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A Low-Cost, Reliable, High-Throughput System for Rodent Behavioral Phenotyping in a Home Cage Environment

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Abstract— **Inexpensive, high-throughput, low maintenance systems for precise temporal and spatial measurement of mouse home cage behavior (including movement, feeding, and drinking) are required to evaluate products from large scale pharmaceutical design and genetic lesion programs. These measurements are also required to interpret results from more focused behavioral assays. We describe the design and validation of a highly-scalable, reliable mouse home cage behavioral monitoring system modeled on a previously described, one-of-a-kind system [1]. Mouse position was determined by solving static equilibrium equations describing the force and torques acting on the system strain gauges; feeding events were detected by a photobeam across the food hopper, and drinking events were detected by a capacitive lick sensor. Validation studies show excellent agreement between mouse position and drinking events measured by the system compared with video-based observation – a gold standard in neuroscience.**

I. Introduction

To understand and treat diseases of the central nervous system (CNS), including those involving motor, sensory, affective, and cognitive functions, ultimately requires an animal model to determine how neural events evoke specific behaviors. The house mouse, *Mus musculus*, fits this purpose, given the many similarities between murine and human brains, and the ease by which pharmacological, genetic, environmental, and surgical interventions can be evaluated in mice.

This kind of behavioral phenotyping is an essential tool to interpret mechanistic studies of gene expression and drug effect [2], [3], [4], [5], [6]. Furthermore, high-throughput technologies developing novel therapeutics, or genome-wide mutation screens, emphasize the need for high-throughput behavioral screens that can identify clinically important phenotypes with high sensitivity [7], [6], [8]. Home cage behaviors (movement, feeding, and drinking) are particularly appealing outcomes for high-throughput assays [1], [9], [10], [5], [6], and are critical for interpreting behavioral effects of any intervention [11].

The home cage attempts to replicate, in a controlled manner, a quasi-naturalistic environment, including a comfortable place for bedding, *ad lib* access to food and water, and defined light/dark cycles [6]. It is particularly important to minimize investigator interaction with the mice, since this contact is a major source of experimental variability [12].

A variety of sensors and technologies have been used to design commercial home cage behavioral monitoring systems. Typically, these systems quantify movement, either by photobeam array [13], [14] or overhead video tracking [15], [16]; feeding, by weighing food hoppers on a precision scale; and drinking, by weighing water bottles on a precision scale or by measuring conductivity across the water bottle sipper tube. However, none of these approaches measure mouse movement, feeding, and drinking with high temporal *and* spatial resolution. These systems are thus unable to discern the fine behavioral patterns that reflect CNS integration of complex internal and external inputs; patterns that provide significant insight into behavioral regulation.

Sophisticated hand-built systems for precise mouse home cage behavioral measures have been developed, e.g. [17]. However, different constraints (video storage, cost, *etc*.) prevent scaling these systems to assay arbitrary numbers of cages. Here, we present an implementation of a home cage monitoring system first described by Goulding and colleagues [1]. The new design, which uses strain gauges for localization [18], capacitive touch sensors [19] for drinking detection, and infrared (IR) photobeam sensors for feeding detection. This system is modular, manufacturable, expandable, and available to other researchers – empowering diverse programs of CNS research. This design improves upon previous work by providing better localization, a highly-robust sensor system, and greater flexibility and ease-of-use.

This paper will explore the design specifications of the system, a method for validating the system's performance, and present results and implications of those evaluations.

II. System Design and Implementation

The design of the home cage monitoring (HCM) system targets a multicage environment, and consists of a mechanical cage apparatus, sensors, a control board, and a data acquisition (DAQ) system. The entire system is modular

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and expandable, allowing it to be reconfigured for different experiments. Each component was designed and iteratively verified in real-world conditions with live mice to ensure ease-of-use, robustness, accuracy, and repeatability.

A. Cage Design

The system (shown in Fig. 1) uses a low-profile mouse cage (Allentown PC1019HT with bonnet). Since strain gauges, and not visible-spectrum cameras, were used for localization, arbitrary environments could be created, including a niche – a small box with dimensions approximating mouse burrows found in the wild. The cage bottom was dimpled to interface with steel pins on the strain gauges (LSB-200, FUTEK), which are in a tripod arrangement on a solid aluminum base. The cage was modified to mount three accessory modules on its front. In each of these three mounting locations, the researcher can install a modified Rodent Café feeding hopper (OYC Americas), a liquid dispenser, or a blank plate to cover the opening. This modular approach allows many different configurations that can be optimized for specific experiments.

B. Cage Modules

While existing systems mount water and feeding stations directly to the cage, the new HCM system uses modules that are physically decoupled from the cage as much as possible. This reduces cage weight and localization errors caused by variations in cage center-of-mass. To maintain physical decoupling required component tolerances that were large enough to adequately prevent accidental mechanical binding while researchers change food or water, but small enough to prevent the mouse from escaping or altering the home cage environment by wedging material in openings.

C. Maintenance

Cage maintenance is a vital concern to researchers working in a facility with dozens of cages, as they must be able to reliably change out water and food during an experimental trial without causing misalignment of the feeder or liquid dispenser. To solve this problem, the modules were implemented with a quick-release self-aligning functionality using rare earth magnets, which snap modules into a routed track in the aluminum base. This functionality drastically reduces the time required at each cage – significantly reducing the overall maintenance time in a multicage environment.

Between experiments, cages must be washed and sanitized. Although plain mouse cages can be run through automatic wash cycles without difficulty, existing home cage systems require careful disassembly and hand-washing of different components. The new HCM system, on the other hand, uses components with high temperature tolerance – withstanding the industry-standard wash temperatures of $85°$C – and standard dimensions to automate its cleaning.

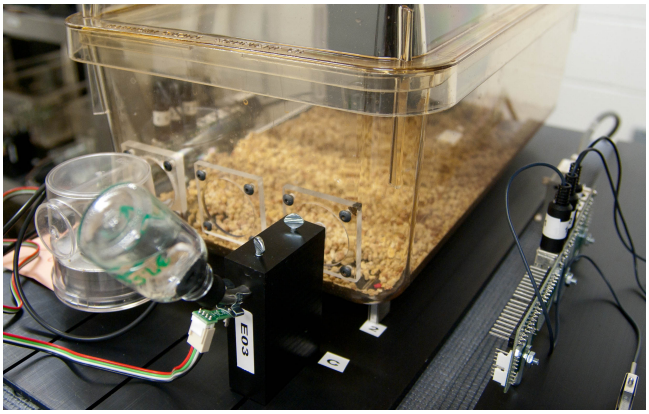

Fig. 1. The system consists of a powder-coated black aluminum base with three indended tracks, allowing any combination of three modules to be used at a time. In the configuration pictured, the feeder module is loaded in the first slot, a blank plate covers the second slot, and the liquid dispenser is installed in the third slot. The control board for the adjacent cage is also visible to the right.

D. Sensors

Each module handles its own sensory requirements. The feeding station uses IR photobeam sensors (HOA6299, Honeywell) to detect animal presence by beam breakage. To detect drinking, the sipper tube measures tongue contact by being electrically coupled to a low-cost, consumer-class capacitive sensor system-on-chip. A problem with [1] was a high susceptibility to EMI (requiring the system to be placed in Faraday cages). The capacitive-touch sensor that was chosen for our design is nearly immune to stray EMI. To set sensor gain required iterative testing of various designs.

Strain gauge output was amplified by the control board's precision buffered instrumentation amplifiers, providing extremely linear response. As the mouse moves about the cage, the center of mass of the entire system shifts, which is reflected in the strain gauge output voltages. The localization algorithm solves the exact torque and force equations for static equilibrium that relates torque and forces at each strain gauge due to cage center of mass and mouse center of mass at a rate of once of per millisecond. Given the cage mass and the mouse mass, we determine the cage center-of-mass position in (x,y) and hence the change in mouse position. Although the system is capable of near-millimeter localization accuracy, resolution finer than 0.5 cm is not required for home cage behavioral phenotyping.

E. Control Board

Each feeding station has a sensor control board that conditions and buffers the signal for transmission. This mixed-signal PCB can amplify up to six low-voltage differential signal inputs, plus provide buffering for 4 digital signals, each with a bandwidth of up to 40 MHz. The control board also has 2 unbuffered pass-through signal inputs. The control board provides six 10 ± 0.001 V independently-regulated power supplies for use by ratiometric sensors, a

clean, one-amp 5V supply, as well as a regulated 12V master supply. To reduce analog noise and signal distortion, the board uses a four-layer stack-up with hashed inner power planes, separate grounding for signal and power distribution, and high-speed routing designed to minimize EM reflection and cross-talk. The board has switch-activated on-board diagnostic capabilities with LED readouts to troubleshoot digital signaling problems as well as power supply short circuiting and overcurrent problems. The board is interfaced to the DAQ system using a single transmission line.

III. Method

Validation was performed using 2-6 month-old male mice of strain C57BL6, C57BL10, or A/J (Jackson Laboratories). Mouse health and HCM food and water were checked daily. All studies were carried out in accordance with Federal and institutional policies governing animal care and use. Mice were recorded overhead (Panasonic, 29.97 frames/sec). We used EthoVision XT 8.5 (Noldus) to convert video data to mouse locomotor trajectories. Parameters were set to track the centroid of a dark colored mouse against a lighter background across the entire home cage, with a hidden zone mapped to the niche. Position data was exported at maximum temporal resolution. These movement trajectories were qualitatively compared to HCM movement trajectories to ensure the system performs similarly to video-based systems. Note that absolute positional accuracy of the system was not studied since it is not as relevant to these behavioral studies as relative movement is.

Mice were water-deprived overnight to increase motivation to drink. We simultaneously collected HCM data and video (Olympus i-Speed 3, 1000 frames/sec), focusing on obtaining a profile of the animal as it accessed the sipper tube. The video camera was triggered when the mouse approached the sipper tube and assumed a position for drinking. Video and HCM data streams were synchronized to the final tongue-off event observed within a series of lick events. Two observers independently scored lick duration and lick on-on intervals by advancing the video one frame at a time, and visually determining when the tongue touched and was withdrawn from the sipper tube.

IV. Results

We focused on validating the two sensor systems that underwent the greatest redesign by studying the HCM's localization and lick event detection against video-based observation. To validate HCM localization, data from a C57BL10 mouse was collected from both the HCM and video camera for 5 minutes. Strain gauge outputs were sampled at 1 KHz each. Strain gauge voltages were converted to cage (x, y) coordinates as above; these positions were then double-filtered using a 250 sample moving window average. Video data was tracked to cage (x, y) coordinates as above. Time series from both systems were aligned, and activity paths created for 5 consecutive 60 second epochs.

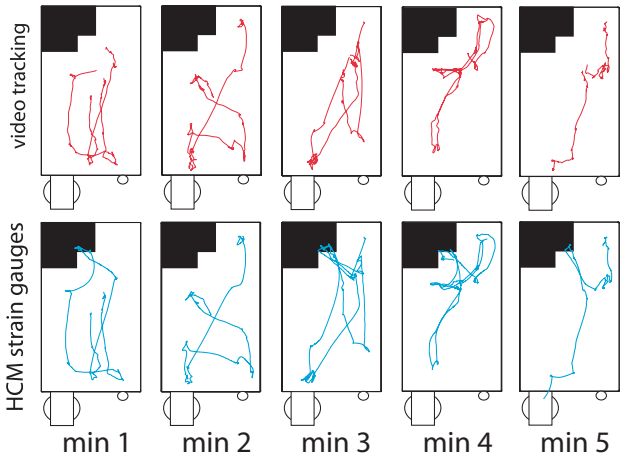

Fig. 2. Validation of strain gauge localization. Top row: mouse movements tracked with EthoVision. Object in cage upper left hand corner is a cartoon of mouse niche showing entrance; in reality, the entire square niche was opaque when observed from above. Object at cage lower left corner is a cartoon of the feeder; object at cage lower right corner is a cartoon of the sipper tube. Bottom row: mouse movements tracked by the HCM.

As shown in Fig. 2, there is excellent mouse movement trajectory agreement between video tracking (top row, red traces) and by our system (bottom row, blue traces). Mice entering or leaving the niche (at cage upper left corner) or the feeder (cage bottom left) can no longer be seen by video camera and are temporarily occluded (minutes 1,3,4,5 in niche, 5 in feeder). By contrast, the strain gauges accurately track the mouse continuously throughout all regions of the cage. Mouse movement trajectories determined by video also are slightly more erratic than the movement trajectories determined by strain gauge (because of filtering).

We validated lick detection by comparing HCM-derived lick events with manual observation of simultaneous high speed video per above (Fig. 3). The difficulty of visually determining when the tongue first and last touched the sipper tube is demonstrated by the significant heterogeneity of called lick-on and lick-off events between the observers. Neither observer was able to distinguish a missed lick from a successful lick. While there was good agreement between the human observers and HCM regarding lickometer on-on intervals (129.6818$\pm$1.571 observer; 130.2273$\pm$2.278 HCM), both observers consistently underestimated lick duration by almost 15% (40.6875$\pm$1.0284 observer; 46.95833$\pm$1.5233 HCM). This error could mask a potentially significant change in water consumption, and clearly justifies using a lickometer to measure this behavior.

V. Conclusion

We have presented a validated system for behavioral phenotyping in a home cage environment using strain gauges for localization, capacitive-touch sensors for detecting water consumption, and photobeam sensors for detecting feeding. This system provides a low-cost, reliable, highly-scalable, high-throughput research platform. Our validation shows

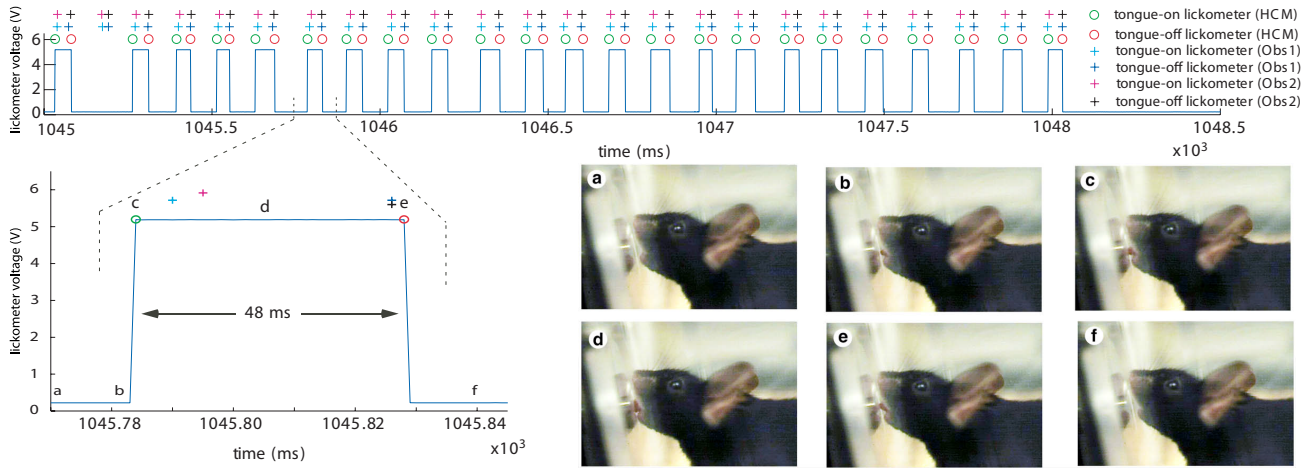

Fig. 3. Series of licks simultaneously observed by HCM and high-speed videography. Open circles depict HCM determination of when the tongue begins (green) and ends (red) contact with the sipper tube. Plus signs depict observer determination (from high speed video frames) of when tongue begins (observer 1 cyan, observer 2 magenta) and ends (observer 1 blue, observer 2 black) contact with the sipper tube. Note HCM determined that the second manually scored event was actually a missed lick. X axis time in ms since data acquisition started; Y axis lickometer voltage. Below, an exploded detail of a single lick event. Lowercase letters along lick trace correspond to frames at right. Note that for frames (a) and (b) that the tongue is protruding from the mouse's mouth but has not contacted the sipper tube; frames (c), (d), and (e) the tongue is in contact with the sipper tube, and frame (f) the tongue has been retracted back into the mouth.

the system performs in a manner intuitively comparable to existing video-based systems, which ensures the system can be used as a drop-in replacement to increase throughput for existing experiments. Further system development will be aimed at increasing the amount of observable behaviors by improving and expanding the set of modules available.

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