An inexpensive and easy-to-assemble device for neural recordings in freely behaving animals

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ABSTRACT

Awake-behaving physiological recordings present manifold technical difficulties. Perhaps the defining challenge of this approach is the tradeoff of the behavior's realism against the stability of the physiological recording; how can one ensure that an electrical probe will remain in place in the brain without physically impeding an animal's movement during natural behavior? A recording setup with long cables would provide slack for an animal to explore a large arena, but long cables are liable to impede behavior. One solution is to effectively shorten the cables, instead using a device that carries the recording setup with the animal as it moves. Short of a wireless recording setup, this option represents the "gold standard" for awake-behaving electrophysiology, but it presents its own set of technical difficulties: one needs a system that can automatically track an animal and adjust the position of the recording equipment, and while these capabilities are relatively commonplace, they have yet to be integrated into a low-cost, closed-loop system for tracking animals. To realize this gold standard, we installed an OpenMV camera onto a repurposed CNC engraver gantry. This camera can identify a high-contrast animal in its field of view and send a control command to the gantry with an efficient communication protocol. Our device tracked an animal in an open arena and in a maze with short walls. It shows promise for tracking animals in a range of environments, including mazes with high walls. This device represents the first open source, low-cost, closed loop animal tracking system with exceptional user friendliness across technical proficiencies. It will allow for neural recordings in freely behaving animals across a range of recording modalities, species, and behavioral tasks.

INTRODUCTION

The ability to record neural activity is an integral part of neuroscience research. However, lots of methods for recording-large scale neural activities require the animal to be restrained, thereby limiting the scope of behaviors that one can investigate with these methods[1]. Moreover, restraint itself can alter brain activities. Previous studies have shown that restraint can elicit stress response in animals, which leads to various possible physiological changes, such as increased Adrenocorticotropic hormone (ACTH) and corticosterone levels, and impaired memory and motivation[2]. Recording neural activities in restrained animals can put us at risk of drawing incorrect conclusions about how the brain works, since we are not observing how the brain works in its natural state.

Wireless electrophysiology is a way to address this issue[3], but it is often unreliable and expensive. It usually saves data to local SD cards, which is limited in space and does not provide real time feedback. A few configurations can transmit in real time via radio or Wi-Fi, but they are unable to transmit large quantities of neural signals over a long range, making them less powerful compared to wired neuropixel probes. In addition to wireless setups, there are other technologies that enable recording in freely behaving animals, such as head-mounted miniscopes[4] and wired microdrives (drivable arrays of electrodes)[5], which usually require the animal to wear a head-mounted device connected to a wire. While these wired technologies are indeed revolutionary, they leave an important practical problem unanswered: cables from these devices are liable to impede natural behavior, especially when animals are put in the kinds of complex environments that they actually inhabit. In a large arena with a cable fixed above the center, as the animal moves toward a wall, the cable will pull on the head up from the floor toward the center. The cable will also act as a pendulum, trying to return to its original position at rest above the center, which the animal will have to bodily resist. Thus, it is imperative for the cable to remain approximately above the head throughout the recording. Both of these failure modes would be eliminated if there were a method to move the cable with the animal as it moves in the environment.

A method to move the cable with the animal as it moves in arbitrary environments would need to be simple, inexpensive, and technically accessible. Here we leverage several existing technologies to realize this vision for awake-behaving rigs and synthesize new design principles that enable with other systems. We built a control system capable of carrying a cable with the animal as the animal moves and tested it on mice moving in open arena and mazes. Our system is inexpensive, easy to assemble, and generalizes across several gantry products besides the ones we tested. Our system is agnostic to recording modality; it can carry cables for electrophysiological or optical probes such as microdrives and miniscopes. To our knowledge, there does not exist an equivalent device for neuroscience.

RESULTS

System design

The device comprised two main elements: an OpenMV Cam H7 microprocessor camera, and a repurposed CNC engraver gantry (Table 1). We successfully implemented this system in two separate CNC engraver products, a smaller (40 cm x 50 cm) model, and a larger (100 cm x 100 cm) model for larger behavioral arenas. The gantry was assembled following all instructions provided by the supplier, but with the laser head replaced with the OpenMV camera mounted via a custom part laser cut from a centimeter-thick acrylic sheet (Figure 1a). An assembled device is shown in Figure 1b.

Table 1. Bill of materials.

Qty	Description	Supplier	Link
1	OpenMV Cam H7	OpenMV	https://openmv.io/products/openmv-cam-h7-r2
1	IR Lens	OpenMV	https://openmv.io/collections/lenses/products/ir-lens
1	40x50cm CNC Engraver Gantry	Bachin	https://www.amazon.com/gp/product/B07YYL2KRN/ref=ppx_yo_dt_b_search_asin_title?ie=UTF8&th=1
1	100x100cm CNC Engraver Gantry	Laseraxe	https://www.aliexpress.com/item/2251832779871127.html?spm=a2g0o.order_detail.0.0.3f31f19c6cPxQF&gatewayAdapt=4itemAdapt

We were able to implement closed-loop control of the gantry due to the surprising accessibility of the gantry's internal firmware via a wired communication protocol. Both gantries described here use the Grbl platform for CNC control as their firmware, which means that we could use the same code to control two different gantry products. To interface the OpenMV camera and the gantry, we needed to access RX and TX pins on the gantry (OpenMV cameras already implement the serial control protocol on their IO pins). For the larger gantry, the RX and TX pins on the OpenMV camera were connected directly to the TX and RX pins on the Arduino located in its control panel. For the smaller gantry, TX and RX pins on its Arduino were not directly accessible but tracing the printed circuit board revealed that these pins were connected to two exposed pins in the offline panel. OpenMV cameras were programmed using MicroPython in OpenMV IDE. Serial communication was implemented via the UART class in the pyb module intended for board control. We sent G-code via the serial communication protocol to the gantry. Grbl is a ubiquitous software in DIY tabletop CNC engraver space, so it is highly likely that users can replicate our system in any CNC gantry that runs Grbl, not limited to the two specific models that we used.

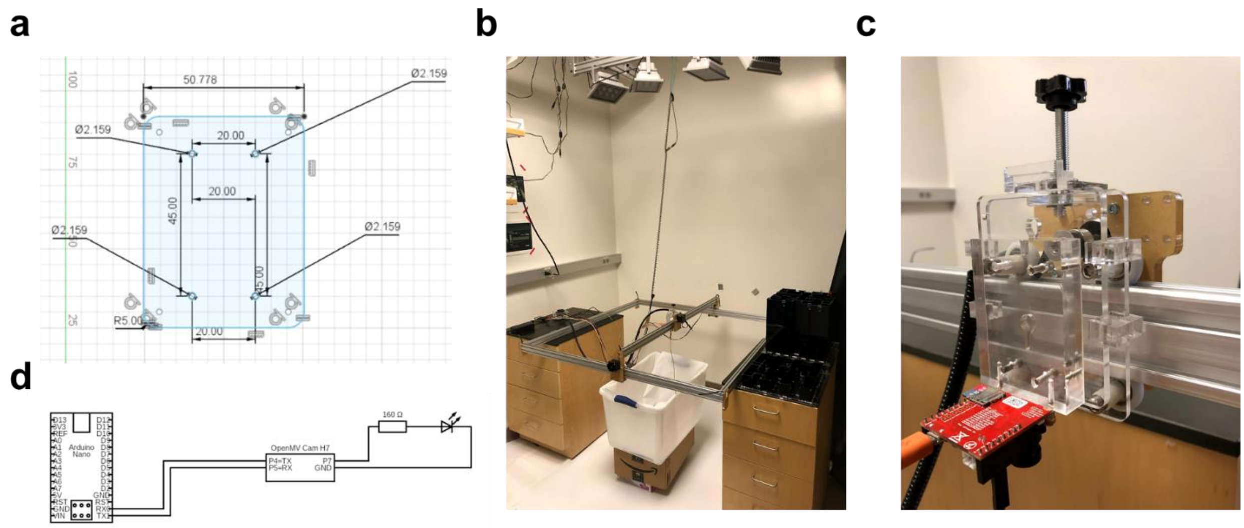


Figure 1. Overview of hardware design. (a) Specifications for the laser cut custom part in millimeters (created in Fusion 360). (b) An assembled device consisting of the gantry and the OpenMV camera. (c) A closer view at the mounted OpenMV camera. (d) Circuit diagram. The gantry is controlled by the Arduino Nano running the Grbl firmware in the diagram. The IR LED light is optional. It is only necessary if the user wants to track an animal carrying an LED light instead of the animal itself.

The image module built-in to the OpenMV camera is designed for scientific machine vision applications, such as blob tracking and distortion correction. We used the blob tracking function to identify all blobs within a certain color range in the image frame and selected the blob with maximal compactness, which gave us the position of the animal. The user should use a different threshold or method depending on the experiment, as the system is flexible enough to allow for several blob tracking configurations in addition to tracking black mice on a white surface. Other high-contrast setups, such as a white/grey zebra finch on a black background, would be equally feasible to track with trivial changes to the code.

To achieve closed-loop control on the gantry’s movement, we used the proportional-integral-derivative (PID) controller, implemented via a third-party built-in library. The protocol is as follows:

Algorithm: closed-loop control with PID controller

Result: keep the animal at the center of the frame.
Initialization:
    Define the pixel coordinates of the center of the frame: rx, ry.
while elapsed time < 60s **do**
    Find the gantry’s current position in absolute coordinates: x0, y0.
    Take a snapshot and find the centroid of the animal in snapshot using OpenMV’s blob detection function: cx, cy.
    if find zero blob in current frame **then**
        Continue to take snapshots until finding at least one blobs in the current snapshot and return cx, cy.

Calculate the error as the difference between the animal's current position and center of the frame: ex = cx-rx, ey = ry-cy[1].
Calculate the proportional, integral, and derivative terms of the error and return a weighted sum: ux = PID(ex), uy = PID(ey).
Convert u in pixels to G-code in millimeters and absolute coordinates using the gantry's current position and send to the gantry via UART: send(gcode(x0+ux, y0+uy)).

end

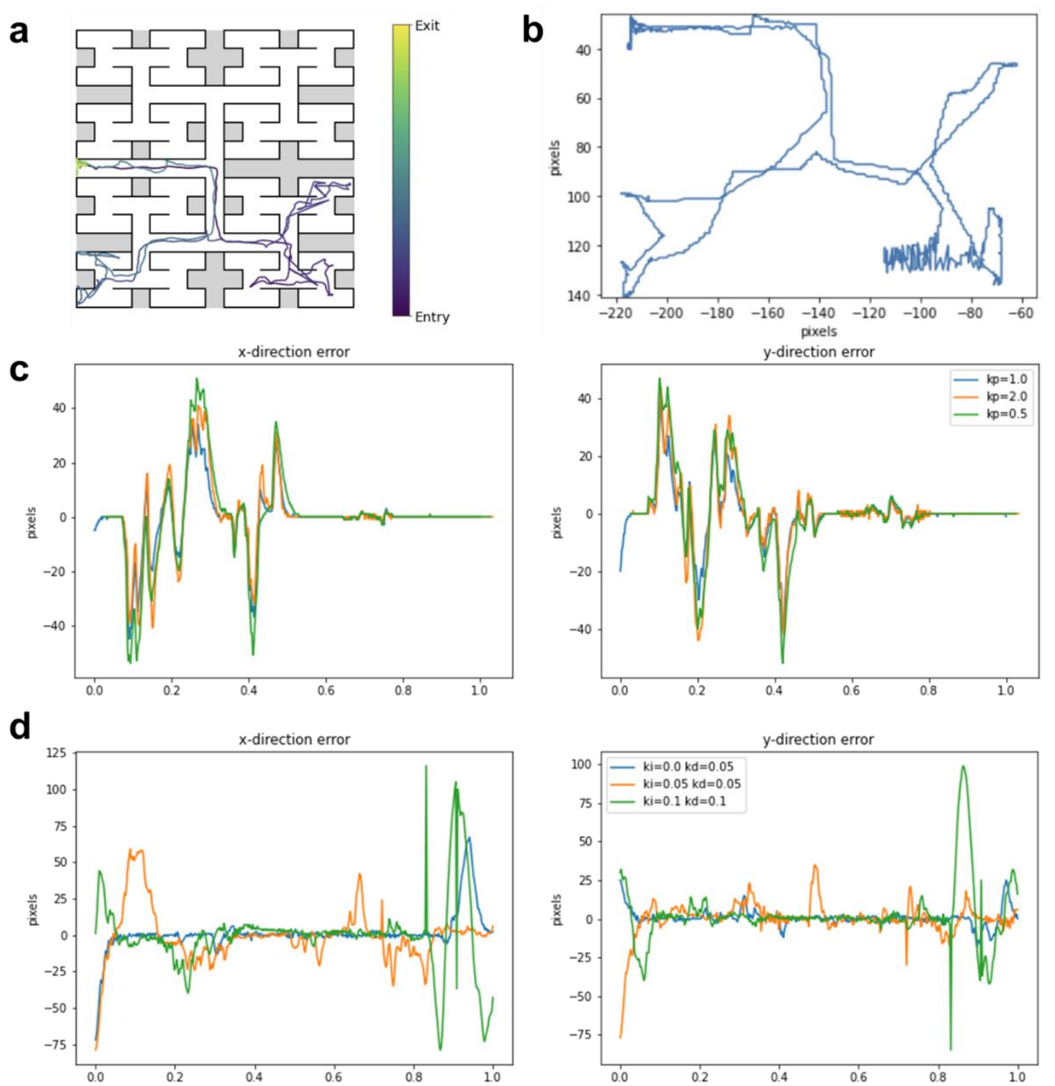


Figure 2. PID tuning. (a) Trajectory of one mouse in a maze[6]. Dimension of maze: 24 x 24 x 2 inches. (b) Mouse trajectory approximated with the small gantry. Movement of the gantry was recorded with a stationary OpenMV camera. (c) Effect of different weights of the proportional term (Kp) on tracking error in x (left) and y (right) directions. The target being tracked was the small gantry approximating the mouse trajectory. If Kp is too large (orange), the system becomes unstable. If Kp is too small (green), the system responds to changes with delay. (d) Effect of different weights of the integral (Ki) and derivate (Kd) terms

[1] Directions of x and y axes of the gantry were inverted compared to that of the camera in our setup, so ex and ey were calculated differently. But users may have a different setup.

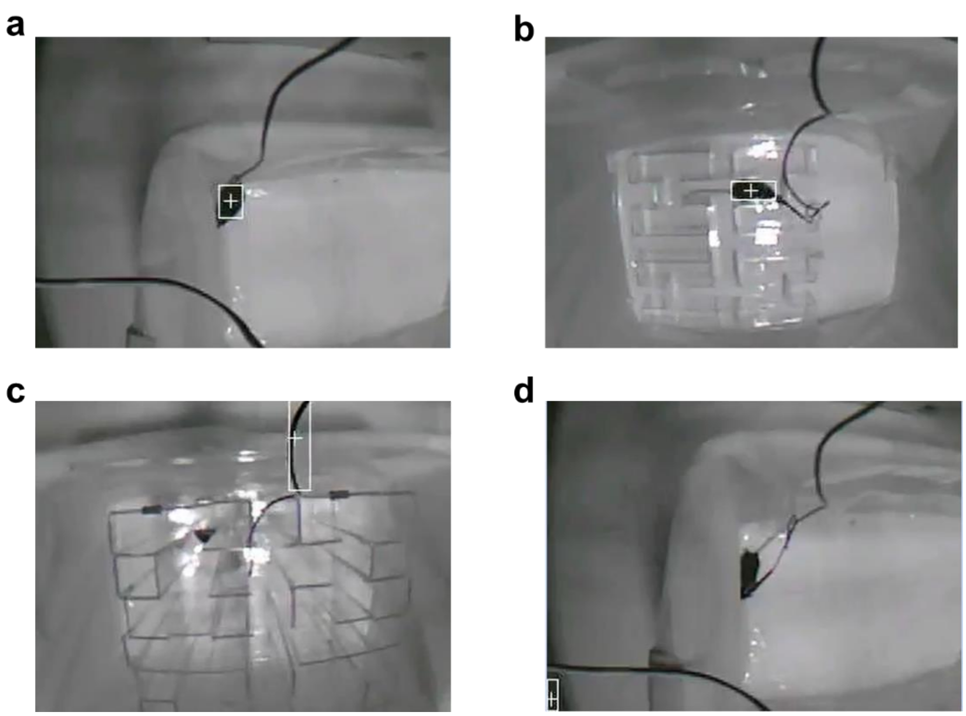
on tracking error in x (left) and y (right) directions. The target being tracked was a mouse moving in open arena. Ki=0 and Kd=0.05 produced the smallest error.

Coefficients that specify the weights of proportional, integral, and derivative terms (Kp, Ki, and Kd) were manually tuned for our tracking purposes. To tune the parameters, we used the smaller gantry to mimic a trajectory of a mouse exploring a maze from a previous study (Figure 2ab)[6] and let our device track its movement. We manually tested different parameters and selected the set that generated a small tracking error, as measured by the pixel differences between the centroid of the mouse and the center of the frame (Figure 2c). An example of the device tracking the small gantry is shown in Supplementary Video 1[2]. We further tuned the parameters on a freely moving mouse in an open arena (Figure 2d). The final parameters were: Kp=1.2, Ki=0, Kd=0.05. Users should tune their own set of parameters to meet specific needs, but these parameters worked well for a wild-type mouse implanted with a miniscope.

Performance

Our device performed well in easy tasks such as carrying a cable connected to a miniscope on a freely moving animal in open arena (Figure 3a) or maze with short walls (Figure 3b). It did not perform well in a maze with tall walls (Figure 3c), as the animal could easily disappear behind the walls and interfere with the tracking. It also tended not to perform well when there was a spot in the frame that was as dark as the animal (Figure 3d), however these issues can easily be addressed with better lighting and vignetting correction.

We also investigated the possibility of letting the camera track an IR LED instead of the animal itself. However, in a maze with short walls, the LED light often generated reflections on the walls. Hence, we did not continue to pursue this option, but future users can try to reduce the reflection by using taller walls or making the light more concentrated.



[2] https://drive.google.com/file/d/1Rqu9ylXmgIdP8Yane__3_929mgeM9tXh/view?usp=sharing

Figure 3. Performance evaluation. (a) An example frame of our device tracking an animal wearing a miniscope under IR illumination (a) in open space under IR illumination. (b) in a maze with shorter walls. (c) in a maze with taller walls. A piece of cable was falsely identified as the mouse in this frame. (d) A dark corner was falsely identified as the mouse. This issue can be resolved by making the illumination more even in an experiment.

DISCUSSION

To study brain activities underlying complex or natural behavior, it is imperative to record in freely moving animals[4]. Here, we presented a device which moves the recording setup cable with the animal, minimizing tug on the animal and allowing for natural movement in a large, complex environment. To our knowledge, there does not exist a cheap functional version of this that is easy to put together.

Several future directions can be taken to further improve our system. First, instead of placing the camera above for maze with tall walls, we could instead track from below as the animal walks on a transparent table using a static camera and send command to the gantry as an open-loop control system. One notable challenge is that pictures taken by the OpenMV cameras are distorted due to the nature of the optics. We need to either correct for the distortion in the images using OpenMV's distortion correction function or learn how pixel distances are mapping differently to gantry coordinates between the center and peripheral of the frame. Second, we could further investigate the option of tracking the animal carrying an IR LED. In addition, tracking via a thermal sensor is also worth trying. OpenMV has products that support thermal tracking.

With our device, complex animal behavior can be studied with simultaneous camera and physiological recordings, for example ethologically realistic hunting bouts[7] and exploration in complex maze environment[6]. Besides carrying recording setup, our system can also be used to deliver certain stimuli based on the animal's position. For example, the gantry can be programmed to mimic a prey or a predator whose decision depends on the animal's movement. It can also be used to modify the environment in real-time, for example, by changing the position of visual or olfactory landmarks based on the experiences of individual animals in the environment.

The prospect of creating a robust, closed-loop control system for tracking animals in real time during physiological recording and natural behavior is likely an intimidating one for many researchers. We have found that this engineering challenge is surprisingly achievable with inexpensive parts. The fact that we were able to set up closed loop PID control in two different gantry models speaks to the generalizability of this closed-loop strategy in the modern DIY robotics ecosystem. Closed loop tracking systems can seem extremely complex, but we have narrowed the requisite components down to three things: a Grbl-powered gantry, and OpenMV camera, and an exposed serial port. We have made our setup and code[3] available to the community and users are encouraged to modify our system and expand its utility.

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[3] https://github.com/zzhong413/mouse-tracker

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