Cabin Air Quality – CAQ3 Project

Animal Study in WP3

January 2023

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How do we test if oil-related fume events are harmful?

Within a team

Sanitätsakademie
der Bundeswehr

Animal experiments are only carried out:

- if no alternatives are available to answer the scientific question
- after the competent authority has approved the animal experiment with the involvement of a commission (according to §15 German Animal Welfare Act)
- in accordance with the European Communities' Council Directive 2010/63/EU

Quality control standards at the German Mouse Clinic (GMC)

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Study design

- Biomarkers of effect/exposure
- Behavioural and neurological symptoms
- Neurotoxicity and neuroinflammation
- Lung toxicity

Basis for design:

- OECD TG 412: Sub acute inhalation study
- 28 days exposure + follow-up
- 5 days per week inhalation exposure
- Peak exposures per day in 2h window
- 3 dose groups + control + positive control
- Sub acute plus delayed effects

1st part: Range finding study

Finding the optimal dose for main experiment

2nd part: Main Study

A) Sub acute effects

What effects do we see right after exposure phase?

B) Long Term Effects

What are the effects on behavior and after a time of non-exposure?

Study design

Main study: Sub-acute effects	Arrival and acclimatization		SHIRPA	Exposition: 4h/day 5 days per week 4 weeks				SHIRPA	Transfer and sacrifice						
Main study: Long–term effects	Arrival and acclimatization		SHIRPA	Exposition: 4h/day 5 days per week 4 weeks				SHIRPA	Transfer and acclimatization	Open Field	CatWalk	ASR/PPI	Y-Maze	Social Discrimination	Sacrifice
Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Sub-acute effects?

SHIRPA – systematic behavioural observation using defined rating scales

General health and autonomous functions
- Weight
- Urination
- Defecation

Reflexes
- Startle reflex
- Contact righting reflex
- Pinna reflex

Behavioral aspects
- Touch escape
- Locomotor activity
- Transfer arousal
- Body position

Posture and movement
- Tail elevation
- Trunk curl
- Gait

Additional observations
- Tremor
- Limb grasping
- Biting
- Vocalisation

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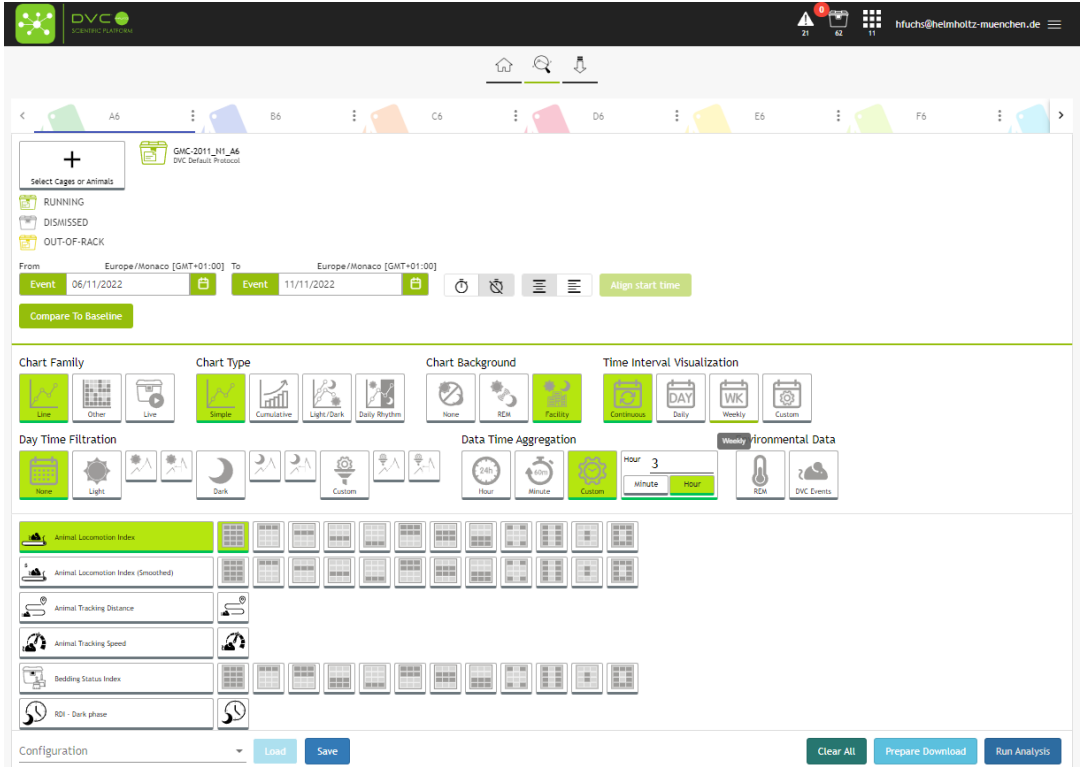
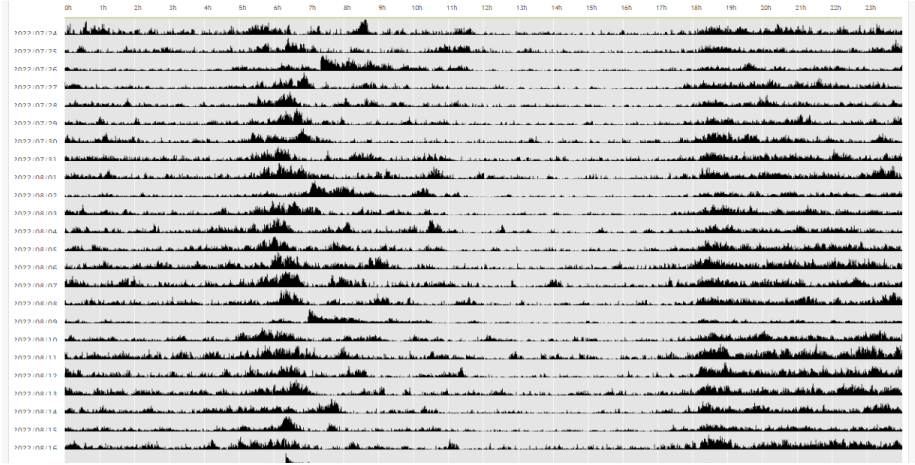
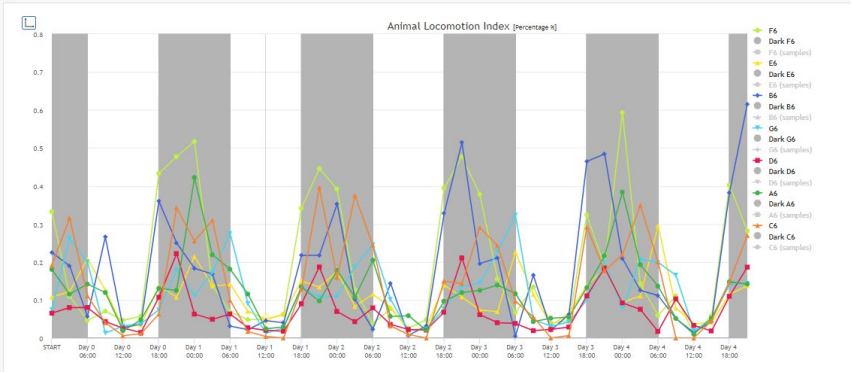
DVC racks

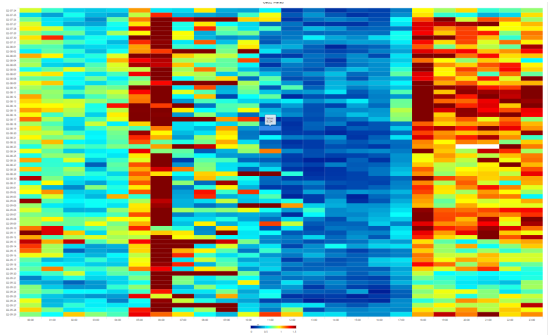

RACK
1

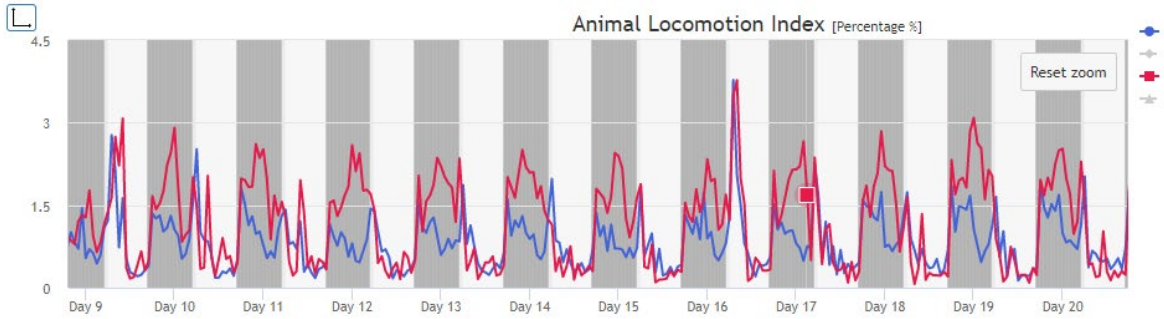

DVC
SCIENTIFIC PLATFORM
hfuchs@helmholtz-muenchen.de
A6
B6
C6
D6
E6
F6
Select Cages or Animals
GMC-2011_N1_A6
DVC Default Protocol
RUNNING
DISMISSED
OUT-OF-RACK
From
Europe/Monaco [GMT+01:00]
To
Europe/Monaco [GMT+01:00]
Event
06/11/2022
Event
11/11/2022
Align start time
Compare To Baseline
Chart Family
Chart Type
Chart Background
Time Interval Visualization
Day Time Filtration
Data Time Aggregation
Hour
3
Minute
Hour
Animal Locomotion Index
Animal Locomotion Index (Smoothed)
Animal Tracking Distance
Animal Tracking Speed
Bedding Status Index
RDI - Dark phase
Configuration
Load
Save
Clear All
Prepare Download
Run Analysis

DVC racks – data analysis



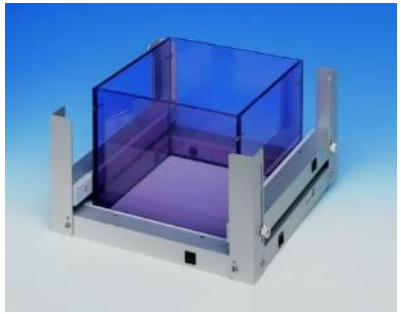






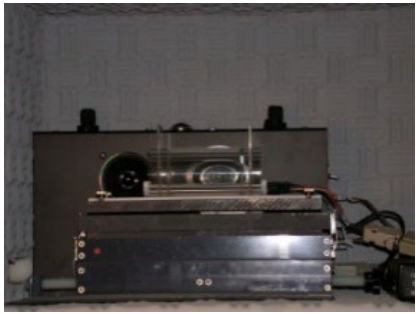
Long-term effects?

Long-term effects on locomotion and cognition?

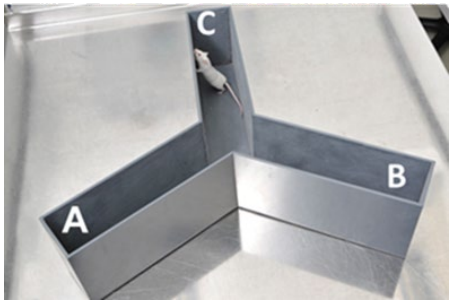


Open Field, TSE

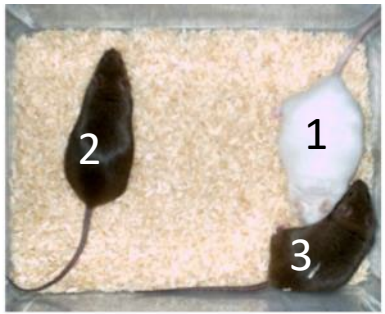
CatWalk, Noldus



Prepulse Inhibition, Med Ass.



Y-Maze



Social Discrimination

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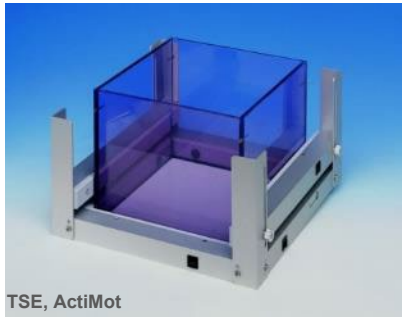
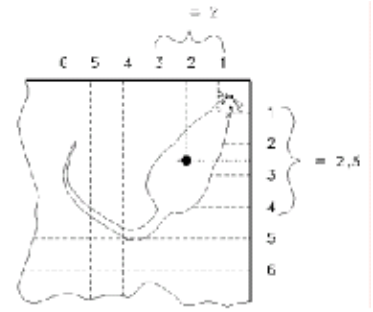
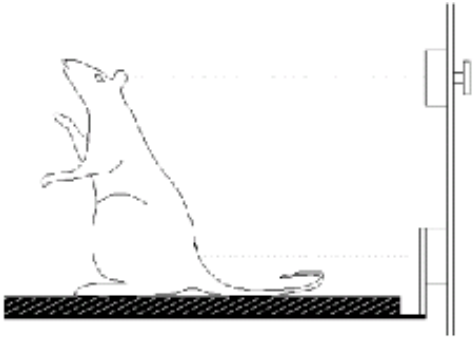
Open Field


TSE, ActiMot



Calculating the center of gravity


Detecting a rearing

www.TSE-Systems.com

- **locomotion**: distance travelled (cm)
- **exploration:** vertical = rearings; horizontal = distance
- **anxiety**-related behavior: time spent in center (%) – more centre time = less anxiety
- **habituation:** time course
- Conditions: length: 45.5 x 45.5 cm, walls: 39.5 cm
  150 – 200 lx, Center: 42%
  Time: 20 min
  Infrared beam breaks

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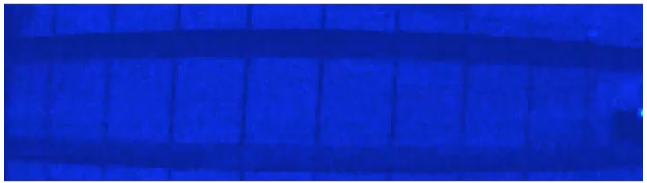
Catwalk® gait and motor coordination analysis

- Quantify locomotion and motor coordination parameters during walkway crossing by visualising foot-floor contacts in time
- Subjects walk across an illuminated glass platform while a video camera records from below.
- Gait related parameters are reported for each animal.



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Catwalk parameters measured

- **Dynamic paw parameters**
  - **Step cycle** – time in seconds between two consecutive initial contacts of the same paw with the glass floor consisting of a stand phase and a swing phase
  - **Stand phase** – duration in seconds of contact of a paw with the glass floor
  - **Swing phase** – duration in seconds of no contact of a paw with the glass floor
  - **Cadence** – how many steps are taken in one minute

  - **Coordination**
  - **Stand index** – speed at which a paw loses contact with the glass floor at the initiation of the swing phase
  - **Print position** – distance in cm between the position of the hind paw and the position of the previously placed front paw on the same side of the body and in the same step cycle.
  - **Initial dual stance** – this is the first time in a step cycle of a front paw or hind paw that the contralateral front or hind paw also makes contact with the glass plate.
  - **Phase dispersion** – this is a measure of inter limb coordination and describes the temporal relationship between placement of two paws within a step cycle.

- **Static paw parameters**
  - **Stride length** – distance between successive placements of the same paw
  - **Print length** – length in cm of the complete print
  - **Print area** – surface area in cm^2 of the complete print

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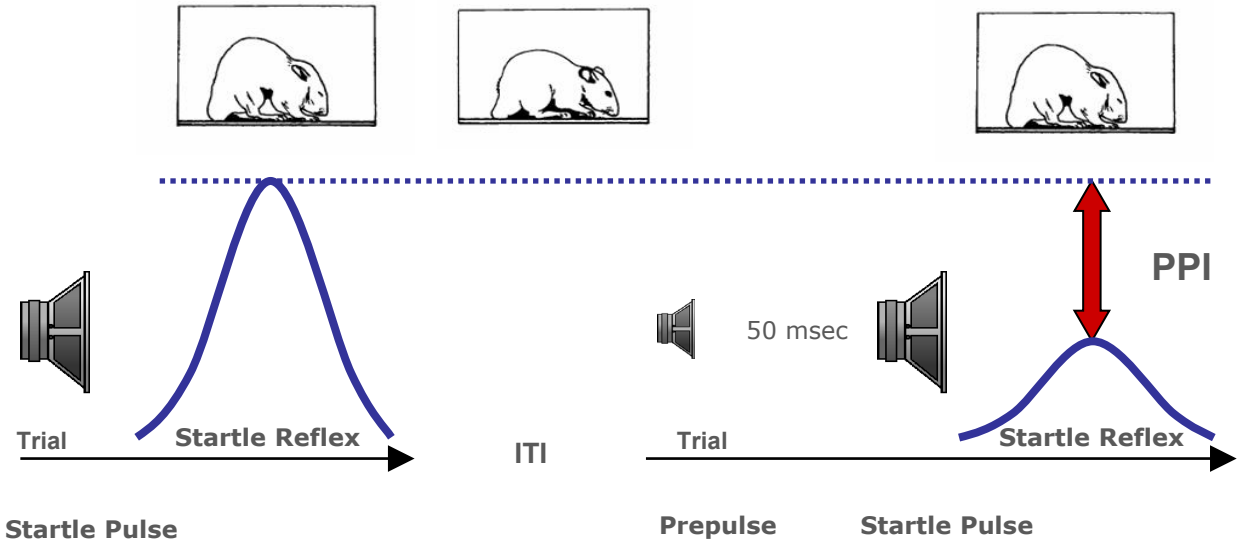
Prepulse inhibition of acoustic startle reflex



- Tests sensorimotor gating and recruitment
- Sensitive to alterations in sensorimotor integration

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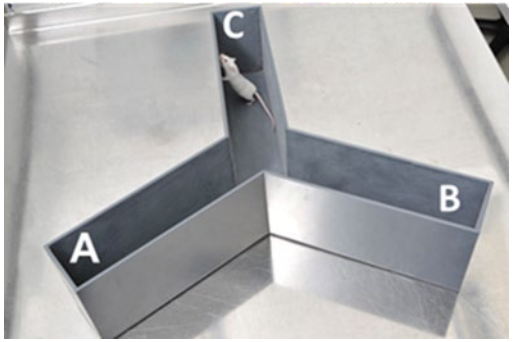
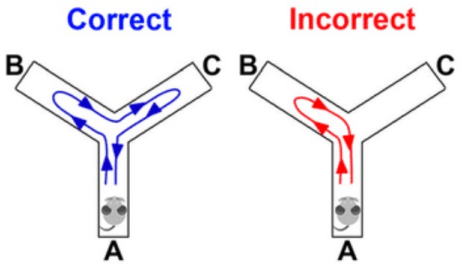
Y maze spontaneous alternation test – working memory



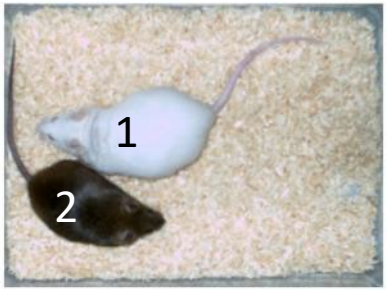


- Spontaneous alternation (SPAs): ABC, BCA, CAB
- Alternate arm returns (AARs): ABA, ACA, BCB, BAB, CAC, CBC
- Same arm returns (SARs): AAA, BBB, CCC

- ↑ % SPAs = enhanced spatial working memory
- ↓ % SPAs = impaired spatial working memory

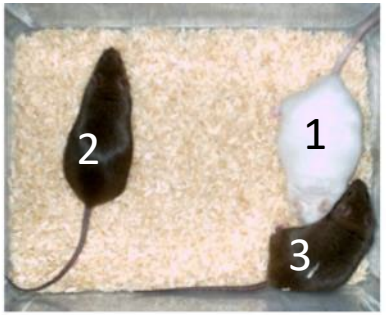
Social Discrimination – social & olfactory memory

2 hours habituation →



Sample phase: familiar subject (2) is introduced to the tested animal (1)

2 hours break →



Test phase: unfamiliar subject (3) together with mouse 1 and 2

Tested Parameters: contact with familiar subject, contact with unfamiliar subject.

Assesses olfactory-based social memory

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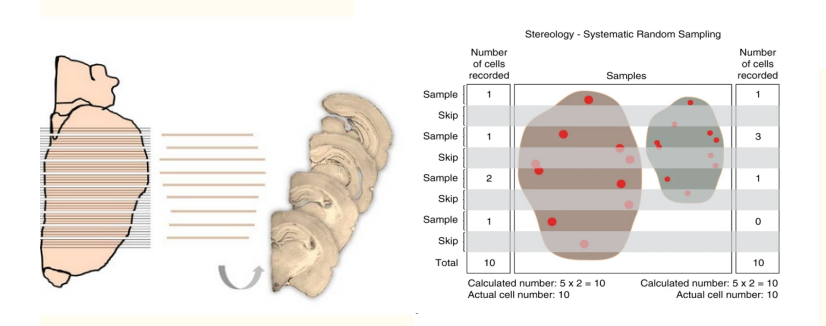
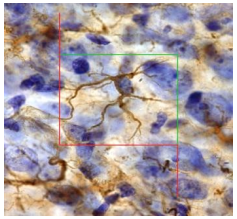
Chronic neuroinflammation in the brain?

Stereological analysis of brain-region specific astrocyte and microglia

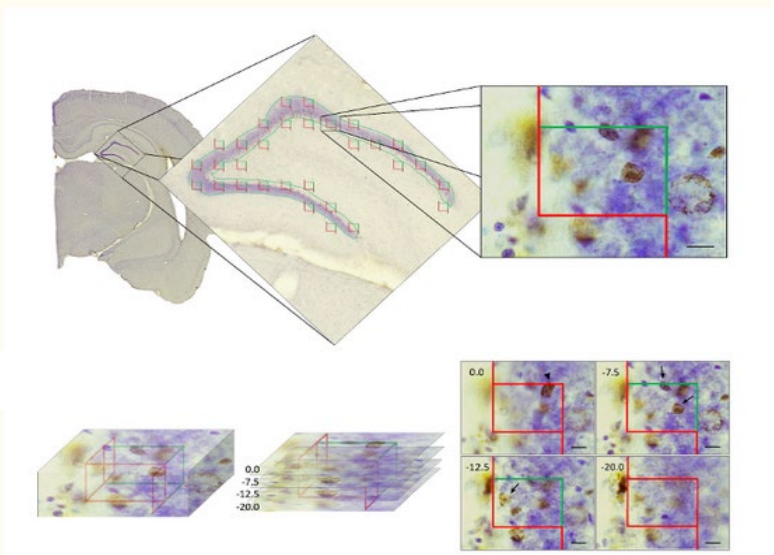




IBA1+ microglia

(Using Stereoinvestigator from MBF Bioscience)

Optical Fractionator cell quantification



(Olesen et al., 2017)

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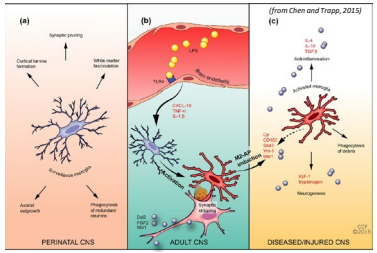
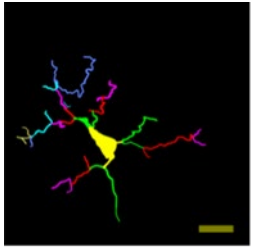
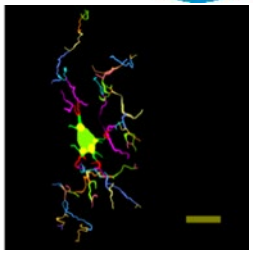
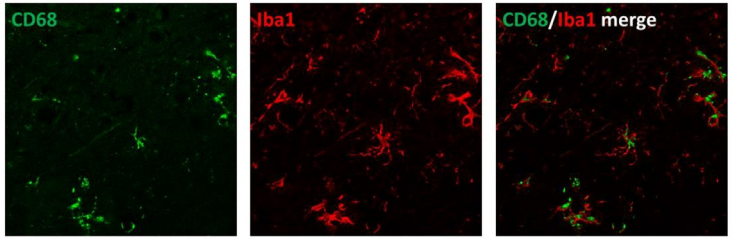
Advanced characterization of glial cells

Microglia morphology analysis – activation state

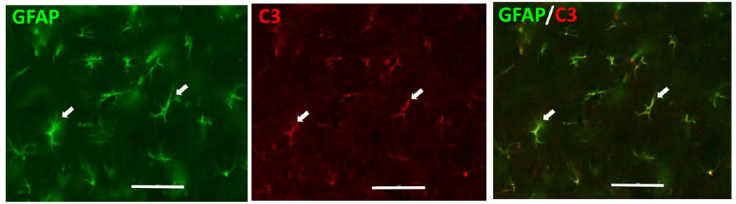








IBA1+ microglia + CD68 =
Actively phagocytic microglia



GFAP+ astrocytes + C3 =
Potentially neurotoxic form of
Activated astrocyte

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Blood	- Targeted Metabolome – ITEM - Inflammation acute phase – NRCWE
Brain	- Neuroinflammation – HMGU
Lung	- Histopathology on lung toxicity – RIVM - Inflammation acute phase – NRCWE
Liver	- Inflammation acute phase – NRCWE

All other organs conserved and stored for further analysis if needed

Acknowledgements

InstPharmToxBw

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Thank you.

https://twitter.com/i/status/1612083675117621248