eNeuro

Research Article: Open Source Tools and Methods

An open-source, automated homecage sipper device for monitoring liquid ingestive behavior in rodents

https://doi.org/10.1523/ENEURO.0292-19.2019

Cite as: eNeuro 2019; 10.1523/ENEURO.0292-19.2019

Received: 25 July 2019
Revised: 26 August 2019
Accepted: 3 September 2019

1. Manuscript Title: An open-source, automated homecage sipper device for monitoring liquid ingestive behavior in rodents

2. Abbreviated Title: Sipper device for rodent ingestive behavior

3. Authors: Elizabeth Godynyuk[1], Maya N. Bluitt[2], Jessica R. Tooley[1], Alexxai V. Kravitz[1,2,3] and Meaghan C. Creed[1,2,3]

1. Department of Anesthesiology, 2. Department of Psychiatry 3. Departments of Neuroscience and Biomedical Engineering, Washington University in St. Louis, 660 South Euclid Avenue, St. Louis, MO. 63108

4. Author Contributions: EG, MNB and JRT performed research. AVK and MCC collected and analyzed data. MCC designed research and wrote the paper with input from all authors.

5. Correspondence should be addressed to:
Meaghan C. Creed, Department of Anesthesiology. CSRB 5567, Washington University in St. Louis. St. Louis, MO, 63108. meaghan.creed@wustl.edu

6. Number of Figures: 4

7. Number of Tables: 1

8. Number of Multimedia: 0

9. Number of words for Abstract: 240

10. Number of words for Significance Statement: 92

11. Number of words for Introduction: 623

12. Number of words for Discussion: 1188

13. Acknowledgements: We thank HHMI GENIE project for gCamp reagents, and M. Christine Stander for excellent technical assistance.

14. Conflict of Interest: The authors report no conflict of interest.

15. Funding sources: Research was supported by internal funds Washington University in St. Louis (AVK, MCC), Brain and Behavior Research Foundation (NARSAD young investigator grant #27461 to AVK, #27197 to MCC), NIH NIDA CEBRA (R21-DA047127), Whitehall Foundation Grant (#2017-12-54) and Rita Allen Scholar Award in Pain to MCC.

Abstract

Measuring ingestive behavior of liquids in rodents is commonly used in studies of reward, metabolism and circadian biology. Common approaches for measuring liquid intake in real time include computer-tethered lickometers, or video-based systems. Additionally, liquids can be measured or weighed to determine the amount consumed without real-time sensing. Here, we built a photobeam-based sipper device that has the following advantages over traditional methods: 1) it is battery powered and fits in vivarium caging to allow home-cage measurements; 2) it quantifies intake of two different liquids simultaneously for preference studies 3) it is low-cost and easily constructed, enabling high-throughput experiments; 4) it is open-source so others can modify it to fit their experimental needs. We validated the performance of this device in three experiments. First, we calibrated our device using time lapse video-based measurements of liquid intake and correlated sipper interactions with liquid intake. Second, we used the sipper device to measure preference for water vs. chocolate milk, demonstrating its utility for two-bottle choice tasks. Third, we integrated the device with fiber photometry, establishing its utility for measuring neural activity in studies of ingestive behavior. This device requires no special equipment or caging, is small, battery powered, and wireless, allowing it to be placed directly in rodent home cages. The total cost of fabrication is less than $100, and all design files and code are open-source. Together, these factors greatly increase scalability and utility for a variety of behavioral neuroscience applications.

Impact Statement

Current methods for measuring liquid consumption in rodents typically require specialized equipment that is tethered to an in-room computer. This makes portability and scalability challenging. We present a device that is small, battery powered, and wireless, allowing it to be placed directly in rodent home cages. Moreover, the total cost of fabrication is less than $100 and the design is open-source. Together, these factors greatly increase scalability, as the devices do not require dedicated experimental space or caging. The battery lasts approximately 2 weeks, enabling studies of long-term intake or circadian biology.

Introduction

Studies of liquid ingestive behavior are widely used in rodent studies of reward-related behavior, metabolism and circadian biology. Most directly, measurements of liquid intake can be used to understand thirst, a powerful motivational state that is critical for survival and regulated by metabolic factors (Camandola & Mattson, 2017; Ho & Chin, 1988; Research & Marriott, 1994). Tracking liquid intake is a passive way of determining circadian cycle (Schwartz & Zimmerman, 1990) , and monitoring the effect of genetic or pharmacological manipulations on circadian biology (Bainier, Mateo, Felder-Schmittbuhl, & Mendoza, 2017; Ho & Chin, 1988; Mistlberger, Antle, Oliverio, Coffman, & Morris, 2001). Moreover, drinking water is a common route of drug administration in behavioral pharmacology studies (Genn, Higgs, & Cooper, 2003; Hsiao & Spencer, 1983), requiring detailed measurements and temporal profile of drinking water consumed to determine drug dosage. Finally, by examining the relative consumption of two fluids, the “preference” can be determined, which relates to a specific motivation for one fluid over another. The strength of a preference for sucrose has been used as a measure of anhedonia (the inability to feel pleasure) in animal models of depression and drug withdrawal (Alkhlaif, Bagdas, Jackson, Park, & Damaj, 2017; Eagle, Mazei-Robison, & Robison, 2016; Liu et al., 2018), while alcohol or drug preference assays (Ackroff, Manza, & Sclafani, 1993; Ackroff & Sclafani, 2013; Adriani, Macrì, Pacifici, & Laviola, 2002; Hwa et al., 2011; Shaham, Alvares, Nespor, & Grunberg, 1992) or liquid self-administration are widely used in addiction neuroscience (Isiegas, Mague, & Blendy, 2009; Peachey, Rogers, Brien, Maclean, & Rogers, 1976; Shaham et al., 1992).

Existing methods of measuring liquid intake fall into two broad categories. The first is the “low tech” method of placing a graduated tube in the cage and visually inspecting it to determine how much liquid was consumed (Tordoff, 2007; Tordoff, Bachmanov, & Reed, 2007). This method is widely as it is inexpensive, robust, and doesn’t require specialized equipment or caging. However, this method does not reveal information on the

116 microstructure of licking or changes in the temporal patterns of ingestive behavior. The
117 second is a more “high tech” method, which involves placing animals in specialized caging
118 that automatically monitors liquid intake over time, for example by continuously sampling the
119 weight of a water bottle, or by registering optical or electrical contacts with a lickometer (for
120 review, see (Weijnen, 1998)). This approach is automatic and provides temporal resolution,
121 but requires wiring(Hayar, Bryant, Boughter, & Heck, 2006), or expensive, specialized
122 equipment and caging, which limit its accessibility and applicability for high-throughput or
123 long-term studies. Moreover, electrical lickometers that rely on capacitative sensing can
124 produce electrical artefacts that limits their use with some in vivo measures of neuronal
125 activity(Hayar et al., 2006; Schoenbaum, Garmon, & Setlow, 2001).

126 Here, we provide detailed instructions for construction of a novel, open-source,
127 home-cage based sipper device, which has all the functionality of the previously described
128 “low-tech” methods, and has several advantages over existing “high-tech” methods. Our
129 device was developed with scalability in mind, and as such was designed to be low-cost
130 (under $100), wireless, and easy to build by users with no previous electronics experience.
131 The device fits in traditional vivarium home-cages, facilitating the study of large populations
132 without requiring additional experimental space or equipment beyond the vivarium racks.
133 The device is also battery powered, enabling continuous data collection over two weeks with
134 minimal experimenter interference. Finally, since the device uses optical sensors
135 (photointerrupters) rather than capacitative sensing to detect interactions with each sipper, it
136 will not introduce electrical artefacts into data acquisition, and is thus compatible with
137 amplifiers for in vivo electrophysiology, fiber photometry systems or behavioral equipment
138 capable of receiving a TTL input.

139 We validated the sipper device across three experiments. First, we validated the
140 sipper devices as a proxy of ingestive behavior and observed a clear circadian rhythm of
141 drinking water consumption. Second, we used the sipper in a two-bottle choice assay, and
142 determined that the device can reliably detect preference for a preferred liquid (chocolate

milk) over water. Finally, we performed a proof-of principle demonstration that the sipper device can easily and successfully integrate with fiber photometry system, establishing its utility in studying the neural substrate of ingestive behavior. Together, these validation experiments confirm that the sipper device can be used to measure ingestive behavior of liquids across multiple behavioral neuroscience paradigms.

Materials and Methods

Animals and Experimental Procedures.

A total of 24 adult mice (C57Bl6/J background, aged 10-16 weeks) were housed in standard mouse vivarium caging and kept on a 12h light/dark cycle (light onset: 7am, light offset 7pm) with ad libitum access to food and water. Both male and female mice were included in the experiments (14 male, 10 female). Mice were single housed after surgery to protect cranial implants. All studies were approved by the internal animal care and use and committee at Washington University in St. Louis.

Experiment 1: To characterize how counts and duration of the sipper device reflects volume of liquid consumed, mice (n = 7 male, 5 female) were individually housed, and sipper devices were run over night as the sole source of drinking water in the homecage. Videos were taken overnight using a time lapse camera (Brinno TLC200), videos were then exported and position of meniscus in the conical tube reservoir of the sipper device was registered using ImageJ, to visually validate the amount of liquid consumed at 1h intervals. As an extension, we ran this experiment overnight for 6 days to determine whether the sipper device detected circadian patterns of activity. The mice used in these validation studies did not undergo any prior surgeries, nor were they used for previous experiments.

Experiment 2: To confirm that the sipper device can robustly detect ingestion of two liquids simultaneously, mice (n = 4 male, 5 female) were individually housed, and given access to both drinking water and chocolate milk (diluted 50% with drinking water) via the sipper device for a 4 hour “two-bottle choice” assay.

Experiment 3: To confirm the device can be successfully integrated with in vivo measurements of neural activity (using population calcium activity as a proxy), we expressed the calcium indicator GCaMP6s (AAVDJ-hsyn-GCaMP6s-eYFP, University of North Carolina Vector Core) in the dorsomedial striatum (DMS; coordinates from Bregma: +0.5 mm AP, ±1.5 mm ML, -2.8 mm DV according to the Atlas of Franklin and Paxinos) and implanted a fiber (200µm core, 0.39 NA, ThorLabs) in the same region in wild-type mice (n = 3m).

Mice were allowed to consume chocolate milk from the sipper device while GCaMP fluorescence was detected through the optic fiber using a fiber photometry system (Neurophotometrics Ltd, San Diego CA), with all data recorded in the open-source visual programming language Bonsai(Lopes et al., 2015). We aligned the photobeam breaks detected with the sipper device to the fiber photometry recording in Bonsai using an Arduino Uno as a digital acquisition device. Support for using the Arduino Uno in this manner is a native feature of Bonsai and required only a minimal modification to the sipper to solder a BNC connector to the sipper device.

Build Instructions

Here, we present a compact, battery-powered, wireless, homecage-compatible sipper device (Fig 1). The device utilizes readily-available building materials, prioritizing “off-the-shelf” electronics such as the Adafruit Feather M0 Adalogger microcontroller (Fig 1A), and 3D printed components (Fig 1B). The sipper device uses infrared (IR) photo-interrupters to sense when the animal interacts with each sipper spout and displays information on a built-in screen to provide real-time data to a user, such as time spent interacting with each individual sipper as well as number of approaches. The Feather M0 Adalogger also records this information at a user-defined frequency to an on-board microSD card (the default code is set to 6 samples/minute). The device is small, battery-powered and wireless, which makes

it compatible with standard rodent vivarium caging (Fig 1C,D) and has a battery-life of over 2 weeks, allowing for long-term monitoring with minimal experimenter interference.

All design files necessary to complete this build (including electronic layout/soldering instructions, Python code, and 3D printing design files) are located at: https://hackaday.io/project/160388-automated-mouse-homecage-two-bottle-choice-test-v2

All Files for 3D printing, along with photographs and instructions for assembling the sipper devices can be found at: https://hackaday.io/project/160388-automated-mouse-homecage-two-bottle-choice-test-v2.

► 3D-Print the two housing components (Fig 1).

► Assemble the sipper tubes. Using a razor or scalpel blade, cut ~1cm of the cylindrical end from each 15mL conical tube. Using hot glue, secure a Hydropac valve into the cut end and allow it to cool and dry. Check for leaks.

► Assemble photo-interrupters: Solder the Sparkfun photo-interrupter and 1K resistor into the Sparkfun breakout board. Finally, solder the 3-pin right-angle JST connecter with jumper wires into the photodetector.

► Solder the short female header pins to the Adalogger M0 board, and short male header pins to the Adafruit OLED wing. Solder the photo-interrupter wires to the OLED shield, with the black wires attached to the ground (GND), and red wires to power (3V), and the signal wires to pins 10 and 11 (Fig 1A).

► Assemble the device by pushing the photodetector units into the slots in the 3d housing and the Feather device into the holder (Fig 1C). Slide the protective cover over the photodetectors, ensuring the wires protrude on the top of the set-up.

► Snap conical tubes into the housing.

► Insert the microSD card into the Feather devices and attach the battery pack.

► Set the on-board real-time clock (RTC). Flash the Adalogger with the code "SipCounter-SetClock.ino" to set the RTC. The RTC will retain the date and time as long as the device doesn't lose power. Alternatively, the date and time can be set on the device itself (details below).

► Flash the Adalogger with the "SipCounter-081118.ino" code. This is the code that controls the operation and function of the device.

Operation Instructions

Videos showing the set-up and operation of the device can be at: https://hackaday.io/project/160388-two-bottle-choice-test-v2.

► When the device is powered up it will start on a welcome screen.

► Holding down the 'B' button in this screen allows for editing the date, time, and device #. These variables will be recorded with the data on the microSD card.

► Pressing 'A' on this screen starts data recording. The number of interactions and the duration of interaction will be displayed on the screen. All data obtained will also be saved in 10 second increments (this frequency can be edited in the code, Fig 1E).

Statistical analysis

Analysis of correlation measurements and photometry experiments were performed in Python (3.7), paired t-tests and Wilcoxon-signed rank tests were done in Prism (version 8). A p-value > 0.05 was considered statistically significant. Non-parametric statistics were used in cases where the dataset did not meet assumptions of normality.

Results

We performed 3 experiments to validate the use of the device in multiple paradigms relevant for behavioral neuroscience. We first validated that device accurately tracked volume of liquid ingested from the sipper device. We next demonstrated that the sipper device can be used to detect preference in a two-bottle choice assay. Finally, we establish that the sipper device can be simply integrated with in vivo measurements of neuronal activity, in this case fiber photometry.

Experiment 1. The sipper device provided the only source of drinking water, the number and duration of photointerrupter beam breaks on the sipper device and volume of liquid consumed (detected via time-lapse video) were measured (Fig 2A). The correlation between volume ingested and sipper counts or duration varied across devices (Sipper Duration: R^2= 0.02 - 0.89, Sipper Counts: R^2= 0.26 - 0.85, Fig 2B). Sipper counts were a better proxy for liquid intake with an average R^2 value of 0.46 (Fig 2C) compared to the average R^2 value of 0.22 between sipper duration and liquid ingested (Fig 2D).

The duration of sipper interaction registered on the sipper device showed the expected circadian rhythm, with drinking water consumption being greater during the dark cycle (78.3% of total sipper duration between 7 pm and 7 am) relative to the light cycle (21.7% of total sipper duration between 7 am and 7 pm). This pattern was confirmed with visual quantification of liquid consumed via time-lapse photography (80.6% of liquid consumed in the dark cycle, R^2 between sipper duration and visual quantification = 0.63, Fig 2E).

Experiment 2. Preference for chocolate milk vs. water was quantified using the sipper device as a two-bottle choice assay for a 4h session (Fig 3A). All mice exhibited longer total duration of sipper interactions (reflecting total drinking time; mean water duration = 6.5 ± 2.0 seconds, mean chocolate duration = 396.5 ± 176.8 seconds, 2-tailed Wilcoxon p=0.0039 Fig 3B), a greater number of sipper counts (reflecting number of interactions with the sipper device; mean water counts = 15.9 ± 7.3, mean chocolate counts = 233.1 ± 105.1, 2-tailed

Wilcoxon p=0.0078, Fig 3C) and a longer average bout duration (total sipper duration / sipper count; mean water bout length = 0.55 ± 0.26s, mean chocolate bout length = 1.85 ± 0.37s, 2-tailed Wilcoxon p=0.0039, Fig 3D) on the sipper containing chocolate milk relative to drinking water. Together, these results confirm the utility of the sipper device for studying liquid preference in a two-bottle choice assay

Experiment 3. We confirmed that the sipper device can be successfully integrated with in vivo measurements of neural by allowing mice to consume chocolate milk from the sipper device while GCaMP fluorescence from the DMS was detected using a fiber photometry system (Fig 4A-B). We chose the DMS since previous studies using fiber photometry have shown that striatal neurons show a ramping activity before reward retrieval and are inhibited during reward consumption (London et al., 2018).

We confirmed that relative to baseline, gCamp fluorescence in the DMS was significantly increased during approach to the sipper (z score = 0.89 standard deviations above baseline), while fluorescence was significantly decreased during liquid ingestion from the sipper device (z score = -0.40 standard deviations below baseline, Fig 4C-D). This result demonstrates that the sipper device can be integrated with in vivo fiber photometry for measuring neural activation during liquid ingestion, and highlights the flexibility and application of open-source hardware for novel applications.

Discussion

Here, we developed and validated a novel two-bottle choice sipper device that is useful for studying ingestive behavior in rodents. Our device has several advantages over traditional approaches to measuring liquid consumption. First, the device is compact, wireless, battery powered, and has a battery life of over two weeks, which enables high-throughput studies in vivarium caging. Second, our device provides data with a high temporal resolution, which allows for detailed analysis of circadian drinking patterns or analysis of drinking bout patterns and structure. Third, by foregoing the need for capacitive

sensors, our device is compatible with amplifiers or photodetectors used in studies to measure neuronal activity *in vivo*. Since the device is based on photointerrupters, animals are not required to 'complete' an electric circuit for licks to be detected, which causes artifacts in electrophysiological recordings. Finally, our device is open-source and all design files and code are freely available. This allows for modifications to enable specific experiments, as well as easy integration with other recording systems, as we demonstrate with our fiber photometry recordings. Despite these strengths, there are some important limitations and areas of future development that must be kept in mind.

Our current design is a two-sipper configuration. However, there are several behavioral neuroscience paradigms in which it may be desirable to simultaneously monitor intake of more than two liquids; for example studies analyzing oral drug or ethanol discrimination. The microcontroller we use can monitor up to 13 sipper tubes with minimal modifications to the design and code, but these modifications would have to be done by an end user. As a second limitation, our current design is built using 15 mL conical tubes as the liquid reservoir. While this supplied sufficient drinking water for at least 3 days, our experiments revealed that mice would frequently consume the entire volume of chocolate milk in the first 6 hours of their dark cycle. Therefore, rate of consumption is an important consideration for designing long-term experiments with the sipper device. Both of these issues can be overcome by modifying the construction of the device, which is readily achievable given the open-source design. In fact, the device has already been expanded by end users to include a 3-sipper design, and a design with larger liquid reservoirs for use in rats(Frie & Khokhar, 2019).

The hardware used in the device also imposes some limitations that merit consideration. When an animal interacts with the sipper device, the photo-interrupters pick up interactions with millisecond resolution, but these interactions are not limited to licks. These interactions include the toungue of the animal, but can also include the snout or other body parts that break the infrared beam below the sipper. For this reason, we describe the

device output as sipper counts or duration and not “licks”. This limitation resulted from a design trade-off that we made to build a wireless, home-cage compatible device, vs. a device that required specialized caging and tethered sensors, such as electrical lickometers. Both the sipper counts and duration were only moderately correlated with volume of intake as measured by time lapse photography (Fig 2). This lack of tight correlation between lick behavior and liquid ingested has been well-documented, and is true of any traditional optical or capacitative lickometer(Buxton & Allison, 1990; Kochanek, 2008; Schultz, Stiemke, & Barber, 1989). However, there are two features of the sipper device that help address this limitation. First, the reservoirs are constructed from 15mL conical tubes with regular gradations. This makes visual inspection of liquid levels in the homecage straightforward, without the need to weigh bottles or otherwise remove traditional sipper tubes (which can be prone to spilling) from homecages. Second, the default code in the sipper device will write photobeam counts and duration to the microSD card every 10 seconds, unless there is an on-going bout (defined as a broken photobeam) at the end of this 10 second interval. In this case, the duration and beam counts will be written at the end of the bout before proceeding to the next 10 second interval. This means that the timestamps can be inspected for outlying photobeam breaks, which could then be removed from the data as outliers. Therefore, while the device does not reliably detect individual licks or electronically measure liquid volumes, the device is still suitable for examining the duration, number and pattern of consumption bouts (Fig 2, 3).

A finally feature is that the sipper devices are equipped with screens that display duration and number of interactions on each sipper from outside of the homecage. Therefore, by inspecting the device screens, potential problems can be detected without interfering with the experiment. Finally, data retrieval requires removal of SD cards and copying data to a computer, which can be time-consuming if many devices are being used. In future versions, wireless communication chips could be added to the device to enable

cloud-based data storage. Such a system would allow multiple users to access data with minimal interference, and would facilitate high-throughput and multi-site experiments.

Beyond these specific concerns, the device is subject to limitations of all two-bottle choice assays. First, positional bias may affect drinking behavior(Ackroff et al., 1993; Gillespie & Lucas, 1957). Therefore, alternating position of liquids in the two sipper tubes across time may be needed to control for this bias in preference assays. Second, the device currently requires animals to be singly-housed, which may limit how many experiments can be run simultaneously due to space constraints, and could introduce stress that may affect behavioral assays (Collins, Pogun, Nesil, & Kanit, 2012; Kamakura, Kovalainen, Leppäluoto, Herzig, & Mäkelä, 2016). However, as the device is open-source, it could be modified to include additional sensors for discriminating multiple animals, such as RFID sensor that have been used in some commercial systems for this purpose(Bains et al., 2016; Galsworthy et al., 2005). While we have tried to optimize the device to prevent damage from mice and with long-term use as the goal, we found that mice sporadically chew on the sipper valves, which can interfere with the function of the device. These valves should be visually examined and replaced if mice chew on them.

In conclusion, we have developed a device for measuring liquid intake in home-cages. The unique strengths of our system are that 1) it is wireless and compatible with vivarium home-cages, 2) it is cost-effective, costing <$100 to build each device and 3) it is open-source, allowing for modifications and flexibility for answering different experimental questions. The device logs interactions with two liquid sippers as detected by photointerrupter beam breaks with high temporal resolution (Fig 1). The device does not require manually weighing bottles and provides temporal resolution over timescales of several weeks without requiring a battery change, which makes the devices suitable for studying liquid preference, circadian biology or resolving long-term patterns of liquid intake (Fig 2-3). Our device was designed with scalability in mind, it requires no specialized equipment and is low-cost and compact enough to allow for high-throughput experiments.

We also provide a proof of concept validation that the device can be integrated with in vivo measurements of neuronal calcium activity (Fig 4). These factors, combined with open-source and off-the shelf nature of the device, ensure that it will be accessible and useful tool for a variety of behavioral neuroscience experiments and studies of liquid ingestion.

References

Ackroff, K., Manza, L., & Sclafani, A. (1993). The Rat's Preference for Sucrose, Polycose and their Mixtures. *Appetite*, *21*(1), 69–80. https://doi.org/10.1006/appe.1993.1037

Ackroff, K., & Sclafani, A. (2013). Flavor Preferences Conditioned by Oral Monosodium Glutamate in Mice. *Chemical Senses*, *38*(9), 745–758. https://doi.org/10.1093/chemse/bjt049

Adriani, W., Macrì, S., Pacifici, R., & Laviola, G. (2002). Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *27*(2), 212–224. https://doi.org/10.1016/S0893-133X(02)00295-6

Alkhlaif, Y., Bagdas, D., Jackson, A., Park, A. J., & Damaj, I. M. (2017). Assessment of nicotine withdrawal-induced changes in sucrose preference in mice. *Pharmacology, Biochemistry, and Behavior*, *161*, 47–52. https://doi.org/10.1016/j.pbb.2017.08.013

Bainier, C., Mateo, M., Felder-Schmittbuhl, M.-P., & Mendoza, J. (2017). Circadian rhythms of hedonic drinking behavior in mice. *Neuroscience*, *349*, 229–238. https://doi.org/10.1016/j.neuroscience.2017.03.002

Bains, R. S., Cater, H. L., Sillito, R. R., Chartsias, A., Sneddon, D., Concas, D., … Armstrong, J. D. (2016). Analysis of Individual Mouse Activity in Group Housed Animals of Different Inbred Strains using a Novel Automated Home Cage Analysis System. *Frontiers in Behavioral Neuroscience*, *10*. https://doi.org/10.3389/fnbeh.2016.00106

411 Buxton, A., & Allison, J. (1990). Efficiency and persistence of licking in rats. *Physiology &*
412 *Behavior*, *47*(2), 239–247. https://doi.org/10.1016/0031-9384(90)90137-s
413 Camandola, S., & Mattson, M. P. (2017). Toll-like receptor 4 mediates fat, sugar, and umami
414 taste preference and food intake and body weight regulation. *Obesity (Silver Spring,*
415 *Md.)*, *25*(7), 1237–1245. https://doi.org/10.1002/oby.21871
416 Collins, A. C., Pogun, S., Nesil, T., & Kanit, L. (2012). Oral Nicotine Self-Administration in
417 Rodents. *Journal of Addiction Research & Therapy*, *Suppl 2*.
418 https://doi.org/10.4172/2155-6105.S2-004
419 Eagle, A., Mazei-Robison, M., & Robison, A. (2016). Sucrose Preference Test to Measure
420 Stress-induced Anhedonia. *BIO-PROTOCOL*, *6*(11).
421 https://doi.org/10.21769/BioProtoc.1822
422 Frie, J. A., & Khokhar, J. Y. (2019). An open source automated two-bottle choice test
423 apparatus for rats. *HardwareX*, *5*, e00061. https://doi.org/10.1016/j.ohx.2019.e00061
424 Galsworthy, M. J., Amrein, I., Kuptsov, P. A., Poletaeva, I. I., Zinn, P., Rau, A., … Lipp, H.-P.
425 (2005). A comparison of wild-caught wood mice and bank voles in the Intellicage:
426 Assessing exploration, daily activity patterns and place learning paradigms.
427 *Behavioural Brain Research*, *157*(2), 211–217.
428 https://doi.org/10.1016/j.bbr.2004.06.021
429 Genn, R. F., Higgs, S., & Cooper, S. J. (2003). The effects of 7-OH-DPAT, quinpirole and
430 raclopride on licking for sucrose solutions in the non-deprived rat. *Behavioural*
431 *Pharmacology*, *14*(8), 609–617. https://doi.org/10.1097/01.fbp.0000104032.08123.4c
432 Gillespie, R. J. G., & Lucas, C. C. (1957). An Unsuspected Factor which influences
433 Consumption of Alcohol by Rats. *Nature*, *180*(4597), 1292.
434 https://doi.org/10.1038/1801292a0
435 Hayar, A., Bryant, J. L., Boughter, J. D., & Heck, D. H. (2006). A low-cost solution to
436 measure mouse licking in an electrophysiological setup with a standard analog-to-
437 digital converter. *Journal of Neuroscience Methods*, *153*(2), 203–207.
438 https://doi.org/10.1016/j.jneumeth.2005.10.023

439 Ho, A., & Chin, A. (1988). Circadian feeding and drinking patterns of genetically obese mice
440 fed solid chow diet. *Physiology & Behavior*, *43*(5), 651–656.
441 https://doi.org/10.1016/0031-9384(88)90221-1
442 Hsiao, S., & Spencer, R. (1983). Analysis of licking responses in rats: Effects of
443 cholecystokinin and bombesin. *Behavioral Neuroscience*, *97*(2), 234–245.
444 Hwa, L. S., Chu, A., Levinson, S. A., Kayyali, T. M., DeBold, J. F., & Miczek, K. A. (2011).
445 Persistent escalation of alcohol drinking in C57BL/6J mice with intermittent access to
446 20% ethanol. *Alcoholism, Clinical and Experimental Research*, *35*(11), 1938–1947.
447 https://doi.org/10.1111/j.1530-0277.2011.01545.x
448 Isiegas, C., Mague, S. D., & Blendy, J. A. (2009). Sex differences in response to nicotine in
449 C57Bl/6:129SvEv mice. *Nicotine & Tobacco Research: Official Journal of the Society*
450 *for Research on Nicotine and Tobacco*, *11*(7), 851–858.
451 https://doi.org/10.1093/ntr/ntp076
452 Kamakura, R., Kovalainen, M., Leppäluoto, J., Herzig, K., & Mäkelä, K. A. (2016). The
453 effects of group and single housing and automated animal monitoring on urinary
454 corticosterone levels in male C57BL/6 mice. *Physiological Reports*, *4*(3).
455 https://doi.org/10.14814/phy2.12703
456 Kochanek, E. A. U. P. D. D. C. and J. (2008, September 30). Measuring Food and Water
457 Intake in Rats and Mice. Retrieved July 21, 2019, from Laboratory Equipment
458 website: https://www.laboratoryequipment.com/article/2008/09/measuring-food-and-
459 water-intake-rats-and-mice
460 Liu, M.-Y., Yin, C.-Y., Zhu, L.-J., Zhu, X.-H., Xu, C., Luo, C.-X., … Zhou, Q.-G. (2018).
461 Sucrose preference test for measurement of stress-induced anhedonia in mice.
462 *Nature Protocols*, *13*(7), 1686–1698. https://doi.org/10.1038/s41596-018-0011-z
463 London, T. D., Licholai, J. A., Szczot, I., Ali, M. A., LeBlanc, K. H., Fobbs, W. C., & Kravitz,
464 A. V. (2018). Coordinated Ramping of Dorsal Striatal Pathways preceding Food
465 Approach and Consumption. *Journal of Neuroscience*, *38*(14), 3547–3558.
466 https://doi.org/10.1523/JNEUROSCI.2693-17.2018

467 Lopes, G., Bonacchi, N., Frazão, J., Neto, J. P., Atallah, B. V., Soares, S., … Kampff, A. R.
468 (2015). Bonsai: An event-based framework for processing and controlling data
469 streams. *Frontiers in Neuroinformatics*, *9*, 7. https://doi.org/10.3389/fninf.2015.00007
470 Mistlberger, R. E., Antle, M. C., Oliverio, M. I., Coffman, T. M., & Morris, M. (2001).
471 Circadian rhythms of activity and drinking in mice lacking angiotensin II 1A receptors.
472 *Physiology & Behavior*, *74*(4–5), 457–464. https://doi.org/10.1016/s0031-
473 9384(01)00591-1
474 Peachey, E., Rogers, B., Brien, J. F., Maclean, A., & Rogers, D. (1976). Measurement of
475 acute and chronic behavioural effects of methamphetamine in the mouse.
476 *Psychopharmacology*, *48*(3), 271–275.
477 Research, I. of M. (US) C. on M. N., & Marriott, B. M. (1994). *Environmental Issues That*
478 *Influence Intake of Replacement Beverages*. Retrieved from
479 https://www.ncbi.nlm.nih.gov/books/NBK231133/
480 Schoenbaum, G., Garmon, J. W., & Setlow, B. (2001). A novel method for detecting licking
481 behavior during recording of electrophysiological signals from the brain. *Journal of*
482 *Neuroscience Methods*, *106*(2), 139–146.
483 Schultz, T. J., Stiemke, T. P., & Barber, B. J. (1989). A device for continuous monitoring of
484 water consumption by rats. *Biomedical Sciences Instrumentation*, *25*, 39–46.
485 Schwartz, W. J., & Zimmerman, P. (1990). Circadian timekeeping in BALB/c and C57BL/6
486 inbred mouse strains. *The Journal of Neuroscience: The Official Journal of the*
487 *Society for Neuroscience*, *10*(11), 3685–3694.
488 Shaham, Y., Alvares, K., Nespor, S. M., & Grunberg, N. E. (1992). Effect of stress on oral
489 morphine and fentanyl self-administration in rats. *Pharmacology, Biochemistry, and*
490 *Behavior*, *41*(3), 615–619. https://doi.org/10.1016/0091-3057(92)90382-p
491 Tordoff, M. G. (2007). Taste Solution Preferences of C57BL/6J and 129X1/SvJ Mice:
492 Influence of Age, Sex, and Diet. *Chemical Senses*, *32*(7), 655–671.
493 https://doi.org/10.1093/chemse/bjm034

494 Tordoff, M. G., Bachmanov, A. A., & Reed, D. R. (2007). Forty mouse strain survey of water
495 and sodium intake. *Physiology & Behavior*, *91*(5), 620–631.
496 https://doi.org/10.1016/j.physbeh.2007.03.025
497 Weijnen, J. A. (1998). Licking behavior in the rat: Measurement and situational control of
498 licking frequency. *Neuroscience and Biobehavioral Reviews*, *22*(6), 751–760.
499
500
501
502
503
504
505
506
507
508

Figure 1. Construction and implantation of home cage drinking monitor. (A) Circuit wiring diagram of electronic components; 3V power is supplied to the photointerupters which are connected to a ground pin. Each photointerrupter is also attached to a digital output pin (shown as D9 and D10). **(B)** 3D rendering of the 3D printed housing for the drinking monitor; views of the front tube assembly (left) and rear battery casing (right) are shown. **(C)** Photo of the assembled device and **(D)** assembled device operating in the home-cage.

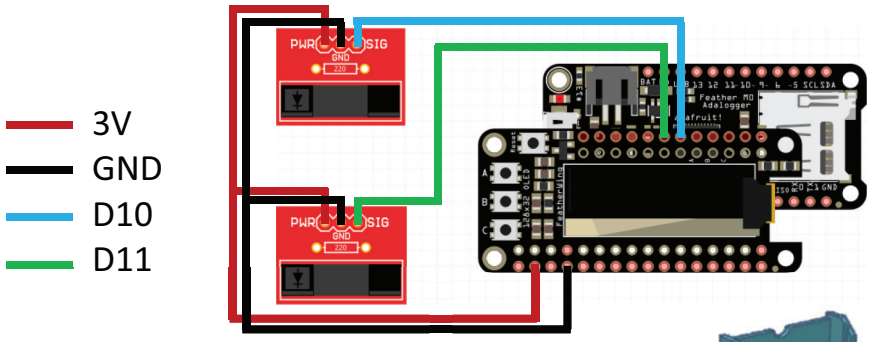
Figure 2. Functional validation of liquid consumption with the homecage drinking monitor. (A) Schematic of the experimental set-up. **(B)** Histogram depicting the distribution of R^2 values of the correlation between measurements (counts or duration) registered on the individual sipper devices with volume of liquid consumed from the same device, as measured via time-lapse video. **(C-D)** Across all 11 mice tested, sipper counts and duration of interactions with the sipper device weakly correlated with the amount of water consumed by visual quantification with time lapse video. **(E)** Circadian rhythms in lick duration and volume of liquid consumed were evident over the 5 days of recording.

Figure 3. Two-bottle choice task with the homecage drinking monitor. (A) Experimental schematic; mice had free access to two liquids in the drinking monitor device: water or chocolate milk. **(B-D)** All mice exhibited a clear preference for chocolate milk over water. Mice exhibited longer total duration of sipper interactions (B), increased duration of sipper interaction bouts (C) and increased sipper approaches (D) for chocolate milk over water. **p<0.01.

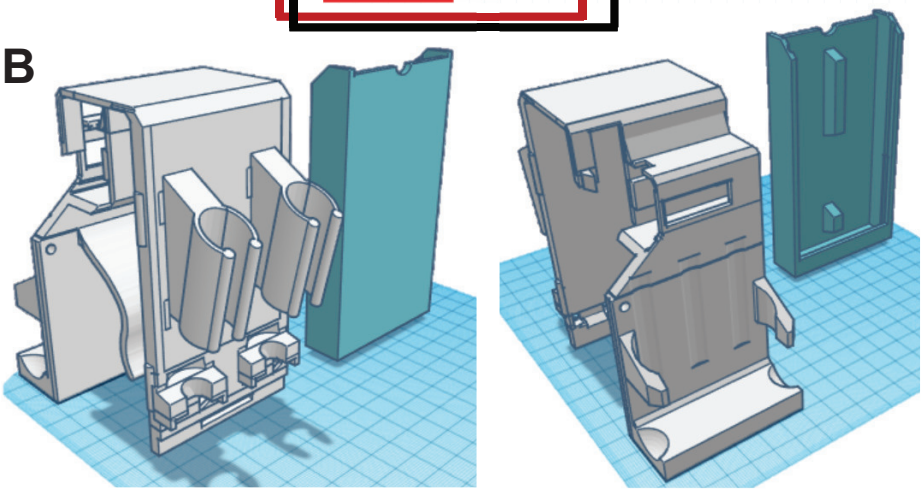
Figure 4. Integration of the drinking monitor with in vivo fiber photometry. (A) Schematic of experiment; photointerrupter beam breaks registered on digital Arduino pins triggered a TTL pulse to an in vivo fiber photometry system. **(B)** Representative raw trace of gCamp signal recorded in the dorsal medial striatum (DMS; top) and concomitant sipper interaction bouts (bottom). **(C)** Normalized gCamp traces were averaged across trials for all mice, and aligned to onset of lick bout. Black line indicates the mean, SEM is depicted in red. **(D)** Duration of sipper interactions is aligned to onset of lick bout, mean probability of a sipper interaction is indicated in black, SEM is depicted in blue. Both C and D are aligned to onset of lick bout.

Table 1. Bill of materials. All materials required for construction of the sipper device are itemized, sourcing and cost are provided.

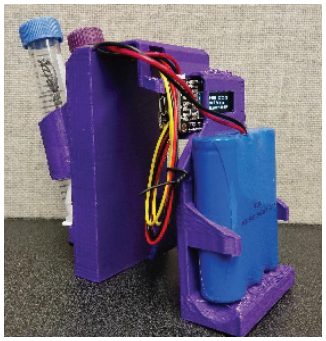
A



B



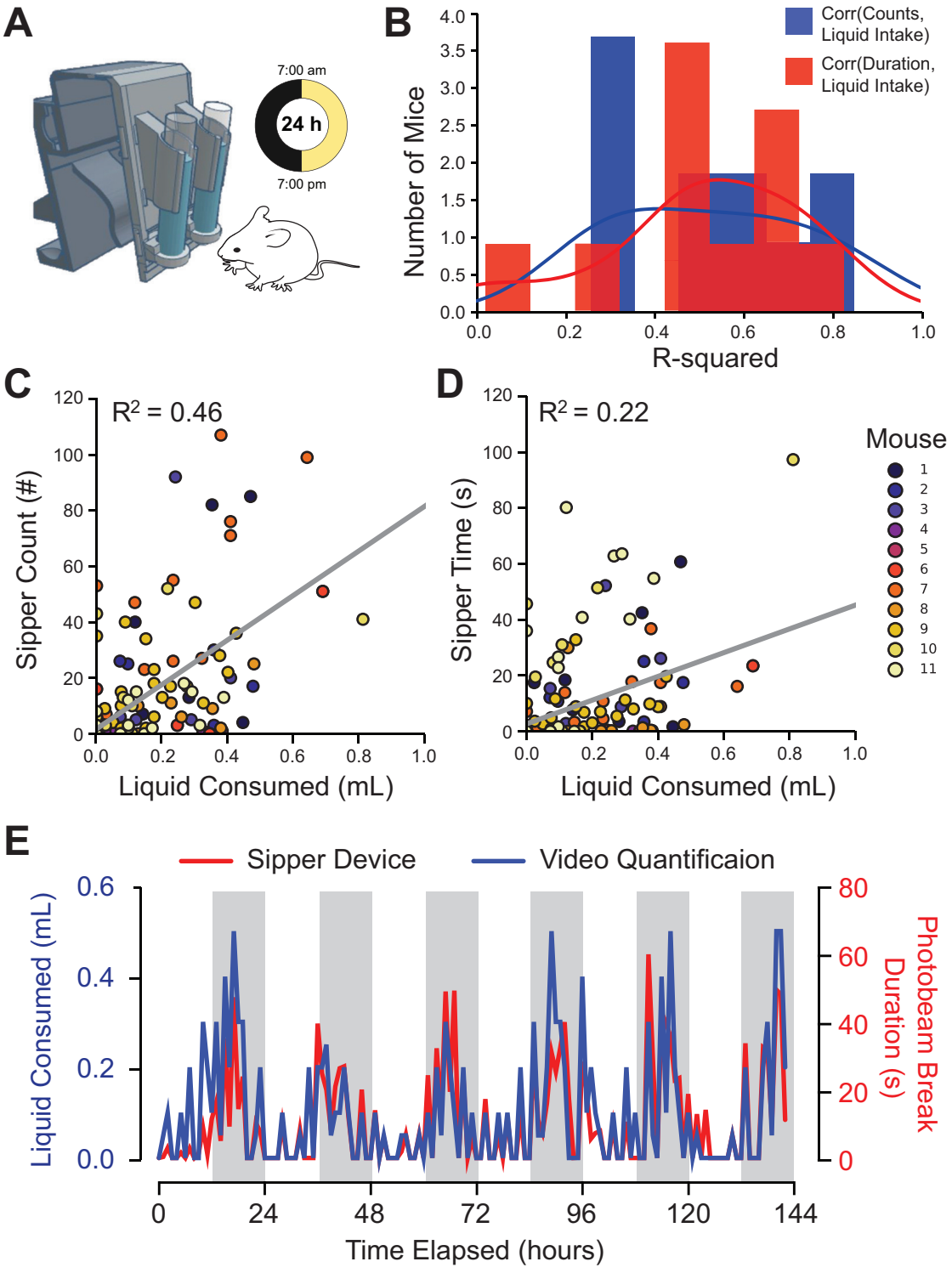
C

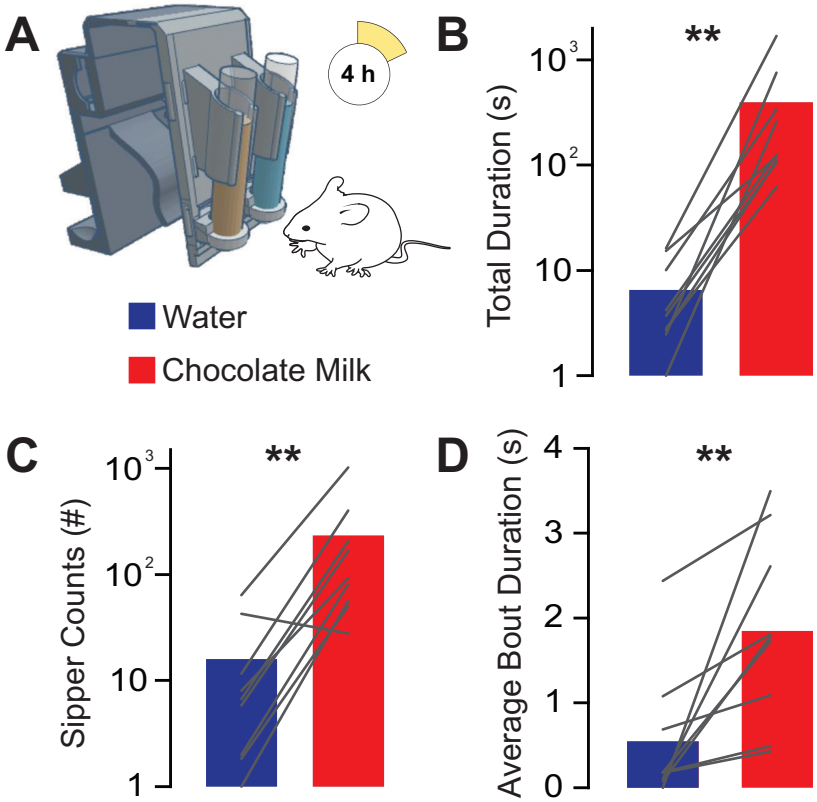


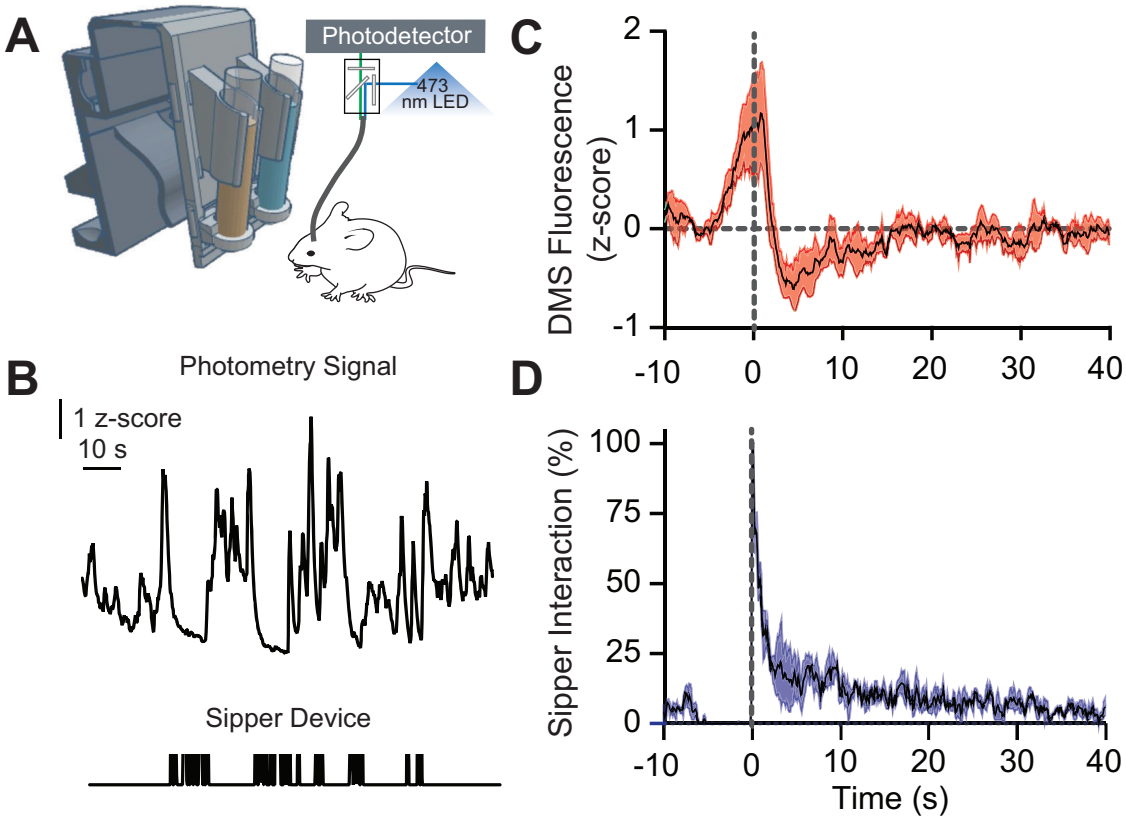
D

E

MM:DD:YYYY hh:mm:ss	Elapsed Time	Device	LeftCount	LeftDuration	RightCount	RightDuration	BatteryVoltage
2/20/2019 14:27:53	01:17:30	1	0	0	0	0	4.05
2/20/2019 14:28:03	01:17:40	1	1	0.15	0	0	4.05
2/20/2019 14:28:16	01:17:53	1	3	10.15	0	0	4.05
2/20/2019 14:28:26	01:18:03	1	3	10.15	0	0	4.05
2/20/2019 14:28:36	01:18:13	1	3	10.15	0	0	4.07
2/20/2019 14:28:46	01:18:23	1	3	10.15	0	0	4.06
2/20/2019 14:28:56	01:18:33	1	3	10.15	0	0	4.04
2/20/2019 14:29:06	01:18:43	1	3	10.15	0	0	4.05
2/20/2019 14:29:18	01:18:54	1	4	13.27	0	0	4.05
2/20/2019 14:29:27	01:19:04	1	5	20.04	0	0	4.05
2/20/2019 14:29:37	01:19:14	1	5	20.04	0	0	4.05







Component	Number	Cost / unit	Total cost	Source of materials
Adafruit Feather M0 Adalogger	1	$19.95	$19.95	https://www.adafruit.com/product/2796
Lithium Ion Battery Pack – 3.7V 6600mAh	1	$29.50	$29.50	https://www.adafruit.com/product/353
Adafruit FeatherWing OLED – 128x32 OLED	1	$14.95	14.95	https://www.adafruit.com/product/2900
Short Headers Kit for Feather – 12-pin + 16-pin Female Headers	1	$1.50	$1.50	https://www.adafruit.com/product/2940
1k Resistor	2	$0.20	$0.40	https://www.sparkfun.com/products/14492
Photo Interrupter GP1A57HRJ00F	2	$2.50	$5.00	https://www.sparkfun.com/products/9299
SparkFun Photo Interrupter Breakout Board - GP1A57HRJ00F	2	$1.50	$3.00	https://www.sparkfun.com/products/9322
MicroSD card	1	$6.00	$6.00	https://www.amazon.com/Kingston-microSDHC-Class-Memory-SDC4/dp/B00200K1TS/
JST Cables	2	$1.50	$3.00	https://vetco.net/products/jst-ph-connector-male-female-pair-pre-wired-3-pin
Plastic Valves	2	$0.50	$1.00	https://labproductsinc.com/product/hydropac-alternative-watering-system/
15mL conical tubes	2			