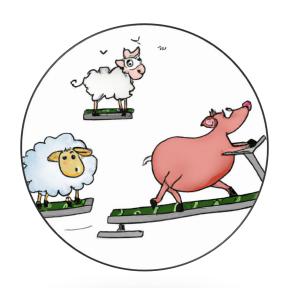
Automated real-time activity monitoring in animal experiments with various livestock species

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Acknowledgements

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Biotechnicians



Animal experiments in the EU

In EU, 8 million animals are used annually for animal experiments*

>400k 'domestic fowl',>120k 'farm mammals'and 10k cats & dogs

Species	Number of animals	Percentage
Mice	4010766	47.86%
Salmon, trout, chars and graylings	1289139	15.38%
Other fish	691587	8.25%
Rats	625777	7.47%
Domestic fowl	417903	4.99%
Rabbits	372239	4.44%
Zebra fish	362449	4.33%
Sea bass	116706	1.39%
Pigs	86953	1.04%
Guinea-Pigs	85167	1.02%
Other birds	80531	0.96%
Guppy, swordtail, molly, platy	78388	0.94%
Hamsters (Syrian)	21613	0.26%
Cattle	21434	0.26%
Turkey	18577	0.22%
Sheep	15909	0.19%
Etc.		





Typical set-up of an (infectious disease) experiment

Timeline:

Day 0: Arrival challenge/treatment

Acclimatisation Post-treatment

- Treatment and control groups
- Especially in weeks after treatment, various parameters are measured



Monitoring behaviour in animal experiments

Why?

To monitor welfare



Refine studies

To better understand effect of treatment



Improve studies

How?

Traditionally, caretaker observations



(bi-)daily snapshots

Increasingly, also **sensor technologies**



Continuous Objective Automated Simultaneous



Many sensor technologies out there, but not all appropriate for our setting of interest...



<u>Aim</u>

Explore technologies and **develop a tool** for automated and real-time monitoring of individual behaviour, particularly activity, in (livestock) experiments

Conditions & requirements

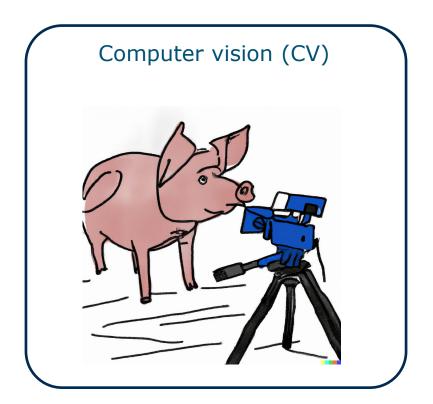


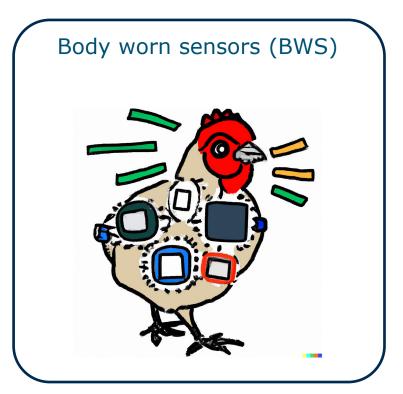
- Multiple (non-rodent) species
- Group housing
- Biosafety levels 2 & 3
- Easy installation

- Duration: 4-8 weeks
- Minimise handling & impact
- Real-time
- Affordable & re-usable



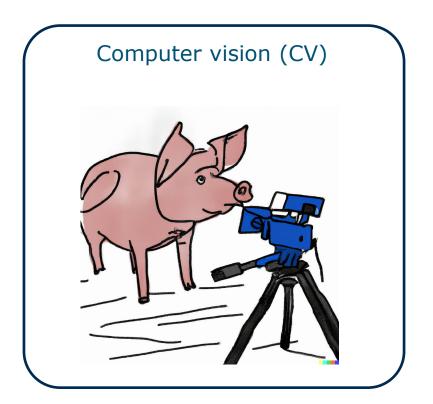
Two types of technologies







Two types of technologies

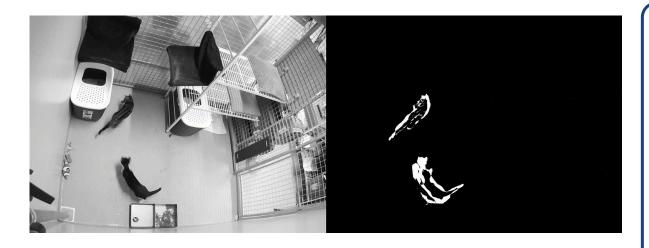






CV example (I): frame differencing

- Pros: simple, cheap, no impact on animal
- Cons: background dependence, no individual data







CV example (II): individual detection and tracking with e.g. neural networks

- Pros: individual data, no impact on animal
- Cons: large effort to train and run, challenges with a.o. (re-)identification, occlusion and multi-camera tracking*





*Wang et al. (2024), EAAP book of abstracts p. 403

CV example (III): Aruco markers

- Pros: individual data, open-source detection tools (no annotation)
- Cons: attachment, visibility (e.g. dirt/feathers), motion blur

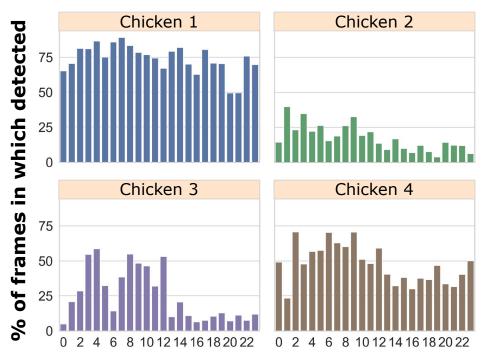








Variation in Aruco detections across animals and time



In cases of few detections, animal could be:

1. Inactive in occluded area;

OR

2. Active and not detected due to motion blur, occlusion due to feathers, etc.

Time (hour of day)



CV example (IV): Aruco & frame differencing

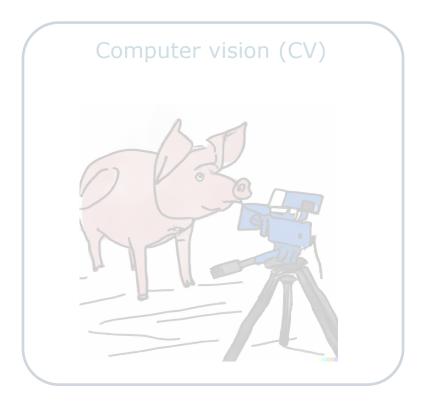
- Combine approaches to better assess specific behaviours
- E.g. 4 days after *T. gondii* infection, rams halved their time drinking

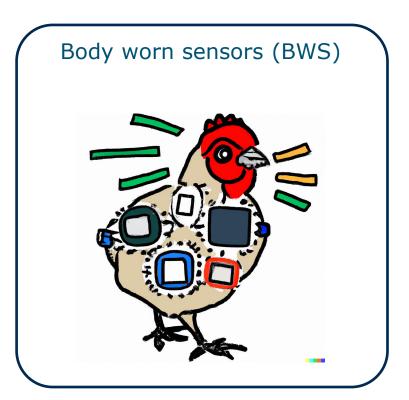






Two types of technologies

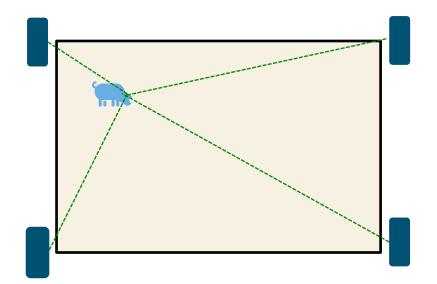






BWS example (I): ultra-wideband

- Pros: individual location data
- Cons: sensor size (~25 g; for animals > 500 grams), laborious installation, error of 20-30 cm, hardware issues → missing data

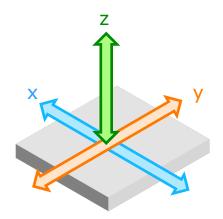




Noldus

BWS example (II): accelerometers

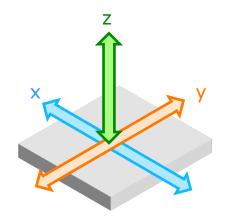
- Pros: small sensor size (e.g. 10g), reliable data stream, relatively simple installation and data processing, affordable
- Cons: acceleration is an indirect measurement of activity/behaviour





Accelerometers

Acceleration in x, y and z



- Acceleration consist of 2 components:
 - 1) Static: gravity, perpendicular to earth
 - 2) Dynamic: resulting from movement

Vectorial Dynamic Body acceleration (VeDBA):

$$VeDBA_{i} = \sqrt{(x_{i} - \overline{x_{i}})^{2} + (y_{i} - \overline{y_{i}})^{2} + (z_{i} - \overline{z_{i}})^{2}}$$

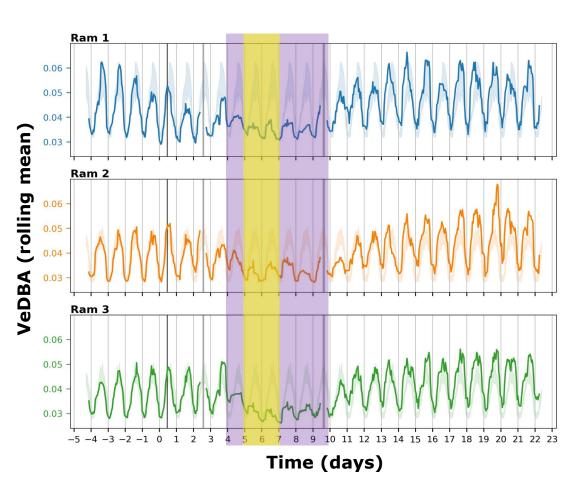


Example: activity of sheep infected with *T. gondii*

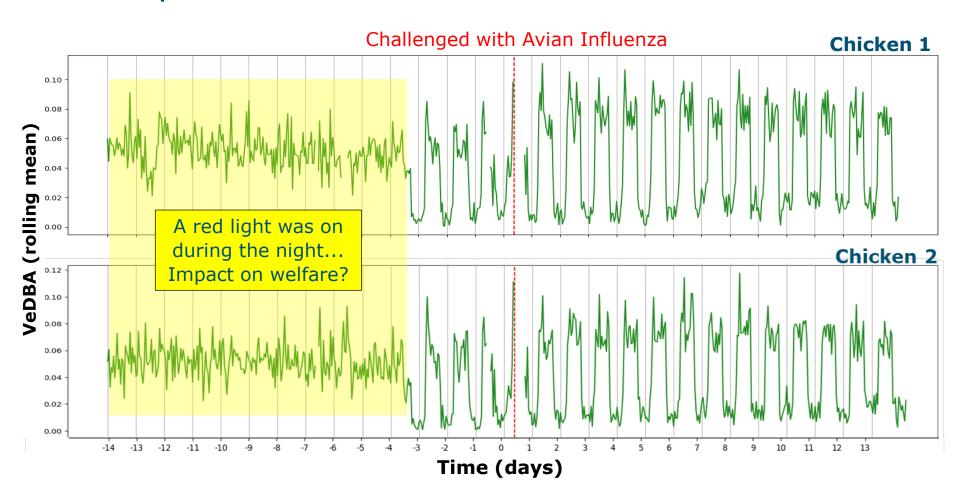
Biotechnicians only recorded a decreased activity in day 5 & 6 (!)





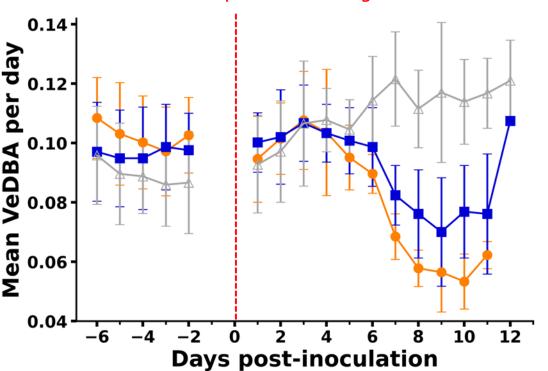


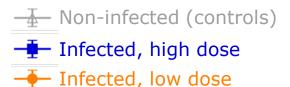
Example: environmental influences



Example: comparing infected and non-infected

Infection of sheep with Bluetongue virus





VeDBA of treatment animals reduced by >30%, while that of controls increased by 30%

No sign. difference between two doses



RAMSMART: a tool for Real-time, Automated and Multi-Species Monitoring of animal Activity in Research Trials



Specs of the device

What?	Specification	
Sensors	Accelerometer (up to ±	The state of the s
	and magnetometer, 12.	5 to 833 Hz. flexibility ©
	Heart rate and non-med	dical ECG.
Size	Diameter 36.6 mm	
	Thickness: 10.6 mm	
Weight	10 gram "5% rule" -	rianimals ≥200 grams ©
Battery	CR2025 coin cell	
Data transmission	BLE (2.4GHz) directly to	o a nearby laptop
Edge computing	Yes no need to trans	smit raw data → battery life ©
Waterproofness	Water resistant to 30m	
Costs	~ € 100 per sensor	affordable & re-usable ☺
	~ € 0,60 per battery	





(1) Build software for edge computing & upload to device







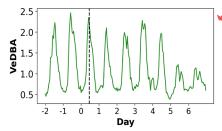
(2) Collect data, compute VeDBA on device and broadcast result

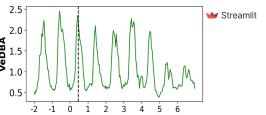


RAM **SMART**



(4) Postprocess and visualise results



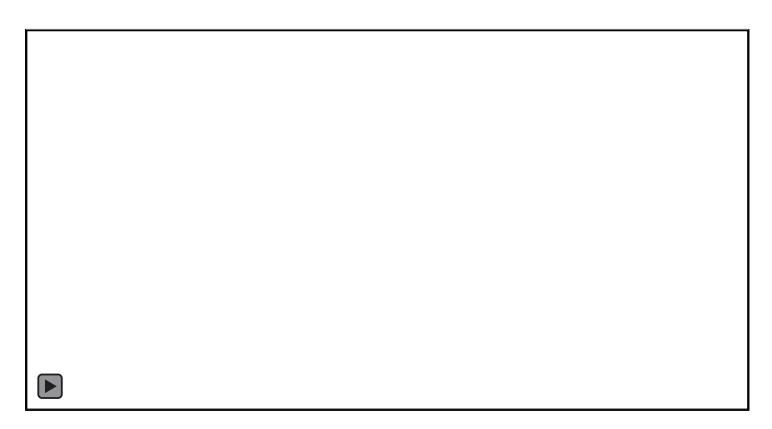




(3) Add timestamp and ID and store in SQLite database



Example visualisation





How long can we monitor?

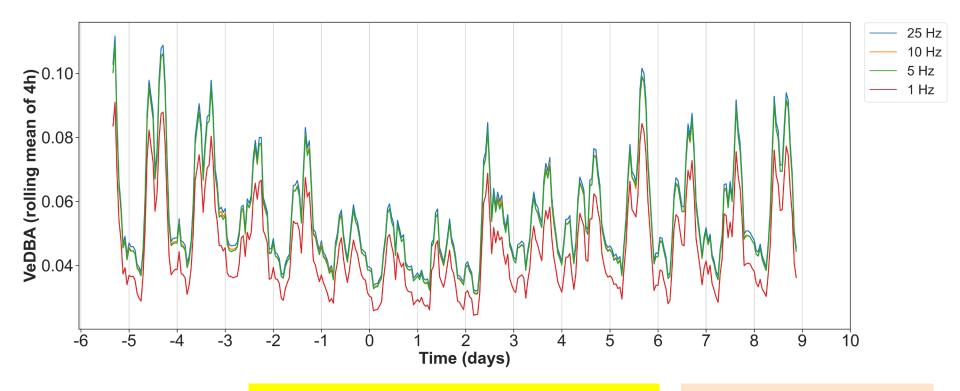
 Depends on frequency of sampling, frequency of updating VeDBA, and frequency of broadcasting data packages

- Current settings:
 - ➤ Sampling at 13 Hz
 - Update VeDBA every minute
 - Broadcast at 4 Hz

Battery life >8 weeks



How frequent should we sample?



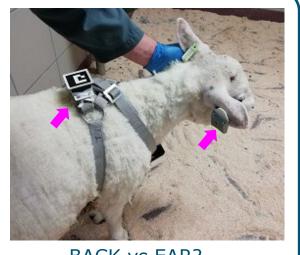


Lower frequency → slightly lower VeDBA, but patterns remain similar

Similar results in cattle, chicken and sheep

Does it matter how we attach the accelerometer?

Sheep



BACK vs EAR?

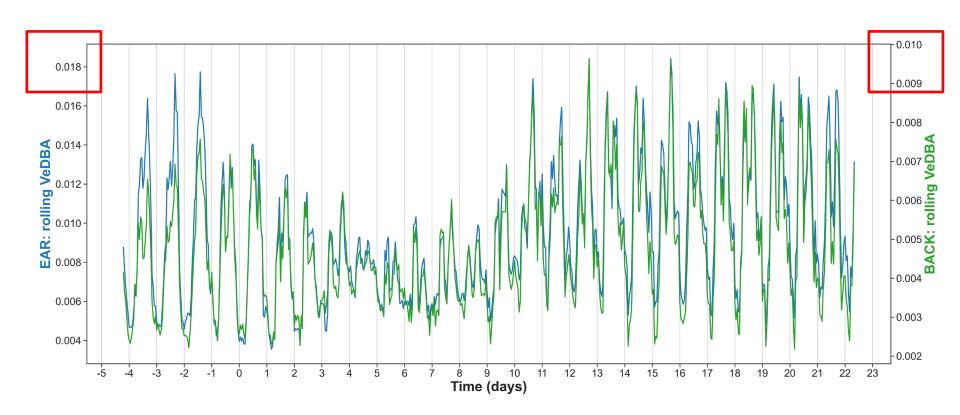
Calves





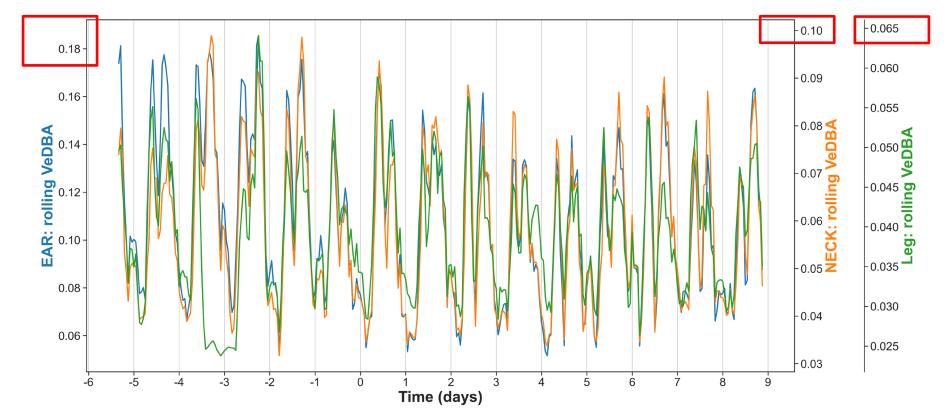
LEG vs BACK vs EAR?

Effect of accelerometer position in a sheep





Effect of accelerometer position in a calf





_EG < NECK < EAR, but patterns fairly similar

Conclusions

- Sensor-based behavioural monitoring has added value in animal experiments:
 - To earlier detect deviations and quantify their severeness
 - To detect (unintended) environmental influences

But always be aware of the pitfalls of each approach

An accelerometery-based tool ticks most of our boxes



Multiple species Real-time >8 weeks Relatively low RAM Plug & play frequency is sufficient **SMART** Re-usable Position independent Affordable **Automated**







Extra slides



VeDBA and oxygen consumption

 ODBA and VeDBA correlate well with rate of oxygen consumption in treadmill tests, e.g. Qasem et al 2012

Humans

	Straight	Skewed
ODBA	$VO_2 = 1132.ODBA + 615 \text{ r}^2 = 0.915$	$VO_2 = 1466.ODBA + 776$ $r^2 = 0.94$
VeDBA	$VO_2 = 1664.VeDBA + 636 \text{ r}^2 = 0.914$	$VO_2 = 1659.VeDBA + 629$ $r^2 = 0.91$

Various species

Species	ODBA	VeDBA
Chaetophractus villosus	0.9775	0.942
Myocastor coypus 1	0.9594	0.94
Myocastor coypus 2	0.7449	0.7019
Myocastor coypus 3	0.9473	0.9486
Myocastor coypus 4	0.8617	0.8568
Cairina moschata	0.9853	0.9841
Anser anser 1	0.9022	0.8904
Anser anser 2	0.9427	0.9242
Spheniscus magellanicus 1	0.975	0.9662
Spheniscus magellanicus 2	0.8979	0.811
Eudyptes chrysocome	0.9914	0.9957
doi:10.1371/journal.pone.00		

doi:10.1371/journal.pone.0031187.t001

Comparing activity of Aruco with VeDBA

- VeDBA of chickens is similar, but Aruco-distance for animal 5 is underestimated
- For both chickens, correlation between approaches is poor to moderate (0.44-0.66)

