. If problem: plug out the ethernet cable and give it a view min
 - *Note 4: If you change something in the cage, like mice or refill food/ water: stop the software*
3. Server and PC
4. Fresh Air Pump
5. ADU Air Drying Unit
 - Detects Calorimetry values from your cages

- Every cage takes 3 minutes to be measured
 - The ADU always reduces the humidity to 30%, independent of the settings
 - The ADU always measures at 5°C. check the blue LED to see if the temperature is stable
 - *Note 5: Water collection clamp: condense water will collect here; empty it daily during a running experiment! Close the clamp after draining it immediately. Otherwise, Calorimetry can't be measured*
6. Air humidity supply
- On top of the Phenomaster
 - Open when you start an experiment and close it afterwards
 - Always refill with VE Water
 - The hotter you set the Phenomaster, the faster you have to refill the water tank
 - Don't let it run dry!
7. Condense Traps
- Right to the server cabinet
 - Drain water by unscrewing
8. Water waste
- Right to the cabinet
 - Empty 1/week during experiments

Before you start an experiment

Before starting an experiment in the Phenomaster, check the following points:

- The mice you want to analyse need to be housed in a facility approved by the TFZ
 - Make a request at the Institute of Comparative Molecular Endocrinology and Physiology via email at both jan.tuckermann@uni-ulm.de and denis.nalbantoglu@uni-ulm.de
 - Provide a hard drive to save your data
 - Inform the animal caretaker 5 workdays before you want to use the phenomaster via Pyrat. This includes:
 - Animal numbers
 - Request for water and food
 - Request for bedding
 - (depending on length of the experiment: reserve food, water and bedding)
 - The animal caretaker provide bedding inside the phenomaster cages. You need to transfer food and water into the phenomaster mounts
 - Weigh the mice before moving them into the phenomaster cages
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- Calibrations before mice are moved into the Phenomaster
 - Turn on Phenomaster and let it run warm for 4 hours, better over night
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 - Gas Calibration
 - Bevor every experiment
 - hit Calibrate> Gas measurment21

- Check if the theoretic values for Gas 1 (20,9% O₂) and Gas 2 (20,0%) are set. If ever other Calibration Gases are ordered, this has to be adjusted
- Click “autom. Calibr.”
- Automated calibration will run. Check if measured values align with theoretical values
 - If calibration is not successful: adjust Drift and Delta values
 - Drift describes difference between measured value and theoretical value; can be adjusted up to a value of 10
 - Delta; can be adjusted up to a value of 75
 - Note 1: CO₂ drift is usually stronger than O₂ drift
 - Note 2: top left window: you need a sample flow of 0.25. if you have a low of 0, your gas tank might be empty or a valve might be closed
- After calibration: close the gas tanks!
- Weight calibrations
 - Every 3-4 month, independent of usage. Check list at the Phenomaster
 - Can take some hours, so take your time, eventually the day before!
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- Take into account that mice need acclimatize in their new environment before you can start your measurements
 - Version 1: 2-4 days of single housing in the phenomaster before measurements start
 - Version 2: 2 days of grouped housing, followed by 2 days of single housing in the phenomaster

Software setup

When ready to run an experiment, set up the software

1. Turn on the power button on the back of the server cabine
2. Open the server cabinet by pushing the handle in
3. Unlock the PC lock at the buttom and turn on the PC
4. Push the Power bottom of the PhenoMaster on the top right side of the cabinet
5. Wait ca. 2 min
6. Start the PhenoMaster software

Setup

Note: You can't change your set-up settings once you started an experiment! Take your time setting everything up.

Experiment

7. Start defining your experiment parameter: File > new> add your file name
8. This will open up the Setup window. If not, hit Setup
 - a. Experiment: Enter Experiment Name, User and additional information
 - b. Cycle interval: defines the interval in which you measure Calorimetry (with all 12 cages active, the tightest possible interval is 39 min, as every cage needs 3 min)

- c. Runtime: is the total time you want to collect data. Max
 - i. Tipp: always set 240 h. you can stop experiment at any time, but you cant prolong a measurement once it started
- d. Decide on what parameter you want to collect
 - i. Grayed fields are parameter we don't have the hardware to detect

Data Backup

- 9. Define a path to save a backup file.
- 10. provide an external hard-drive for the single purpose to immediately create a backup

Boxes

- 11. Pick the cages you want to collect data from
- 12. If you not use all cages, you can leave them in the cabinet and deselect them here
 - a. To change selection, click into "enable" and type yes/no
- 13. Add animal identification number in "animal number"
- 14. "weight": add starting weight of your mice
- 15. Text 2 & Text 3: You can add additional information like injuries or gender

Alarm

- 16. Our System does not have an alarm module, so leave everything alone

Activity

- 17. Measures movement of the mouse
- 18. Default: you get an activity measurement every 39 minutes (every time the calorimetry measurement is made for that cage)
- 19. If you want more information: chose "write binary file"
 - a. Define the time interval you want to collect data
- 20. If you want to collect data every time a sensor is triggered: chose "write RAW data fine"
 - a. Collected data might be convoluted
 - b. Refractory period: time that has to pass until a record is made to sort out unspecific measurements
 - c. Default: 0.8 sec to not detect breathing rhythm
- 21. Smooth coordinates: enables diagonal movement, otherwise you get only x & y coordinates
- 22. Light Beam Configuration: allows you to define center areas in which you are particular interested in movement, i.e. around food/ water
 - a. Note: running wheels might interrupt sensors
- 23. Enable light beams: sensors: X axis: 64; y axis: 32
 - a. Green: defined areas of interest
 - b. Checked: active sensors

Climate

- 24. Leave all settings on off

Calorimetry

- 25. Continuous mode: chose only if you work with only 1 mouse in 1 cage
- 26. Flow: gives the air exchange rate = Luftwechselrate

- a. Determines how fast air flows out of the cage and how fast it is refilled
 - b. The faster the exchange rate, the smaller the values that can be detected by the sensor
 - c. Default value: 0.4 L/min
27. Smoothing ADC: gives average of given time to not be surprised by outlayers
- a. Default value: 10s
28. Reference measurement scrambled
- a. If active, the order of reference measurement is randomized over the course of all cages
29. Calorimetry is measured in “heat”
- a. Default: watt/kg
 - b. Alternatively:
30. Default: you get an calorimetry measurement every 39 minutes (every time the calorimetry measurement is made for that cage)
31. If you want more information: chose “write binary file”
- a. Define the time interval you want to collect data
32. Calculation of VO₂ and VCO₂
- a. Defined as volume O₂/ volume CO₂
 - b. These values/ their ratio are used to calculate the lean body mass of the mice

Drink/Feed/Bodyweight

33. Default: you get an information every 39 minutes (every time the calorimetry measurement is made for that cage)
34. If you want more information: chose “write binary file”
- a. Define the time interval you want to collect data
35. Only positive consumption
- a. If checked, the software will not detect added weight
 - b. i.e. the mouse eats their food, so the weight has to decline. In case the mouse climbs the food reservoir, the weight will increase. This will then not be detected by the software
36. Min. consumption and Max. consumption
- a. This defines minimal and maximal values that qualify as food/ water consumption to be recognized by the software
 - b. Sometimes mice lean towards the reservoirs. This can result in the software to detect an unusual high decline in weight
 - c. Attention: the default values might be too sensitive and might be adjusted

Time Manager

37. Control panel to regulate Climate, Humidity, Light, treadmill
38. Table control:
- a. Date: date when you want to trigger an event
 - b. Time:
 - i. Absolute time: type time in this format, i.e.: 06:00 (6 am); 18:00 (6 pm) when to trigger an event
 - 1. Absolute time of the time of the PC
 - ii. Relative time: type in this format, i.e. +06:00 (triggers 6h after you started an experiment); +00:00 (triggers event at the start of the experiment)
 - c. Actions:
 - i. Clim.Temp.Set.Val: set a temperature: in °C

- ii. Clim.Hum.Set.Val: Set humidity: in
- iii. Clim.Light.Set.Val: set white light intensity in % for dark/ light cycle
- iv. Rmp settings: to change a value over time, i.e. to simulate Sunrise/sunset
- v. Enable wheel function: define time intervals when wheel can be used
- d. Parameters:
 - i. Set the values you want to have for your actions

39. Notes:

- a. Your humidity will drop while the temperature decreases. When wished temperature is reached, humidity will regulate itself
- b. If you want to start an experiment and measurements at 4°C, cool down the cabinet to 4 °C before starting manually on the cabinet

Running wheel

- 40. Displayed unit of the distance: rotation, cm, inch, feet
- 41. Threshold for run detection: measurement does not start until a certain velocity is reached, to not detect a mice just jumping on the treadmill
- 42. Default: you get an information every 39 minutes (every time the calorimetry measurement is made for that cage)
- 43. If you want more information: chose “write binary file”

If all settings are set, go to Measurement> Start

When your experiment is done, hit view to see collected data

- 44. You can pick table parameters to visualize

Current Status

To see current values of the cages, hit Status and chose parameter you are interested, i.e. calo/climate

Refill Food/ water Reservoir

- If you need to refill food/ water reservoir during an experiment, you have to pause measurements. Otherwise, this will intervene with your measurements
- Hit “Trial Monitor Drink/Feed”; right click on the box> pause box > refill> click continue box

After the experiment

Cleaning

- Cages can be autoclaved
- Sensors and Lid: with a towel
- If mice are not to be sacrificed: transfer them back to fresh housing cages and arrange transfer with the animal caretakers