



The 9 to 5 rodent – Time for change?



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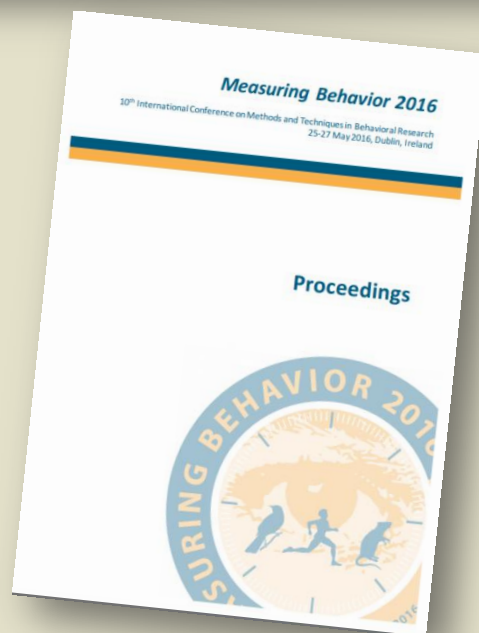
The 9 to 5 rodent: time for change?

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The 9 to 5 Rodent – Time for Change? Scientific and animal welfare implications of circadian and light effects on laboratory mice and rats

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HIGHLIGHTS

- Time of day and lighting can affect data obtained from rodents in neuroscience studies.
- Studying animals during their inactive period may be detrimental to their welfare.
- Where possible nocturnal animals should be tested during their active phase (night time).
- Studying behaviour in the active phase requires solutions such as automated testing.
- The time of day and lighting conditions should be reported for all studies.

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ABSTRACT

Rodents, particularly rats and mice, are the most commonly used laboratory animals and are extensively used in neuroscience research, including as translational models for human disorders. It is common practice to carry out scientific procedures on rats and mice during the daytime, which is the inactive period for these nocturnal species. However, there is increasing evidence for circadian and light-induced effects on rodent physiology and behaviour which may affect the validity of results obtained from mice and rats in neuroscience studies. For example, testing animals during their inactive periods may produce abnormal results due to cognitive deficits, lack of motivation to perform the task or stress from being disturbed during the resting period. In addition, conducting procedures during an animal's resting period may also pose an animal welfare issue, as procedures may be experienced as more stressful than if these were done during the active phase.

In this paper we set out the need to consider the impact of time of day and lighting conditions, when scientific procedures or routine husbandry are performed, on both the welfare of mice and rats used in neuroscience research and on data quality. Wherever possible, husbandry and experimental procedures should be conducted at times of day when the animals would be active, and under naturalistic lighting conditions, to minimise stress and maximise data quality and translatability.

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1. Introduction

Mice and rats are widely used in research and testing, in a wide range of disciplines that examine both normal and pathological behaviour and physiology. However, rodents are often housed and tested in laboratories in environments which differ significantly from their living conditions in the wild (Burn, 2008; Castelblanco-Carlos and Baumann, 2009; Latham and Mason, 2004).

Although it may be tempting to conclude that strains bred in the laboratory for many generations are adapted to that environment, this is not borne out by the animals' behaviour or by experimental evidence. Laboratory rodents rapidly exhibit naturalistic behaviours when placed in an environment which allows these to be expressed. For instance, mice exhibit a strong urge to build nests when provided with nesting material (Caskill et al., 2012), and rats are highly motivated to dig burrows (Makowska and Weary, 2016). Laboratory rats have also been shown to exhibit natural social, exploratory and foraging behaviours when housed in semi-wild conditions and, importantly, these animals also rapidly revert to a predominantly nocturnal activity pattern (Berdy, 2002).

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measuringbehavior.org/files/2016/MB2016_Proceedings.pdf pages 361-78
sciencedirect.com/science/article/pii/S0165027017301334



Why is this an issue?



- Mice and rats are the majority of lab animals
 - EU statistics for 2011: mice (61 %) and rats (14 %) are most commonly used
- Wild-type behaviours are innate in lab animals – if they can express these, e.g. *Ratlife* video
- Laboratory housing, husbandry and care can conflict with wild-type behaviours

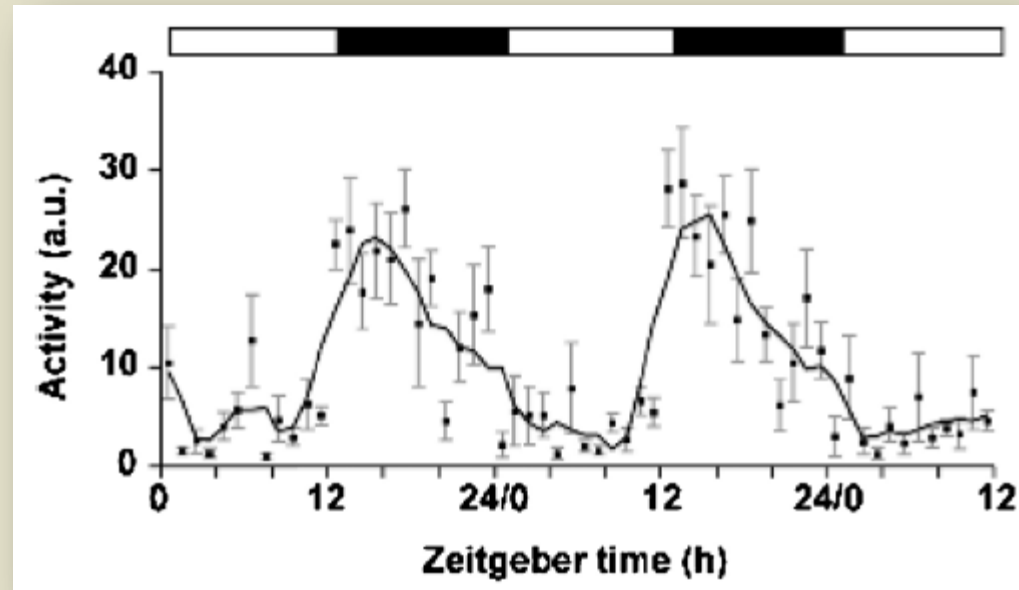
Conflicting needs ...

Mice and rats are but
Nocturnal and crepuscular	they are housed in bright light and used during the day when they would normally be sleeping
Highly dependent on smell and scent markings	their markings are completely destroyed whenever the cage is cleaned
Made extremely anxious when captured by the tail (mice)	most people catch them by the base of the tail
Most comfortable at a temperature of 26 to 34 °C (mice)	many facilities house them at colder temperatures, and/or do not provide sufficient nesting material



Mouse and rat circadian rhythms

- Nocturnal and crepuscular
- Multiphasic sleep - many short bouts, more in the light phase
- Husbandry and experimental procedures usually done in the light phase



Male C57BL/6 mice over 60 hours

Better welfare = better science

- What are the implications for **animal welfare and science**, when nocturnal animals are used in the light phase and during the human working day?



Animal welfare implications of using mice and rats in the light phase



Sleep and welfare

- Mice sleep deeply at the beginning of the light phase – the beginning of the human working day
- Disrupting the light cycle increases aggression and stress in mice
- Rats are more susceptible to chronic mild stressors when they occur during the resting period
- **Conducting procedures when nocturnal animals would normally be asleep is a welfare issue**

Out like a light ...?



EFFECTS OF SLEEP DISRUPTION AND NESTING MATERIAL

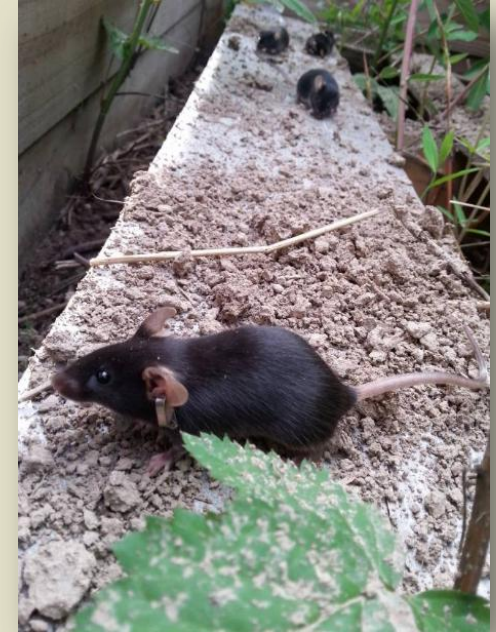
- Cage clean and welfare checks at 10.00 or 22.00 (lights on 07.00 to 19.00)
- Mice appeared to anticipate and acclimate
- Stressors were mild & predictable, animals singly housed
- Mice given 9 g or 12 g nesting material slept in longer bouts (than 3g or 6g)

Care and accommodation

DIRECTIVE 2010/63/EU ARTICLE 33(1B)

“Member States shall ... ensure that ... any restrictions on the extent to which an animal can satisfy its physiological or ethological needs are kept to a minimum.”





Leung JM, Budischak SA, Chung The H, Hansen C, Bowcutt R, et al. (2018) Rapid environmental effects on gut nematode susceptibility in rewilded mice. PLOS Biology 16(3): e2004108. doi.org/10.1371/journal.pbio.2004108

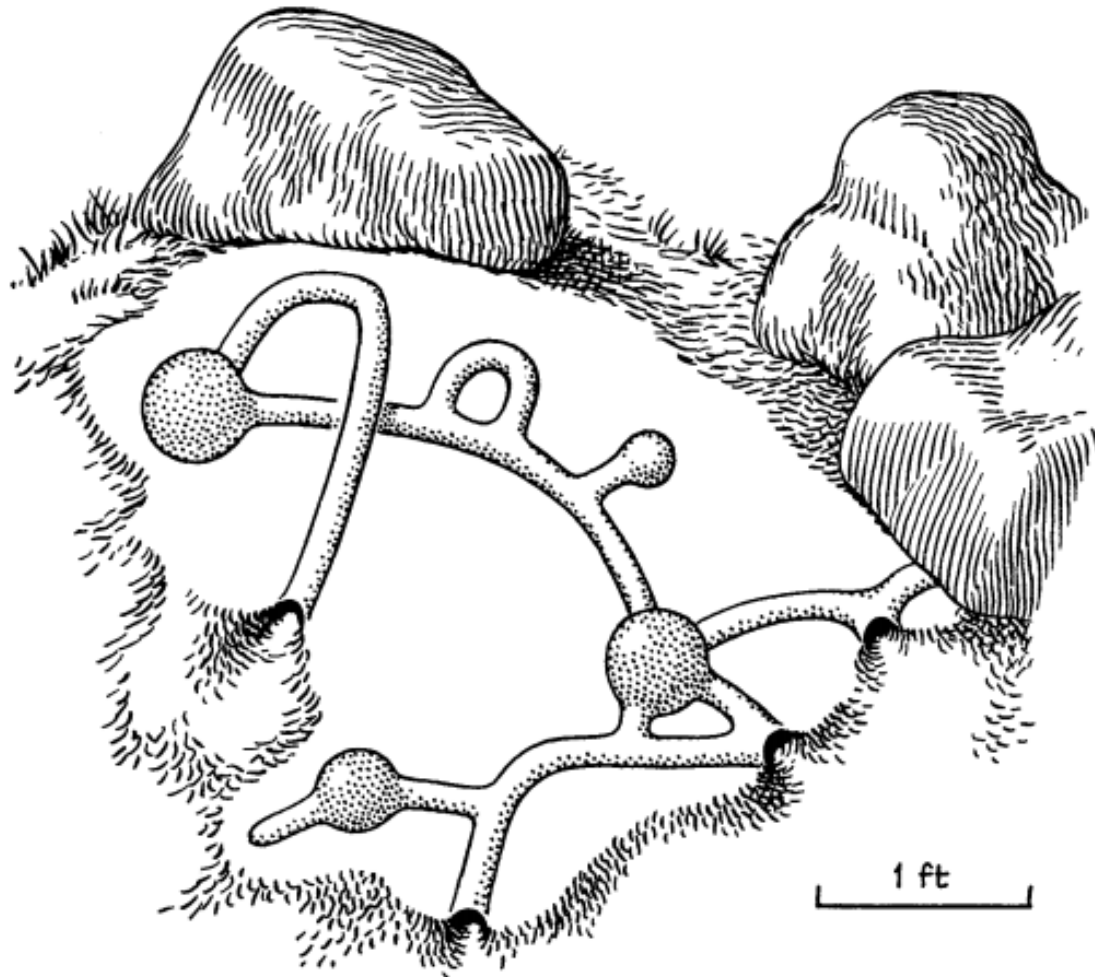
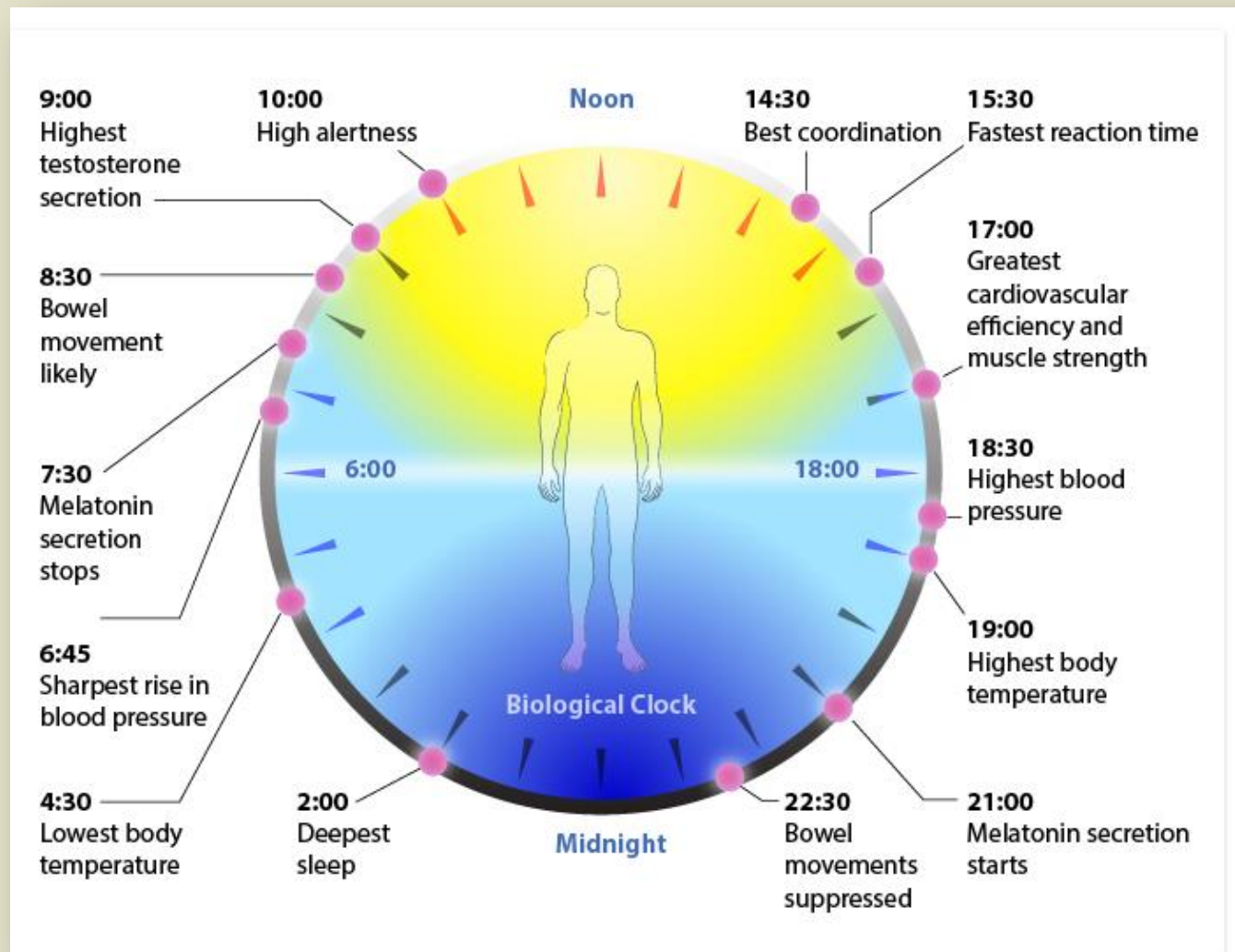


FIG. 5

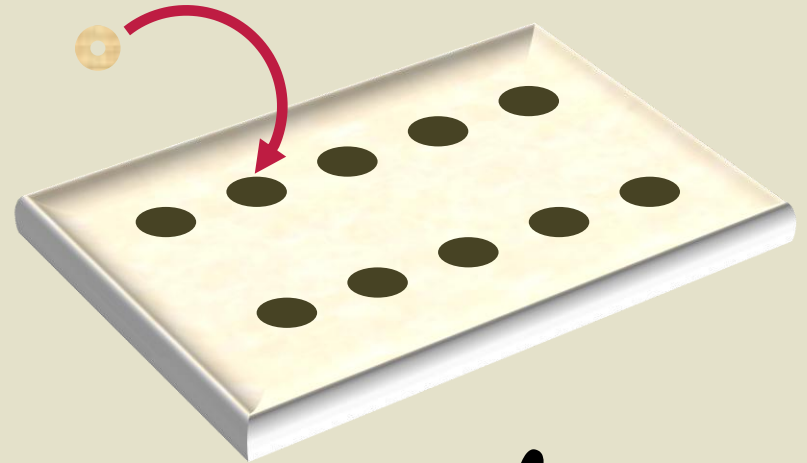
Excavation of a typical mouse burrow in earth on Skokholm. The runway system was in a 30° grassy slope, with the actual runways up to about 9 in. below the surface (from Berry, 1968a).

Scientific implications of using mice and rats in the light phase



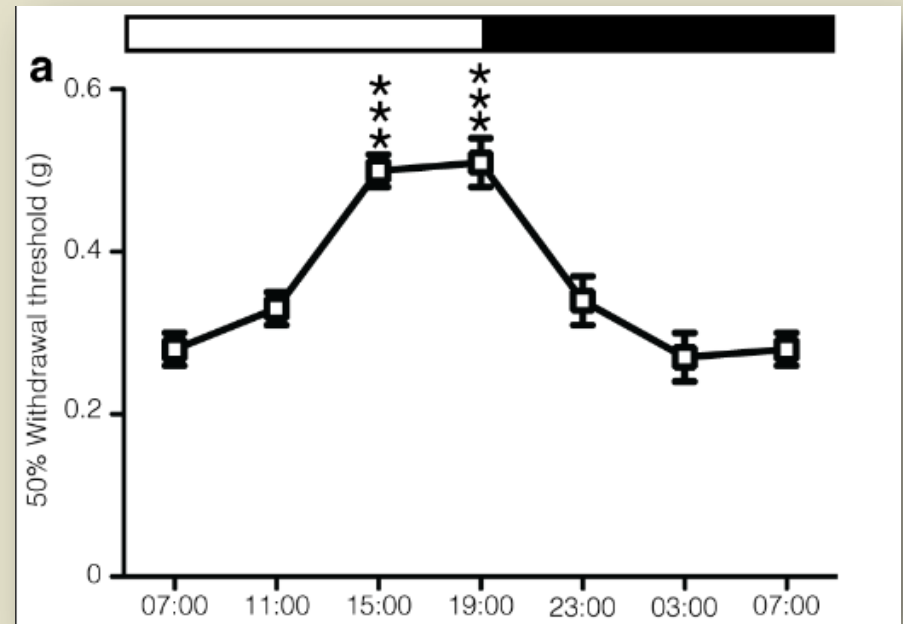
Outcomes of a cognitive test

- DBA mice were tested using a modified hole board
- Behavioural inhibition and cognitive disruption under white light, in comparison with dark/red light



Pain responses

- Significant increase in 50 % withdrawal threshold to mechanical paw stimulation in C57BL/6 during light phase
- Pain behavioural phenotypes may be misinterpreted, or missed altogether



Behavioural responses to von Frey hairs applied to the hindpaw over a 24 h period

Time windows and reproducibility

BEHAVIOURAL TESTS, E.G. ANXIETY, LEARNING

- Lights off at 09.00, on at 21.00
- Tests at 08.15-08.45, 10.45-11.15, 16.30-17.00
- Significant strain effects depending on testing time
- Tests included elevated plus, open field – applying this data could have significant consequences



It's complicated ...

- Some behavioural studies have found no effects of timing regarding circadian rhythms
- Or better performance during the light phase, driven by circadian rhythms
 - Why? Possibly effects of preceding sleep and arousal, especially if tests are especially anxiogenic
- Visual function may be impaired during the active (dark) phase, e.g. contrast sensitivity is reduced
- **A nocturnal species at night \neq a diurnal species in the day**

How to address these animal welfare and scientific issues?

1. Working assumption

Disturbing, and using, mice and rats in the light phase is an animal welfare issue and may also affect the data



2. Research current knowledge on:

- Sleep-wake cycles and sleep behaviour of the species and strain – including timing and physical environment
- Welfare impact of disturbing, and using, the animals at different times within the sleep-wake cycle
- Effects on the science and implications for the 'model'

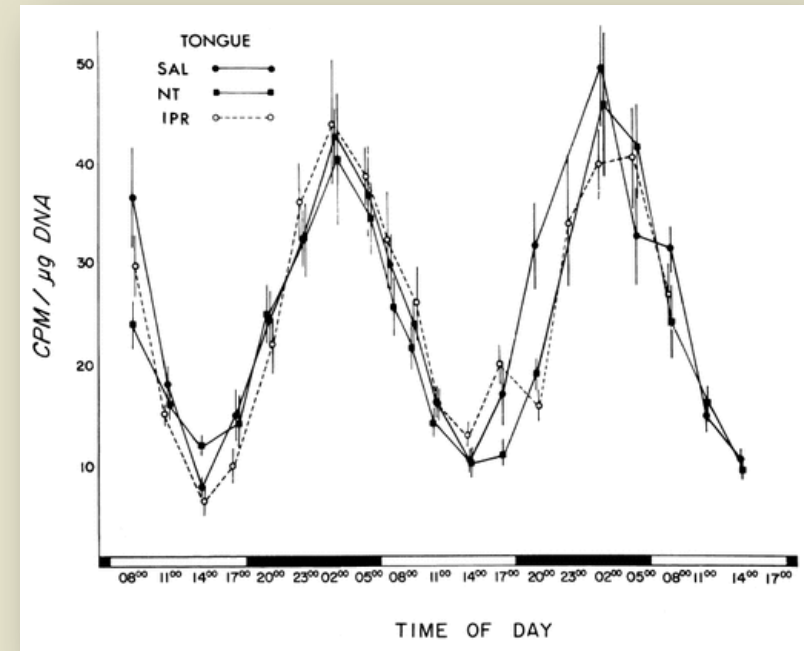
PREPARE

- ☐ The relevance of the species, its biology and suitability to answer the experimental questions with the least suffering, and its welfare needs
- ☐ Acclimatization, optimal housing conditions and procedures, environmental factors and any experimental limitations on these

3. Reconsider protocols

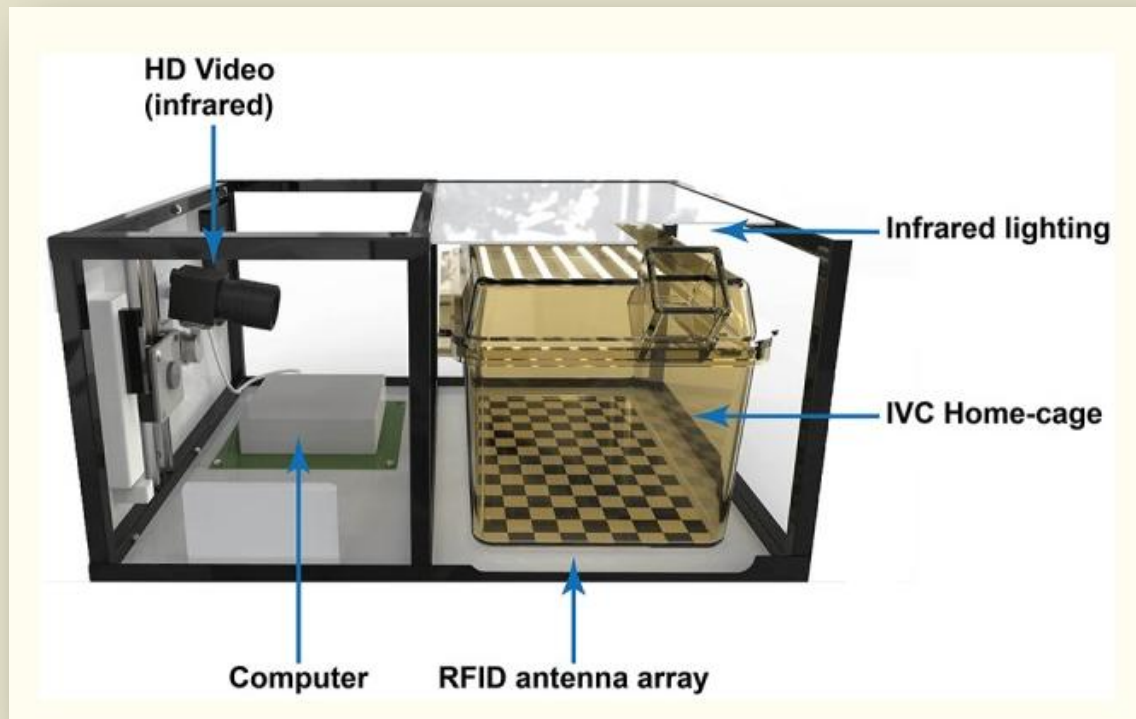
TIMING OF PROCEDURES

- Repeat tests every 2-4 hours
(Burns 2000, search for '[Biological time and in vivo research: A field guide to pitfalls](#)')
- Systematically include two different testing times ([Bodden et al. 2019](#))



4. Reconsider test paradigms

AUTOMATED BEHAVIOURAL TEST SYSTEMS THAT ANIMALS
ACCESS VOLUNTARILY



5. Reverse the dark and light phases?

THINGS TO CONSIDER

- Practical factors – health checks and procedures
- Just a flash of light can disrupt the rhythm
- Mice CAN see in red light! (and sodium vapour light)
- Dim light at night affects mood, immune function
- Facility will be noisy when animals are active – another welfare issue?



5a. An alternative – time shifting?

- Lights on well before human working day begins
 - Working day begins at 0700, staggered 12:12 light cycles with lights off at 0800, 0900 and 1000
 - 12:12 cycle with lights out at midday
 - 12:12 cycle with lights out at midnight



6. Reporting standards

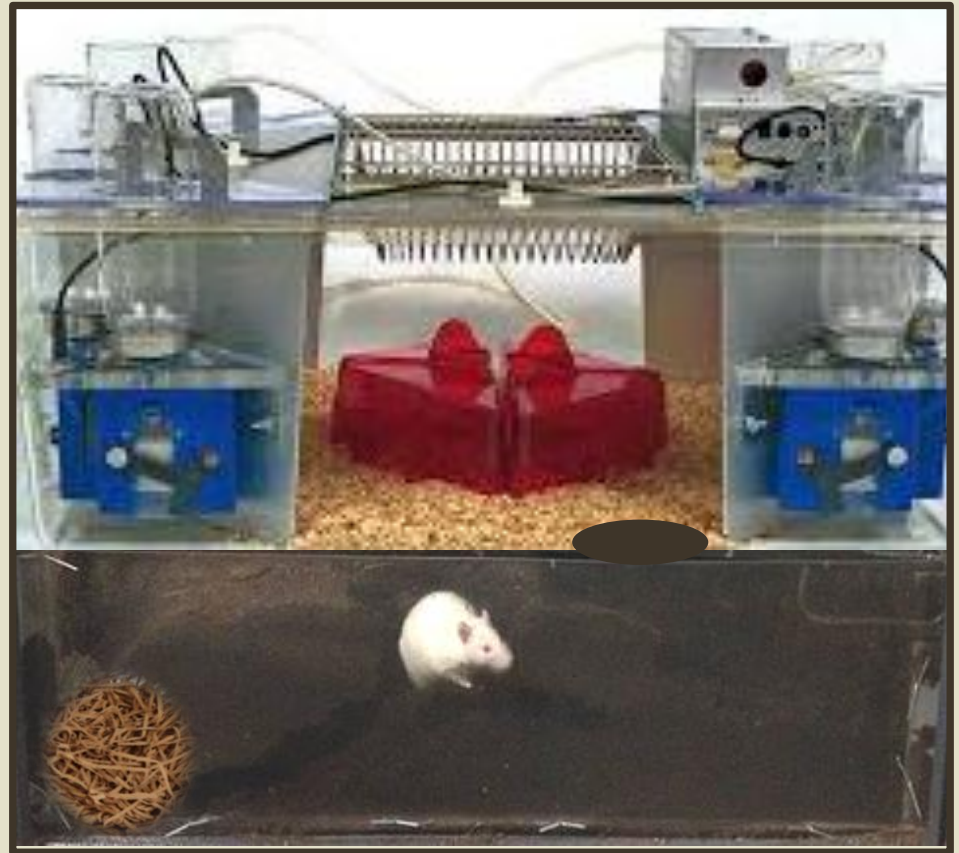
- Good practice guidelines for reporting, e.g. Gold Standard checklist/ARRIVE
 - tinyurl.com/3Rs-GoldStandardChecklist
 - NC3Rs.org.uk/ARRIVE
- Sufficient information
 - Light:dark, light levels, time and day, whether ramping at dawn and dusk ...?

ARRIVE

- ☐ When (e.g. time of day)
- ☐ Husbandry conditions (e.g. light/dark cycle)
- ☐ Comment on any limitations of the animal model

Ideal scenario?

- Large cages with deep litter for burrowing
- Automated tests that animals could choose to do
- Continuous behaviour and welfare monitoring
- Continuous data collection



Action points

- 'Time stamp' all your experiments
- Aim to test in dark (active) phase for the sake of the animals' welfare
 - Unless there is scientific justification otherwise
- Consider setting at least two time points per day for obtaining data, e.g. in dark (active) and light phase
- Consider changing to paradigms in which animals can choose when to access tests
- Include light phases and times when reporting animal use and share data!

Thank you!

