Assessing the impact of environmental conditions on animal behaviour

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Abstract

Pioneers of ethology Tinbergen and Lorenz proposed that instinctive and innate animal behaviours are built from simpler stereotyped forms based on observations by eye. Their work relied on behaviour patterns drawn from the life cycle of animals, in contrast to modern laboratory situations. To resolve this discrepancy, we assessed the impact of environmental conditions on laboratory mice. We collected large video image datasets of untrained untethered animals. In parallel, we recorded data on light, temperature, and sound. We found correlations between sleep/ wake cycles of mice with behavioural task performance. In summary, we systematically describe the relationship between mouse behaviour and environmental conditions using machine vision and learning techniques.

Introduction

Behaviour is a mirror in which everyone shows his image.

Johann Wolfgang von Goethe [1]

Mice are the unlikely heroes of their own stories. From *Flowers for Algernon* to *The Rats of NIMH*, mice use craft and cunning to emerge victorious while living in close proximity to us. We desire to understand mouse behaviour because it illuminates the human condition. They have similar genes and suffer from many of the same diseases as us. When Charles Darwin published *On the Origin of Species*, *The Descent of Man*, and *The Expression of Emotions in Man and Animals* in the nineteenth century, it provided compelling evidence that the mind and behaviour of animals and man were intimately related and bonded by common ancestry [2-3]. Since Darwin, many scientific disciplines ranging from ethology to neurobiology continue to study how animal behaviour is generated.

We studied untrained untethered animals to better understand the environment of a laboratory mouse. On one hand, the life of a laboratory mouse is unnaturally stressful [4]. The standard temperature for mouse facilities (20-26 °C) is comfortable for researchers but lower than optimal for mice [5-6]. On the other hand, a laboratory mouse has no competition for food or territory. We provide insight on how environmental stressors can influence mouse behaviour.

We were motivated to pursue this question because of consistent poor behavioural performance in mice. For eight months, ninety percent of head-fixed mice did not run continuously on cylindrical wheels for food when they were food-deprived. Moreover, there was a dropout rate as high as two months where mice that successfully displayed running behaviour and consumed food reward no longer performed the task. This suggested that there was an active process by which mice lost motivation to run for food reward.

We hypothesized that the laboratory environment was stressful for the mouse and led to poor behavioural performance by disrupting its sleep/wake cycle. To test this hypothesis, we analysed six mice for seven days over the course of training while monitoring light, temperature, humidity, vibration, and ultrasound. We demonstrate that the sleep/wake cycles of mice are correlated with their behavioural performance. Moreover, we identified the source of stress to be fluorescent light fixtures in the animal room, which generate ultrasonic noise above 20kHz. We recommend refinements in laboratory environments to promote the welfare of animals and improve the quality of scientific data.

Results

The following external factors and environmental variables can impact animal behaviour:

- Light [light control unit, dark light cycle, power of light]
- Temperature [temperature control unit, climate]
- Humidty
- Barometric pressure
- Visual effect [what we see]
- Airflow [air-conditioning system]
- Sounds [equipment generating sounds, voices]
- Vibration [refrigerators, ventilators, PCs, specific equipment]
- Electromagnetic waves [transmitting equipment, magnets, mobile phones]
- Smell [specific gases, perfumes]

Animal experimentation must be preceded by controlling the above external factors and environmental variables. We explored these variables and identified extremely high levels of ultrasound in animal rooms. This can be caused by devices (measurement equipment, switching power adaptors) or general infrastructure (tube lights (TL) or gas-discharge lamps, ventilation, air-conditioning system). Some animal facilities attempt to avoid this issue by putting on a speaker connected to a radio, which is tuned to a music station. However, the frequency range of the speaker is often limited from 500 Hz to 12 kHz, which covers only 10% of the frequency range that can be heard by a standard mouse. In addition, the volume of the speaker is far lower than some of the sounds in the ultrasonic range. Another potential source of stress is vibration, high air cycles, and air pressure drops.

Light

Colour temperature [wavelength], intensity [lux], and duration [photoperiod] of light affect mice [9]. Mice are nocturnal animals and are active during dark light. They preferably play in sheltered places. The presence of enrichment objects promotes hiding behaviour. If light had a role in our study, we expected to see a difference in the performance of the top cage compared to the bottom cage. To test this, we hypothesized that the mouse in the top cage would perform worse than its counterpart in the bottom cage because it receives a greater amount of light. Our data did not support this hypothesis and we did not find any statistically significant difference between the mice. We concluded that light levels did not disrupt mouse behaviour. The lighting conditions in both animal rooms had a stable intensity over 24 hours at 325 lux (30 ft-candle). Moreover, the IVC racks and open-shelves are located far from the light fixtures such that the mice do not receive excessive light.

Both animal rooms studied use light from warm-white fluorescent tubes. Fluorescent strip lighting emits an even illumination, are easily accessible, and have a long tube life.11 To promote normal physiologic function, the light is cyclical. The rooms are on a 12-hour light – 12-hour dark cycle without any apparent problems. Consistency of the light-dark cycle is ensured by the use of automatic timers. There is a data logger for light, temperature, and humidity done at all times to prevent any mechanical or electrical failures.

Light is critical for the regulation of animal behaviour and physiology. It maintains circadian rhythms and stimulates and synchronizes breeding cycles [12]. The hypothalamic suprachiasmatic nucleus (SCN) neuronal activity is directly regulated by environmental light via the retino-hypothalamic tract [14]. Although the housing photoperiod is stable, we found that the circadian rhythms of each set of three mice in the animal rooms are not in sync. Moreover, the mouse that is awake during the white light cycle often does voluntary wheel-running and fails to perform the behavioural task the following day. This implies that an environmental condition is compromising their sleep/wake cycle, which in turn affects their behavioural performance.

In both animal rooms, dimmers are used to create twilight periods between the light and dark cycles to mimic dawn and dusk. We believe these dimmers contribute to ultrasonic noise and advise the use of timers providing a gradual light transition to better mimic natural light.

Temperature and Humidity

Ventilation affects animal welfare. The air movement in the cage is related to the temperature (affects the thermoregulatory capacity of animals) and relative humidity [10].

Vibration

The animal rooms contain fire alarms, which generate excess vibration from 8 – 8:30 AM every Monday. The alarm is sound-proof and produces a noise that disturbs humans but does not awaken mice from sleep. When the silent alarm was switched on, the animals already awake did not show a startle response, ear twitching, or any other indication of auditory disturbance. This device produces pure tones alternating between 430 and 470 Hz, below the optimal hearing range for mice and rats [14,17].

Sound

Sound is measured by its frequency or oscillations per second [Hz] and by its intensity [dB] or pressure level [SPL]. Both humans and animals respond directly to changes in intensity as opposed to frequency. Humans are aware of sounds in the 20-20,000 Hz (0.02 – 20 kHz) range. Mouse hearing is optimal between 10 – 20 kHz but can be as high as 110 kHz. Ultrasound production by mice is recognized within the range of 33 to 140 kHz and represent songs during mating. In rats, ultrasound vocalizations at 50kHz and 22kHz correspond to a positive and negative emotional state, respectively [18]. Hearing playback at 22 kHz slowed down the activity of the rats and decreased their likelihood of staying in the open arena.14 Meaningful sounds at low-intensity levels can have a considerable impact on animal physiology and behaviour by engaging limbic structures and higher centres involved in determining context and meaning [10,13]. Since ultrasound wavelength is short, it does not easily pass through a barrier such as the mouse plastic cage. Although the ultrasound microphone was kept inside an empty cage, there was a small space due to the wire extending out for the computer. One caveat of our experiment is that this small space may have been enough to allow high-frequency content from the environment to enter.

To an extent, high noise levels in animal facilities are unavoidable. The sources of sound are cage feeding and cleaning operations as well as technical devices (air-conditioners, air handlers, ventilated rack systems, electronics, video monitors, and laboratory equipment) [10]. Movement of cages and racks generated additional noise. Animals also generate noise by climbing and chewing on accessories. However, electronic appliances that generate ultrasonic noise can be removed to promote animal welfare.

Ultrasounds can be produced by electronic equipment in the animal facility. In the animal rooms studied, there were five appliances being powered:

- Laptop
- Vacuum
- Ventilation system
- Infra-red motion sensor
- Fluorescent strip lighting

Physiologic effects of noise on the mice are rarely considered [8]. However, the adverse biologic effects of noise on rodents is well-studied.

Discussion

Noise is a critical environmental variable in an animal room. It activates the sympathetic division of the autonomic nervous system, which produces gastrointestinal, immunological, reproductive, nervous and cardiovascular effects [14]. We believe that ultrasonic noise interferes with sleep/wake cycles in mice. Previous research suggests that acoustic noise can slow brain oscillations [15-16], which implies disruption of the hippocampal theta rhythm in rodents.

We identified fluorescent light fixtures to be the source of ultrasonic noise in the animal rooms. To solve this issue, we intend to mount a thin sheet of plastic for sound-proofing. Ultrasound frequencies can be reflected with a dense material such as a plastic shield or medium-density fibre (MDF) board. This way the ultrasound is trapped inside.

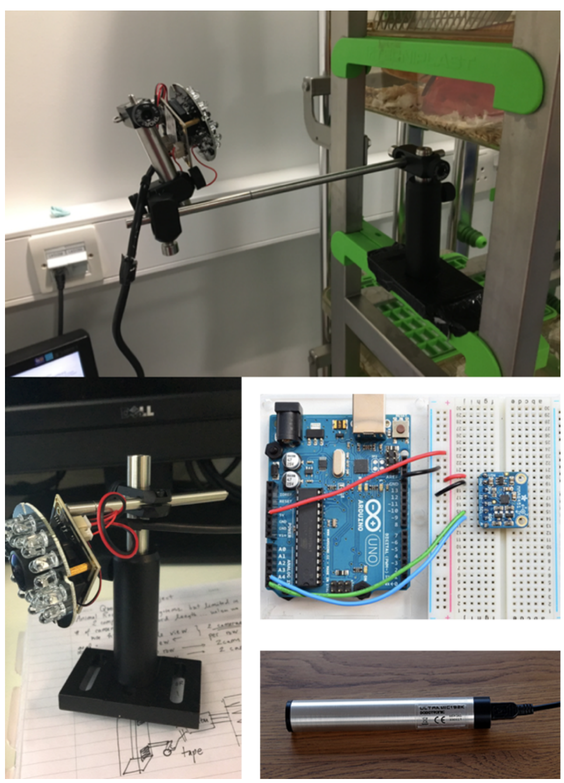
An alternative solution is refitting the fluorescent strip lighting with light-emitting diodes (LEDs). LEDs are as efficient and generate less heat. In a study that compared the effect of LED and cool white fluorescent light of equal intensity on Sprague Dawley rats, both light sources similarly suppressed melatonin produced by the pineal gland. Retinal function and morphology were similar in both groups. During light periods, the standard fluorescent light in animal facilities provides a narrower range of wavelengths than sunlight [14]. We recommend the use of lights with a full spectrum to provide a more natural environment in the laboratory.

We advise that acoustics engineers are part of a facility planning team, and that they consider animal and human frequencies when designing and testing the lab animal facility [14].

Methods

Video Imaging

Videos were recorded using a night-vision IR LED USB camera clamped to an IVC rack. Videos were acquired at 15 or 30 frames per second. By recording a side-view, we did not capture any humans on screen and were HIPAA-compliant.



Experimental set-up to analyse behaviour. Major elements of the set-up are an ultrasonic microphone, an accelerometer, and IR LED cameras mounted for a side view.

Environmental Monitoring

An MMA8451 accelerometer (Adafruit) and Ultramic200K (Dodotronic) were used to record vibration and ultrasound, respectively. Both devices were placed inside an empty cage on an IVC rack to capture the experience of a laboratory mouse. Light, temperature, and humidity data was supplied by the building management system.

Data Acquisition

We used Bonsai, an open-source visual programming language, to acquire all video and audio data. All video files were saved as compressed AVI files using FFMpeg software. The audio files were saved as uncompressed WAV files to capture high-frequency data (>30kHz). We used CoolTerm to record accelerometer data from an Arduino Uno. This was saved as a TXT file.

No syncing was necessary as we used AutoHotKey to start-up Bonsai and CoolTerm at the same time on a single machine. Data was acquired every hour for 24 hours at a time. We generated 230GB of data after each run and continuously transferred all recordings to an external server.

Data acquisition was done for six cages over seven days in two animal rooms. Three mice were in IVC racks and three mice were on open-shelves.

Video Analysis

We used the OpenCV library in Python to perform video tracking post hoc. The algorithm is a simple delta frame calculation with an optional minimum area argument (to avoid tracking smaller objects such as wood chips in the mouse cage). This avoids the need to adjust brightness and contrast levels in each frame. The output is a TXT file that contains the delta frame difference and number of bounding boxes (a proxy for range of motion, where higher numbers reflect a wide degree of motion). Analysis is done on each frame, which represents 1/30 seconds. To scale up video analysis, we used a High-Performance Cluster (HPC) to analyse 1008 videos (24 hours x 6 cages x 7 days) using Bash scripting.

Figures

All figures for this report are under revision at this time. We aim to have this available by October 1, 2019. Please see the corresponding presentation slides.

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