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NoSeMaze: Refinement of animal testing in behavioral science through high sensor integration

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Introduction

While in many fields of science in-silico or in-vitro modelling has replaced animal experiments, in some studies an observed interaction of living animals is still necessary. These require a regular measurement of physiological parameters. In order to minimize the burden for the animals and prevent a distortion of experimental results through such interactions, a preferably automatic and unobtrusive measurement of is necessary. In this work, we present an automated habitat with multimodal sensors for physiological and environmental parameters.

Modern animal experiments should be designed in the accordance of the 3R published by W. Russel and R. Burch in 1959 [1]. The 3R defined by W. Russel and R. Burch are replacement of the animal, reduction of the animals in use and refinement of the experiment itself [1]. Refinement is necessary for many fields of research, where questions can only be addressed by living animals like cognitive and behavioral science. According to W. Russel and R. Burch refinement is defined by a “humane” experiment [1], in which as little harm as possible is experienced by the animals. Aims for a refined experiment are:

- The animals are not manipulated or touched by the experimenter
- The animals are able to live in a natural way, e.g. as part of a group of animals and not solitarily
- The validity of the collected data is ensured by the arrangement of the test setup

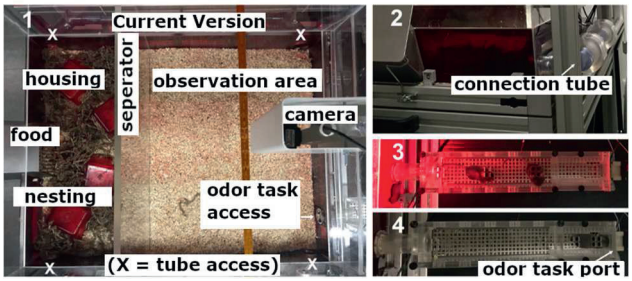

Fig. 1: Overview NoSeMaze. 1) Top view of the NoSeMaze habitat. 2) Tubes connecting the nesting and observation area. 3) + 4) Area where the mice have to solve odor based tasks.

We are working on a fully automated and sensor-rich mouse habitat (NoSeMaze) to achieve these goals, see Fig. 1. The idea of NoSeMaze is observing a mouse cohort, which is able to live in the habitat naturally without any external intervention. The data collection takes place unobtrusively by various non-invasive senor modules.

Currently, NoSeMaze aims at experiments in the field of neurobehavioral science but is not limited to this field of research. Any research involving rodent colony tracking could be applicable in NoSeMaze.

In this work, we will present the environmental sensor node and the planned physiological sensor for NoSeMaze. The environmental sensor node collects data on air quality and temperature but also data regarding external stimuli like sound or light. Especially the external stimuli could lead to a bias in the animals, e.g. by loud sounds. The physiological sensor shall assess the breathing rate of the animals during odor-based tasks.

Methods and Materials

Environmental sensor node

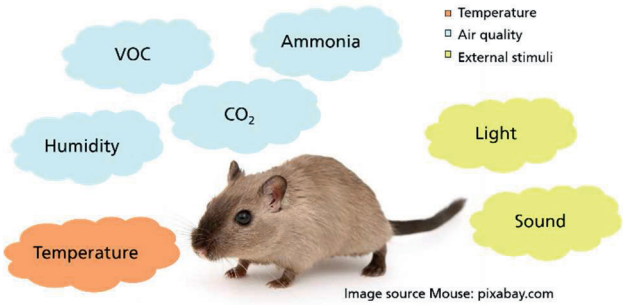

Fig. 2: Data collected by the environmental sensor node

The data collected by the environmental sensor node is depicted in Fig. 2. A sensor node collects the data. Core of the sensor node is the microcontroller board ESP32-S2-DevKitM-1 (Espressif). The different sensors are connected to the microcontroller board by I2C or provide an analog value which is sampled by the ESP32-S2 internal ADC. A SCD41 (Sensirion) is used to collect data about temperature, humidity and CO_2. A SGP40 (Sensirion) measures the volatile organic compounds (VOC) concentration in the air. A MiCS-6814 (SGX Sensortech) is used to

measure ammonia concentrations. The MiCS-6814 uses a heated detecting layer, which changes its resistance in the presence of ammonia. As the resistance is quite large for the ammonia sensor switchable resistor form a potential divider with sensor for proper assessment. An APDS-9151 (Broadcom) measures the ambient light. An electret condenser microphone model CMEJ-0415-42-P (CUI Devices) is used to record sound. Only the loudest noise together with its timestamp is stored and reset after read out. The sensor nodes are connected to a host PC by a USB-to-serial connection. Fig. 3 shows the block diagram of a single sensor node.

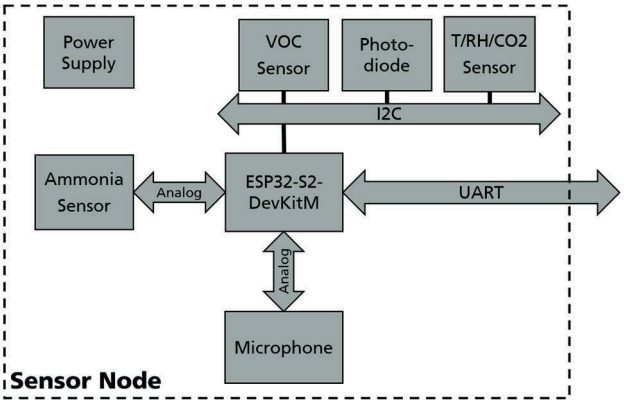

Fig. 3: Block diagram of the sensor node.

Placed in casings two of the sensor nodes are distributed across the mouse habitat to assess whether different micro climates exist within the habitat. One sensor node is placed above the nesting area, the other sensor node is placed in the observation area.

A Python program on the host PC collects the data from all sensor nodes every five minutes and stores the data in CSV files on a Cloud server.

Two different mouse cohorts were placed inside the habitat for six days each while environmental data was recorded.

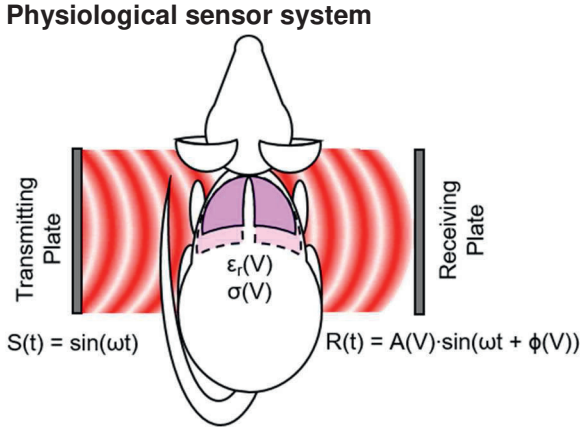

Fig. 4: Principle of the physiological sensor. The mouse's cardiorespiratory acitvity modulates an electromagnetic field.

The principle of our physiological sensor is shown in Fig. 4. The physiological sensor consist of two antennas, one transmits an electromagnetic field, the second antenna receives the field after it passed through the animal. The receiving signal is modulated due to the cardiorespiratory activity of the animal. Originally, we designed this sensor as a capacitive modality with sensor plates instead of antennas. In the original version we used a lock-In amplifier as transmitter and receiver. In preliminary trials we tested our sensor sysem on fixated mice, an additional pressure sensor with nose mask was used as reference sensor for the respiration, see Fig. 5.

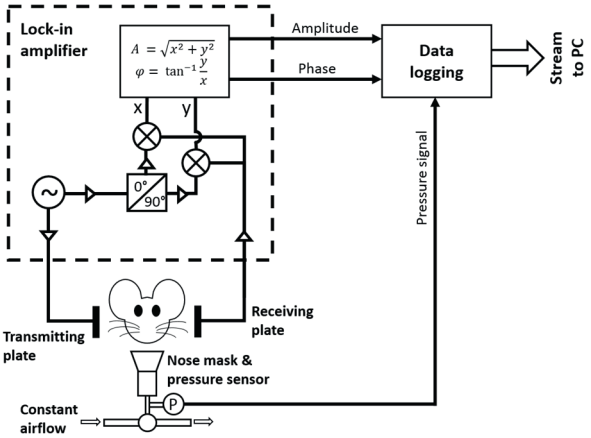

Fig. 5: The mice were fastened using a head-bar and a nose mask was placed directly on the animal's snout to ensure as little leakage as possible. A constant airflow bypassed the nose mask. The mice created an increase in pressure during expiration and a decrease in pressure during inspiration, which was captured by a pressure sensor (HDIM050GBY8H3, SensorTechnics). The sensor plates were placed laterally on the mouse's thorax. The distance between the two plates was about 2.7cm. The plates were connected to a lock-in amplifier (HF2LI, Zurich Instruments). A signal generator in the lock-in amplifier generated a 30 MHz sine signal to drive the transmitting plate. The receive signal is mixed in the base band using the generator signal and a 90° shifted version of the signal. This results in an I/Q demodulation and leads to the representation of the receive signal as a complex value: x[t] + j·y[t]. The complex value is then used to calculate the amplitude and phase of the receive signal separately. The data from the lock-in amplifier and the pressure sensor were synchronously logged and streamed to a PC.

For the free-running trials in NoSeMaze, we used an enhanced version of the physiological sensor described by part of the authors in [2]. The sensor is part of a whole sensor system, which includes a distance sensors (Sharp 0A41SK F 1X) and a Camera (OpenMV Cam), see Fig. 6. The distance sensor starts the measurement as soon as a mouse is present, the camera shoots a video with low frame rate to provide additional meta data. This is especially relevant if more than one mouse is visible in the video or if the mouse moves a lot. The whole sensor system is placed in front of the area for the odor port. This

area is narrow so that there is only room for one mouse. Furthermore, the mouse has to remain still to perform the odor-reward task.

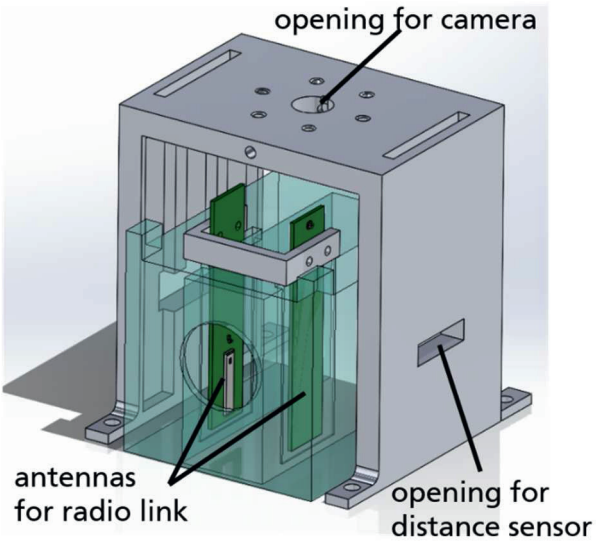

Fig. 6: CAD plot of the physiological sensor system

The raw data of every measurement is stored together with the corresponding video on a Cloud server.

During the writing of this contribution to the Dresdner Sensor Symposium the final physiological sensor system was not yet integrated in the NoSeMaze. Therefore, only preliminary data was obtained with one mouse to test the operation of the sensor system.

All animal experiments were approved by the Regierungspräsidium Karlsruhe and the local welfare authorities.

Results

Enviromental data

As mentioned in the previous section two mouse cohorts were placed inside the habitat while environmental data was recorded. The first cohort was placed inside the habitat around 11 a.m. on 10th June 2022 and removed from the habitat around 11 a.m. on 16th June 2022. The second cohort entered the habitat on 17th June 2022 around 11 a.m. as well and left around 1 p.m. on 23rd June 2022.

Fig. 7 shows the output of the ammonia sensors. The output of the sensor is the resistivity of its detecting layer. Because the resistivity of the detecting layer has a rather wide spread from 10 kΩ to 1500 kΩ [3], we normalized it to the resistivity at the beginning of the experiment. The resistivity decreases with an increase in ammonia concentration. The ammonia concentration rises roughly one day after the animals are placed in the habitat. The ammonia concentration seems to be slightly lower in the nesting area compared to the observation area especially in the first cohort.

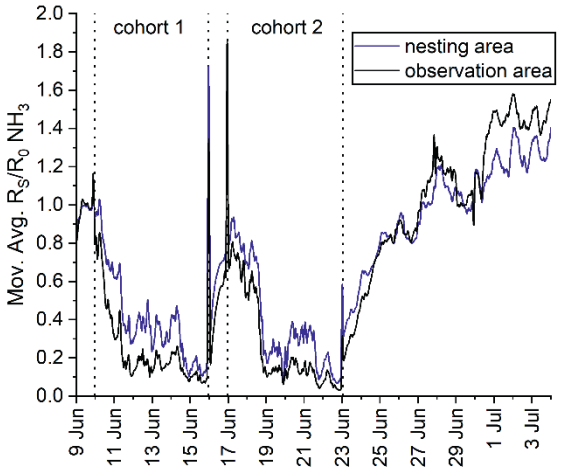

Fig. 7: Resistance of the NH_3 sensors detecting layer in relation to its resistance at the start of the experiment. A decrease in resistivity corresponds with an increase in ammonia concentration. The ammonia concentration increases roughly one day after the mice are placed in the habitat. This makes sense as animals' urine has to cumulate. In the first cohort there seems to be a lower concentration of ammonia in the nesting area compared to the observation area- However, in the second cohort the difference seems to be less significant.

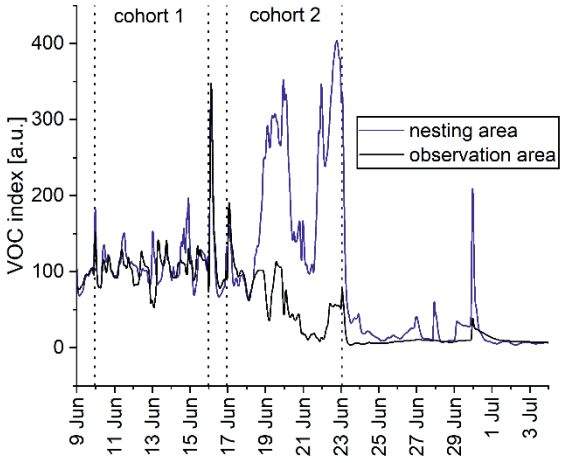

Fig. 8: VOC index derived by the Sensirion Gas Index algorithm. A value of 100 corresponds to normal air quality. A value below 100 indicates an improvement of the air quality. Whereas a value above 100 indicates a decline of air quality. It can be seen that for the first cohort the air quality remains on an average level throughout the experiment. However, the experiment with the second cohort shows a decline in air quality over the course of the six days in the nesting area and an improvement in the observation area.

The data of the VOC sensor is shown in Fig. 8. We use the Sensirion Gas Index Algorithm [4] to assess the air quality in regard to VOC. A value of 100 corresponds to standard air quality, values above 100 correspond to a decline in quality and values below 100 correspond to an improvement. During the experiment with the first cohort there is no significant

change in standard air quality in regard to VOC. However, with the second cohort there is an improvement in the observation area visible and a decline in the nesting area.

Regarding the temperature, we see an increase of about 1.5 K when animals were in the habitat. Apart from that the temperature follows the regular day-and-night rhythm. Regarding the external stimuli there were no events regarding the light and regarding the sounds the events can be traced back to the experimenter working on the habitat.

Physiological sensor system

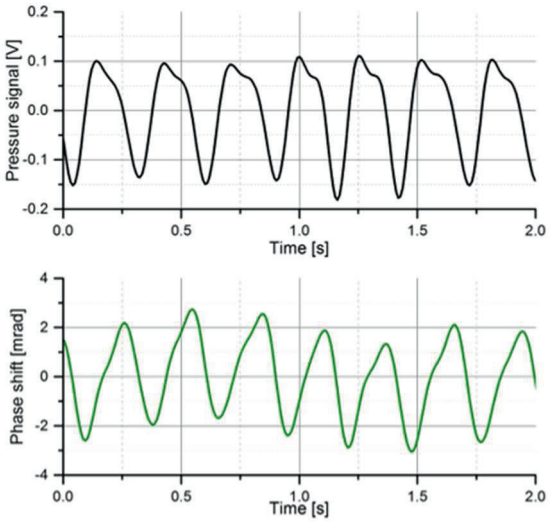

Fig. 9: Preliminary trials: Pressure signal (black) vs. the phase (green) over 2 seconds. The high synchronity is clearly visible. However, the signal shapes of the sensor differ.

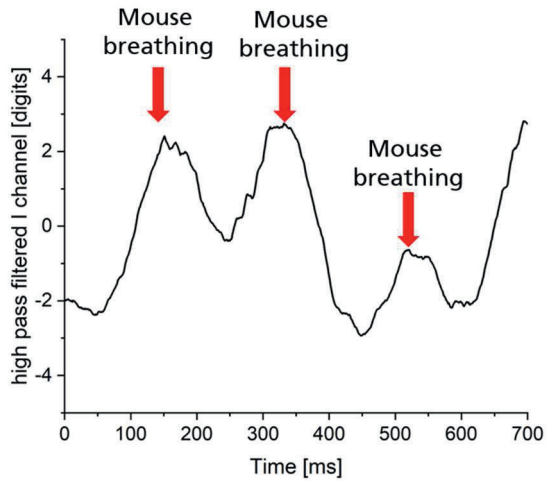

Fig. 10: Excerpt of the physiological sensor signal during the free moving trials. Shown is a short time interval where the mouse rested for a short while between the sensor's antennas. Clearly visible are three peaks in a time frame of 500 ms. If they correspond to breaths, this is in good accordance to reported values.

Our preliminary trials with the original version of the physiological sensors showed a high synchronity with the data derived from the pressure sensor, see Fig. 9.

While writing this contribution to the Dresdner Sensor Symposium the physiological sensor system was not completely integrated into NoSeMaze, yet. So far, we could only test the basic principle of our system on a single individual, which was freely moving in a large cage. In contrast to the actual NoSeMaze setup, the mouse had not to solve any task. Therefore, we only tested the overall systems functionality. However, the mouse rested between the antennas for a short duration while exploring. The data is shown in Fig. 10 and looks highly plausible with about 3 breaths in a time frame of 500 ms. This is in good accordance with published values [5, 6].

Discussion

Regarding the environmental data it became obvious in our first trials that a thorough calibration and modeling is needed to derive reliable and quantifiable values of the gas concentrations. This holds especially true for the NH3 sensors. The manufacture already specifies a spread of one magnitude of the sensor's sensitivity [3]. Ideally, each sensor would have to be calibrated on its own. However, if we will be able to derive quantifiable values in the near future, we could offer a valuable element for refinement by closely monitoring the living conditions of laboratory animals. Apart from that, the current system for the environmental data can be used to collect valuable covariates to assess the quality of the behavioral data and to derive possible biases in the data. Also, the sensor node allows an easy upscaling to monitor different areas of the habitat. In our future works, we especially want to enhance this point and also monitor the micro environment closer to the ground level. Currently the sensor nodes are mounted close to the habitat top to prevent the mice from reaching them.

The preliminary trials with out physiological sensor already showed the feasibility of this system to correctly assess the respiration rate. However, the signal shape between the pressure signal and our physiological sensor differ. We assume that the signal shape for pressure and phase shift are different because of their different physical origin. The pressure signal is correlated to the airflow and therefore, to the change of air volume in the lung over time. In contrast, the phase shift is correlated to the air volume in the lung. Thus, the time derivative of the phase shift shows a higher similarity to the pressure signal (see Fig. 11). No significant delay is visible between the pressure signal and the time derivative of the phase signal. We assume that the difference from local minimum to local maximum of the amplitude and phase shift correlate to the tidal volume for each breath. However, a correlation between the exact tidal volume for each breath and the sensor signals could not

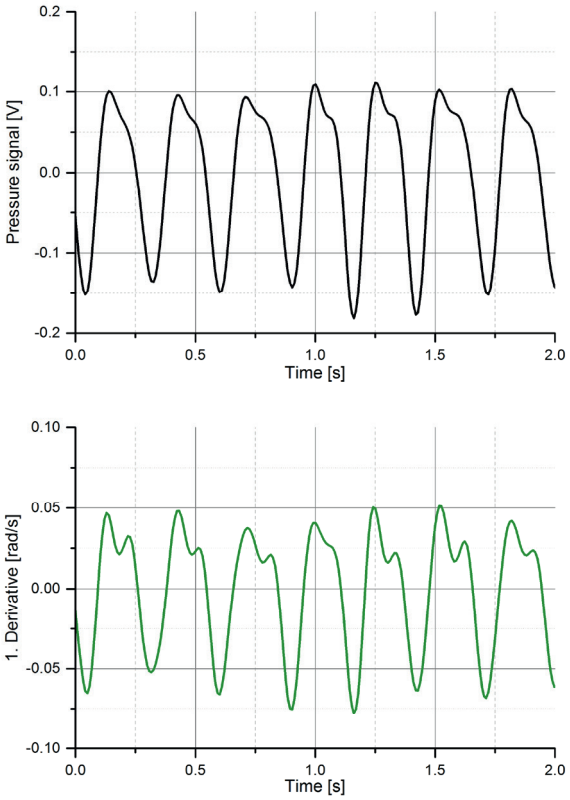

Fig. 11: Pressure signal (black) vs the first time derivative of the phase shift (green). The signal shapes are very similar (Pearson coefficient: 0.87)

be investigated because the reference sensor is not capable of a reproducible measurement of such small volumes.

Our physiological sensor system could be a valuable element in the refinement of the monitoring of mouse respiration. In the current state of the art, monitoring of the mouse respiration in a free-moving setup is only possible with implants. Here we want to show a way to highly refine this setup and increase the animals' well-being.

Funding

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