Wireless Monitoring of Liquid Consumption in Rodents using BLE Technology

Sikandar Ali[1], Roberto Ciccocioppo[2], Massimo Ubaldi[2], Fabio Casarola[3], and Marco Giammarini[3]

***Abstract*— Drinkometer is device for automated assessment of liquid consumption in laboratory animals, widely used in neuroscience and biomedical research. It allows researchers to track and automatically record the drinking patterns of animal during behavioral experiments. Despite being a very useful instrument, it is currently very expensive, which hampers the possibility of its widespread use. Alternatively, animals drinking behavior can be recorded manually, which doesnt provide detailed information and also involves human intervention by weighing the bottle after giving intervals of time. Human involvement, in addition to being labor-intensive, may influence experimental results because when the experimenter enters the room, it can interfere with the natural behavior patterns of the animal, including drinking behavior. Here, we describe the development of a cost-effective, easy-to-use automated system that offers a significant solution. In this paper we describe a development of a Bluetooth low energy consuming capacitance-based device to monitor laboratory animals drinking behaviors. The firmware of the device was written in C sharp, where python based script was written to communicate with device, a raspberry pi was used for data collection. The system showed high level of stability, stable data acquisition rates with average device operating for 48 to 50 hours, from single charge.**

I. INTRODUCTION

Laboratory rodents, rats and mice rats are the most common species used for scientific research. Physiological parameters associated with animals' drinking behavior are important measures not only in ingestive behavior experiments but also for the real-time, continous evalution of animal health [1], [2], [3]. There are systems available in the market for automated recording of drinking behavior called home cage monitoring system [3]. From a video camera-based system [4], [5] to beam braking system in which rats lick the spout of the bottle, triggering a sensor to record each drinking event. This system logs detailed information like duration and interlick intervals providing precise data on drinking behavior. It helps researcher to study thirst regulation, motivation and drugs effects on drinking [6]. Other techniques like force transducer in which force transducer sensors attached to spout to detect the force and convert into an electrical signal [7], passive infrared techniques to microwave and piezoelectric [8], [9], [10]. Each system comes with its unique set of advantages. Most of the system works on the principle of lick sensing circuit, when its tongue touches the spout, it grounds the circuit through animal body causing voltage drop. However this method has limitations as current could be sensed by animal or it can change the taste of liquid, which would affect its drinking behavior. Traditional methods, in which liquid consumption is monitored by weighing bottles at least once every 24 hours, have limitations. The human interaction scared the animal, which effects its drinking pattern. [11]. In this paper the proposed system used FD1004 sensors with two electrodes called ref electrodes and level electrodes. The sensor will calculate the data and transfer to the micro-controller for further processing and then sent to device through Bluetooth as this approach overcome the limitation and enabling more accurate and stress-free data collection as no human intervention will be involved [12]. Few systems are available on the market that come with its own caging system, which makes them more expensive. In this paper, we describe the development of a Bluetooth based wireless system for monitoring the liquid consumption of rats/mice, The proposed system works on the principle of capacitive sensing and works with existing standard cages available in lab. It uses FD1004 sensors with two electrodes, called reference electrodes and level electrodes. The sensor capture the data and transfers them to the micro controller for further processing, data are then send to a computer via Bluetooth. This approach enables an accurate and stress-free data collection, as no human intervention is involved and overcomes multiple limitation of existing system. This paper has the following structure: section 2 will explain the literature review. Section 3 will briefly discuss the methodology, where section 4 presents the results and discussion. Section 5 concludes the research.

II. LITERATURE REVIEW

'Liquid monitoring systems are very important in ingestive research and animal research laboratory facilities, especially rats and mice to control animal health and to monitor many experimental data. There are multipile systems currently available. Among the most popular there was a home cage based Drinko-meter system was able to measure the liquid consumption with precision up to 10 micro liter that allows 24/7 recording. The system was able to measure liquid consumption through a high precision sensor attached to the drinking bottles [12]. Another system that was developed, based on the high throughput and low-cost lick meter system had been proposed to analyze mice drinking behavior.

*This work was supported by AM Microsystems Srl

[1]Sikandar Ali is with the School of Advanced Studies, Center of Neuroscience, University of Camerino, Camerino, Italy. sikandar.ali@unicam.it

[2]Roberto Ciccocioppo and Massimo Ubaldi are with the School of Pharmacy, Center for Neuroscience, Pharmacology Unit, University of Camerino, Camerino, Italy. {roberto.ciccocioppo, massimo.ubaldi}@unicam.it

[3]Fabio Casarola and Marco Giammarini are with AM Microsystems Srl. info@am-microsystems.com

The proposed system was equipped with data processing, integrated filtering and could measures lick count. With high resolution monitoring system, the proposed system was able to do analysis of complex licking patterns in multiple mice within their home-cage over the extended period of time [13]. A third system largely in use was high precision optical fiber based licko-meter system. The proposed system has photo-diodes, which related to photo detection circuits with a 3mm valve holder. The nose entry has been designed in a way that only mice/rats could interrupt the beam. System starts recording data as beam interrupted by mince/rats. The device was able to detect nose pokes and presented visual cues in behavioral tasks. The system was helped full to study lick micro-structure [14]. In their study, researcher has proposed low-cost open source lickometer system. The proposed system can hold one or more drinking bottles depending. The bottle holder could be made of 3D or plastic materials to hold the bottle. The bottle spouts were wired to sound card microphone input to capture the signal. As computer sound card were designed to detect a tiny voltage change from microphone input. Instead of microphone, the proposed system used lick circuit. Upon licking every time, a small pulse was sent to sound card, which record them as spikes in audio wave form. [15]. An alternative method was presented by researchers, where an efficient technique was used to record the licking behavior in rodents, by connecting a metal sipper tube. 100 to 800mv DC voltage signal was recorded each time rodent licks the spout. This voltage change occurred due to the contact between water and metal spout, creating metal to water junction potential and this behavior lasted as long as tongue contacted with spouts. The proposed system helps researchers to understand both neural mechanisms behind it and patterns of licking [16]. In another relevant study a Raspberry pi-based device for operant licking in rats was proposed. The proposed device uses raspberry-pi computer to gather data from sensors and could be transferred to a remote server via wireless network. The proposed device has a 3D frame which has two lick spouts that are linked to a touch sensitive sensor. Capacitive touch sensor was connected to drinking spout of the bottle, to record the licking behavior of rodents. When the numbers of licks on a spout meet the predetermined criteria, the system delivers a fixed amount of liquid by triggering the syringe pump. Visual cue turned on every time solution was delivered [17].In existing methods liquid consumption is monitored through by weighing the bottle after specific intervals in which researcher lost critical details, where as there are some automatic system available in market but it comes in with huge price tag as it doesn't work with existing system available in lab for example cages. There is a huge need for a wireless system, which could monitor the liquid consumption in rats without human involvement for data gathering and work with existing cages available in lab. In this work we have proposed a wireless system for collecting data related to liquid consumption by rats. The proposed system works on the principle of capacitive technology and works with standard cages.

III. Methodology

The proposed system on chips has used FD1004 sensor for measuring liquid consumption. It works on the principle of capacitance sensing. It has 4 channels capacitance to digital converter. The system is powered by BLE technologies, which means it consume low energy and highly efficient when it comes to consumption of power and can last for a longer period then class Bluetooth. Different soft tests have been conducted at AM Microsystems head quarter before conducting in real time environment at university of Camerino animal facility. The performance of different key parameters was observed at each test for example system average operating time on battery, sensor output and system stability.

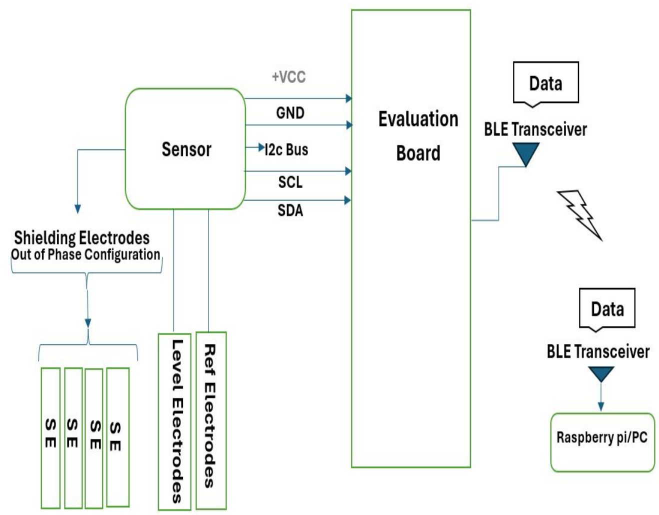

Fig. 1. Shows Methodology of Proposed work.

Figure 1 shows the Bluetooth based liquid assessment system. The proposed system has used fd1004 sensors to collect information related to real time liquid level inside bottles. The capacitive sensor FD1004 connected with different electrodes, one is called reference electrode, level electrode and shielding electrodes. The reference electrodes make the constant reference point for the capacitance, whereas ratio between reference electrodes and level electrodes refers to the actual water level inside the bottle. The sensor has been configured out of phase for data collection. The sensor has 4 pins, which are VCC, GND,SDA, SCL and GND. The VCC pin is used for power, GND is used for ground, SDA pin used for data and SCL pin is used for clock. These input pins are connected with an evaluation board called FDC1004EVM. I2C communication protocol has been used for data acquisition from sensors. The sensor will collect data and send to nrf52840 micro-controller for processing by using I2C communication protocols. NRF 52840 evaluation board is supported by Bluetooth low energy. The data is sent through towards client computer by using nrf52840 ble transceiver. The data sending rate was set at around 50 samples per second. The firmware of the device was written in C sharp to continuously sending data at 50 hertz. Different UUID was written for each feature. Python based script was written to communicate with multiple Bluetooth devices at high speed. Linux based service has been written on raspberry pi, which was continuously running in back-ground as soon as raspberry pi get powered on. Linux triggers the python script, which starts scanning around for the devices and as soon as devices are in range it establishes connection and starts storing data in csv files. Which would be later analyzed. The system works efficiently. The custom bottle holder was designed to hold the bottle.

Fig. 2. Shows the custom bottle holder for holding the bottle and Micro-Controller based SOC for monitoring liquid Consumption.

Figure 2 shows the custom bottle holder which is specially designed to hold bottles as well as the sensor and circuitry of the device. The electrodes have been placed inside the bottle holder, which is connected to micro-controller to gather information and measure the real time change in liquid level inside bottle. The custom bottle holder can hold the standard laboratory size bottle 250 ml. The bottle holder was designed by using software called solid edge and then printed out by using photolithography 3D printing. The custom design allows easy replacement and removal of the bottles during the experiment, and it ensures the tight fit to prevent the licks. There is empty space Infront of bottle holder, which allows user to see the real time water level.

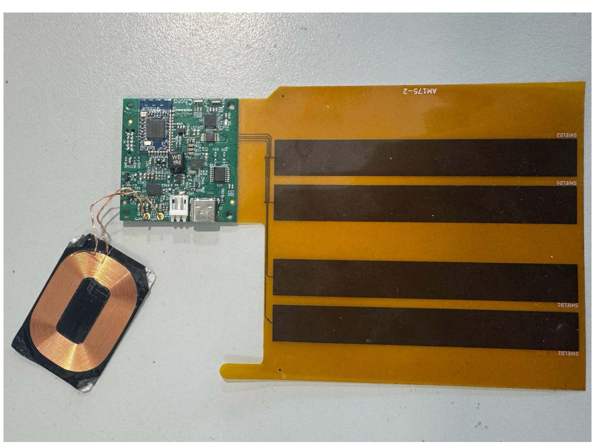

Fig. 3. Shows a BLE and Micro-Controller based System on chip for monitoring liquid Consumption.

Figure 3 Shows the low-energy Bluetooth and micro controller-based system on chips used to monitor liquid consumption. The system works on 4 channel capacitances to digital converter. The device is powered by the micro-controller nrf52840 to process all the information collected by the sensor called FD1004 and then send it to the client device by using low energy Bluetooth. The proposed device supported wireless and USB-C charging to charge the batteries. These devices are powered by a 5v battery and could last up to 40 hours. The device has a battery connector to power the system. As soon as the battery is attached to the system, it turns on the system and starts gathering data from sensor, Process the data, and then send to receiving device at 50 samples per second.

IV. Device Validation

Bluetooth based device has been tested at AM Microsystems Headquarters to check the basic parameters like system stability, data acquisition rate, and battery timing. After these soft tests at AM Microsystems, an experimental test was conducted at University of Camerino. The experiment was carried out in ventilated rooms of the animal facility of the pharmacy school. The standard GM500 and conventional 1500U cages from techniplast were used with dust free wooden litters and standard food pallets for these experiments. The room was held at control temperature of 22°C and humidity (30-40 percent RH relative humidity). The bottles used in these experiments were standard 250ml techniplast bottles.

Fig. 4. Shows a real time working prototype system for real time monitoring of liquid intake in lab animals, using sensors based SOC equipped with stainless steel and carbon peek spout of the bottles and data acquisition device mounted on the cage .

Figure 4 shows transparent rodent cages containing laboratory rats. Each cage equipped with an electronic monitoring device mounted top. The device was mounted on a bottle with at least two types of spouts, one made up of stainless steel and the other spout was made up by using carbon peek spout. The most effective outcomes were achieved with the stainless-steel spout with rats.

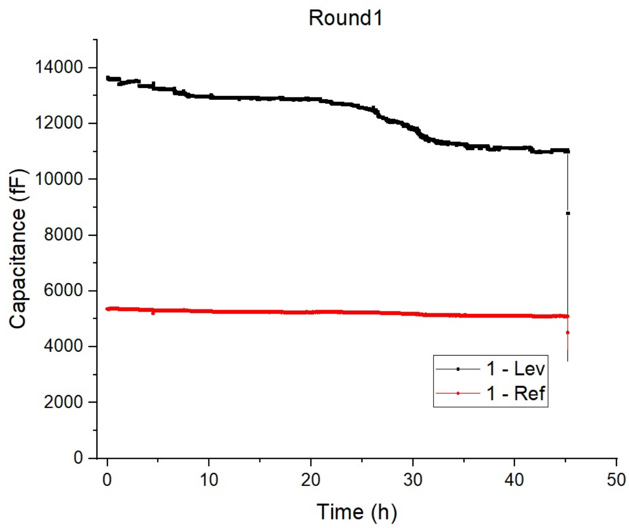

Fig. 5. Round 1 First charge /Recharge cycle of capacitance-based monitoring of liquid consumption in rats showing steady decrease over time.

Figure 5 demonstrates the monitoring of liquid consumption in rats over time using capacitance-based sensor. The graph represents capacitive-based monitoring over a period of approximately 50 hours. The black line (1-Lev) represents decreasing behavior of the liquid inside the bottle, which steadily declines as the rat's drink. The red lines (1-Ref) serve as a reference value useful for final calculation to detect the real time liquid level inside the bottle. The data demonstrates a consistent decrease in liquid level, confirming the effectiveness of the monitoring system. The lipo battery (3.7volt, 850mAh) provided a continues power to device over 45 to 50 hours ensuring reliable and continuous monitoring.

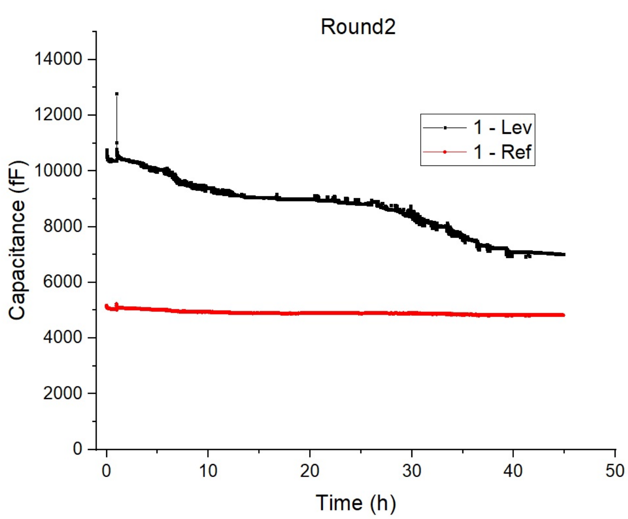

Fig. 6. Round 2 Second charge /Recharge cycle of capacitance-based monitoring of liquid consumption in rats showing steady decrease over time.

Figure 6 shows the collection of data for another 50 hours. The starting capacitance of round 2 matched the final value of round 11, which conforms to the seamless monitoring continuity. The black line (1-Lev) reflects the capacitance tied to the liquid level, showing a steady decline as the rats consumed the liquid. Early fluctuation like due to the system reactivation or could be due to shaking when the bottle is placed back in the cage, which stabilized over time, highlighting the reliability of the setup. The red line (1-Ref) remand constant serves as a reference to detect slow movement on the dataset just like temperature drift or other environmental parameters.

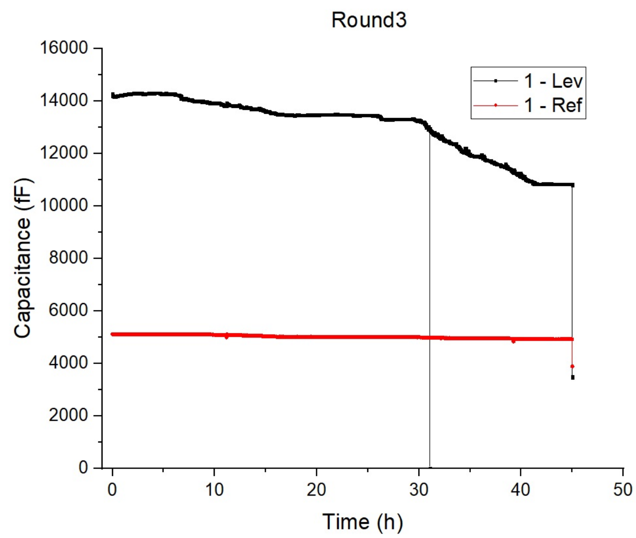

Fig. 7. Round 3 Second charge /Recharge cycle of capacitance-based monitoring of liquid consumption in rats showing steady decrease over time.

Figure 7 shows that the third round of data collection was conducted with fully battery, extending the monitoring from the previous cycle, tracking liquid consumption of rats for approximately 40 to 50 hours. This round also marks the end of our 7-day test, during which we refilled the bottle to its maximum level (250 ml) before starting this final phase. The black line (1-Lev) representing the capacitance related to the liquid level calculated by performing ratiometric or differential algorithm between level and reference electrodes (1-Ref), shows the gradual decline as the rats consumed the water. A key observation in this round is the notable shift in capacitance just after the vertical line at around 30 hours, which indicates midnight. Following this point, capacitance drops more sharply, reflecting increased liquid consumption. This suggested heightened activity in rats during their nocturnal phase, consistent with their natural circadian rhythm(being more active at night) thus drinking more water.

V. Conclusion

This study was conducted using our patented monitoring system (International Patent No. PCT/EP2019/066453) to investigate rodent ingestive behaviors to support preclinical research on drinking behaviors in laboratory rodents. A low-cost Bluetooth Low Energy (BLE)-based system was developed to accurately monitor liquid consumption. The system was rigorously validated through multiple experiments that demonstrated reliable performance in tracking water level within standard rodent cages. The device firmware was implemented in C sharp, while Linux based service managed data acquisition by triggering python script for Bluetooth communication in raspberry pi. The system successfully displayed the decreasing water level in real time, confirming its functionality. Designed for scalability. The device is compatible with various types of cages, ensuring adaptability across experimental setups. Key performance metrics include a stable data acquisition rate and an average battery life of 45 to 50 hours, highlighting the suitability of the system for long-term monitoring. Capacitive sensing effectively tracked liquid consumption, validating the proposed method for continuous measurement. To reduce noise and optimize sensor performance, stainless steel and carbon peek were tested. Although both materials were viable, the stainless steel spouts demonstrated superior effectiveness in maintaining signal accuracy and consistency. In the current prototype, Kapton material was tested with an FD1004 sensor was tested. Initial expectations suggested significant signal sensitivity, but empirical testing revealed suboptimal performance with the out-of-phase technique. In future experiments, the system will replace Kapton with SR4 material, which preliminary tests indicate superior results for this sensing methodology.

VI. Acknowledgment

We thank AM Microsystems for the collaboration to this study. The author Sikandar Ali was supported by fellowship from the Eureka program of the Region Marche, Italy. The work was supported by PRIN-PNRR2022 -P202274WPN (to RC), PRIN-2022 20227HRFPJ (to RC), MNESYS (PE0000006)- Project AMSUD 2024, PRIN 2022X9X5MS (to MU).

References

[1] V. Baumans, “Science-based assessment of animal welfare: laboratory animals,” *Rev Sci Tech*, vol. 24, no. 2, pp. 503–513, August 2005.

[2] K. Kramer and L. B. Kinter, “Evaluation and applications of radiotelemetry in small laboratory animals,” *Physiological genomics*, vol. 13, no. 3, pp. 197–205, 2003.

[3] C. A. Richardson, “The power of automated behavioural homecage technologies in characterizing disease progression in laboratory mice: A review,” *Applied Animal Behaviour Science*, vol. 163, pp. 19–27, 2015.

[4] H. Jhuang, E. Garrote, X. Yu, V. Khilnani, T. Poggio, A. D. Steele, and T. Serre, “Automated home-cage behavioural phenotyping of mice,” *Nature communications*, vol. 1, no. 1, p. 68, 2010.

[5] A. I. Pack, R. J. Galante, G. Maislin, J. Cater, D. Metaxas, S. Lu, L. Zhang, R. V. Smith, T. Kay, J. Lian *et al.*, “Novel method for high-throughput phenotyping of sleep in mice,” *Physiological genomics*, vol. 28, no. 2, pp. 232–238, 2007.

[6] TSE Systems, “TSE PhenoMaster,” 2018, (Accessed 17 November 2018). [Online]. Available: https://www.tse-systems.com/product-details/phenomaster

[7] Metris B.V., “LABORAS,” 2018, (Accessed 17 November 2018). [Online]. Available: https://www.metris.nl/en/products/laboras

[8] L. A. Brown, S. Hasan, R. G. Foster, and S. N. Peirson, “Compass: continuous open mouse phenotyping of activity and sleep status,” *Wellcome open research*, vol. 1, p. 2, 2017.

[9] A. E. Flores, J. E. Flores, H. Deshpande, J. A. Picazo, X. Xie, P. Franken, H. C. Heller, D. A. Grahn, and B. F. O’Hara, “Pattern recognition of sleep in rodents using piezoelectric signals generated by gross body movements,” *IEEE transactions on biomedical engineering*, vol. 54, no. 2, pp. 225–233, 2007.

[10] A. Genewsky, D. E. Heinz, P. M. Kaplick, K. Kilonzo, and C. T. Wotjak, “A simplified microwave-based motion detector for home cage activity monitoring in mice,” *Journal of biological engineering*, vol. 11, pp. 1–12, 2017.

[11] R. F. Leeman, M. Heilig, C. L. Cunningham, D. N. Stephens, T. Duka, and S. S. O’Malley, “Ethanol consumption: how should we measure it? achieving consilience between human and animal phenotypes,” *Addiction biology*, vol. 15, no. 2, pp. 109–124, 2010.

[12] V. Vengeliene, H. R. Noori, and R. Spanagel, “The use of a novel drinkometer system for assessing pharmacological treatment effects on ethanol consumption in rats,” *Alcoholism: Clinical and Experimental Research*, vol. 37, pp. E322–E328, 2013.

[13] M. Monfared, Q. Mascret, A. Marroquin-Rivera, L. Blanc-Árabe, Q. Lebouleux, J. Lévesque, B. Gosselin, and B. Labonté, “High-throughput low-cost digital lickometer system for the assessment of licking behaviours in mice,” *Journal of Neuroscience Methods*, vol. 410, p. 110221, 2024.

[14] A. Silva, P. Carriço, A. B. Fernandes, T. Saraiva, A. J. Oliveira-Maia, and J. A. da Silva, “High-precision optical fiber-based lickometer,” *eneuro*, vol. 11, no. 7, 2024.

[15] T. G. Mast, J. M. Breza *et al.*, “An open-source lickometer and microstructure analysis program,” *HardwareX*, 2018.

[16] A. Hayar, J. L. Bryant, J. D. Boughter, and D. H. Heck, “A low-cost solution to measure mouse licking in an electrophysiological setup with a standard analog-to-digital converter,” *Journal of neuroscience methods*, vol. 153, no. 2, pp. 203–207, 2006.

[17] M. Longley, E. L. Willis, C. X. Tay, and H. Chen, “An open source device for operant licking in rats,” *PeerJ*, vol. 5, p. e2981, 2017.