The Crunchometer: A Low-Cost, Open-Source Acoustic Analysis of Feeding Microstructure

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Abstract

Elucidating the neuronal circuits that govern appetite requires a detailed analysis of the microstructure of solid food consumption. A significant barrier in this field is that existing techniques for monitoring feeding are either prohibitively expensive, limiting their use, or lack the high temporal resolution necessary to align feeding events with neuronal activity. To overcome these limitations and democratize this technology, we developed the Crunchometer, an open-source sound-based system that utilizes a cost-effective condenser microphone and video recordings, along with two mathematical methods (supervised thresholding and an unsupervised Support Vector Machine, SVM), to precisely capture bite sounds with unprecedented temporal resolution and built a feeding ethogram for both Chow and High-Fat Diet (HFD) pellets. While human observers tend to estimate longer, more frequent feeding bouts, our automated methods more precisely and reliably detect the start and end times of most feeding bouts. In consequence, both automated methods fragmented feeding bouts more frequently than human observers. We demonstrate the Crunchometer's utility by monitoring feeding patterns across different energy states, hunger (fasted), and satiety, and by characterizing the pharmacological effects of the anti-obesity drug semaglutide. This revealed that semaglutide not only suppresses solid food intake but also decreases the preference for HFD pellets. Additionally, we differentiate the genetically-evoked gnawing from consummatory feeding behaviors involving GABAergic neurons in the Lateral Hypothalamus (LH). Importantly, the Crunchometer integrates seamlessly with in vivo electrophysiology and calcium imaging in freely behaving mice. The Crunchometer, paired with recordings of LH activity, revealed the presence of meal-related neurons that monitor the entire meal, rather than just individual feeding bouts. Microendoscopes and calcium imaging on Vgat-IRES-cre and Vglut2-IRES-cre mice further suggest that GABA neurons in LH (but not glutamatergic neurons) are also strongly modulated by eating a solid food pellet. Notably, the acts of licking liquid sucrose and eating solid food activate distinct sets of neurons. Its compatibility with electrophysiology and calcium imaging makes the Crunchometer an ideal tool for elucidating the neuronal correlates of feeding behavior for solid foods.

Introduction

For decades, neuroscience research into appetite, taste, and reward has heavily relied on liquid food models, often reducing the study of ingestive behavior to simple licking measurements (Davis, 1989; Davis and Smith, 1992). The reasons for this are largely practical: liquids are easy to deliver with precision in experimental settings, such as fMRI scanners (Smeets et al., 2019), in humans, and are well-suited for basic research on rodent studies (Gutierrez et al., 2006; Tellez et al., 2012). This methodological convenience, however, has fostered a narrow view of eating, oversimplifying the complex sensory and motor challenges unique to solid food. In rodents, eating solid food is a fundamentally different neurobiological event than sipping a liquid. The process begins with motor actions, such as biting and mastication (chewing), which in turn trigger a complex cascade of trigeminal (touch and texture) and gustatory (taste) stimulation. This is not a fixed mechanical action, but rather a dynamic, fine-grained sensory-motor feedback loop (Jacquin and Zeigler, 1983). Within this loop, sensory properties such as taste, texture, temperature, and hardness generate a rich stream of information known as “mouthfeel,” which is continuously relayed to the brain to influence eating rate, bite size, and ultimately, satiety (Gutierrez and Simon, 2021). Despite these critical differences, neuroscience has scarcely explored the neuronal correlates of the microstructure of solid food, focusing on liquid diets, operating under the largely untested assumption that both food forms recruit the same neuronal circuits, see (Pilato et al., 2024; Yamamoto et al., 1989), for exceptions. This lack of knowledge is mainly due to the high cost of current technologies and limitations of traditional techniques for monitoring food intake in laboratory animals.

Current methods to monitor feeding behavior could be classified into four different classes: 1) *Manual Weighing*: The most traditional and straightforward method involves manually weighing the food hopper or a dish of food at regular intervals. 2) *Automated Weighing Systems and Home Cage Monitoring Systems (Smart Cages)*: These systems build upon the engineering prowess of Curt Richter. In 1922, by continuously monitoring the food intake and activity of rats, Richter discovered the homeostatic functions of ingestive behavior (Richter, 1922). This equipment provides continuous monitoring through automated weighing scales, utilizing load cells or other methods, such as Snacker Tracker (Mueller et al., 2025). Home cage monitoring systems are the current state-of-the-art for tracking feeding behavior in a minimally

invasive and ethologically relevant environment. Commercial systems, such as the BioDAQ (Research Diets) (Farley et al., 2003; Yang et al., 2025), OxyletProTM System (PANLAB, Cornellà, Spain) (Mariné-Casadó et al., 2018), Oxymax CLAMS-HC (Columbus Instruments), Promethion metabolic cage system (Sable Systems International, Las Vegas, NV, USA) (Ye et al., 2024), TSE PhenoMaster/IntelliCage system (TSE Systems, Bad Homburg, Germany) (Bake et al., 2014), PhenoTyper (Noldus IT) (Acosta-Rodríguez et al., 2017; Robinson and Riedel, 2014), employ sophisticated (and costly) automated weighing scales. These systems achieve high temporal resolution, enabling the analysis of meal patterns defined by bouts of grams consumed (Farley et al., 2003). The IntelliCage system excels in simultaneously monitoring the feeding behavior of multiple mice. But it is not compatible with recordings. Still, they often find it challenging to precisely differentiate actual consumption from spillage or other feeding-related behaviors, such as biting, gnawing, licking, and general locomotor activity. 3) *Automated Pellet Dispensers*: Often integrated into operant conditioning chambers, these devices provide a controlled way of delivering food pellets. While devices like the open-source Feeding Experimentation Device (FED3) (Ali and Kravitz, 2018; Matikainen-Ankney et al., 2021), a pellet dispenser, are useful for measuring reinforcement, they alter the natural feeding patterns of mice, for example requiring a simple action such as a nose-poke can reduce overeating and weight gain in mice (Barrett et al., 2025). A significant strength of this method is its capacity to facilitate closed-loop optogenetic stimulation concurrently with neuronal recordings. 4) *Video-Based Analysis*: While often used in conjunction with other methods, video analysis can provide unique insights into feeding behavior, but requires large video storage capacity and computer power, and frequently fails to distinguish crossing from a food zone from actually eating (Jennings et al., 2015). Moreover, direct human observation is labor-intensive and inherently subjective, compromising data quality.

Audio-based methods have been largely unexplored and underutilized (Ali and Kravitz, 2018), as they have mainly focused on using expensive microphones to detect rodents' ultrasonic vocalizations (USVs) (Champeil-Potokar et al., 2023; Wardak et al., 2024), such as the 40 kHz vocalization associated with rat food consumption (Champeil-Potokar et al., 2023) or the 50 kHz vocalization linked to positive reinforcement (Wardak et al., 2024). Their inconsistent occurrence limits the utility of

USVs, as they are not observed with every bite or in all subjects. Beyond these technical limitations, behavioral complexities further hinder accurate quantification. A further complication arises from the common hoarding behavior of mice. This practice of transporting food to other locations presents a considerable challenge to the precise assessment of spillage and the quantification of what is consumed. Consequently, most research has relied on liquid diets (e.g., sucrose or Ensure), where lickometers allow for precise and easy measurement of licking microstructure (Gutierrez et al., 2006; Spector et al., 1998; Tellez et al., 2012; Zhu et al., 2025). In contrast, the microstructure of eating solid food and its neuronal correlates remain poorly understood (Décarie-Spain et al., 2025), primarily due to technical limitations that impede detailed analysis of bite count and food chewing in mice (Stuber et al., 2025). To overcome these challenges, we developed the "Crunchometer," a novel, cost-effective acoustic system for monitoring feeding. Unlike methods that rely on expensive microphones for USV detection, the Crunchometer utilizes an economical condenser microphone (Fifine K669 Amplitank, priced under $63) to analyze the temporal dynamics of feeding behavior by extracting and identifying bite sounds. This will enable scientists to create detailed feeding ethograms, thereby establishing an acoustic-based method to investigate the microstructure of solid food intake. This study introduces and validates the Crunchometer, a novel, cost-effective acoustic system that precisely analyzes the microstructure of solid food intake in mice. We demonstrate its utility by comparing automated bite detection with human observation, monitoring feeding patterns in various physiological states (hunger/satiety), characterizing pharmacological effects (semaglutide), and differentiating distinct feeding-related behaviors such as gnawing (i.e., biting an inedible object) vs. consumption induced via chemogenetics (Roth, 2016). Furthermore, we highlight its seamless integration with *in vivo* multichannel electrophysiology and microendoscope calcium imaging to elucidate the neural correlates of solid food consumption.

Results

The Crunchometer: An Open-Source Sound-Based Method for Studying the Microstructure of Feeding Behavior

The setup of the Crunchometer is shown in **Figure 1A,** and an exploded view of its components is presented in **Figure 1B**. The Crunchometer consists of an acrylic box (1) with 11 holes drilled on one lateral wall (see Inset **Figure 1A**). During each session, two food pellets—one HFD (2) and one standard Chow (3) —were placed on a wall near the condenser microphone (4). A bottom-view camera (5) continuously records the mice. Additionally, a 10% sucrose solution was dispensed to ensure mice had *ad libitum* access to both food and liquid. A Med Associates contact lickometer (6) could measure each lick and dispensing a 1 µL drop of sucrose per lick (7). The video was recorded using Open Broadcaster Software (OBS) (OBS Project, 2025) in the .mkv format.

Before the feeding sessions, mice were habituated to the behavioral box over two consecutive days with 20-minute sessions to reduce stress. During the feeding session, audio (ogg) and video (.mkv) recordings were obtained. Potential sound bite events were identified by detecting the amplitude of sound frequencies between 500 and 950 Hz (**Figure 1C**). The power spectrum was averaged within this frequency band to isolate a potential sound bite. The audio snippets with frequencies within this interval were binarized: audio frames identified as putative bites were labeled “1,” while non-bite frames were labeled “0”. To improve bite detection accuracy, we calculated the mean power within the frequency band and applied a fixed threshold set at -85 dB (**Figure 1D**). Although the optimal dB threshold can vary across setups, we utilized a fixed threshold calibrated for each one. This approach was chosen because, unlike dynamic thresholds (e.g., z-score and 3 standard deviations above average noise), a fixed threshold more accurately detects when a mouse is not biting at all, such as during a state of satiety. Using these binary-labeled sound bites, we extracted the corresponding video frames (termed “video snippets”) for detecting feeding bouts. Given that microphones more reliably detect the abrupt noise of a broken pellet (i.e., a bite) but do not consistently capture chewing sounds, we opted to define feeding bouts in a way that incorporates chewing times as much as possible. Accordingly, feeding bouts were defined as sequences containing one or more bites with Inter-Bout-Intervals (IBIs) of less than 5 seconds. Pauses longer than 5 seconds marked the start of a new feeding bout (**Figure 1E**). Using the onset and offset times of these video

snippets, we then automatically sorted them into different behaviors: Artifact, Chow, HFD, and Gnawing (**Figure 1F**), based on the motion energy of two ROIs drawn over the pellets, which essentially indicated whether the mice crossed any of the ROIs. The final and most crucial step in our classification pipeline was human validation. A human observer supervised the sorting of all video snippets. In the event of a misclassification, snippets can be manually moved to the correct folder, and the corresponding matrix of trials (containing the onset and offsets of each bite) can be relabeled (using the homemade Matlab script `sorted_snippets`). Common errors included misclassifications that occurred, for example, when the mouse's tail touched a pellet, or, more frequently, when the mouse engaged in gnawing behavior on a non-food object, such as the plastic lid. This human validation was essential for ensuring the high fidelity of our behavioral database and mitigating the inherent limitations of automated classification. Finally, an ethogram was constructed to visualize the temporal distribution of these behaviors throughout the session. This was achieved using both supervised thresholding and an unsupervised Support Vector Machine (SVM) approach (**Figure 1G**). The Crunchometer successfully captured individual sound bites from mice, allowing us to construct detailed feeding ethograms for Chow, HFD pellets, and gnawing behavior. In summary, the Crunchometer enables the systematic detection of individual bites, providing a robust platform for monitoring complex feeding dynamics.

The Crunchometer

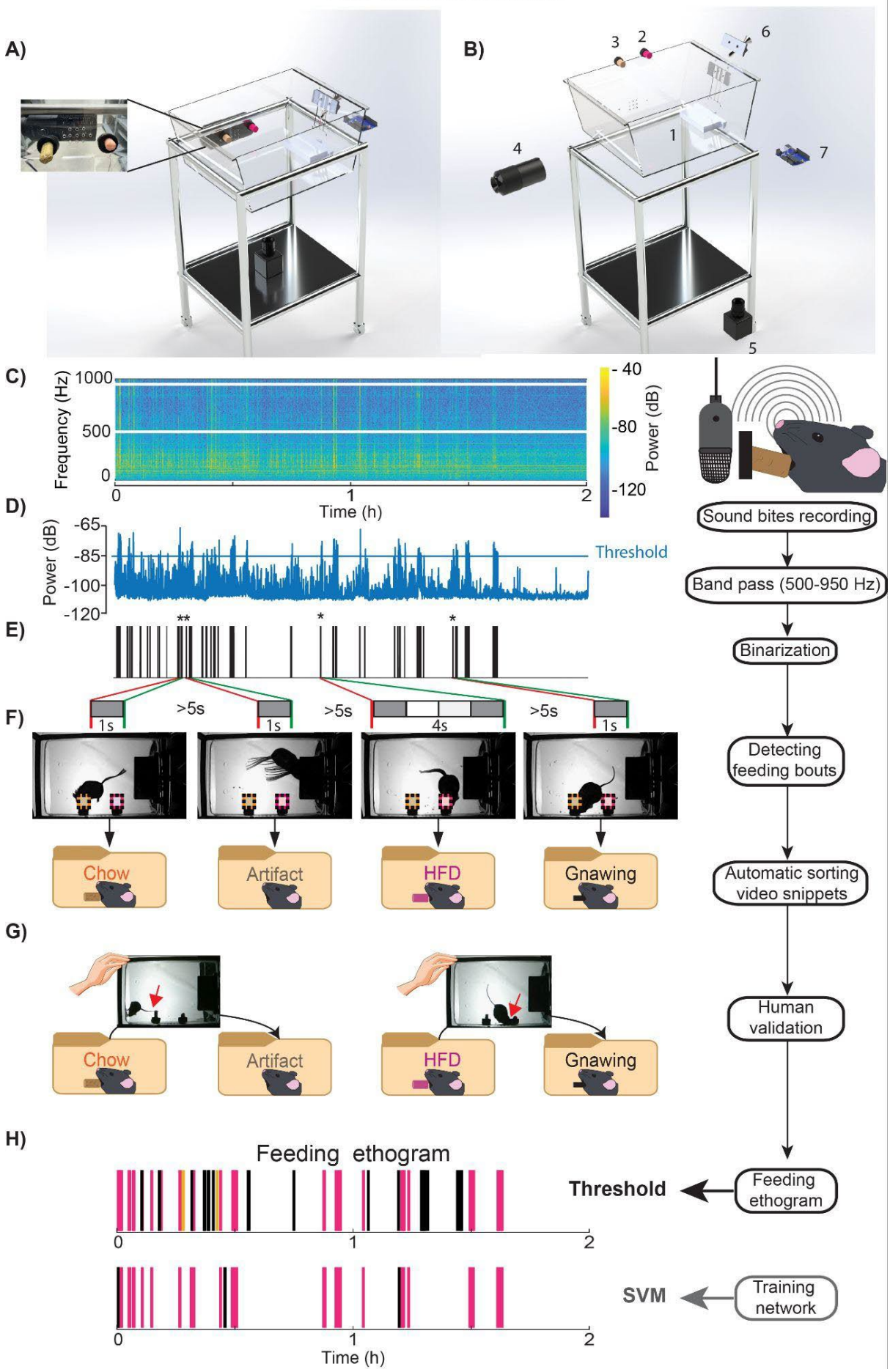

Figure 1. The Crunchometer System and Workflow for Acoustic Feeding Analysis.
A) Schematic of the Crunchometer setup for behavioral feeding studies. This diagram illustrates the system's integrated components for the acquisition and analysis of acoustic feeding data in mice. The inset details the microphone's optimized placement, within the outer wall of the 11-hole box, and the precise locations of the food pellets. **B)** Exploded view of Crunchometer components. The acrylic box (1) in which a High-fat diet (HFD) (2) and Chow (3) pellets were positioned on the inner wall of the behavioral box (See **Video 1** for pellet preparation), at the same height as the condenser microphone (4). The microphone is mounted on the adjacent outer wall. A bottom-view camera (5) is installed beneath the box to record mouse locomotor activity (an optional top-view camera is not shown). A contact lickometer (6), controlled by an Arduino device or MedAssociate system (7), provides 1 µL of 10% sucrose per lick. **C)** Sound recording and processing workflow. Audio was sampled at 44.1 kHz, and video was recorded at 30 frames per second (fps). The spectrogram (1 s resolution) is also shown with the color bar indicating the power in dB. **D)** Power spectrum filtering. The power spectrum of the recorded audio was averaged between the 500 and 950 Hz bands to isolate potential bite sounds. The average dB in this band is plotted. A fixed threshold at -85 dB (found by trial and error) was used for our setup. Every 1 s bin exceeding the threshold was assigned a value of 1; otherwise, it was assigned a value of 0. **E)** Identification of bite frames and feeding bouts. Black lines indicate putative bites, obtained through signal binarization and labeled as bite frames. Asterisks mark representative examples of feeding bouts, with red lines indicating bout onset and green lines indicating bout offset. Gray boxes represent 1-second time bins. Each feeding bout consists of one or more bites, separated by pauses of less than 5 seconds. Feeding bouts were detected within two defined Regions of Interest (ROIs) on the video recording, where the area corresponding to each pellet was outlined (yellow box for Chow, pink box for HFD). **F)** After identifying feeding bouts, the software automatically classified each video snippet by sorting them into four folders: “Chow,” “HFD,” “Gnawing,” or “Artifact.” The primary classification was determined by motion energy within two regions of interest (ROIs) drawn over the food: a yellow square for the Chow pellet and a pink square for the HFD pellet. For example, if motion energy was greater in the Chow ROI, the snippet was classified as 'Chow.' Snippets were labeled 'Gnawing' if visual inspection showed mouth movements without food consumption, such as biting the plastic cap that holds the pellet (or any other non-edible object). Finally, a snippet was labeled as ‘Artifact' if a noise occurred while the mouse was outside both food ROIs. **G)** Human validation is a critical step to correcting automated classification errors. For example, the system may misclassify a snippet as "feeding" simply because the mouse is positioned within a feeding region of interest (ROI), even if it isn't eating. The examples highlighted by the red arrows show such cases. During the manual review, a user corrects these errors by moving the misclassified snippets from the diet folders (Chow or HFD) to their proper category, such as "artifact" or "gnawing." **H)** The final ethogram displays feeding bouts identified by two methods: supervised thresholding and a Support Vector Machine (SVM). In the graph, colored lines represent feeding on Chow pellets (yellow), HFD pellets (pink), and gnawing behavior (black). While the SVM method is more efficient at distinguishing bite-like sounds and less prone to artifacts, this precision comes at the cost of underestimating gnawing behavior. In **Supplementary Figure 1-1**, the bill of materials is provided.

The Crunchometer was more precise and reliable than humans in detecting the start and end of a feeding bout

After defining the Crunchometer's signal processing, we evaluated our sound-based feeding bout detection method by comparing its performance with that of human observers to identify key differences. We analyzed the feeding behavior of six mice using the Crunchometer. Separately, seven human observers independently annotated feeding bouts using the open-source software BORIS (Behavioral Observation Research Interactive Software) (Friard and Gamba, 2016). Both the Crunchometer and human observers consistently detected feeding bouts (**Figure 2A**, top panel). However, some feeding bouts were more challenging to detect and were only identified by the Crunchometer or by human observers (see black arrows, **Figure 2A**, bottom panel). We quantified the agreement between human observers and the Crunchometer using Normalized Mutual Information (NMI). This metric, which ranges from 0 (no overlap) to 1 (100% similarity), provides a measure of their shared information. Initially, four of the seven human observers (Humans 2, 3, 4, 6; pairs human 3 vs. 6 (NMI = 0.53), 3 vs. 4 (NMI = 0.55), 2 vs. 4 (NMI = 0.61), and 2 vs. 3 (NMI = 0.64)) achieved higher agreement NMI values than the remaining three humans (7, 5, and 1). This variability within humans likely reflects limitations in attention span, reaction times, or fatigue, potentially leading to delayed or missed detection of feeding bouts (**Figure 2B**). Overall, the within-human agreement achieved a mean ± standard deviation of NMI = 0.38 ± 0.14. In contrast, both the Threshold and SVM methods exhibited higher similarity between them in detecting feeding bouts (**Figure 2B**; NMI = 0.58). However, the similarity between human and Crunchometer was consistently lower compared to within-group comparisons (**Figure 2B**, blue rectangle; max = 0.36, mean ± std = 0.27 ± 0.05), highlighting notable differences in how the Crunchometer and human observers identified feeding bouts.

To further explore the differences between humans and the Crunchometer, we examined the overlap in feeding bout detections across sessions. The SVM method demonstrated greater overlaps with the Threshold method (an overlap probability above 0.8, indicating a likelihood of at least one or more bins overlapping) than with human observers (**Figure 2C**, top panel). This means that SVM frequently identified the same moments as eating times as those of the Threshold. Humans also agree with the feeding bout intervals detected by the Threshold method (overlap probability humans vs Threshold around 0.7). In contrast, both Crunchometer methods exhibited less overlap when compared to human observers (**Figure 2C**, bottom panel, with an

overlap probability of less than 0.5 for both methods). This result indicates that the SVM and Threshold automated methods for detecting feeding bouts are more similar to each other compared to human observation.

We quantified the response times for detecting the bout onset and offset by both the SVM method and human observers relative to the detection of the Threshold method (Time = 0 s). Human observers exhibited longer delays in identifying the feeding bouts (average response relative to Start: -8.19 s and End: +10.46 s) compared to the SVM method (Start: -3.36 s and End: +3.01 s; **Figure 2D**). Consequently, human observers exhibited lower precision in detecting both the onset and offset of feeding bouts when compared to the more reliable performance of the Threshold and SVM methods.

Next, we quantified the number and size of bouts detected by Crunchometer methods compared to those detected by humans. The Threshold method detected a similar number of bouts as human observers, but these bouts had shorter durations (**Figure 2E**, left panels). In contrast, the SVM method identified fewer bouts with intermediate durations (**Figure 2E**, right panels). Overall, human observers detected more and longer feeding bouts than both Crunchometer methods. This was because the Threshold method tended to fragment feeding events, while the SVM method captured fewer bouts (**Figure 2F**).

We finally examined whether the number of detected bouts could predict the actual food intake (in grams) of the mice during the 2-hour feeding session. Initially, we observed a low Pearson coefficient of determination across all conditions between the number of feeding bouts and total intake (**Figure 2G**, top panel; see R^2 in green) or between bout size and total intake (**Figure 2G**, bottom panel; see R^2 in green). This lack of correlation stemmed from one subject (mouse 5), which consumed approximately 0.8 g but exhibited very few bites of short duration, suggesting a highly efficient bite pattern. Notably, the detection of feeding behavior in this specific mouse presented difficulties for both the Crunchometer and human observers. Consequently, after excluding this mouse, we recalculated the correlations and found that the best predictor for food intake depended on the method used. When using the number of feeding bouts (**Figure 2G**, top panel), the Threshold method showed the strongest correlation with food intake ($R^2 = 0.53$, dashed black line), surpassing both the SVM method ($R^2 = 0.46$) and human observations ($R^2 = 0.31$). However, when using the

total feeding time as the predictor (**Figure 2G**, bottom panel), human observers were most effective, achieving the highest correlation (R^2 = 0.61) compared to both the Threshold and SVM methods (R^2 = 0.43 for both). This highlights the fundamental distinction in detecting when a mouse eats: the Crunchometer excels at detecting discrete, sound-producing bites, making it a precise event counter. In contrast, by integrating both auditory and visual cues, like quiet chewing and eating spilled crumbs, humans are better able to estimate total food intake over a continuous feeding period.

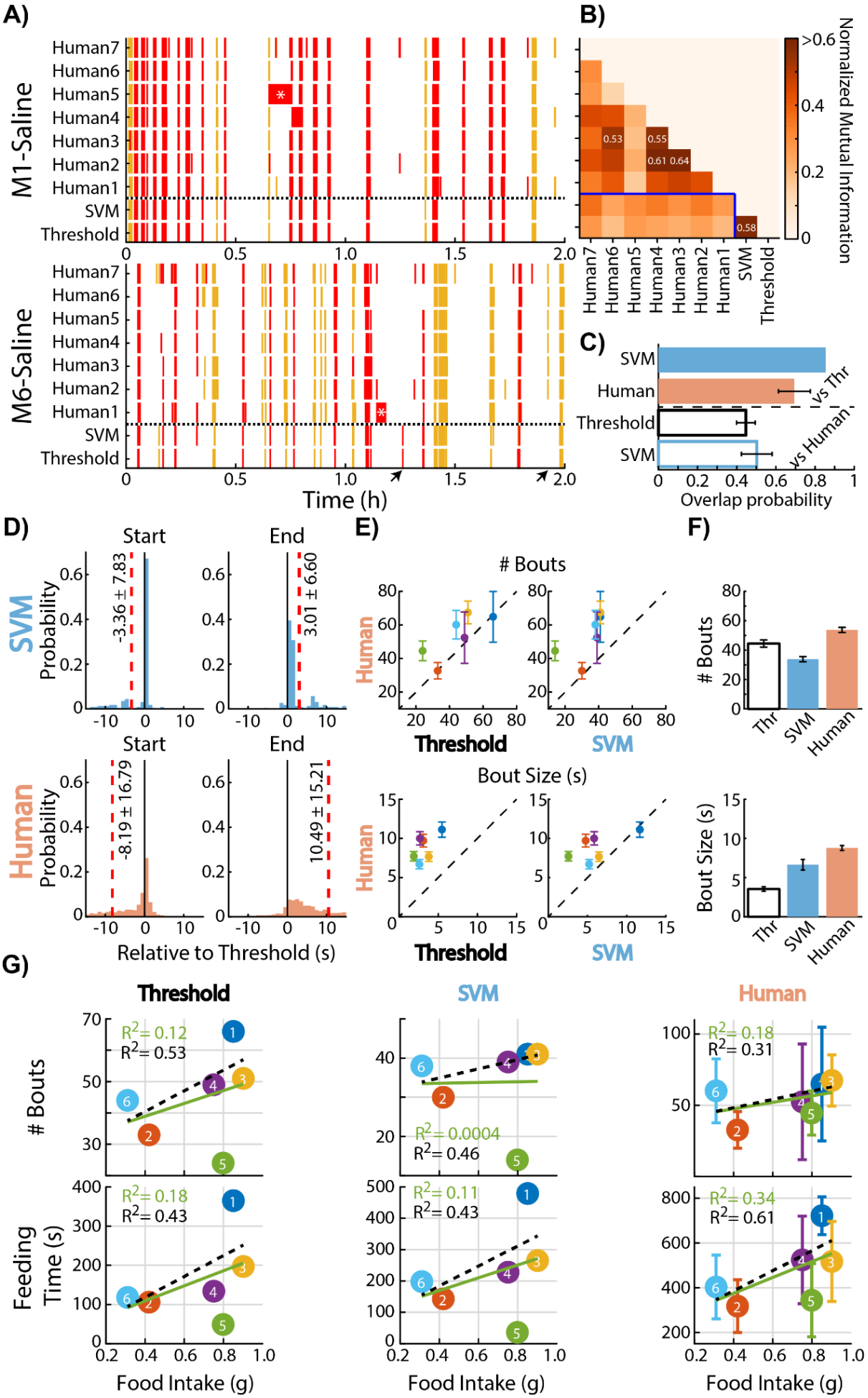
A)
M1-Saline
Human7
Human6
Human5
Human4
Human3
Human2
Human1
SVM
Threshold
M6-Saline
Time (h)
B)
Normalized Mutual Information
>0.6
0.4
0.2
0
0.53
0.55
0.61
0.64
0.58
C)
SVM
Human
Threshold
SVM
vs Thr
vs Human
Overlap probability
D)
Start
End
SVM
Probability
-3.36 ± 7.83
3.01 ± 6.60
Human
-8.19 ± 16.79
10.49 ± 15.21
Relative to Threshold (s)
E)
Bouts
Bout Size (s)
Threshold
SVM
Human
F)
Bouts
Bout Size (s)
Thr
SVM
Human
G)
Threshold
SVM
Human
Bouts
Feeding Time (s)
R2= 0.12
R2= 0.53
R2= 0.18
R2= 0.43
R2= 0.0004
R2= 0.46
R2= 0.11
R2= 0.43
R2= 0.18
R2= 0.31
R2= 0.34
R2= 0.61
Food Intake (g)

Figure 2. The Threshold and SVM methods were more precise and reliable than humans in detecting the start and end of a feeding bout; however, these methods tend to fragment feeding bouts more than humans do, as they estimate more events and more extended feeding periods. A) Representative ethograms illustrate the feeding behavior of two fasted mice. Red and yellow marks denote feeding bouts related to HFD and Chow pellets, respectively. A horizontal dashed black line separates human annotations from method-detected feeding bouts. The experiment on the top panel (M1-Saline) demonstrates greater consistency between human observations and Crunchometer detection, whereas the black arrows in the bottom panel (M6-Saline) highlight two feeding bouts that were uniquely detected by either mathematical methods or by human observers. Also, the asterisk (*) indicates periods in which a human failed to detect a feeding bout correctly. **B)** To evaluate the similarity between the automated methods and human detections, Normalized Mutual Information (NMI) was used, with varying shades of orange indicating different NMI values. While both human observers and the methods showed high within-group correlation, there was notably reduced similarity between human and method detections (highlighted by the blue rectangle). **C)** To assess the overlap probability between the Crunchometer methods and human observers, we first concatenated all feeding bouts detected across the six experiments. Subsequently, we quantified the total number of feeding bouts identified by human observers and by each Crunchometer method. Solid bars represent the overlap probability that an SVM and seven human observers agreed on the detection of a single feeding bout, relative to the 267 total bouts detected by the Threshold method across all six mice. Empty bars display the overlap probability between Threshold and SVM detections relative to each of the seven human observers (with total feeding bouts detected by each observer being 251, 286, 228, 248, 583, 215, and 444 for human 1 through 7, respectively). Horizontal black lines indicate the mean ± SEM. **D)** Time delay in detections by SVM and Human observers relative to Threshold-based onsets. The probability distribution of detection times by the SVM (top panels) and Human observers (bottom panels) is exhibited relative to the start (left panels) and end (right panels) of Threshold-based detections. The solid black line indicates the Threshold onset, while the red dashed line depicts the mean detection delay. Delay values are presented as mean ± std. **E)** Scatter plots illustrate the number and size of feeding bouts detected by human observers and the Crunchometer methods. Points falling on the diagonal dashed line indicate similar detection rates between human and automated methods. Deviations below the line (lower right) suggest greater detection by the Crunchometer methods, while deviations above the line (upper left) signify more detections by human observers. The left panels compare human detections to the threshold-based method, and the right panels present comparisons with the SVM-based method. Errors indicate the mean ± SEM. **F)** Total number (top) and size (bottom) of feeding bouts detected by human observers and the Crunchometer methods. Bars represent the mean for each detection source, with error bars indicating the mean ± SEM. **G)** Pearson coefficient of determination, R-squared, of mouse intake with bout size and bout number from automated methods and human observers. These scatter plots display the correlation between food intake and either feeding size or the number of bouts across all experiments. The black dashed line represents the linear regression excluding one subject (mouse number 5), whereas the green solid line indicates the regression including all subjects. For human detections, error bars are the mean ± SEM.

The Crunchometer System Distinguishes Meal Patterns of Fed and Fasted Mice

We initially validated our novel food intake monitoring system, the "Crunchometer," by measuring consumption in six naive wild-type mice under both fed and 18-hour fasted conditions. Behavioral observations were conducted over a two-hour timeframe, a duration sufficient for the expression of the entire behavioral satiety sequence following feeding (Halford et al., 1998a; Tejas-Juárez et al., 2014). A visual inspection of the feeding ethograms revealed that fasted mice exhibited a greater number of feeding bouts compared to their sated counterparts (**Figure 3A**). Notably, one mouse (number 5) displayed robust gnawing behavior, indicated by black ticks in **Figure 3A**, specifically while in a fasted state. Supporting these observations, the cumulative feeding time was significantly higher in the fasted group, nearly doubling that of the fed group (Two-sample Kolmogorov-Smirnov test: D = 0.92500, $p < 0.0001$; **Figure 3A**, bottom panel). As predicted by the behavioral satiety sequence (Halford et al., 1998b), fasted mice initially exhibited short IBIs that progressively lengthened as they approached satiety. This pattern is illustrated in the upper panel of **Figure 3B**, which plots the average IBI in 10-minute increments. We observed a significant increase in IBI time bin at 1.16 h (Mann–Whitney U test: U = 2, $p = 0.0317$). Likewise, the feeding rate increased significantly within the first 10 minutes of the session (two-way ANOVA, time effect: $F_{(11,110)} = 2.663$, $p = 0.0046$, physiological state effect: $F_{(1,10)} = 4.293$, $p = 0.0651$, and interaction: $F_{(11,110)} = 3.474$, $p = 0.0003$, *post hoc* analysis: time bin = 0.17 h $p < 0.0001$; time bin = 0.58 h $p = 0.0349$; **Figure 3B**, lower panel). Finally, we found a significant correlation between the total grams of food consumed and total feeding time for both fed and fasted mice (**Figure 3B**, right panel). This positive correlation indicates that longer feeding durations were associated with greater food intake.

The Crunchometer could also quantify various behaviors related to the microstructure of feeding. These metrics include not only total food consumption (in grams) and feeding time (in seconds), but also the number and size of feeding bouts, latency to the first bite, and the IBI. As expected, total food intake was significantly different between the groups: fasted mice consumed more grams of food than fed mice (Mann–Whitney U test: U = 2, $p = 0.0043$). Likewise, compared to fed mice, fasted mice exhibited a significant increase
in total feeding time (Mann–Whitney U test: U = 7, $p = 0.0465$; **Figure 3C**) and in the number of feeding bouts (Mann–Whitney U test: U = 4, $p = 0.0130$; **Figure 3C**). However, the average bout size was not significantly different between the groups

(Mann–Whitney U test: U = 12, p = 0.1970; **Figure 3C**). The latency to the first bite was significantly shorter in the fasted group, suggesting an increased motivation to eat (Mann–Whitney U test: U = 0, p = 0.0011; **Figure 3C**). Similarly, the IBIs across the entire session were significantly shorter in fasted mice than in fed mice (Mann–Whitney U test: U = 3, p = 0.0152; **Figure 3C**). Taken together, these results demonstrate that the Crunchometer can distinguish between the physiological energy states of hunger and satiety. These states were differentiated primarily by the overall feeding pattern (total feeding time), an increased initial feeding rate, and a higher number of bouts separated by short IBIs. In simple terms, hungry mice eat more frequently, especially at the beginning of the session.

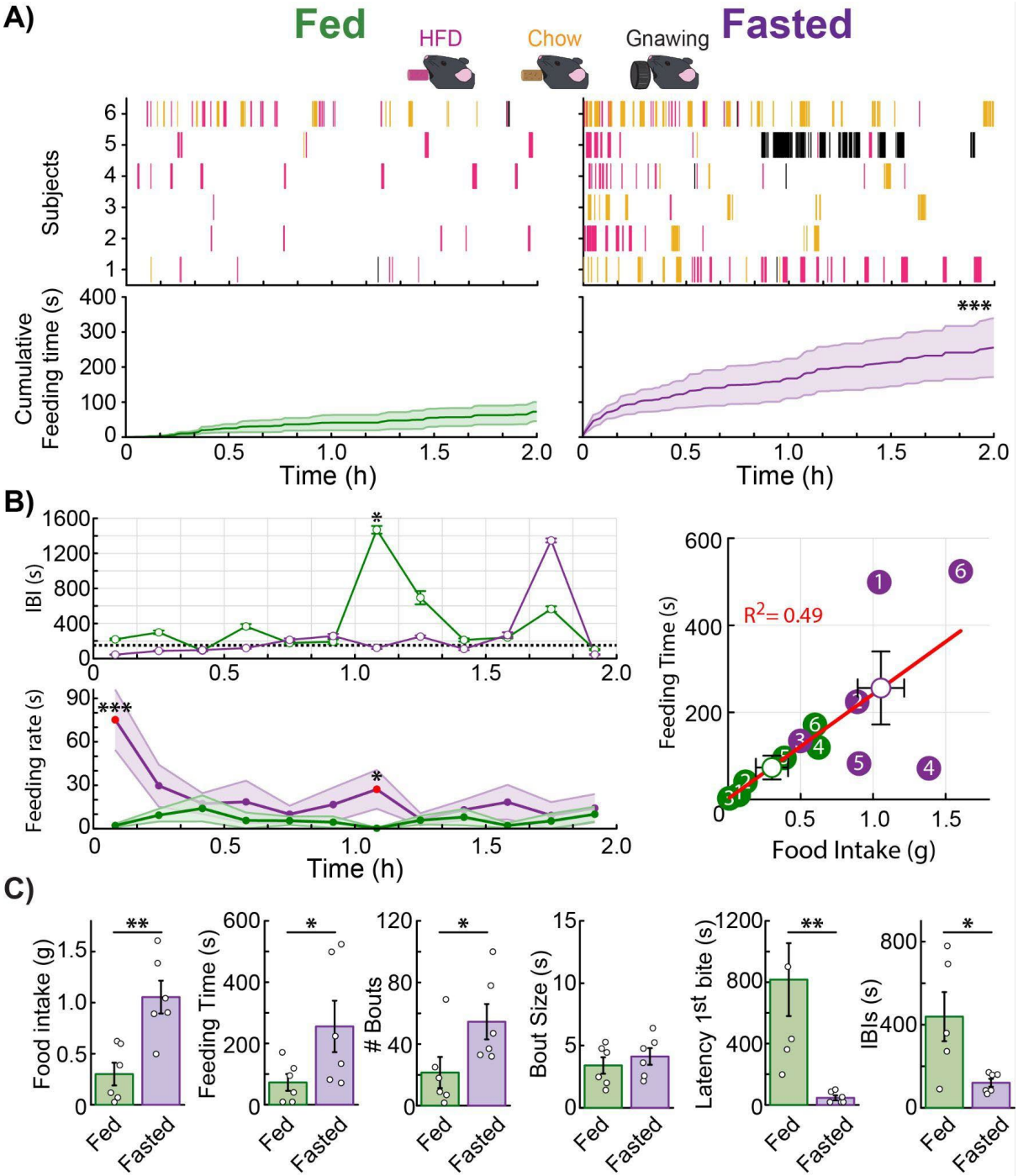

Figure 3. The Crunchometer captures detailed differences in feeding microstructure between satiated and fasted states. A) Representative feeding ethograms from a satiated (Fed) and a food-deprived (Fasted) mouse. Lines indicate bouts of HFD consumption (pink), standard Chow consumption (yellow), and non-edible gnawing (black). The lower panel shows the cumulative feeding time over a two-hour session for the fed (green) and fasted (purple) groups (n = 6 mice). Shaded areas indicate the standard error of the mean (± SEM). **B)** The top panel shows the mean Inter-Bout Interval (IBI) in 10-minute bins, illustrating the progression toward satiety. The bottom panel shows the corresponding feeding rate; red dots indicate a significant difference between fed and fasted states. The scatter plot (right) shows a significant correlation between total feeding time and food intake ($R^2 = 0.49$, $p = 0.011$). **C)** Quantification of feeding parameters, including total feeding time, number of bouts, bout size,

latency to first bite, and mean IBI. Data are expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Semaglutide Suppresses Feeding and Reduces Preference for a High-Fat Diet

To characterize the acute anorexigenic effects of semaglutide, a widely used GLP-1 receptor agonist (Huang et al., 2024; Kim et al., 2024; Knudsen and Lau, 2019; Teixidor-Deulofeu et al., 2025), we used the Crunchometer to analyze detailed feeding microstructure. Mice were administered saline or semaglutide via subcutaneous injection immediately before the start of the behavioral experiment. Under baseline conditions (fasted, saline-treated), mice exhibited robust feeding with a clear preference for the HFD over standard Chow (Two-sample Kolmogorov-Smirnov test: D = 0.91667, $p < 0.0001$) (**Figure 4A**). In sharp contrast, following the administration of semaglutide (0.123 mg/kg) (Zhang et al., 2023), the same fasted mice displayed markedly suppressed feeding. Surprisingly, they also lost their strong preference for the HFD pellet (**Figure 4B**). This reduction in feeding behavior persisted in a follow-up test 24 hours later, even when mice had *ad libitum* access to food (**Figure 4C**). semaglutide treatment led to a significant decrease in total food intake and a sharp reduction in preference for HFD (two-way ANOVA, treatment effect: $F_{(2,30)} = 26.88$, $p < 0.0001$, diet effect: $F_{(1,30)} = 16.74$, $p = 0.0003$ and interaction: $F_{(2,30)} = 23.53$, $p = < 0.0001$; *post hoc* analysis: Ctrl Chow vs. Ctrl HFD, $p < 0.0001$; Sem Chow vs Sem HFD, $p = 0.8731$; Post sem Chow vs Post sem HFD p = 0.4857; **Figure 4D**). Likewise, mice spent significantly less feeding time under semaglutide treatment, and the next day, followed up test (two-way ANOVA, treatment effect: $F_{(2,30)} = 16.96$ $p < 0.0001$, diet effect: $F_{(1,30)} = 0.7130$, $p = 0.4051$ and interaction: $F_{(2,30)} = 10.85$, $p = 0.0003$; *post hoc* analysis: Ctrl vs. Sem, p = 0.0028; Ctrl vs Post sem $p = 0.0019$; **Figure 4D,** right panel). Quantitative analysis of the feeding microstructure confirmed and extended these observations: Acute semaglutide administration significantly reduced both the number of feeding bouts (two-way ANOVA, treatment effect: $F_{(2,30)} = 16.96$, $p < 0.0001$, diet effect: $F_{(1,30)} = 0.7130$, $p = 0.4051$ and interaction: $F_{(2,30)} = 10.85$, $p = 0.0003$; *post hoc* analysis: Ctrl vs Sem $p < 0.0001$, Ctrl vs Post sem $p < 0.0001$; **Figure 4D**) and the size of each bout compared to saline-treated controls but only in the Post sem day (one-way ANOVA, $F_{(2,15)} = 4.394$, $p = 0.0315$; *post hoc* analysis: Ctrl vs Post sem $p = 0.0097$; **Figure 4E**). No significant difference was found in latency to the first bout.

Consistent with the induction of satiety, the IBIs were significantly longer in mice treated with semaglutide (one-way ANOVA, $F_{(2,15)} = 5.929$, $p = 0.0127$; *post hoc* revealed a significant difference Ctrl vs. Sem, $p = 0.0036$; **Figure 4E**). The anorexigenic effect of semaglutide extended to other palatable stimuli, the consumption of 10% sucrose solution was also significantly reduced (one-way ANOVA, $F_{(2,15)} = 21.37$, $p < 0.0001$; *post hoc* analysis: Ctrl vs Sem $p = < 0.0001$, Ctrl vs Post sem $p = < 0.0001$; **Figure 4E**, right panel). Finally, analysis across the session revealed that semaglutide-treated mice took significantly longer pauses between feeding bouts (IBIs) (Mann–Whitney U test: U = 0, time bin = 1.16 h Ctrl vs Sem, $p = 0.0119$; U = 0, time bin = 1.5 h Ctrl vs Post sem, $p = 0.0357$; U = 0, time bin = 1.66 h Ctrl vs Post sem, $p = 0.0179$; **Figure 4F**, left panel) and exhibited a correspondingly lower feeding rate compared to saline-treated controls (two-way ANOVA, time effect: $F_{(11,165)} = 1.386$, $p = 0.1837$, Treatment effect: $F_{(2,15)} = 7.310$, $p = 0.0061$, and interaction: $F_{(22,165)} = 0.8404$, $p = 0.6721$; *post hoc* analysis: time bin = 0.17 h Ctrl vs Post sem, $p = 0.0005$; time bin = 0.33 h Ctrl vs Sem, $p = 0.0056$; time bin = 0.33 h Ctrl vs Post sem, $p = 0.0065$; time bin = 1 h Ctrl vs Sem, $p = 0.0014$; time bin = 1 h Ctrl vs Post sem, $p = 0.0033$; **Figure 4F**, middle panel). Additionally, feeding time exhibited a significant correlation with food intake (**Figure 4F**, right panel). These findings are consistent with the induction of a satiety-like state. Notably, the semaglutide-treated mice clustered together with the satiated (fed) and post-semaglutide groups, indicating they all shared a similar low-feeding phenotype. In summary, these results demonstrate that acute semaglutide administration produces a powerful satiety-like state, characterized by a smaller number of feeding bouts and longer IBIs. Furthermore, the Crunchometer analysis reveals that semaglutide also markedly reduces the preference for a palatable high-fat diet (**Video 2**). These findings validate the Crunchometer as a sensitive tool for dissecting the complex behavioral effects of anorexigenic drugs.

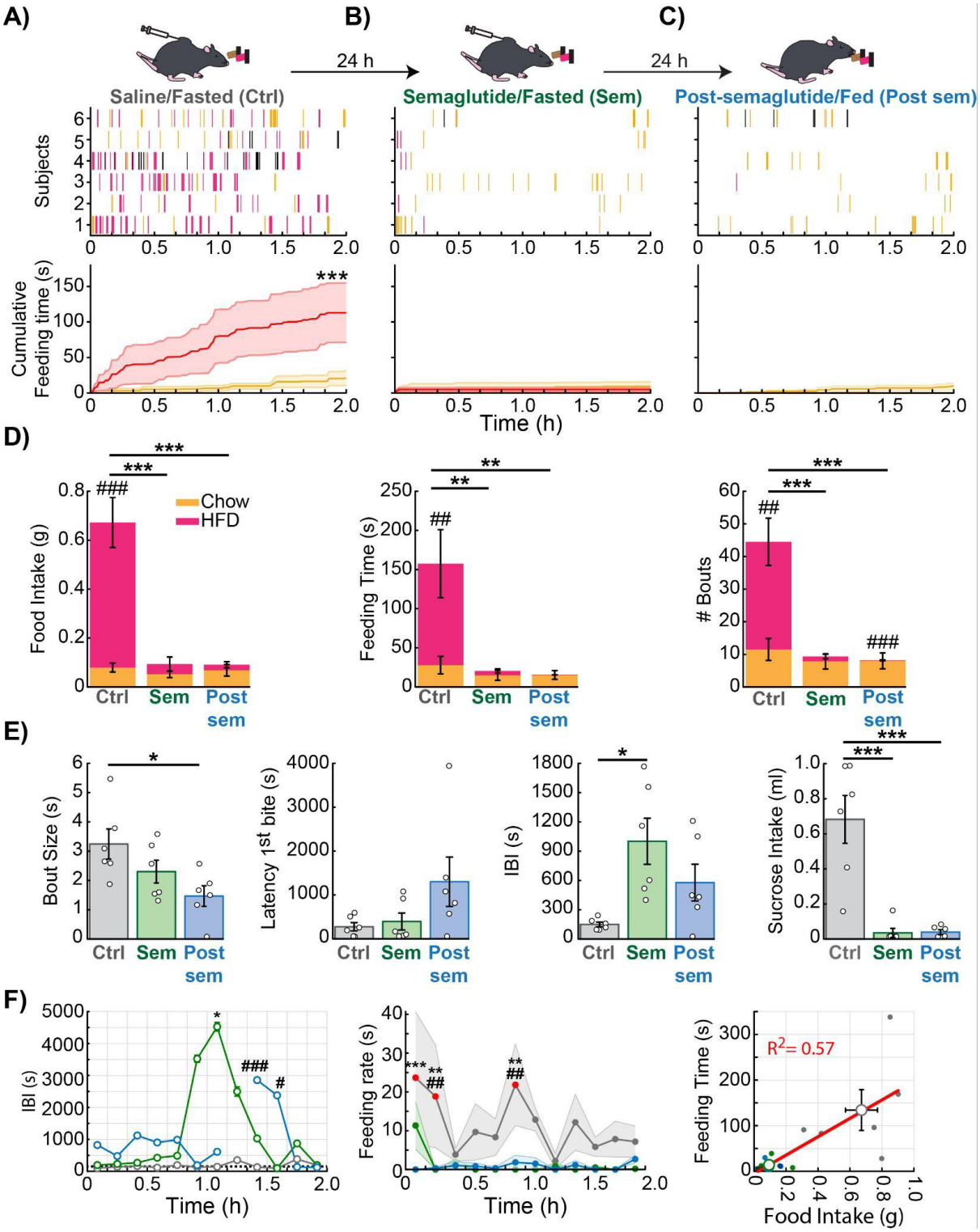

Figure 4. **Semaglutide suppresses appetite and reduces the preference for a high-fat diet.** **A)** Feeding bouts and cumulative intake in fasted mice following subcutaneous administration of saline (control group, Ctrl). **B)** The same parameters were measured in the same mice 24 hours later, under fasting conditions, following subcutaneous semaglutide administration (Sem group). **C)** Feeding behavior recorded 24 hours after semaglutide administration under fed conditions (Post-Sem group). In the ethograms, pink lines represent HFD pellet consumption, yellow lines indicate pellet consumption, and black lines denote gnawing events. In cumulative intake plots, solid lines show group averages (n = 6), and

shaded areas represent the ± SEM. All three experimental conditions (Ctrl, Sem, and Post-Sem) were tested sequentially in the same animals, with 24-hour intervals between sessions, as indicated by the arrow in the experimental timeline. **D)** Bar plots of food intake, feeding time, and number of bouts for Chow pellet (yellow bars) and HFD pellet (pink bars) across the three experimental groups. The sum of the yellow and pink bars represents the total food intake. Asterisks indicate statistically significant differences between experimental groups (Ctrl (Sal/Fasted), Sem (Sem/Fasted), and Post sem (Post-semaglutide/Fed)), while the hash symbol (#) denotes significant differences between Chow and HFD pellets within the same group. **E)** Quantitative analysis of feeding variables measured using the Crunchometer: latency to the first bite, bout size, IBI, and intake of a 10% sucrose solution. Data are expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. **F)** Average IBIs and feeding rate calculated in 10-minute bins across the session. For the feeding rate, red dots indicate a significant difference between groups. Asterisks indicate statistically significant differences between Ctrl vs Sem (Sal/Fasted vs Sem/Fasted), while a hash symbol denotes significant differences between Ctrl vs Post sem (Sal/Fasted vs Post-semaglutide/Fed). The scatter plot in the right panel shows the correlation between feeding time and total food intake, $R^2 = 0.57$, $p = 0.00031$.

The Crunchometer Distinguishes Food Intake from Gnawing Behavior Induced by LH GABAergic Neuron Activation

We next sought to determine the effect of chemogenetic activation of GABAergic neurons in the LH on feeding behavior. To this end, an AAV8-hSyn-DIO-hM3D(Gq)-mCherry virus was bilaterally injected into the LH of Vgat-IRES-Cre mice (n=4), allowing for the expression of the activating DREADD transgene over three weeks. The reporter protein mCherry was expressed in the neuronal somas of the LH, confirming localized transfection (**Figure 5A**). Under baseline satiety conditions, mice injected intraperitoneally with saline exhibited fewer feeding bouts than fasted mice; however, both groups showed a preference for the HFD pellet (**Figures 5B-C**). In contrast, chemogenetic activation of LH GABAergic neurons in satiated mice via CNO administration (Clozapine N-Oxide) led to a paradoxical behavioral pattern. It increased overall consummatory actions, particularly towards both Chow and HFD pellets, and exacerbated spillage and gnawing behavior (**Figure 5D;** see **Video 3**).
Analysis of the IBI revealed that Saline/Fasted mice initially exhibited short IBIs, which lengthened as they approached satiety. In contrast, CNO-treated fed mice (CNO/Fed) maintained short IBIs for over an hour (**Figure 5E**, top panel). Similarly, the feeding rate of CNO/Fed mice was sustained above the Saline/Fasted group for up to 50 minutes, indicating that chemogenetic activation of LH GABAergic neurons promotes

an intense period of continuous consummatory behavior (two-way ANOVA, time effect: $F_{(11,108)} = 4.942$, $p < 0.0001$, Treatment effect: $F_{(2,108)} = 15.83$, $p < 0.0001$, and interaction: $F_{(22,108)} = 2.262$, $p = 0.0030$; *post hoc* analysis: time bin = 0.17 h Fed Saline vs. Fed CNO, $p < 0.0001$, Fasted Saline vs. Fed CNO, $p = 0.0136$; time bin = 0.33 h Fed Saline vs. Fed CNO, $p < 0.0001$, Fasted Saline vs. Fed CNO, $p < 0.0001$; time bin = 0.5 h Fed Saline vs. Fed CNO, $p = 0.0185$, Fasted Saline vs. Fed CNO, $p = 0.0248$; time bin = 0.83 h Fasted Saline vs. Fed CNO, $p = 0.0401$; **Figure 5E**, middle panel). Interestingly, this intense feeding episode did not directly correspond to the consumption of more grams of food. The correlation between feeding time and food intake was weak ($R^2 = 0.32$, red line, **Figure 5E**, lower left panel) when the CNO/Fed group was included in the analysis. However, this correlation increased dramatically ($R^2 = 0.79$, red line) when food spillage was considered (**Figure 5E**, right bottom panel), suggesting that CNO-treated fed mice were not consuming all the food. A quantitative analysis of feeding microstructure revealed that activating LH GABAergic neurons promoted feeding-related motor patterns without increasing overall food ingestion. Although total food intake was similar across all groups, CNO-treated mice exhibited significantly more food spillage (one-way ANOVA, $F_{(2,9)} = 48.72$, $p = < 0.0001$; *post hoc* analysis: Fed saline vs Fed CNO $p = < 0.0001$, Fasted saline vs Fed CNO $p = < 0.0001$; **Figure 5F**) and gnawing (one-way ANOVA, $F_{(2,9)} = 19.42$, $p = 0.0005$; *post hoc* analysis: Fed saline vs Fed CNO $p = 0.0004$, Fasted saline vs Fed CNO $p = 0.0005$; **Figure 5F**). Consequently, these mice spent significantly more time engaged in feeding behaviors (one-way ANOVA, $F_{(2,9)} = 7.333$, $p = 0.0129$; *post hoc* analysis: Fed saline vs Fed CNO $p = 0.0049$, Fasted saline vs Fed CNO $p = 0.0238$; **Figure 5F**), despite being satiated. This was driven by a change in feeding frequency, as CNO-treated fed mice initiated significantly more feeding bouts (one-way ANOVA, $F_{(2,9)} = 3.631$, $p = 0.0698$; *post hoc* analysis: Fed Saline vs. Fed CNO, $p = 0.0367$) and had shorter IBIs (one-way ANOVA, $F_{(2,9)} = 3.346$, $p = 0.0820$; *post hoc* analysis: Fed Saline vs. Fed CNO, $p = 0.0294$). In contrast, the size of each bout (one-way ANOVA, $F_{(2,9)} = 1.133$, $p = 0.3641$) and the latency to the first bite were unaffected (one-way ANOVA, $F_{(2,9)} = 0.107$, $p = 0.8992$).

Given that these LH GABAergic neurons have been implicated in processing reward, we next tested if their activation would specifically increase the consumption of a highly palatable food. In agreement with previous findings (Garcia et al., 2021; Ha et al.,

2024; Jennings et al., 2015; Navarro et al., 2016), CNO administration prompted a significant increase in liquid sugar intake compared to control groups (one-way ANOVA, $F_{(2,9)} = 9.754$, $p = 0.0056$; *post hoc* analysis: Fed Saline vs. Fed CNO, $p = 0.0025$, Fasted saline vs Fed CNO $p = 0.0081$; **Figure 5F**). Together, these results suggest that while activating this neuronal population drives a robust motivation to eat, this drive is most effectively translated into consumption when the palatable food is liquid sucrose.

In summary, activating LH GABAergic neurons promoted a general increase in consummatory behaviors—including liquid sucrose intake, feeding time, spillage, and gnawing—without increasing solid food ingestion. These results demonstrate that the Crunchometer can successfully differentiate actual food consumption from other neuronally induced oromotor behaviors, highlighting its value in dissecting complex feeding-related circuits. This aligns with previous findings that these neurons increase consummatory behavior toward any available stimulus, including caloric (e.g., food, sucrose), non-caloric (e.g., saccharin), and non-nutritive items (e.g., wood, cork) (Garcia et al., 2021; Jennings et al., 2015; Navarro et al., 2016; Nieh et al., 2015).

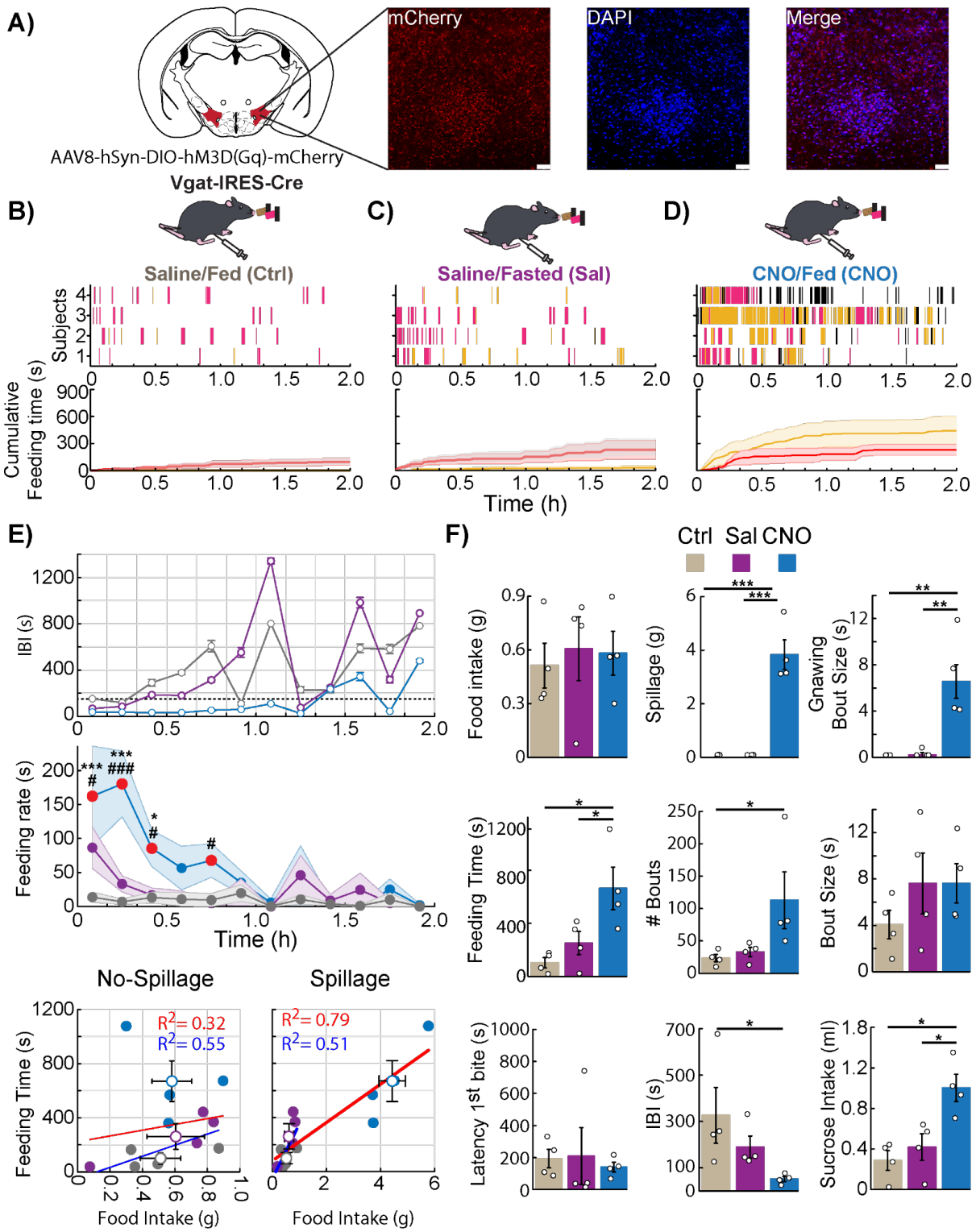

Figure 5. Chemogenetic activation of GABAergic neurons in the LH promotes spillage and gnawing behavior. A) Schematic of viral infection and representative immunofluorescence images (right panels) showing expression of red fluorescent protein (mCherry, red) and nuclear labeling with 4',6-diamidino-2'-phenylindole (DAPI, blue) in neuronal somata within LH of Vgat-IRES-Cre mice. The white scale bar in the lower right corner represents 10 μm. Cumulative feeding time in **B)** fed (control group, Ctrl) and **C)** fasted (Sal) mice following intraperitoneal administration of saline. **D)** Feeding behavior in fed mice

following intraperitoneal injection of Clozapine-N-oxide (CNO), a ligand for hM3D(Gq). In the feeding ethograms, pink lines represent HFD pellet consumption, yellow lines indicate Chow pellet consumption, and black lines denote gnawing events. In cumulative feeding time plots, solid lines show group averages (n = 4), and shaded areas represent the ± SEM. **E)** Average IBIs and feeding rate were calculated in 10-minute bins across the session (top and middle panels, respectively). In the feeding rate measure, red dots indicate a significant difference between groups. * Indicate statistically significant differences between Ctrl vs CNO (Saline/Fed vs CNO/Fed), and a # symbol denotes significant differences between Sal vs CNO (Saline/Fasted vs CNO/Fed). Bottom panel: Scatter plots display correlations between feeding time and food intake with or without spillage (left and right panels, respectively). Red lines indicate the correlation considering all groups (No spillage $R^2 = 0.32$, $p = 0.5782$; Spillage $R^2 = 0.79$, $p = 0.0001$), while blue lines denote the correlation excluding the CNO group (No-Spillage $R^2 = 0.55$, $p = 0.0354$; Spillage $R^2 = 0.51$, $p = 0.0462$). **F)** Quantitative analysis of feeding behavior, including total food intake, spillage, gnawing bout size, number of feeding bouts, bout size, latency to the first bite, IBIs, and intake of a 10% sucrose solution. Data are shown as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

The Crunchometer can be utilized in electrophysiological experiments to align neuronal responses to feeding behavior

After demonstrating that the Crunchometer enables us to capture and quantify hunger and satiety states, as well as how these feeding patterns are disturbed by pharmacological appetite suppressants and chemogenetic manipulations, we employed the Crunchometer to uncover the neuronal responses of the LH associated with feeding behavior at a millisecond timescale (Garcia et al., 2021). We implanted an OptoDrive electrode array in the LH of two mice to record extracellular activity (Caballero-Ruiz et al., 2025). To synchronize signals, a pulse generator system was implemented, whose output was recorded both through the OpenEphys electrophysiology system's analog input and via an LED that blinked on and off (1 Hz, 55% duty cycle), captured frame by frame in the video. This setup allowed alignment of the analog signal, LED blinking, and raw audio using the onset of the first pulse as a common reference (**Figure 6A-B**).

We recorded a total of 68 LH neurons from two freely behaving mice (two sessions per mouse) during the feeding behavior. LH neurons exhibited either increases or decreases in firing rates during feeding and remained modulated specifically throughout sequences of closely timed feeding bouts, defined as a meal (a collection of feeding bouts punctuated by pauses larger than 2.5 min; **Figure 6C**). Interestingly, this analysis revealed for the first time that individual feeding bouts *per se* do not

modulate LH neurons; instead, they exhibited neuronal modulation that spanned the entire meal. Accordingly, we named these LH neurons meal-related neurons. Previously, we reported similar meal-related neurons associated with Ensure intake in the nucleus accumbens shell (Tellez et al., 2012). Then, we identified that 63.24% of neurons were activated (n = 43), 22.06% were inhibited (n = 15), and 14.70% exhibited no modulation by feeding behavior (n = 10; **Figure 6D**), suggesting that LH activity mainly increased during the consumption of solid food. The high-temporal resolution of audio signals emphasizes the reliability of the Crunchometer in precisely aligning neuronal activity with feeding bouts. Impressively, LH responses were robust enough to detect feeding behavior even under a food hoarding event (see asterisk and horizontal line in **Figure 6D**), when the Crunchometer did not detect bite sounds, such as when the mouse cut off a large chunk of a HFD pellet and chew it away from the microphone, reducing the accuracy of the sound-based detection (see **Video 4**). Thus, our data demonstrates that the act of chewing food robustly modulates LH neurons, even when the mice are eating and moving around the box simultaneously.

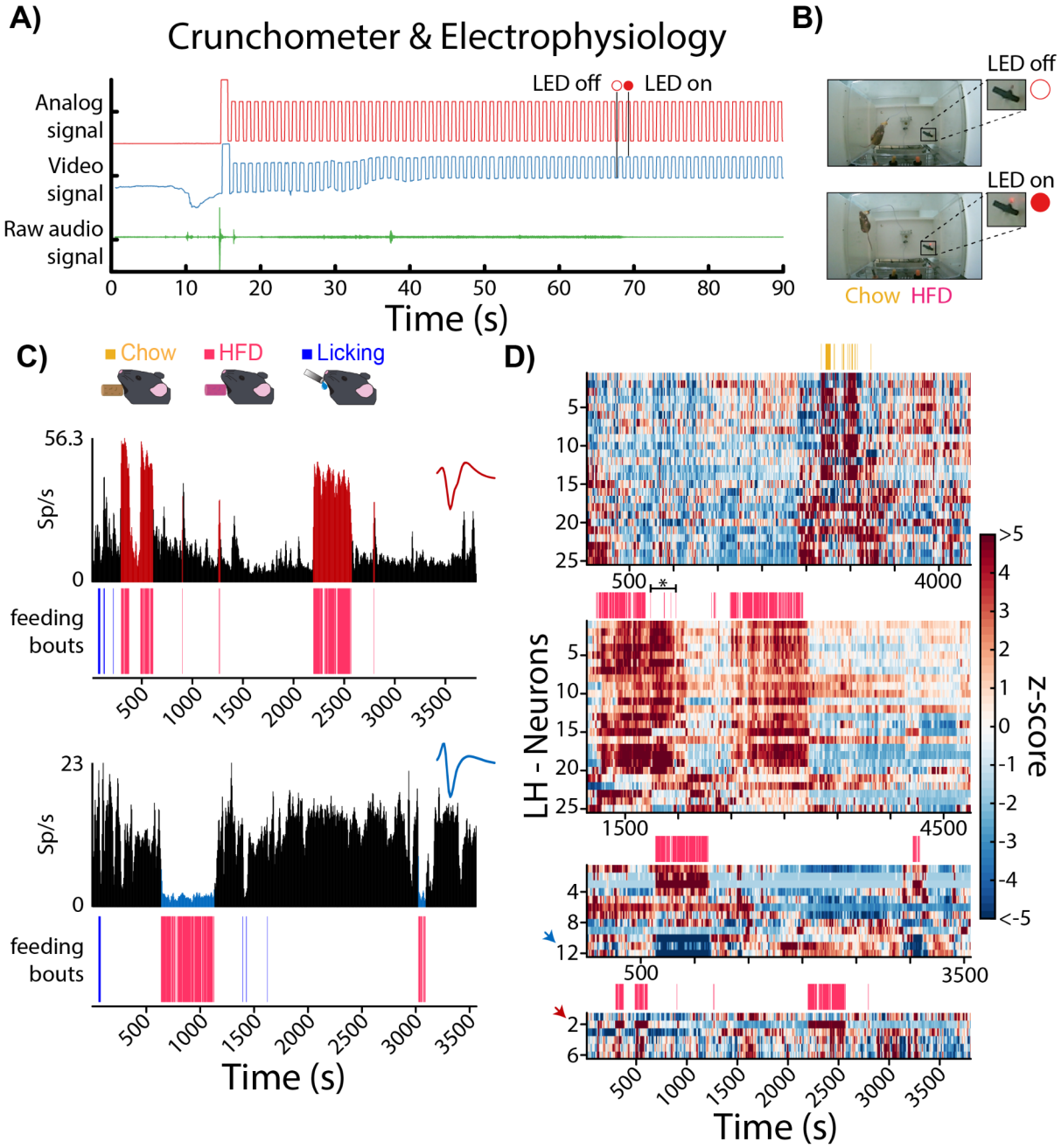

Figure 6. The Cruchometer and electrophysiological responses of LH neurons in freely behaving mice. A) To record electrophysiological responses with the Crunchometer, a pulse generator system synchronizes the Crunchometer data with neuronal recordings. Video and audio signals captured by the Crunchometer are aligned with the electrophysiological recordings using a pulse generator, whose output is simultaneously recorded through the electrophysiology analog input (red line) and used to blink an LED (blue line), which is captured frame by frame by the camera during the session. **B)** Example of LED blinking used to synchronize video and audio signals with electrophysiological recordings. Frames showing the LED off (top) and on (bottom) were subtracted from the video to generate a signal resembling the analog input; these frames correspond in time to the horizontal black lines in panel **A**. **C)** Representative activity of LH neurons during feeding behavior. The firing rates of individual LH neurons were calculated (top plots) and aligned to feeding bouts (bottom plots). The top panel exhibits a neuron that increased its firing rate during feeding bouts (red bars), whereas the bottom panel displays a neuron that decreased its firing rate during the feeding bouts (blue

bars). Feeding bouts for Chow and HFD pellets are marked by yellow and pink squares, respectively; licking behavior is indicated by blue squares. The inset depicts the waveform of the action potential in two neurons. **D)** Population activity from four LH neuronal recording sessions. Top plots display feeding bouts in yellow and pink for Chow and HFD pellets, respectively; bottom plots exhibit the z-scored neuronal population activity of the LH across sessions. Red and blue arrows highlight the representative neurons in panel **C** that were selectively activated or inhibited during food intake, respectively.

Synchronizing Calcium Recordings with Feeding Bouts

To test the utility of the Crunchometer to uncover the neuronal correlates of eating behavior using calcium dynamics, we performed pilot experiments of simultaneous calcium imaging and behavioral recordings in freely moving mice expressing GCaMP7s in either GABAergic (VGat-Cre mice, n = 2) or glutamatergic (VGlut2-Cre mice, n = 2) neurons of the lateral hypothalamus (LH). Mice were allowed to freely explore the Crunchometer's box for 30 minutes, with access to Chow pellets, HFD pellets, and a licking chamber delivering water or sucrose solutions (**Figure 7A**, right panel; **Figure 8A**, right panel). Neurons were identified, and their calcium signals were extracted and normalized to a z-score. The temporal dynamics of neural activity were then aligned to feeding and drinking events (**Figure 7B**, top and bottom). We identified approximately 20 neurons per mouse. In VGAT mice, we found diverse neural activity patterns, including cells that were positively or negatively correlated with feeding bouts (**Figure 7C**). Some neurons were inhibited during feeding, potentially reflecting local inhibition from other LH GABAergic neurons (a kind of mirror activity). This motif resembles the ONsemble/OFFsemble mirror interactions recently described in cortical circuits (Pérez-Ortega et al., 2024). In particular, neurons 6, 7, and 8 in **Figure 7C** (right experiment) form part of a feeding ONsemble, while neurons 3 and 14 exhibit inverse patterns consistent with an OFFsemble dynamic. These observations suggest the existence of structured, opposing ensembles within the LH GABAergic population that are differentially engaged during food consumption. (**Figure 7C**). In contrast, in VGlut2-cre mice, very few, if any, of the glutamatergic neurons exhibited clear correlations with feeding bouts (**Figure 8B**, top and bottom). A few glutamatergic neurons exhibited a clear correlation with licking events, but no response to solid food. Most neurons exhibited activity unrelated to consummatory behavior: feeding or licking (**Figure 8C**).

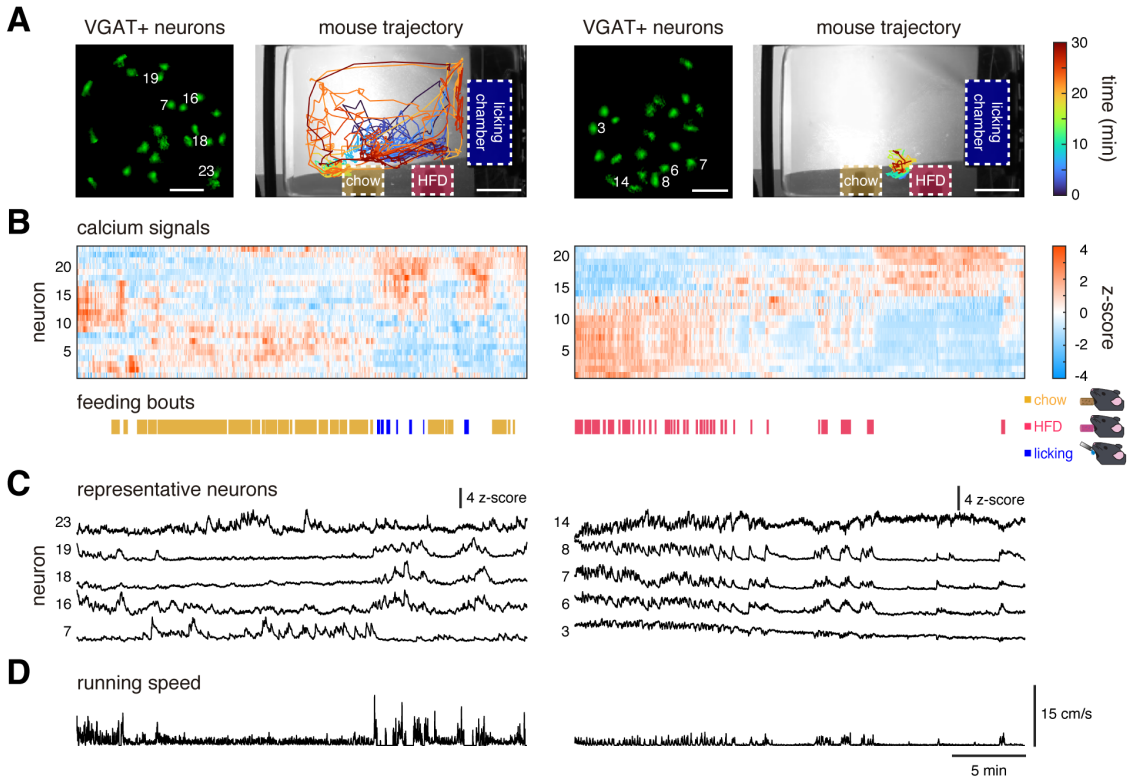

Figure 7. Microendoscope calcium imaging of LH GABAergic neurons during natural feeding behavior. A) Two pilot experiments are shown (left and right panels). Left: GCaMP7s fluorescence images displaying ROIs of individual GABAergic neurons in the LH. Scale bar = 20 µm. Right: Mouse trajectories over a 30-minute session with access to Chow pellets, HFD pellets, and a licking chamber delivering water or sucrose solutions. Color gradient indicates time progression (blue to red). Dashed boxes indicate the locations of Chow, HFD, and licking zones. Scale bar = 5 cm. **B)** Top: z-scored calcium traces from all detected neurons for each experiment, sorted by their similarity in activity patterns (Coss et al., 2022; Pérez-Ortega et al., 2024). Bottom: Feeding and licking bouts automatically detected by the Crunchometer, color-coded by food type (Chow: yellow; HFD: pink; licking: blue). **C)** Calcium traces from representative GABAergic neurons from each experiment. Neurons display diverse activity profiles, including activation and suppression during feeding bouts. In the left panel, neuron 7 exhibited more activity during feeding but no activity during licking sucrose; in contrast, neurons 16, 18, and 19 mainly responded to liquid sucrose but not to solid food. Neuron 23 exhibited activity during both licking and solid food intake, suggesting that it may participate in processing both types of feeding-related behaviors. In the panel at right, during feeding periods, neurons 6, 7, and 8 become active, while neuron 3 is inhibited, exhibiting a mirror-like pattern, and neuron 14 shows rebound disinhibition. In this session, the mouse did not lick sucrose. **D)** Running speed over time, estimated from video tracking.

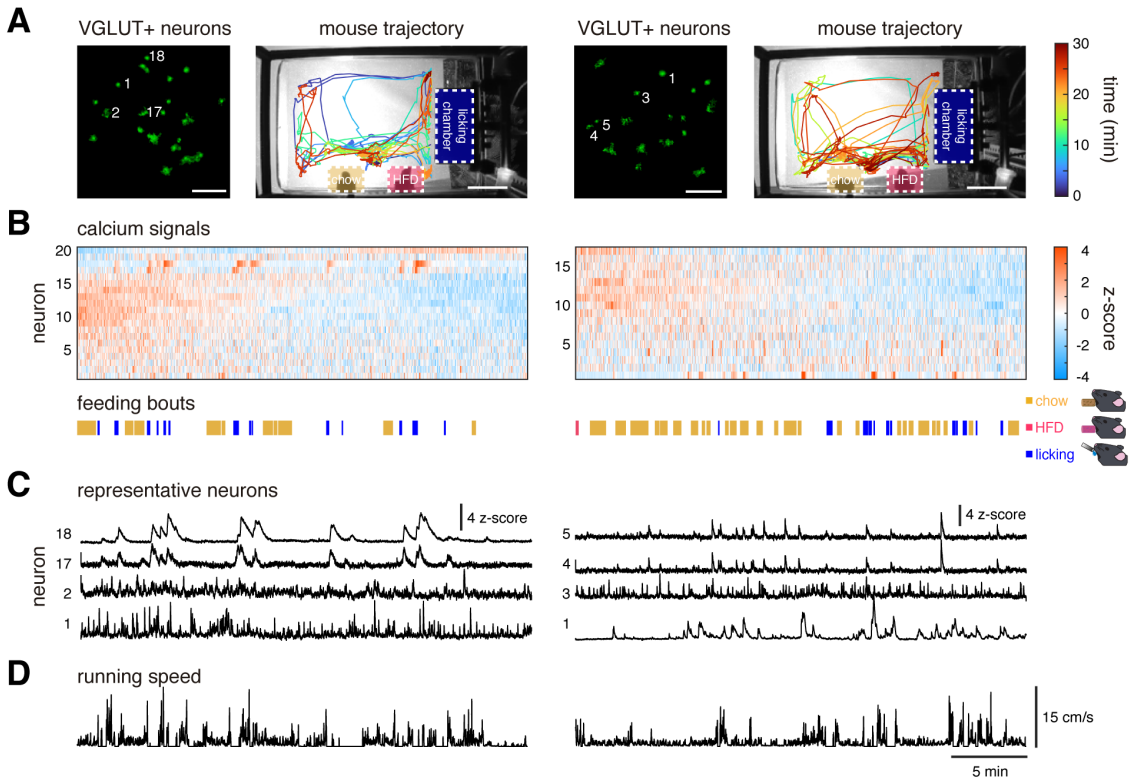

Figure 8. Activity of LH glutamatergic neurons does not correlate with feeding bouts. **A)** Two pilot experiments are shown (left and right panels). Left: GCaMP7s fluorescence image showing ROIs of individual glutamatergic neurons in the lateral hypothalamus. Scale bar = 20 µm. Right: Mouse trajectories during 30-minute sessions with access to Chow pellets, HFD pellets, and a licking sipper (with a metal floor for contact lickometer) delivering water or sucrose solutions. Colors represent time progression (blue to red). Dashed boxes indicate the locations of Chow (yellow ticks), HFD (pink), and licking (blue) zones. Scale bar = 5 cm. **B)** Top: Z-scored calcium traces from all detected glutamatergic neurons in each experiment. Bottom: Automatically detected feeding and licking bouts as in **Figure 7**. **C)** Analysis of calcium transients in glutamatergic neurons showed that most recorded cells were non-selectively responsive to feeding bouts. However, a small population of neurons (including neurons 17 and 18) exhibited activity that was tightly coupled to licking for liquid sucrose yet remained unresponsive during the intake of solid food. **D)** Running speed traces from both experiments.

Discussion

This study demonstrates that analyzing bite sounds provides a novel method for characterizing feeding behavior, offering unprecedented temporal resolution and precision for measuring its microstructure with solid foods. This improved temporal resolution for detecting the onset and offset of feeding bouts, based on bite sounds, paves the way for unveiling neuronal modulation phase-locked to feeding, thereby

accelerating the discovery of the neural circuits controlling appetite. We propose the Crunchometer as a valuable, low-cost, open-source tool that can be easily implemented in any standard laboratory. To facilitate its adoption, we provide accompanying software along with detailed setup and installation instructions (Arroyo et al., 2025). We also provided a benchmark database (comprising six 2-hour-long recordings of hungry mice, used in **Figure 2** to validate the Crunchometer for further refinement by the scientific community; see the Open Science Frame database folder Benchmark (Arroyo et al., 2025)).

Our recent research using the Crunchometer (previously named Crunch Master (Liu et al., 2025)) has revealed that the gut can 'taste' microbial patterns of bacteria, playing a key role in regulating feeding behavior. We discovered that specialized sensory cells in the colon, known as neuropods, utilize a receptor (TLR5) to detect flagellin, a protein produced by certain microbes. This detection triggers the release of the appetite-suppressing hormone PYY. In mice, this signal reduced food intake and prevented obesity, revealing a new sense named the "neurobiotic sense" (Liu et al., 2025). The device's precision in tracking the consumption of an individual Chow pellet was essential for this detailed microstructural analysis. In this work, we extend the capabilities of the Crunchometer (Liu et al., 2025). By enabling the simultaneous monitoring of two different solid foods (standard Chow and a high-fat diet) alongside a liquid option (sugar water), the system can now be used to assess food preference in addition to the fine-grained analysis of feeding microstructure.

Microstructure of feeding under different fed and fasted energy states

This study demonstrates that the Crunchometer system accurately differentiates the meal patterns of fed and fasted wildtype mice by quantifying their feeding microstructure. Fasted mice exhibited shorter latencies to eat, higher initial feeding rates, and more frequent bouts, resulting in a doubling of their cumulative feeding time and food intake compared to their satiated counterparts. Furthermore, the initial increase in feeding rate followed by a progressive lengthening of the IBI in fasted mice illustrated the transition to satiety. This ability to resolve significant differences between fed and fasted energy states validates the Crunchometer as a sensitive tool for investigating the neurobiological and pharmacological regulation of appetite (Shrivastava et al., 2025; Wang et al., 2024).

Semaglutide: more than just appetite suppression, it reshapes fatty, energy-dense food preferences

The Crunchometer analysis revealed the rapid and robust appetite suppressant effects of acute semaglutide administration, consistent with the idea that this GLP-1 agonist induces a robust satiety sensation (Drucker, 2025). More striking was the reduction in preference for the HFD observed on the administration day, which was maintained until the next day (post-semaglutide; **Figure 4,** see **Video 2**). This result aligns with observations in humans, where semaglutide was also associated with reduced hunger and food cravings, improved control overeating, and, more importantly, a lower preference for fatty, energy-dense, non-sweet foods (Blundell et al., 2017).

Chemogenetics activation of LH GABAergic neurons triggers a stress-induced eating pattern

Activating GABAergic neurons in LH via chemogenetics induces a striking behavioral phenotype. Instead of normal, homeostatic eating, this stimulation triggers a frantic, stress-like pattern of consumption, characterized by a significant surge in biting and gnawing directed at the food source (**Video 3**). The Cruchometer, the Threshold methods, could successfully capture this frenetic feeding pattern with unprecedented precision. This feeding pattern involved hypertrophic mastication and gnawing directed at all proximal stimuli (palatable, non-palatable, or inedible), resulting in a large amount of food spillage and increased sucrose liquid intake**.** In agreement with previous findings (Garcia et al., 2021; Ha et al., 2024; Jennings et al., 2015; Navarro et al., 2016).

Beyond the lick, a new world of possibilities for the neuronal correlates of eating solid food: Meal-related neurons in the lateral hypothalamus

Extracellular multichannel recordings and calcium imaging were easily synchronized with the Crunchometer, allowing for alignment of neuronal responses to the onset and offset of a feeding bout with unprecedented resolution. The study of neuronal correlates of eating solid food has been rarely investigated, see (Pilato et al., 2024; Yamamoto et al., 1989), for exceptions. Here, we found that LH neurons exhibit robust

modulation during the consumption of solid foods. We named these responses LH meal-related neurons, in agreement with the well-established role of LH on ingestive behavior. Unexpectedly. Our pilot data (see below for calcium imaging) suggest that licking vs eating solid food may rely upon distinct neuronal circuits, in agreement with Dilorenzo's finding in rostral NTS neurons, in which they found little correspondence between liquid taste-evoked responses and those evoked by eating solid food. Pat Dilorenzo wrote, "What is striking is that we see no correspondence whatsoever, not even a weak one." Here we also observed a partial separation of neurons responding to solid food and liquid sucrose (more of this below). The fact that licking vs. eating solid food seems to recruit distinct neuronal ensembles also opens the possibility of labeling them using TRAP or Tet-tagging systems (Guenthner et al., 2013; Zhang et al., 2015) for further testing of their specific contribution to feeding.

LH GABAergic neurons are modulated by eating solid food, and a different subset responds to liquid sucrose

Our calcium imaging data from VGat::GCaMP7s mice revealed that lateral hypothalamic (LH) GABAergic neurons are heterogeneously modulated during feeding behavior. Distinct subsets of neurons increased or decreased their activity in response to the consumption of Chow or high-fat pellets, suggesting functional diversity within this population. Interestingly, licking behavior, used to access liquid sucrose solutions, engaged a separate set of neurons, with minimal overlap between neurons modulated by solid food and those responsive to licking. This suggests that the LH GABAergic network encodes feeding modality through partially segregated neuronal ensembles, potentially supporting parallel pathways for controlling ingestive behaviors toward solids and liquids. Although our sample size was small (only two pilot experiments per condition), these preliminary observations demonstrate the utility of combining the Crunchometer with calcium imaging to identify behaviorally relevant neural dynamics. Future studies with larger cohorts and controlled stimulus delivery will be needed to characterize these ensemble responses in detail.

A few LH Glutamatergic neurons were modulated during sucrose intake

In our recordings from VGlut2::GCaMP6 mice, we found little evidence that LH glutamatergic neurons are modulated during feeding behavior. Across conditions

involving solid food intake, licking behavior, and general movement, most of these neurons showed no clear changes in activity. Only a couple of neurons exhibited a robust correlation with licking events, suggesting that, at least under our experimental conditions, LH glutamatergic neurons were poorly modulated during consumption-related behaviors, except for a few neurons that exhibited large increases on calcium transients related to licking liquid sucrose as seen before (Gordon-Fennell et al., 2025; Rossi et al., 2019), interestingly they also do not respond to solid food intake. While these findings are limited by our small sample size (two pilot recordings in this condition), they support the preliminary view that LH glutamatergic neurons are not primarily engaged during naturalistic feeding of solid food, in contrast to their GABAergic counterparts. These observations, although not conclusive, highlight the value of our approach and suggest that future studies with expanded datasets will be crucial to determine whether specific subsets of LH glutamatergic neurons contribute under other behavioral contexts, particularly those more related to an aversive context (Gordon-Fennell and Stuber, 2021).

Limitations and Future Directions

Our current SVM model reliably distinguishes between bites, non-bites (silence), and artifacts; however, it does not accurately differentiate between biting and gnawing, likely due to highly similar acoustic profiles recorded by our microphone. As a result, gnawing periods were manually reviewed. Nevertheless, our current SVM model for the Crunchometer provides a fully automated and scalable method for identifying bite events from audio recordings, with minimal human intervention limited to initial model training and fewer artifact detections. Future iterations of the classifier could incorporate finer spectral features or unsupervised pre-clustering to disambiguate subtle behaviors. Another limitation of our trained SVM model is that it is expected to generalize poorly across different Crunchometer setups (not shown). Consequently, retraining a new model is likely required for each laboratory or for any setup that uses a microphone different from the one standardized here. In this regard, the Threshold method is more reliable and easier to tune for each setup. That said, to our knowledge, this is the first application of machine learning to detect mouse feeding microstructure acoustically across varied experimental paradigms. Other impressive deep-learning methods have been previously developed, but they were designed to classify and sort

distinct vocalization types, not to detect feeding bouts (Coffey et al., 2019). Closed-loop optogenetic experiments were not tested here, but they could also be easily implemented by detecting bites in real time and sending TTL pulses to control laser activation. For these closed-loop experiments, our SVM model's predictions are ideal for rapidly detecting feeding bouts with a low artifact rate. Finally, long-term recordings: the size of the video files, though not the audio files, makes continuous 24-hour recording more challenging to process (but not impossible) due to the large storage space required. While technically possible, a more feasible approach for long-term studies would be to adapt the Crunchometer to an event-triggered recording, limited to feeding events (saving video and sound snippets) in real time. We anticipate that future versions of the Crunchometer will incorporate this capability, contributing a powerful new behavioral tool towards achieving the long-term dream of Curt P. Richter. We anticipate the Crunchometer will allow the neuroscience field to “freely” move forward from head-fixed approaches and further enrich the new era of the “behavioristic study of the activity of rodents (Richter, 1922).”

Conclusion

The Crunchometer offers a transformative and accessible tool for high-resolution studies of solid food consumption, paving the way for democratizing the use of these technologies (Marzullo and Gage, 2012). By integrating effortlessly with essential techniques such as *in vivo* electrophysiology and calcium imaging, it has already yielded novel insights into the neural basis of feeding in freely behaving mice. These findings include the discovery of how bacteria’s microbial patterns regulate mice’s feeding behavior (Liu et al., 2025) and the specific role of LH GABAergic neurons in feeding, as well as the distinct neural encoding for solid versus liquid food rewards. As a powerful, ready-to-deploy solution, this low-cost and open-source technology promises to accelerate the precise dissection of appetite circuits and the development of new therapies for obesity and eating disorders.

Methods

Subjects. Adult male and female C57BL/6J mice (30–40 g) were used to study energy states (hunger and satiety) and the anorexigenic effects of semaglutide. Separately,

adult male and female Vgat-IRES-Cre mice (20–30 g) were used for chemogenetic activation of GABAergic neurons or for electrophysiological recordings. Vgat-IRES-Cre and Vglut2-IRES-Cre adult mice were used for calcium imaging recordings. All mice were individually housed in standard laboratory cages under controlled conditions: a 12:12 h light/dark cycle (lights on at 06:00), a temperature of 22 ± 2°C, and a relative humidity of 50 ± 5%. Mice had *ad libitum* access to water and standard Chow diet (LabDiet 5008) unless otherwise stated. No sex differences were observed in any of the experiments; therefore, data from male and female mice were pooled. All behavioral experiments were carried out during the light phase of the cycle. All procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC) of CINVESTAV.

Viral vector. The Cre-inducible adeno-associated virus (AAVs) were purchased from Addgene (Watertown, MA, USA). The viral concentration was 4×10^{12} viral genomes ml^{-1} for AAV8-hSyn-DIO-hM3D(Gq)-mCherry (#44361). pAG-AAV-syn-FLEX-jGCaMP7s-WPRE (#104491) virus was used for calcium imaging experiments. Viruses were divided into aliquots and stored at -80 °C until use.

Stereotaxic surgery. Mice were anesthetized with isoflurane (induction, 5%; maintenance, 1–1.5%; ViP 3000 Matrix). The microinjection needles (29-G) were connected to a 10 µl Hamilton syringe and filled with AAV. For all experiments, the mice were bilaterally injected into the LH (from Bregma (mm): −1.4 AP, ±1.0 ML, and −5.8 DV) with AAV (0.25 µl) at a rate of 0.1 µl min−1 with an additional 5 min for a complete diffusion. Coordinates were taken from Allen's reference atlas of the mouse brain. Following the suturing of the mouse's head, a three-week recovery period was allowed to enable healing and transgene expression. For electrophysiology, in two VGat-IRES-cre mice, a custom-made 16-tungsten OptoDrive array was implanted targeting the LH (from Bregma (mm): −1.3 AP, ±1.1 ML, and −5.3 DV)(Caballero-Ruiz et al., 2025). The OptoDrive was fixed to the skull using dental acrylic and anchored with a grounding screw. After surgery, mice were allowed to recover for one week, and ketoprofen (45 mg/kg, i.p.) was administered for three consecutive days to manage postoperative pain. For calcium imaging experiments, two VGat-IRES-Cre and two VGlut2-IRES-Cre mice were used. To label neurons with a genetically encoded calcium indicator, an adeno-associated virus (AAV) encoding GCaMP7s was injected

into the LH (from Bregma (mm): lateral hypothalamus at coordinates −1.3 AP, ±1.1 ML, and −5.3 DV). A total volume of 300 nL was delivered at a rate of 30 nL/min, and the injection needle was left in place for an additional 10 minutes to allow for viral diffusion. Following surgery, mice were allowed to recover for three weeks to ensure robust GCaMP7s expression. After the recovery period, a gradient refractive index (GRIN) lens (ProView Integrated Lens, 7.3 mm length, 0.6 mm diameter; Inscopix) was implanted at a final depth of DV –5.1 to –5.2 mm, approximately 200 µm above the injection site. During implantation, the nVISTA microendoscope system (Inscopix) was temporarily connected and activated to verify both GCaMP7s expression and an appropriate field of view. To minimize tissue compression and accommodate displacement caused by the lens, it was advanced in 300 µm increments, with a 100 µm upward retraction after each step. After lens implantation, mice were allowed to recover for an additional five days before the start of behavioral and calcium imaging experiments. To manage postoperative pain, Ketoprofen (45 mg/kg, i.p.) was administered once daily for three consecutive days following both the viral injection and the lens implantation procedures.

Drugs. Semaglutide was diluted in 0.9% saline to a final concentration of 0.067 mg/mL. Saline and semaglutide were administered via subcutaneous injection at a dose of 0.123 mg/kg immediately before the start of the behavioral experiment. Clozapine-N-Oxide (CNO) was also prepared in 0.9% saline at a final concentration of 2 mg/mL. Both saline and CNO were administered via intraperitoneal injection at a dose of 3 mg/kg immediately before the start of the behavioral protocol.

Behavioral protocol. All mice were habituated for two days before the recording session in the Crunchometer. Each habituation session lasted 30 minutes, during which two food pellets were placed in the chamber: one standard Chow pellet (LabDiet 5008) and one highly palatable high-fat diet (HFD) pellet (Research Diet, D12451). The pellets were positioned on the chamber walls in the same location described in detail in **Figure 1**. During habituation, the mice were in a fed state and allowed to explore both food types freely (no video recordings were performed). This was done to reduce stress and novelty on the day of the behavioral recording. On the recording day, each Crunchometer session lasted two hours. Throughout this period, mice had *ad libitum* access to both standard Chow and HFD pellets. In addition, a 10% sucrose

solution (from Sigma-Aldrich Mexico) was provided as a liquid source during the feeding behavior. All fasted mice, regardless of the experimental group, were food-deprived 18 hours before the session, with only solid food removed. In contrast, fed mice had *ad libitum* access to both food and water. For pharmacological and chemogenetic experiments, drug administration was performed immediately before placing the mice in the recording chamber. At the end of each session, mice were returned to their home cages. Food pellets were weighed before and after each session, and any spillage was collected and weighed to calculate precise intake. For calcium imaging, mice were trained in a behavioral chamber containing five stimuli: three liquid solutions (water, 3% sucrose, and 18% sucrose) and two types of food pellets (standard Chow and HFD). Before the calcium imaging recording session, mice underwent a 3-day habituation period (30 minutes/day). On the day of calcium imaging, mice were food-deprived for 18 hours before the session. Each imaging session lasted 30 minutes, during which mice again had free access to the same liquid and solid stimuli.

Electrophysiological recordings. Electrophysiological recordings were performed using the OpenEphys Acquisition Board (OpenEphys Production Site) and GUI (Siegle et al., 2017) during feeding behavior in freely moving mice. The neural signals were sampled at 30 KHz and band-pass filtered between 0.5 and 8 KHz. Action potentials exceeding 50 μV were recorded, and putative units were identified online using voltage-time windows. Subsequently, offline spike sorting was implemented using an unsupervised algorithm (Chaure et al., 2018) to isolate single units. Only the timestamps of these sorted units were used for further analysis.

Immunofluorescence. Mice were initially sedated with isoflurane (5%, ViP 3000 Matrix) for 1 minute and subsequently euthanized using a lethal dose of embutramide and mebezonium iodide (T61) (2 ml/kg). Intracardiac perfusion was performed using phosphate-buffered saline (PBS), followed by fixation with 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed and fixed in 4% paraformaldehyde and then stored for three days. Brain tissues were dehydrated by gradually increasing the sucrose concentration (10%, 20%, and 30%) before slicing. Brains were sliced into 40 μm sections using a cryostat (Thermo Scientific HM525). Free-floating sections were incubated with 300 nM 4’, 6-diamidino-2-phenylindole (DAPI, D9542, Sigma-

Aldrich) for 1 min. After a final wash with TBST, sections were mounted in Dako fluorescence mounting medium. Immunofluorescence was observed using a LEICA Stellaris 5 confocal microscope.

Signal processing pipeline of the Crunchometer. In each recording session, we obtained an ".mkv" file containing both video and audio signals. From the video file, the audio was extracted into an ".ogg" file for further analysis using a custom-made MATLAB function (`extractAudio.xml`). To analyze the audio, the power spectrum was calculated using a short-time Fourier transform (using MATLAB's `spectrogram` function). This produced a matrix in which each column represented a bin of a 1-second time window (with no overlap), and each row corresponded to a specific frequency. We focused on the 500-950 Hz frequency band, which was associated with bite sounds produced by the mice. The bite-related power was computed as follows:

$$Power_{sound-bites} = 10 * log_{10}(mean(spectrum_{500-950\ Hz}))$$

To detect sound bites, we set a threshold based on the distribution of power values across sessions. This threshold was defined as:

$$Threshold = \mu_{power} + \sigma_{power} \approx -85\ dB$$

This threshold value was consistent across sessions, given our standardized experimental conditions. Bins exceeding the threshold were classified as detections and grouped into sequences with less than 5 seconds between detections, referred to as feeding bouts. At a macrostructural level, a meal was defined as a pause between feeding bouts lasting longer than 2.5 minutes. Once the time intervals of these bouts were identified, they were used to generate "video snippets.mp4" video files containing both audio and video around each possible feeding event. For automated sorting, each snippet was placed into a labeled folder (e.g., Artifact, Chow, HFD, Gnawing) based on the mouse's proximity to either the Chow or HFD pellet locations during the event. If the mouse was not near either pellet source, the detection was classified as an Artifact. Then, all snippets were reviewed to confirm or correct their categorization. Misclassified snippets were manually reassigned to their respective folders.

Statistical Analysis. Data on cumulative feeding time were analyzed with a two-sample Kolmogorov–Smirnov test (two-tailed) to assess distribution differences. The

one-tailed Mann–Whitney U test for group comparisons, as well as one-way and two-way ANOVA, was applied where appropriate to the crunchometer metrics. Fisher´s LSD post hoc analysis was performed. α was always set $p < 0.05$.

SVM model of the Crunchometer: automatic detection of biting from audio. We developed a supervised classification algorithm based on a multi-class support vector machine (SVM), implemented in MATLAB, to automatically detect biting events from audio recordings. The classifier was trained on data from a single 1-hour session recorded from one mouse. Audio segments were manually annotated into three categories: “bite”, “artifact”, and “no sound”. Recordings were acquired at 44,1 kHz using a mono microphone placed inside the behavioral chamber. Spectrograms were computed using 1-second non-overlapping windows with a frequency resolution of 5.4 Hz (nfft = 2^{13}). Each time window was represented as a column vector of spectral features. Pairwise Euclidean distances between these vectors were calculated and used for hierarchical clustering with the Ward linkage method. The resulting clusters were manually assigned to one of the three sound categories (bite, artifact, or no sound) based on an inspection of the spectrogram patterns, associated audio, and synchronized video clips. This labeled dataset was then used to train the multi-class SVM. Remarkably, this model, trained on data from a single mouse, generalized robustly across all mice recorded under similar conditions, without requiring additional retraining. For subsequent recordings (sampled at 44,1 kHz), spectrograms were computed using the same parameters, and each 1-second window was classified as a bite, artifact, or silence using the trained model.

Definition of feeding bouts. Feeding bouts were defined as sequences of bite events separated by less than 5 seconds. If more than 5 seconds elapsed between bite detections, a new bout was initiated. Feeding bouts were categorized as “Chow” or “HFD” based on video analysis of mouse location and movement (see below). Licking events were identified separately using a lickometer and were not considered part of feeding bouts.

Identification of Chow vs. HFD consumption. To identify the type of pellet consumed during each feeding bout, we initially analyzed the spectrogram within the frequency band associated with sound bites. However, no difference was found

between pellets based on their acoustic features. Therefore, we examined the video clips corresponding to the detected feeding intervals. Two regions of interest (ROIs), defined by the location of the pellets in the video, were manually defined over the Chow and HFD pellet dispensers. Motion energy was computed for each ROI using the MoussionEnergy algorithm (Pérez-Ortega, 2023a), based on the mean absolute difference between consecutive video frames. If the motion energy within an ROI exceeded a manually set threshold (determined to exclude noise), the bout was classified accordingly: “Chow” if the Chow ROI had greater motion energy, “HFD” if the HFD ROI did. Bouts with low or ambiguous motion were excluded.

Calcium imaging experiments and analysis. GCaMP7s-expressing VGat-IRES-cre or VGlut2-IRES-cre mice were implanted with Inscopix microendoscopes targeting the lateral hypothalamus. Calcium imaging was conducted using the Inscopix nVISTA microendoscope system (Palo Alto, CA, USA). Neural activity was recorded continuously for 30 minutes at a sampling rate of 30 Hz (Inscopix Data Processing Software). To enable synchronization with behavior, a trigger signal was delivered at the onset of the experiment. Behavioral events, including individual licks, were precisely timestamped. Additionally, a low-frequency auditory tone (250 Hz, dB = 67.1) paired with a visible LED flash in the video, both synchronized and recorded, provided a reference point to ensure alignment between the behavioral video and neural datasets. This LED/tone pairing was also timestamped within the nVISTA system (Inscopix), facilitating accurate temporal correlation between calcium dynamics and specific behavioral actions. Raw calcium videos were preprocessed by subtracting a Gaussian-blurred version of each frame ($\sigma \approx 16\,\mu m$) to remove background fluorescence. Rigid-body motion correction (translation only) was then applied. Following the procedure described in (Pérez-Ortega et al., 2024), neuronal regions of interest (ROIs) and $\Delta F/F_0$ fluorescence traces were extracted using the Xsembles2P algorithm (Pérez-Ortega, 2023b). Neurons with a peak signal-to-noise ratio (SNR) below 10 dB were excluded from further analysis. The remaining fluorescence traces were normalized using z-scoring.

Tracking trajectory and running speed. Mouse position was tracked from grayscale video recordings. A static background was estimated by computing the maximum projection across all frames, resulting in a bright background image. Each frame of the

original video was then subtracted from this background, highlighting regions where the mouse was present as darker areas. Foreground segmentation was performed using global thresholding (Otsu's method), followed by morphological erosion with a disk-shaped structuring element (radius = 2 pixels) to reduce noise. The centroid of the resulting binary mask was used to estimate the mouse's (x, y) position in each frame. Running speed was calculated as the frame-to-frame displacement, scaled by the video's sampling rate. Tracking was not possible while the mouse was inside the licking chamber due to occlusion. This was implemented with a homemade MATLAB function `Mass_Center_From_MP4.m`.

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Data and Code Availability

Benchmark and Crunchometer software will be available on OSF (Arroyo et al., 2025) using the following link:
https://osf.io/bmkdc/?view_only=913feef710714d2bbd4cfb45960fb7be

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Supplementary information

Video 1. Preparation of food pellets

Video 2. Change in HFD preference during acute semaglutide administration. This video was made with the MATLAB function `Run_AllBites`

Video 3. Chemogenetic activation of LH GABAergic neurons induced a stress-like feeding pattern. This video was made with the MATLAB function `Run_AllBites`

Video 4. Food Hoarding event during LH multichannel recordings.

Video 5. Tutorial: Installing the ffmpeg software before running the Crunchometer.

Supplementary Fig 1-1. Bill of materials

Supplementary Fig 2. Installation Guide for FFMpeg and audio extraction for the Crunchometer

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Supplementary Fig. 1-1. Bill of materials

Crunchometer Bill of Materials							
Item	**Description**	**Image**	**Quantity required**	**unit price USD**	**Webpage seller**	**Model**	**Seller**
1	**Condenser Microphone with USB Cable**		**1**	**$ 63.88**	**https://a.co/d/evaasBZ** (Accessed 03-07-2025)	**K669**	**FIFINE**
2	**Acrylic Box**		**1**	**N/A**	**N/A**	**N/A**	**N/A**
3	**Global Shutter High Speed 120fps at 1280 x 720p USB Camera with Mini Case with CS mount 2.8-12mm varifocal lens**		**1**	**$ 107.24**	**https://a.co/d/22F4TgV** (Accessed 03-07-2025)	**KYT-U100-MCS2812GS1**	**Kayeton**
4	**ATmega2560 microcontroller board with USB cable.**		**1**	**~$ 29.4**	**https://a.cod/d/B01H4ZLZLQ** (Accessed 3-07-2025)	**Mega 2560 R3**	**ELEGOO**

5	**Contact lickometer controller with a 28 VDC controlled MED output**		**1**	**~$350 - 400**	**-lickometer-controller** **(Accessed 3-07-2025)**	**ENV-250C**	**Med Associates inc**
6	UHU Patafix Glue Pad Removable Reusable White		**1**	**~$8-10**	**https://www.amazon.com/dp/B01DNWS4YK** **(Accessed 25-07-2025)**	**N/A**	**N/A**

Supplementary Fig 2. Installation Guide for FFMpeg and audio extraction for the Crunchometer

To successfully extract audio snippets using the Crunchometer program, it is imperative to install the FFmpeg library. FFmpeg will enable MATLAB to process video files and extract audio components. Note: failure to install FFmpeg will result in the output videos lacking audio tracks (**Video 5**).

Procedure

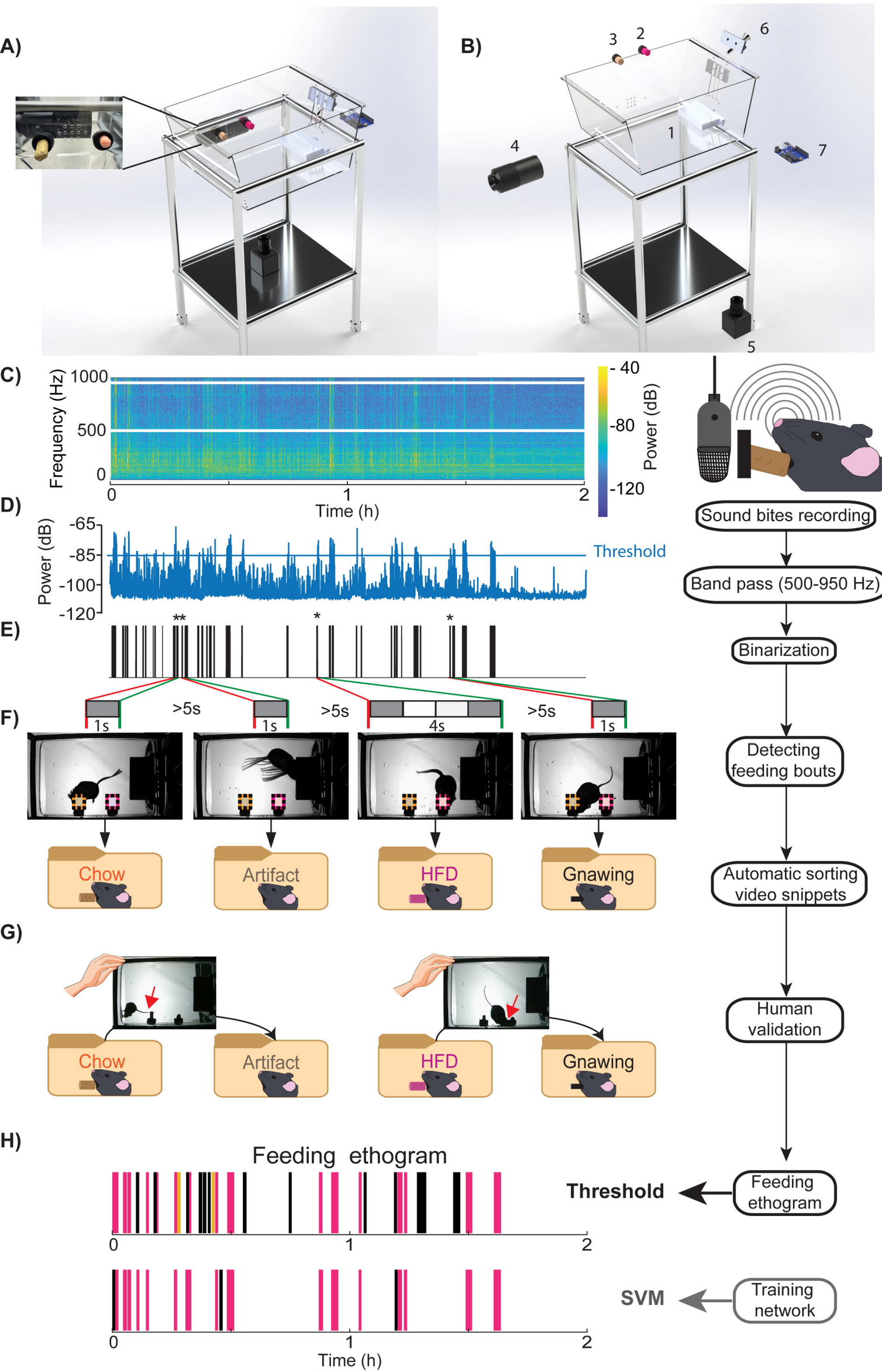
1. Download FFmpeg:
    - Navigate to the official FFmpeg website: https://ffmpeg.org/download.html
    - Download the latest stable version of FFmpeg that is compatible with your operating system.
2. Extract the Downloaded File:
    - Locate the downloaded FFmpeg archive file (usually a .zip or .7z file).
    - Right-click on the file and select "Extract All".
    - Choose the root directory of your C drive as the extraction destination. For example, C:\ffmpeg.
3. Identify the FFmpeg Executable:
    - The FFmpeg executable (ffmpeg.exe) is typically located within the bin subdirectory of the extracted folder.
    - Copy the full path name to the ffmpeg.exe file. For instance,
    - C:\ffmpeg-2024-10-17-git-e1d1ba4cbc-full_build\bin
4. Configure Environment Variables:
    - Right-click on the "Start" button and select "System".
    - Go to "About" and then click on "Advanced system settings".
    - In the "System Properties" window, navigate to the "Advanced" tab and click on "Environment Variables".
    - Under "System variables", find the "Path" variable and click "Edit".
    - Click "New" and paste the full path to the directory containing ffmpeg.exe (e.g., C:\ffmpeg\bin).
    - Click "OK" to save the changes.
5. Verify Installation:
    - Open a command prompt or terminal window.
    - Type the following command and press Enter: ffmpeg -version
    - If the installation was successful, you will see the installed version of FFmpeg displayed.

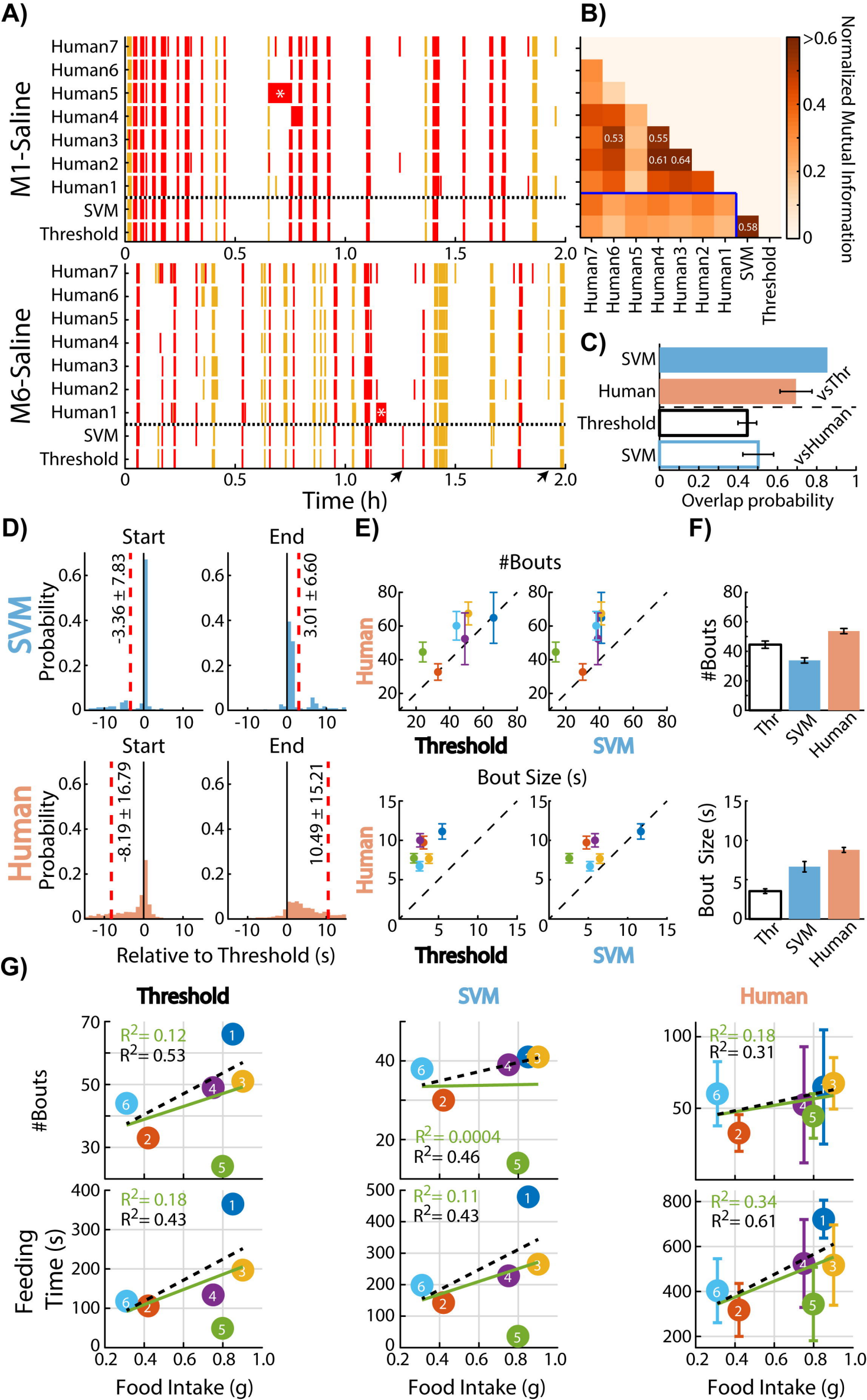
Additional Notes

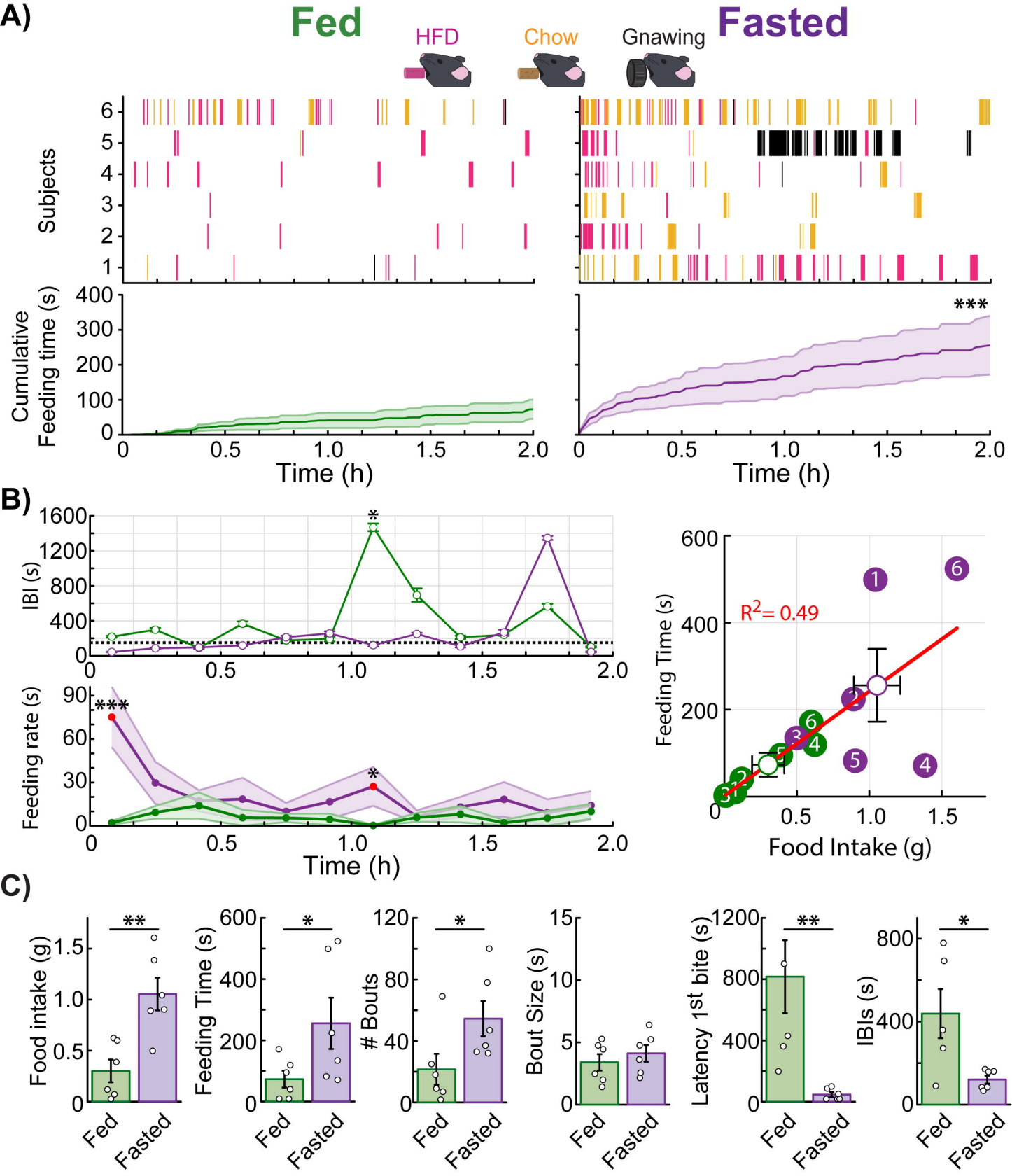
- Compatibility: Ensure that the downloaded FFmpeg version is compatible with your MATLAB installation.

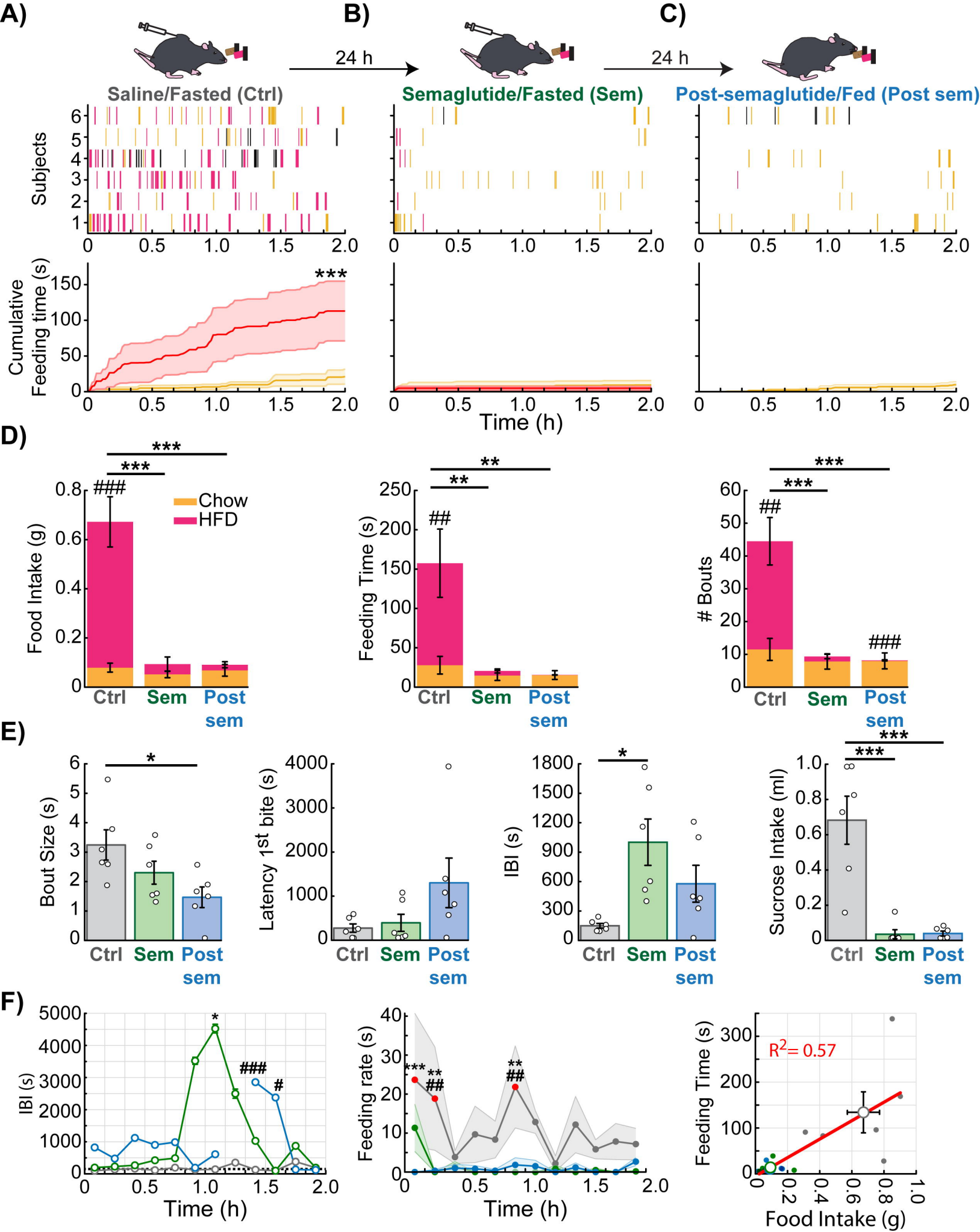
- Path Variable: Adding the FFmpeg directory to the system's PATH variable allows you to execute the ffmpeg command from any directory in your command prompt or terminal.
- Troubleshooting: If you encounter any issues, refer to the FFmpeg documentation for troubleshooting tips.

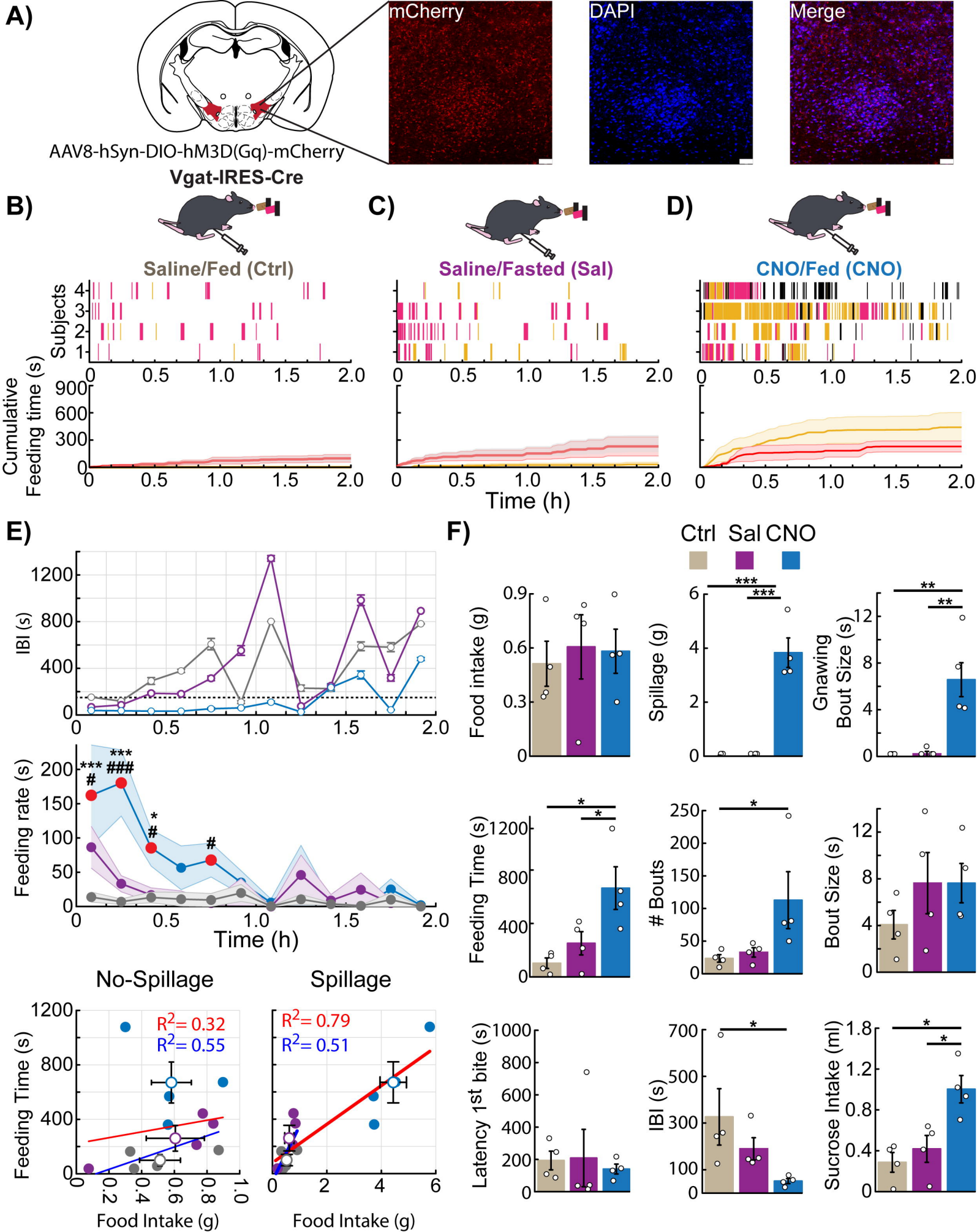
The Crunchometer

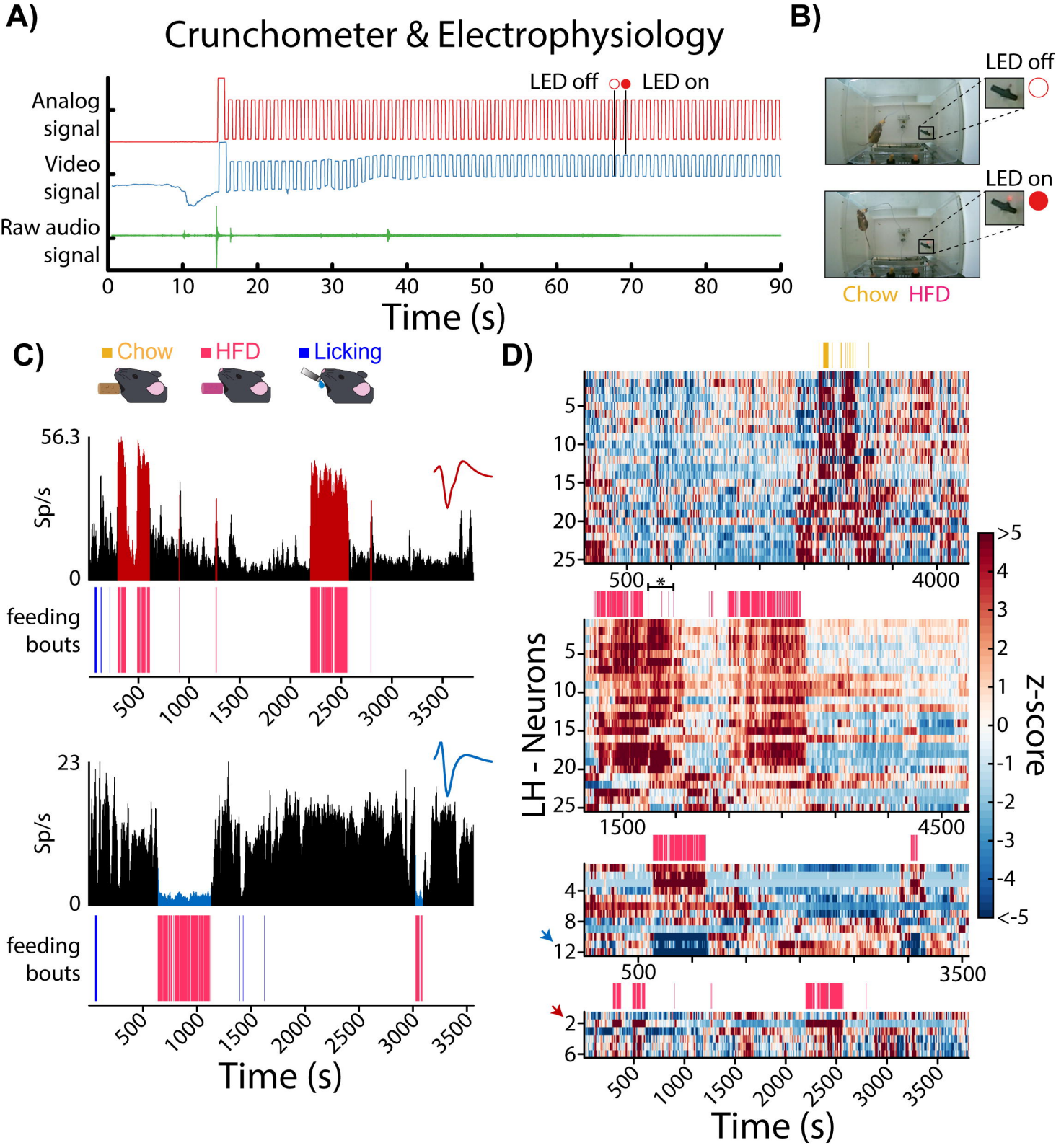


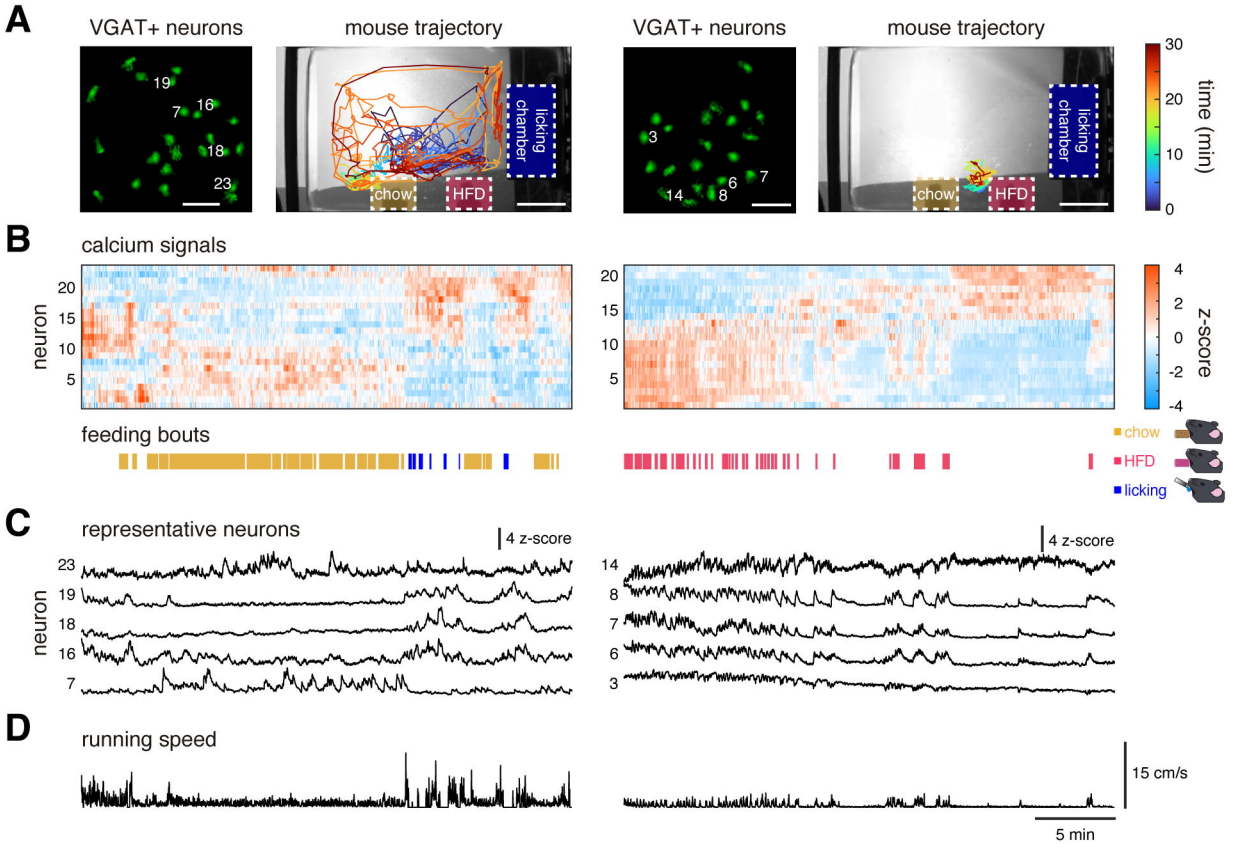

A)
M1-Saline
Human7
Human6
Human5
Human4
Human3
Human2
Human1
SVM
Threshold
M6-Saline
Time (h)
B)
Normalized Mutual Information
>0.6
0.4
0.2
0
0.53
0.55
0.61
0.64
0.58
C)
SVM
Human
Threshold
SVM
vsThr
vsHuman
Overlap probability
D)
Start
End
SVM
Human
Probability
-3.36 ± 7.83
3.01 ± 6.60
-8.19 ± 16.79
10.49 ± 15.21
Relative to Threshold (s)
E)
#Bouts
Bout Size (s)
Human
Threshold
SVM
F)
#Bouts
Bout Size (s)
Thr
SVM
Human
G)
Threshold
SVM
Human
#Bouts
Feeding Time (s)
R2= 0.12
R2= 0.53
R2= 0.0004
R2= 0.46
R2= 0.18
R2= 0.31
R2= 0.18
R2= 0.43
R2= 0.11
R2= 0.43
R2= 0.34
R2= 0.61
Food Intake (g)

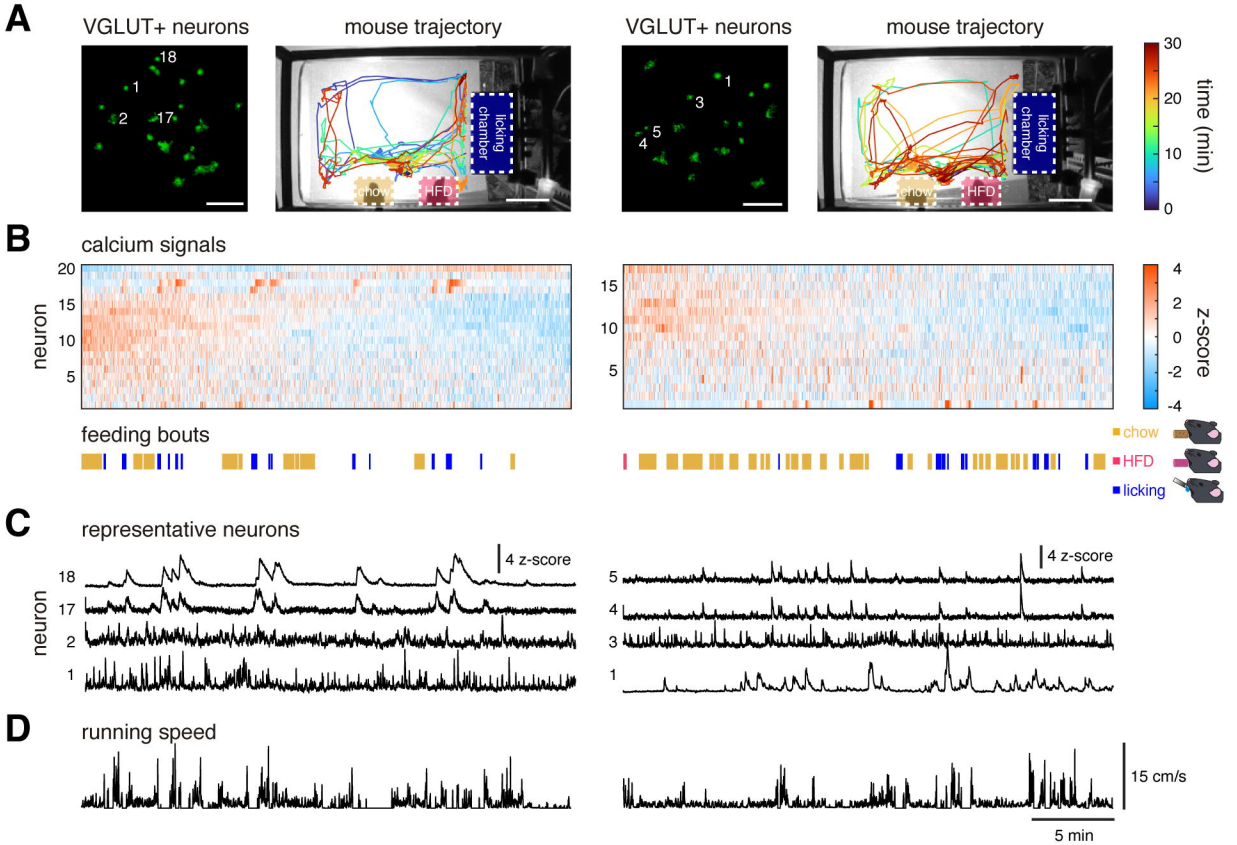

A)
Fed
Fasted
HFD
Chow
Gnawing
Subjects
Cumulative Feeding time (s)
Time (h)
***
B)
IBI (s)
Feeding rate (s)
Time (h)
Feeding Time (s)
Food Intake (g)
R^2= 0.49
C)
Food intake (g)
Feeding Time (s)
Bouts
Bout Size (s)
Latency 1st bite (s)
IBIs (s)
Fed
Fasted


A)
B)
C)
24 h
24 h
Saline/Fasted (Ctrl)
Semaglutide/Fasted (Sem)
Post-semaglutide/Fed (Post sem)
Subjects
Cumulative Feeding time (s)
Time (h)
D)
Food Intake (g)
Feeding Time (s)
Bouts
Chow
HFD
Ctrl
Sem
Post sem
E)
Bout Size (s)
Latency 1st bite (s)
IBI (s)
Sucrose Intake (ml)
F)
IBI (s)
Feeding rate (s)
Feeding Time (s)
Food Intake (g)
R2= 0.57


A)
mCherry
DAPI
Merge
AAV8-hSyn-DIO-hM3D(Gq)-mCherry
Vgat-IRES-Cre
B)
C)
D)
Saline/Fed (Ctrl)
Saline/Fasted (Sal)
CNO/Fed (CNO)
Subjects
Cumulative Feeding time (s)
Time (h)
E)
IBI (s)
Feeding rate (s)
Time (h)
F)
Ctrl Sal CNO
Food intake (g)
Spillage (g)
Gnawing Bout Size (s)
Feeding Time (s)
Bouts
Bout Size (s)
Latency 1st bite (s)
IBI (s)
Sucrose Intake (ml)
No-Spillage
Spillage
R2= 0.32
R2= 0.55
R2= 0.79
R2= 0.51
Feeding Time (s)
Food Intake (g)
Food Intake (g)


A)
Crunchometer & Electrophysiology
LED off
LED on
Analog signal
Video signal
Raw audio signal
0
10
20
30
40
50
60
70
80
90
Time (s)
B)
LED off
LED on
Chow
HFD
C)
Chow
HFD
Licking
56.3
Sp/s
0
feeding bouts
500
1000
1500
2000
2500
3000
3500
23
Sp/s
0
feeding bouts
500
1000
1500
2000
2500
3000
3500
Time (s)
D)
LH - Neurons
5
10
15
20
25
500
4000
*
1500
4500
4
8
12
500
3500
2
6
500
1000
1500
2000
2500
3000
3500
Time (s)
z-score
>5
4
3
2
1
0
-1
-2
-3
-4
<-5


A
VGAT+ neurons
mouse trajectory
VGAT+ neurons
mouse trajectory
19
7
16
18
23
licking chamber
chow
HFD
3
14
8
6
7
licking chamber
chow
HFD
30
20
10
0
time (min)
B
calcium signals
neuron
20
15
10
5
20
15
10
5
4
2
0
-2
-4
z-score
feeding bouts
chow
HFD
licking
C
representative neurons
4 z-score
neuron
23
19
18
16
7
4 z-score
14
8
7
6
3
D
running speed
15 cm/s
5 min


A
VGLUT+ neurons
mouse trajectory
VGLUT+ neurons
mouse trajectory
18
1
2
17
1
3
5
4
chow
HFD
licking chamber
time (min)
30
20
10
0
B
calcium signals
neuron
20
15
10
5
z-score
4
2
0
-2
-4
feeding bouts
chow
HFD
licking
C
representative neurons
neuron
18
17
2
1
4 z-score
5
4
3
1
D
running speed
15 cm/s
5 min