Striatal signaling tracks naturalistic short-term fluctuations in hunger-satiety

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Abstract

The neuromodulator dopamine is integral to feeding behavior, believed to modulate food pursuit and satiety. Here, we examine how dopamine and spiny projection neurons (SPNs) signaling in the nucleus accumbens changes during consumption as animals transition from hunger to satiety in naturalistic feeding. Both dopamine and SPNs transiently increase during food approach; however, the magnitude of this approach-related increase diminishes across progressive pellet ingestion, reflecting short-term satiation. Fasting dissociates the regulation of meal size and frequency-- termination and initiation, respectively-- with altered dopamine corresponding to changes in meal size but not frequency. Despite substantially decreasing feeding, pharmacological satiation via a glucagon-like peptide-1(GLP-1) agonist had no impact on dopamine signaling, suggesting that the GLP-1 agonist disengaged or decoupled dopamine from its modulatory role in food seeking.

Introduction

Appetition and satiation are critical to energy management, are highly regulated and complex, and contain many redundant systems (Fazzino, 2022; Valassi et al., 2008). A negative energy balance can lead to malnutrition and death, while a surplus increases the risk of obesity (Nakamura and Nakamura, 2018). While motivation for food pursuit is critical, limiting consumption is equally necessary.

Dopamine (DA) is widely believed to play a central role in driving appetitive pursuit, with evidence spanning multiple methodological approaches. Early findings from microdialysis studies suggest increased dopamine levels during feeding (Hernandez and Hoebel, 1988a, 1988b); however, the technique lacks sufficient temporal resolution to capture rapid fluctuations. Research utilizing pharmacological and genetic manipulations further demonstrated a crucial role for dopamine in feeding behavior (Cannon and Palmiter, 2003; Szczypka et al., 1999; Zigmond and Stricker, 1973). For example, dopamine deficient mice will not eat and will die unless administered levodopa (Zhou and Palmiter, 1995). While considerable data suggest that dopamine facilitates food-seeking, it has also been proposed conversely that diminution in dopamine activity can reduce food intake, some hypothesizing that diminished dopamine activity mediates satiety and disengagement from eating (Cassidy and Tong, 2017).

Midbrain DA is sensitive to regulation via circulating signals that reflect the animals current energetic state and convey post-ingestive feedback, including insulin, leptin, ghrelin and numerous others (Cassidy and Tong, 2017; Liu and Borgland, 2015). This sensitivity provides a mechanism whereby modulation of dopamine could link behavioral activation or disengagement to changing physiological energy states. Insulin resistance, a common feature of obesity can disrupt this feedback loop, potentially impairing a putative role for dopamine in stopping eating in response to satiation (Speed et al., 2011). The effects of these energy signals on dopamine can be complex and difficult to characterize. For example, insulin effects on dopamine vary in a regional and time-dependent manner (Liu and Borgland, 2015; Sallam and Borgland, 2021), often exerting both positive and negative modulation. The extent to which the complex effects of energy signals might be integrated in the dopamine system to signal the transition from hunger to satiety and, in turn, modulate consumption and meal patterning, has not been evaluated.

Prior studies have examined DA in awake behaving animals during feeding, but these studies have been in structured operant tasks, employed food restriction, and typically focused on reward-related behavior rather than changes in appetitive drive or satiation (e.g., Amin and Mercer, 2016; Avena et al., 2013; Li et al., 2020). Indeed, by employing food restriction and limiting the number of pellets an animal can obtain during a single session of such tasks, the goal is specifically to eliminate the confound of changes in DA or behavior due to satiation.

Moreover, persistent food restriction induces profound changes across neurophysiological regulatory systems, including increased insulin sensitivity, decreased circulating leptin, chronic stress activation, and many others (Nolan et al., 2020; Zhen et al., 2006). Such changes can have significant functional consequences, including altering brain reward stimulation (Cabeza de Vaca et al., 2004; Elder et al., 1965; Gnazzo et al., 2021). Carr and colleagues have demonstrated that food restriction alters both the dopamine system and target regions and can create greater vulnerability to drugs of abuse (Carr, 2007, 2002). Whether real-time DA release and spiny projection neuron (SPN) activity track the transition from naturally occurring hunger to satiety has not been examined. Thus, to date, there has not been a careful study of how DA signaling changes in non-food restricted/naturalistic feeding for the purposes of determining how DA mediates both appetitive behavior and the reduction of that appetitive behavior, i.e, satiation.

Here we use fiber photometry in a naturalistic feeding paradigm to track real-time DA and SPN activity across feeding, providing a view into how these signals change with progressive satiation. We then examine how DA signaling across feeding is altered by overnight fasting and pharmacological satiation with a glucagon-like peptide-1(GLP-1) agonist. DA and direct spiny projection neuron (dSPN)/indirect spiny projection neuron (iSPN) activity increases during food approach, as expected. However, transients in both DA and dSPN activity on approach vary within and between meals, primarily in the nucleus accumbens core (NAcc), reflecting short-term fluctuations in hunger and satiety with progressive ingestion. Fasting revealed a dissociation between initiating and terminating meals, suggesting that dopamine, at least as we observe and measure it here, modulates termination but not initiation of meals. Pharmacological satiety induced by a GLP-1 agonist, in contrast, blunted variability in DA transients associated with short-term fluctuations in hunger and satiety and effectively decoupled DA from meal patterning.

Materials and Methods

Animals

Mice of both sexes of approximately 120 days of age were used for all experiments. We used selective cre-recombinase lines to target GCaMP to indirect pathway spiny projection neurons (iSPNs; Adora-cre: B6.FVB(Cg)-Tg(Adora2a-Cre)KG139Gsat/Mmucd; GENSAT, 036158-UCD) or direct pathway SPNs (dSPNs; D1-cre: B6.FVB(Cg)-Tg(Drd1a-cre)EY262Gsat/Mmucd; GENSAT, 030989-UCD). Mice were singly housed after surgery to protect cranial implants. Mice were maintained on a 12:12 hour light-dark cycle and provided ad libitum water and food (either freely available on cage floor before testing or freely available via automated feeder

during testing). All procedures were approved by the Queens College Institutional Animal Care and Use Committee.

Automated feeding paradigm

Mice were habituated to the experimental room and grain pellets for five days prior to testing. Mice were then moved to chambers with automated feeders (FED3, Matikainen-Ankney et al., 2020) that dispensed a single 20 mg grain pellet at a time (Bioserv, cat# F0163). Mice could retrieve pellets *ad libitum* with no work requirement; after each pellet is retrieved, another is immediately made available. Every pellet retrieval was time-stamped and recorded. Mice were allowed 1-2 days to adjust to the automated feeder and then feeding was monitored for 4 days to assess meal patterns. We recorded with fiber photometry the first four hours after the onset of the active/dark cycle of the 4th day. For fasting experiments, the pellet dispenser was turned off and no food pellets were available for the fasted group on day three of baseline prior to recording. At the onset of the active cycle the following day, the pellet dispensers were turned on and fiber photometry recorded.

Pharmacological satiety

Exendin-4 (Ex-4), a GLP-1 agonist, was used to induce pharmacological satiety (Kjaergaard et al., 2019). Ex-4 (Cat# E7144, Sigma-Aldrich, Saint Louis, MO) was dissolved in 0.9% saline and given either 90 µg/kg (pharmacological satiety group) or saline controls (Veh) via subcutaneous injections 45 minutes prior to onset of the dark cycle. To reduce stress of injections, mice were handled and given 'mock' injections (touch tip of capped syringe to domed skin) for 3 days prior to Ex-4 administration. In this experiment, we recorded only the first two hours of feeding. Based on previous findings, we expected the lead time to be forty-five minutes post s.c. injection and the effects of Ex-4 on hunger and satiety to last about two hours (Kjaergaard et al., 2019).

Fiber photometry

We co-expressed the red dopamine sensor RdLight1 (AAV9-Syn-RdLight1; Cat# 1646-AAV9) and the green calcium sensor GCaMP6f (AAV5.Syn.Flex.GCaMP6f.WPRE.SV40; Cat# 100833-AAV5), targeting GCaMP to either d- or i- SPNs using the D1-cre or Adora-cre mouse lines noted above. 350 nL of the RdLight1/GCaMP6f virus mixture were injected into either the nucleus accumbens core (NAcc; AP: +1.3mm, LAT: +.9mm, DV: −4.25) or shell (NAcSH; AP: +1.3mm, LAT: +.7mm, DV: −4.4) and mice were allowed three weeks for recovery and expression of the sensors. Fluorescent signals were recorded using a Neurophotometrics dual-color fiber photometry system.

For signal processing, RdLight1, GCaMP6f, and isosbestic signals were de-interleaved. We denoise each signal by computing the simple moving average with a 10-data-point window. We correct for photobleaching with a moving mean algorithm using reweighted penalized least squares (Martianova et al., 2019; Zhang et al., 2010). Signals are standardized using Z-scores. To calculate ΔF/F signals and correct for movement artifacts, isosbestic control signals were fitted, scaled, and subtracted from RdLight1 and GCaMP6f signals, then divided by the fitted isosbestic signal (Martianova et al., 2019). Due to the extended 4-hour recording period, we adjusted the LED's excitation power to 40 μW, reduced the sampling rate to 20 Hz, and incorporated blank frames into the duty cycle to enhance our signal-to-noise ratio and minimize photobleaching. We used only fiber optic implants with a greater than 85% light efficiency (Li et al., 2019; London et al., 2018).

Statistical Analyses: Signal processing was performed using a custom-written script in MATLAB (RRID:SCR_001622, Mathworks Inc., MA). All other analyses, statistics, and graphs were generated using R Project for Statistical Computing, v. 4.4.1 (RRID:SCR_001905). ANOVA was used to test the significance of the behavior data, followed by a Tukey post-hoc pairwise comparison. The statistical significance of fiber photometry data was evaluated using linear mixed-effects models (LMM) with the lme4 R package (RRID:SCR_015654, Bates et al., 2015). The LMM included individual mice as a random variable. *p* values and degrees of freedom were calculated from LMM models using Satterthwaite's degrees of freedom method in the lmerTest R package. We used a mixed logistic regression (MLR, estimated using maximum likelihood and the Nelder-Mead optimizer) to predict binary outcomes while needed to account for within-subjects data, again including individual mice as a random variable. 95% Confidence Intervals (CIs) and p-values were computed using a Wald z-distribution approximation.

Results

Mice exhibit stable free-feeding patterns with automated feeders

We used dual color fiber photometry in mice to measure real-time changes in extracellular DA (red signals) and either direct or indirect pathway spiny projection neuron activity (green signals) in the striatum across phases of feeding, including approach, consumption, and post-consumption (recovery) in an automated free-feeding paradigm (Figure 1A). Mice could collect a 20 mg pellet at will, after which a new pellet was immediately dispensed and available. The phases of feeding were defined as windows of time relative to pellet retrieval (time 0). The baseline was defined as -10 to -2 s before pellet retrieval; food approach as the 2s immediately prior to pellet retrieval (-2 to 0), consumption was 1 to 3 s post pellet retrieval, and recovery was 6 to 10 s post pellet retrieval, similar to London et al. (2018).

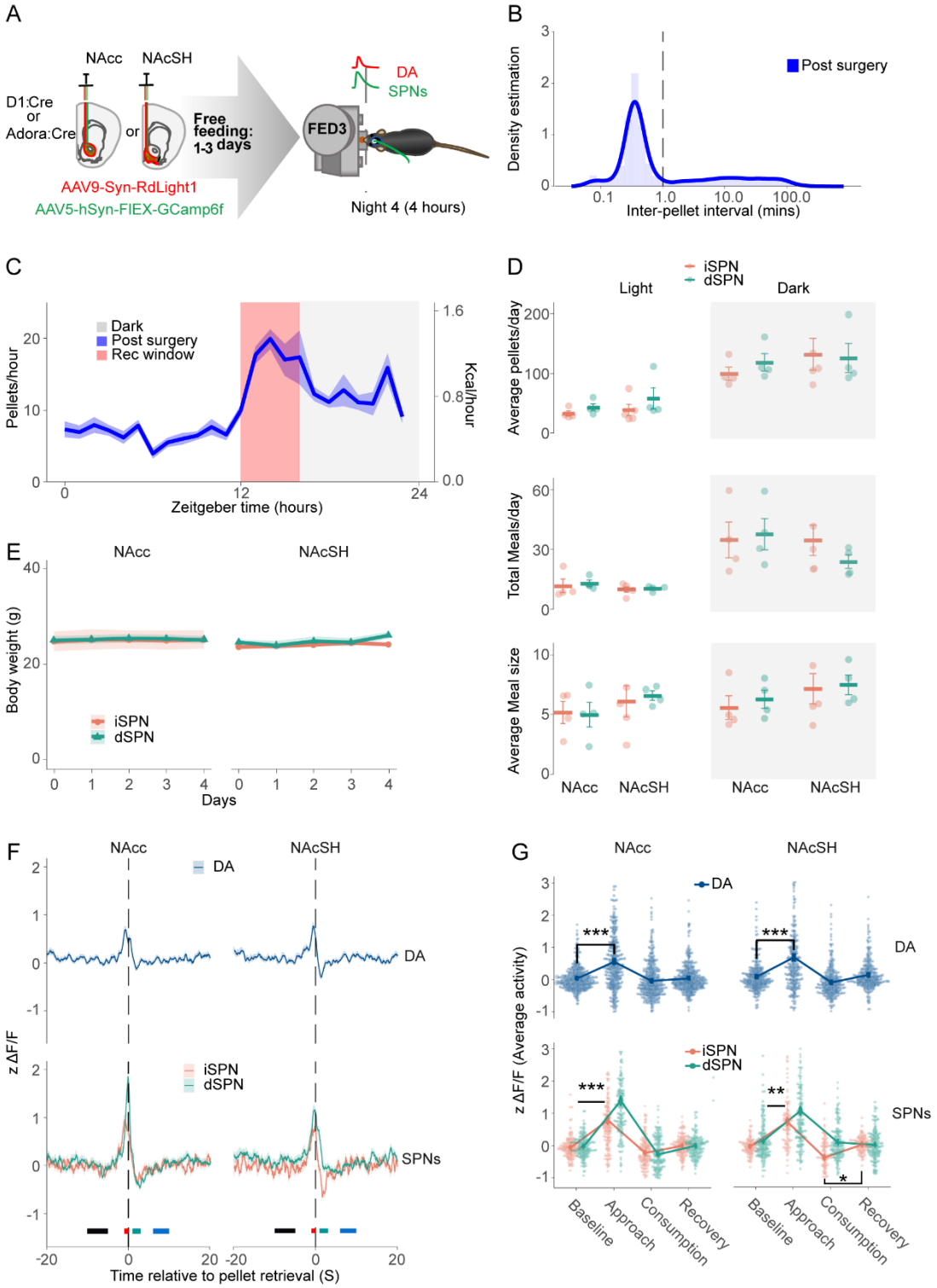

Figure 1. DA and MSN activity increases during food approach in natural feeding. **A)** Schematic of concurrent recordings of DA and SPN activity for 4 hours at the onset of the dark cycle in free-feeding mice. **B)** Interpellet interval density plots for free-feeding (defining a meal). **C)** Mean number of pellets/hr across a 24-hr period (all mice). **D)** The total number of pellets, meals, and average meal size for all four days of feeding by groups and cycle. **E)** Body weights over four days by groups. **F)** Average dopamine release (Z scores) relative to pellet retrieval for direct and indirect SPN activity grouped by pathway and sub-region of the nucleus accumbens. Black, red, green, and blue rectangles indicate feeding stages: baseline, approach, consumption, and recovery, respectively. **G)** Average activity of DA and d/iSPNs during feeding stages for all groups. Data are presented as the mean ± s.e.m. in *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Examining the interpellet interval across all mice (Figure 1B), most pellets were consumed within a minute. We defined a 'meal' as a string of consecutive pellet retrievals with interpellet intervals of less than one minute. A break longer than one minute from retrieval/eating was considered the start of a new meal (Matikainen-Ankney et al., 2021).

Consistent with prior studies (Matikainen-Ankney et al., 2021), food intake at the onset of the dark cycle peaked at approximately 20 pellets/hour, during which mice consume, on average, 44.76 $\pm$ 3.5 pellets over the first 4 hours of the night cycle. There was little variation across mice in pellets eaten during 24-hour periods (Figure 1C, $F_{(16,284)} = 0.99$, $p = 0.47$). We recorded this first 4 hours (shaded red rectangle, Figure 1C).

We compared four groups of mice distinguished by the population and location of cells from which we recorded GCaMP calcium signals (see methods): (1) dSPNs in the nucleus accumbens core (NAcc), (2) dSPNs in the nucleus accumbens shell (NAcSH), (3) iSPNs in the NAcc, and (4) iSPNs in the NAcSH, referred to as dSPN-core, dSPN-shell, iSPN-core, and iSPN-shell, respectively. We concurrently recorded red RdLight DA signals and pathway-specific SPN calcium signals from all mice. Total consumption (pellets per day), the number of meals per day, and average meal size did not differ significantly across the defined groups of mice (Figure 1D), nor did their body weights (Figure 1E). The mice exhibited consistent, stable feeding patterns across all groups and days.

Dopamine and striatal spiny-projection neuron activity increase during food approach

No differences in DA release were observed between the Adora-cre and D1-cre mice, which targeted GCaMP to iSPN and dSPN cells, respectively ($F_{(1,12)} = 0.11$, $p = 0.75$); therefore, we combined the DA data from these lines. DA release increased during approach, i.e., immediately preceding pellet retrieval, in both the NAcc and NAcSH in all mice (Figure 1F-G, top blue traces, approach vs. baseline, $F_{(3, 2706)} = 126.6$, $p < .001$). Comparing DA release between the NAcc and NAcSH, both show increased activity on the approach to food pellets in both d- and i-SPN cells, with no significant differences between the regions.

Similarly, SPN activity in both the direct and indirect pathways increased robustly during food approach immediately preceding pellet retrieval in both the NAcc and NAcSH (Figure 1F-G, lower panels, $F_{(3, 2366)} = 341.8$, $p < .001$), consistent with prior reports (London et al., 2018). During consumption, a decrease was observed in the indirect pathway in the shell only (Figure 1F, Tukey post-hoc: $t_{(2366)} = -4.14$, $p < 0.01$). These data indicate that transient increases in DA and dSPN activity correlates to food approach rather than consumption.

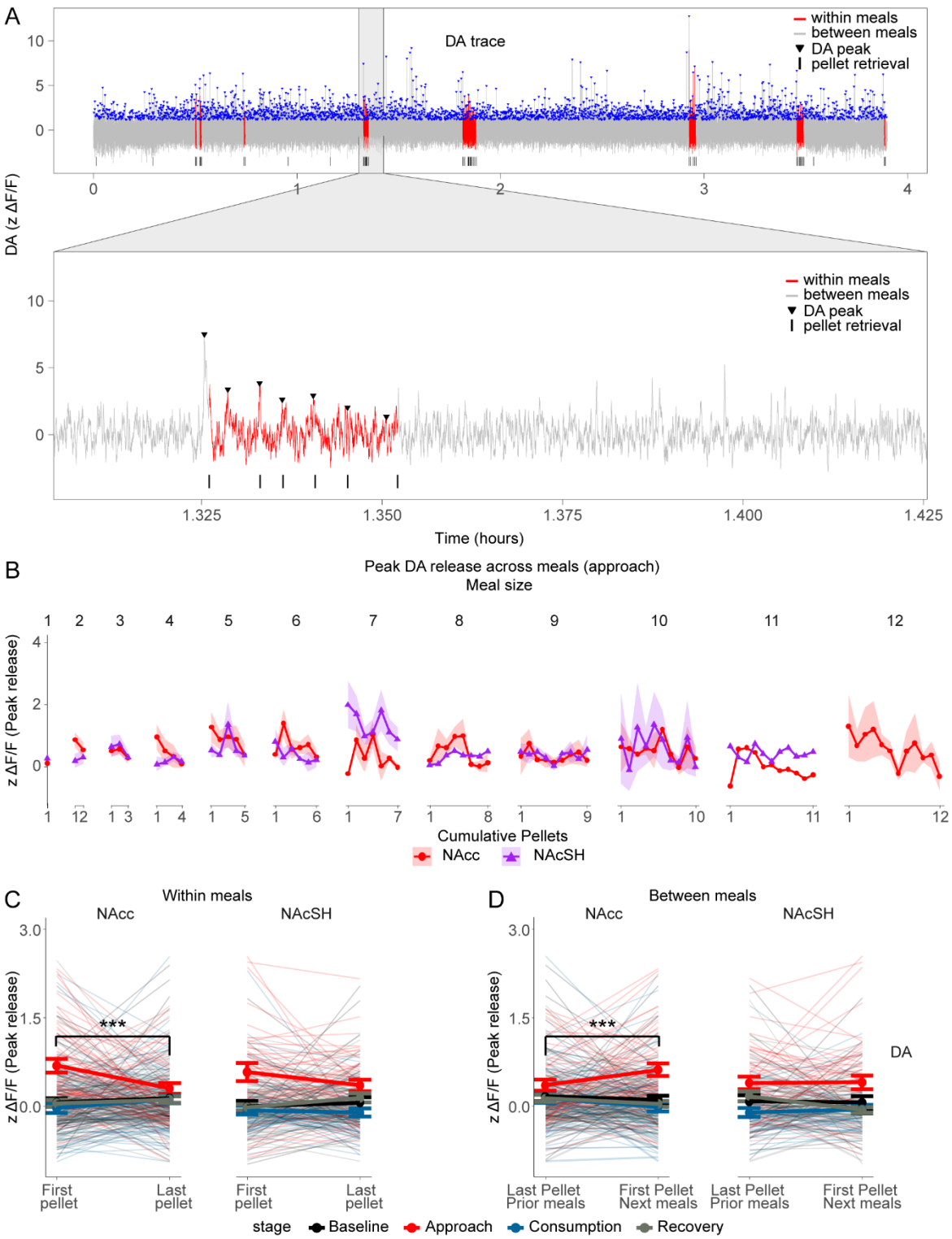

Figure 2. Dopamine activity attenuated as mice reached satiation in the NAcc. **A)** Top: An example DA trace across time (top), red and grey, indicating that DA occurred within or between meals, respectively. Blue down-facing triangles indicated DA peaks above the threshold of 1. Bottom: A zoomed-in plot showing the progressive decline of DA release; the vertical black line indicates pellet revival. **B**) The average DA release in the NAcc and NAcSH was grouped by meal size across cumulative pellets during food approach. **C**) Average DA activity from the first and last pellet of a meal grouped by the NAcc and NAcSh. **D**) Average DA release from the last (prior meal) and first pellet (next meal) between meals grouped by the NAcc and NAcSH.

Dopamine activity attenuates as mice reach satiation in the NAcc

We inspected the quality of the DA traces for artifacts and an appropriate signal-to-noise ratio; most transient peaks exceeded one z-score (blue triangles in example trace, Figure 2A). We observed progressive changes in DA release occurring during food approach within individual meals. Specifically, DA release declines within a meal (Figure 2A, red traces) across consecutive food approaches (Figure 2A, bottom panel, black triangles). As meal lengths varied, we summarized changes in dopamine signals at each pellet retrieval across consecutive pellets within a meal, separating meals of different sizes (Figure 2B). In the NAcc, we observe a general pattern of peak DA release at food approach, which declines across individual meals (red trace), an effect most prominent in both shorter and longer meals. In contrast, in the NAcSH (purple trace), DA release at food approach did not exhibit a consistent pattern across pellets within individual meals. We quantified these changes in peak DA by comparing the first and last pellets of meals (Figure 2C). Consistent with the patterns observed visually in Figure 2A, we found a significant difference in peak DA release at food approach from the first to the last pellet of a meal in the NAcc (Tukey: $t_{(539)} = 6.77$, $p < .001$), but not the NAcSH (Tukey: $t_{(539)} = 1.29$, $p = 0.90$). These data demonstrate a short-term, progressive decrease in peak DA during pellet approach from initiation to termination of a meal, consistent with short-term satiety; that is, progressive ingestion of pellets attenuates DA signaling during food approach, suggesting declining appetitive motivation.

To determine if the decline in DA between meals recovered, we compared peak DA at pellet approach between the last pellet of a meal to the first pellet of the subsequent meal. Peak DA release recovered between meals; DA at the first pellet of a subsequent meal was increased compared to the last pellet of the previous meal (Figure 2D). Again, this was observed only in the core (Tukey: $t_{(911)} = -4.04$, $p < .01$) and not the shell (Tukey: $t_{(911)} = -2.61$, $p = 0.391$). This recovery of peak DA suggests an underlying diminution of short-term satiety that restores appetitive food pursuit.

We examined whether the magnitude of peak DA release on the first pellet of a meal corresponds to the length of that meal, i.e., whether a higher initial peak leads to longer meals. We first compared this DA peak from all meals larger than one pellet to single pellet meals. Peak DA at approach is significantly higher in multi-pellet compared to single-pellet meals in the NAcc (Figure 3A, Tukey: $t_{(222)} = 3.22$, $p < 0.01$) but not the NAcSH (Figure 3A, Tukey: $t_{(222)} = 1.58$, $p = 0.392$). This suggests that the magnitude of DA release at the beginning of a meal could influence meal length.

We tested whether the initial DA peak correlates to meal length for each feeding stage. Unexpectedly, it does not for approach ($r = -0.03$, $p = 0.726$) nor the other feeding stages. To

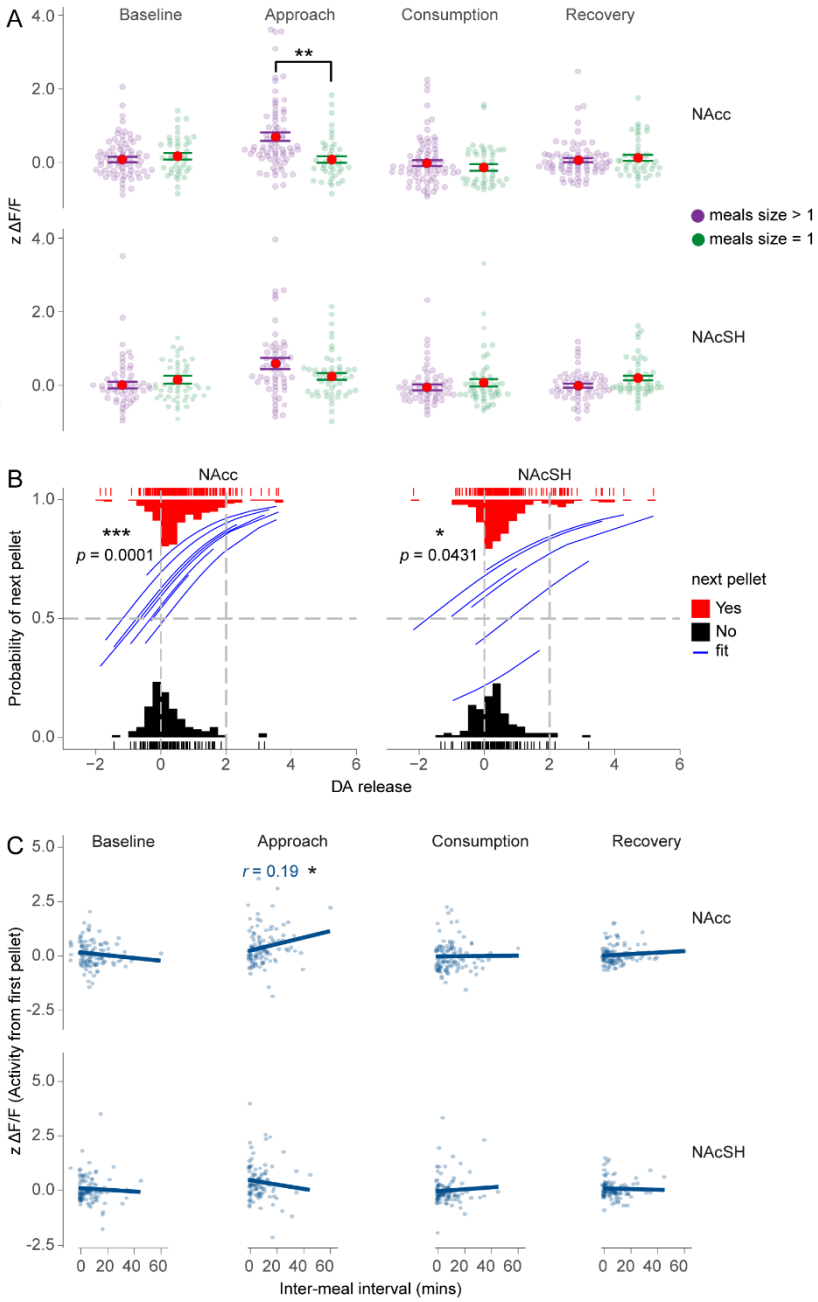

Figure 3. Dopamine release influences meal patterns**. A)** The DA release of meals larger than one pellet compared to meals that are exactly one pellet for baseline, approach, consumption, and recovery. **B)** A mixed logistic regression model showing that peak DA release significantly predicts the probability of the next food pellet in the NAcc. Blue traces represent the fit of each mouse. **C)** Linear regression of the average DA release for the first pellet of a meal during food approach predicted by the inter-meal interval. Error bars: ± s.e.m.; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

further probe the relationship between DA release and meal size/length, we tested whether DA release facilitates subsequent pellets, leading to longer meals. To achieve this, we estimated the relationship between DA and the binary outcome of taking or not taking the next pellet, accounting for the within-subject nature of our data using a mixed logistic regression. Higher DA release on approach to pellet retrieval significantly increased the probability of a next food pellet being taken (blue traces indicate each mouse predicted fit) in the same meal for both the NAcc (Figure 3B, MLR: Conditional R^2 = 0.17, 95% CI [0.33, 0.87], β = 0.6, $p < 0.001$) and NAcSH (Figure 3B, MLR: Conditional R^2 = 0.18, 95% CI [0.008, 0.56], β = 0.28, $p = 0.043$).

We examined whether the initial DA peak at the beginning of a meal was influenced by the length of time since the last meal. Indeed, the recovery of dopamine peak at the first pellet of a meal (Figure 2D) does increase with time since termination of previous meal in the NAcc (Figure 3C, $F_{(1, 108)} = 4.09$, $p < 0.05$) but not in the shell (Figure 3E, $F_{(1, 101)} = 1.06$, $p = 0.306$). No

relationship was observed between DA signals and the length of time since the last meal in other segments of the signal (i.e., baseline, consumption, or recovery).

These data demonstrate a relationship between the magnitude of DA transients associated with food approach and the regulation of eating on a minute-to-minute timescale. Within a meal, peak dopamine declines with successive pellet consumption, consistent with short-term satiety. The longer the mouse waits to eat, the higher the DA release is at the start of a meal. Moreover, greater DA release facilitates taking a next pellet, extending the size of the meal, though this effect is probabilistic. Thus, overall, the higher the dopamine release, the more likely the mouse is to continue eating.

dSPNs activity attenuated as mice reached satiation in the Nacc

We similarly evaluated progressive changes in SPN activity at pellet approach within and between meals. In both the NAcc and NAcSH, dSPN activity gradually decreased with successive pellet retrieval within a meal (Figure 4A, green traces), an effect less clear in iSPNs (Figure 4A, orange traces). Comparing the first and last pellets of meals as above, we found in that both dSPN activity (Figure 4B, Tukey: $t_{(880)} = 5.53$, $p < .001$) and iSPN activity (Figure 4B, Tukey: $t_{(880)} = 3.85$, $p < .01$) significantly decreased on approach across pellets within a meal in the NAcc. In NAcSH, there was a trend toward reducing dSPN activity (Figure 4B, Tukey: $t_{(880)} = 4.39$, $p = 0.079$), but no decrease was observed in iSPN activity (Figure 4B, Tukey: $t_{(880)} = 0.69$, $p = .99$).

Examining changes in SPN between meals, dSPN activity increases from the last pellet of a preceding meal to the first pellet of the next meal in the NAcc (Figure 4C, Tukey: $t_{(768)} = 4.65$, $p < 001$) but not in the NAcSH (Figure 4C, Tukey: $t_{(768)} = 3.33$, $p = 0.20$). This suggests, in the NAcc, that the diminution of dSPN activity at pellet retrieval across successive pellets within a meal recovers between meals, similar to the recovery observed in DA. There is not a significant recovery in iSPNs in the NAcc (Figure 4C, Tukey: $t_{(768)} = 3.22$, $p = 0.26$) or NAcSH (Figure 4C, Tukey: $t_{(768)} = 0.01$, $p = 0.99$).

To determine if a higher initial peak of SPN activity for the first pellet of a meal corresponds to longer meals, we compared d/iSPN activity from all meals larger than one pellet to single pellet meals across feeding stages. Consistent with DA release in the NAcc, we found that only dSPN activity during pellet approach is significantly higher in meals larger than a single pellet approach in the NAcc (Figure 4D, top, dSPNs on approach, Tukey: $t_{(266)} = 3.97$, $p < 0.01$; iSPNs, Tukey: $t_{(260)} = 2.11$, $p = 0.41$) but not in the NAcSH (Figure 2D, bottom, dSPNs on approach, Tukey: $t_{(266)} = 0.796$, $p = 0.99$).

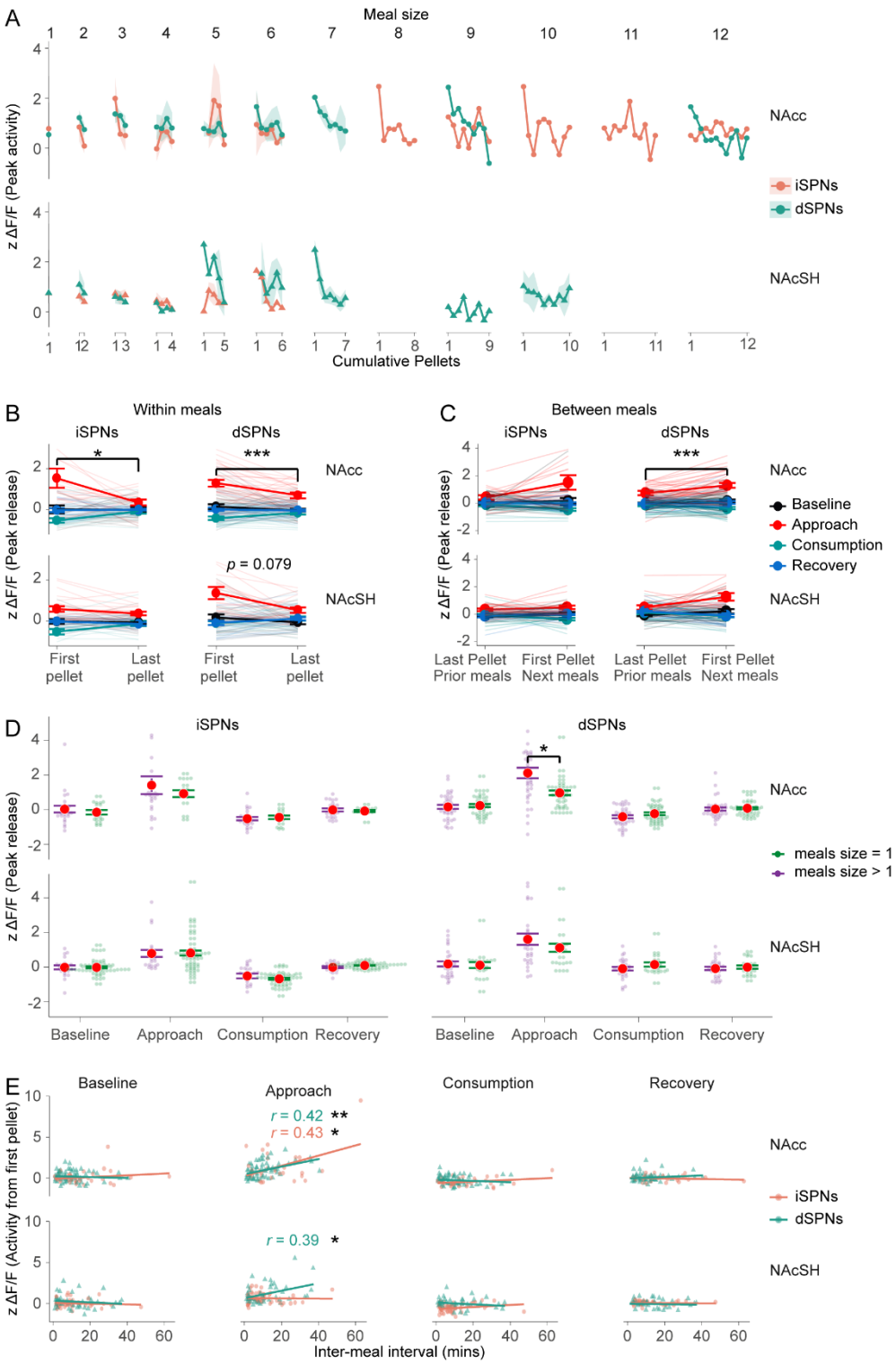

Figure 4. dSPNs activity attenuated as mice reached satiation in the NAcc**. A)** The average d/iSPNs activity in the NAcc and NAcSh was grouped by meal size across cumulative pellets during the approach. **B)** Average d/iSPNs activity from the first and last pellet of a meal grouped by the NAcc and NAcSH. **C)** Average d/iSPNs activity from the last (prior meal) and first (next meal) pellet between meals grouped by the NAcc and NAcSh. **D)** Average SPN activity from the last and first pellet between meals grouped by the NAcc and NAcSH. **E)** For each feed stage, a best-fit line is drawn for the inter-meal interval in relation to the d/iSPN activity. Activity is at the first pellet of a meal.

We examined whether recovery of the initial peak in SPN activity at meal onset was influenced by the time elapsed since the last meal. In the NAcc, recovery of both dSPN and iSPN activity peaks on approach for the first pellet of the next meal was increased by the length of time since the last meal (Figure 4E, dSPNs: $F_{(1, 57)} = 11.9$, $p < 0.001$; iSPNs: $F_{(1, 30)} = 6.74$, $p < 0.05$). In the NAcSH, only the dSPNs demonstrated a relationship between peak activity at first pellet in a meal and time elapsed (Figure 4E, dSPNs: $F_{(1, 38)} = 6.88$, $p < 0.05$; iSPNs: $F_{(1, 51)} = 0.029$, $p = 0.865$). As with DA signals, time elapsed between meals had no effect on other behavioral segments, i.e., baseline, consumption, and recovery. To test whether DA and SPN activity correlate, we performed cross-correlations for each mouse. In all mice, DA correlated significantly with both dSPNs and iSPNs in the NAcc both within and between meals and within in dSPNs but not iSPNs in the NAcSH (supplemental Figure 3 A-C).

Overall, dSPN activity mirrors DA activity exhibiting clear modulation in signaling in the NAcc with regard to pellet approach and changes across and between meals. This dSPN eating related modulation is also evident in the NAcSH though less robustly. iSPN activity also shows eating related modulation, similar to DA and dSPNs, though only in the core and less robustly. Taken together, these data indicate that dopamine and SPNs in the striatum contribute to modulating on-going meal patterning. We next tested the effects of either a 24-hour fast or pharmacological satiety (GLP-1 agonist) on these signals and their relationship to meal patterns.

Hunger modulates DA activity associated with food approach

To assess how hunger modulates eating behavior and underlying striatal dopamine signaling, we fasted mice for 24 hours (Figure 5A), resulting in a short-term, modest loss of body weight that recovered the following day (Figure 5B). We compared the non-fasted controls from this experiment to those in the first experiment (Figure 2, reported above) and observed no significant differences in their meal patterns (data not shown). We compared them on DA signals and found no significant difference in DA signaling (pellet approach, Welch's t-test; $t_{(3.05)} = 0.01$, $_{(3.05)}$, $p = 0.99$). Therefore, we combined the non-fasted control data with the data from the prior experiment for comparison with fasted mice. The patterning of meals, i.e., interpellet interval distribution, was similar between the fasted and non-fasted mice and aligned with the previous experiment (Figure 5C). However, the small peak at a longer interval, presumably reflecting the most common intermeal interval, is slightly left-shifted and higher in fasted mice, suggesting a shorter period between meals and an increase in meal frequency. There was no difference in the average interpellet interval within meals as defined here. Increased hunger led to higher food intake. The 24-hour fasted mice consumed nearly double the pellets in 3 hours

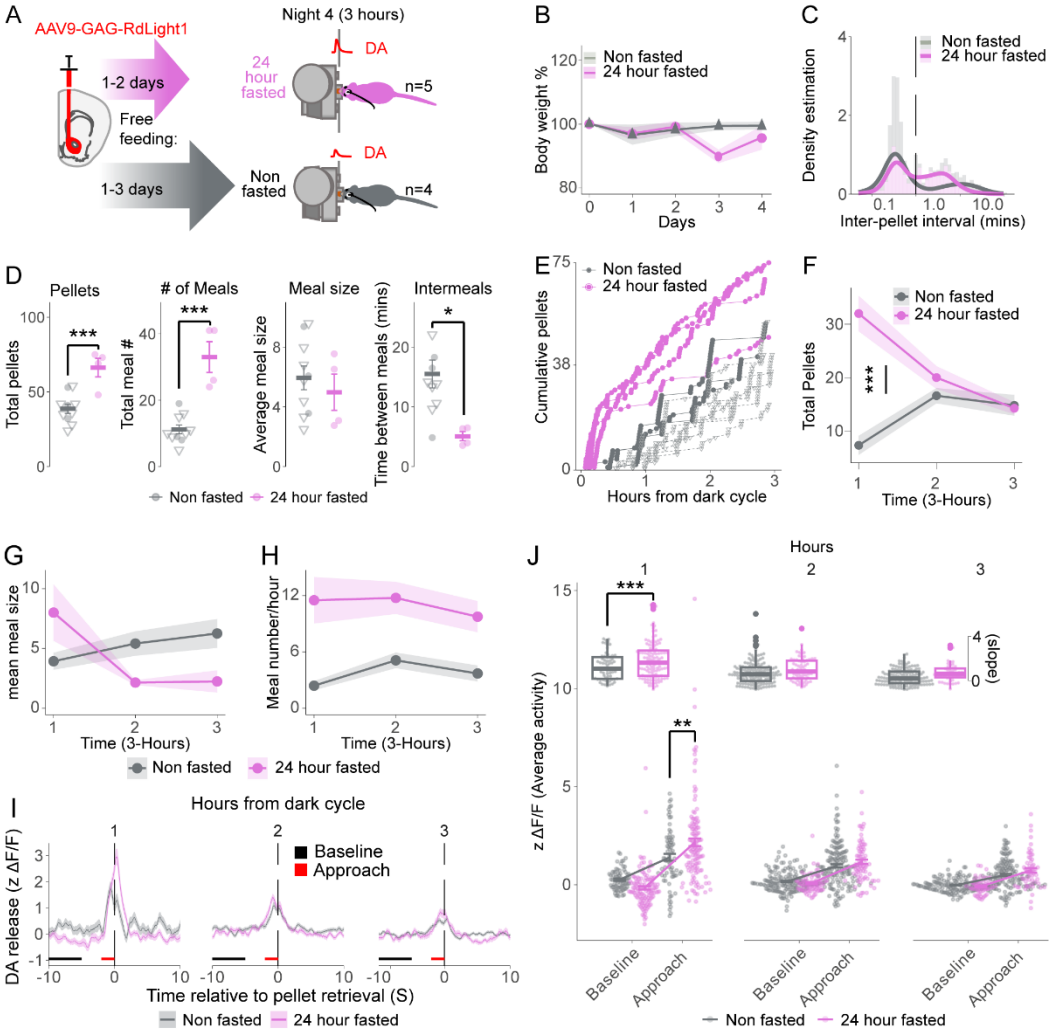

Figure 5. 24-hour fast increases DA release during the first hour of feeding. **A)** (left) Surgery for expressing RdLight1 in WTB6 mice in the NAcc, (right) Meal patterns assessed using FED3 for 24 hours across two days, then half the mice fasted 24 hours, and the other half were left on free feeding. On night 4, at the onset of the dark cycle, DA release was measured in both fast and 24-hour fast mice for 3 hours. **B)** Bodyweight percent by groups across days. **C)** Interpellet interval density during recordings (2 hours). **D)** The total number of pellets, meals, average meal size, and intermeal time for 3 hours of recordings. **E)** Cumulative pellets eaten across 3 days for each mouse, grouped by whether they were fasted. **F)** Total pellet intake by the hour for each group. **G)** Mean meal size across hours by group. **H)** Meal frequency across hours by group. **I)** The average DA release in Z scores, grouped by whether the mice were fasted, by hour. **J)** Average activity of DA release during baseline and food approach for both groups by the hour. The inset is the slope of the baseline and approach. Error bars: ± s.e.m.; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. 24-hour fast, n=5, Not fasted, n=10. Triangle markers indicate previous controls.

compared to the non-fasted mice (Figure 5D, pellets: $F_{(1, 12)} = 19.64$ $p < .001$), eating twice as many meals (Figure 5D, meals: $F_{(1, 12)} = 39.02$, $p < .001$). The average size of individual meals appeared unchanged during the 3-hour period (Figure 5D, meal size: $F_{(1, 12)} = 0.42$, $p = 0.52$). Consistent with a greater frequency of meals, the intermeal interval was significantly decreased in the fasted mice (Figure 5D, intermeals: $F_{(1, 12)} = 8.34$, $p < .05$).

We evaluated whether fasted and non-fasted mice exhibited different meal patterns over successive hours, reflecting accumulated consumption. The increased consumption in the fasted mice occurred almost entirely within the first hour (Figure 5E-F, group x hour: $F_{(1, 38)}$ = 29.24, $p < .001$). Although meal size averaged across 3 hours is unchanged between groups, meal size substantially varied by hour in the fasted but not the non-fasted mice (Figure 5G, LMM: group x hour: $F_{(2, 24)} = 8.85$, $p < .01$). Specifically, the fasted mice consumed larger meals in the first hour and then smaller meals in the second two hours (Figure 5G, Tukey: $t_{(24)} = 3.14$, $p < 0.05$). Despite these changes in meal size by hour, the number of meals taken remained consistently elevated across all three hours in the fasted compared to non-fasted mice (Figure 5H, LMM: $F_{(1, 37)} = 17.86$, $p < .001$). Thus, in the first hour, both meal size and meal number are contributing to greatly increased consumption, while in hours 2-3 meal number remains elevated while meal size decreases to less than that seen in non-fasted mice, resulting in comparable consumption between the groups in those hours (Figure 5F, hr 2, Tukey: $t_{(36)} = -1.05$, $p = 0.90$; hr 3, Tukey: $t_{(36)} = 0.116$, $p = 1.0$).

These data suggest that fasting induced an elevated energy deficit at the beginning of the active cycle that drove compensatory consumption and altered meal patterning, i.e., the starting and stopping of meals. This observed compensatory eating comprises two dissociable components. First, we observe an increase in meal frequency across the entire 3-hour period recorded, suggesting that the energy deficit generated by overnight fasting leads to more rapid recovery of hunger after short-term satiation resulting in an increased tendency to initiate a new meal; that is, the mice *start* eating again sooner. Second, we observe changes in meal size that vary hour-by-hour. In the initial hour, meal size is substantially larger, reflecting delayed termination of meals. This, in combination with more frequent meals, mediates substantial compensatory eating. In hours 2-3, however, meal size decreases substantially, reflecting an *earlier* termination of eating. This earlier termination of meals resulted in smaller meals than non-fasted mice. The impression is of two separate processes: one mediating 'interest' in eating (meal initiation), which appears to be elevated in a sustained way and the other mediating feedback on the necessity of continued eating, i.e., satiety. In the second and third hours, elevated 'interest' and greater frequency of meal initiation is apparently offset by more rapid satiety resulting in a 'check' on fasting induced compensatory (over-)eating.

DA release differed between fasted and non-fasted mice only on approach during the first hour (Figures 5I-J, LMM: hour x group x feeding stage: $F_{(2, 937)} = 5.60$, $p < .01$). We quantified differences between the fasted and non-fasted mice by comparing the slopes of DA increases from baseline to approach across hours 1 through 3. The 24 hour fasted mice exhibit significant greater slopes compared to non-fasted mice in the first hour (Figure 5J, top inset,

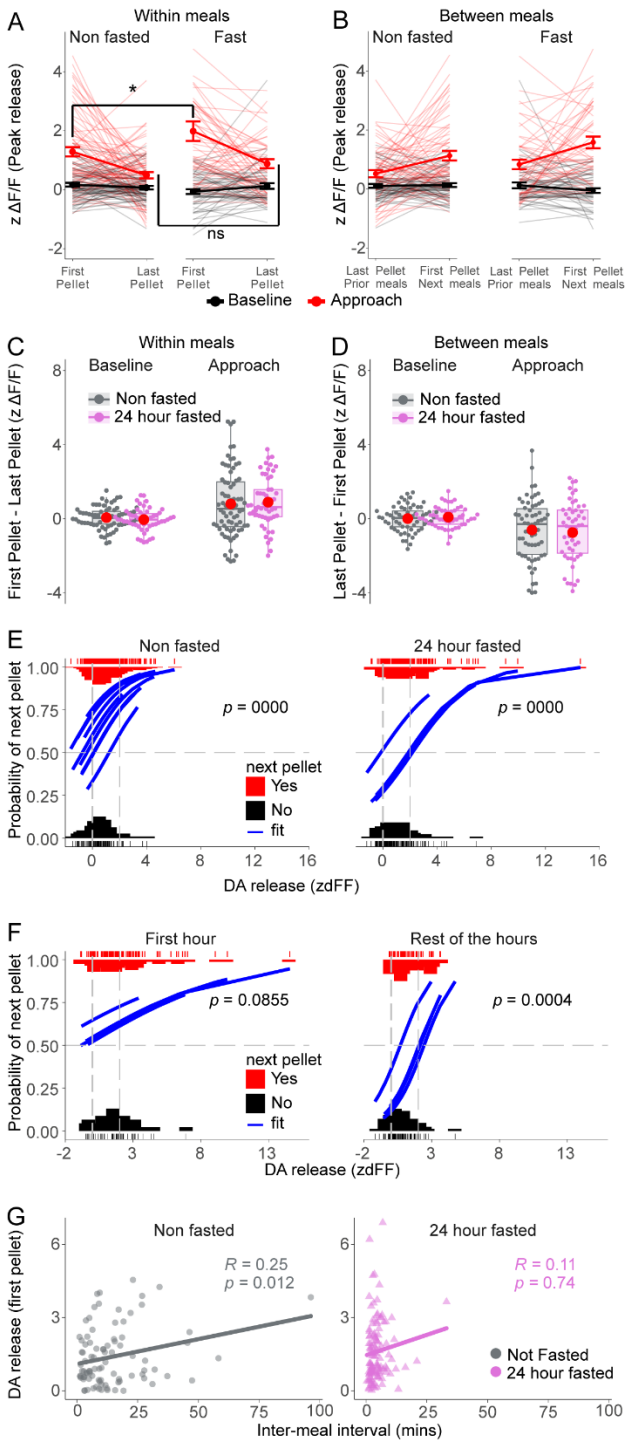

Figure 6. A 24-hour fast reduces the influence of dopamine on meal size. **A)** Average DA activity from the first and last pellet of a meal, grouped by the fasted condition. **B**) Average DA release from the last and first pellet between meals grouped by the fasted condition. **C)** Difference between first and last pellet within meals, the red dot indicates the mean. **D)** Difference between last pellet of prior and first pellet of next meal. **E)** A mixed logistic regression showing peak DA release significantly predicts the probability of the next food pellet for the NAcc. Blue traces represent the fit of each mouse. **F)** A mixed logistic regression analysis showing that the peak DA release probability is shifted according to the fasted condition. **(G)** DA release at first pellet regressed against time since last meal. Blue traces represent the fit of each mouse. Error bars: ± s.e.m. in *p < 0.05; **p < 0.01; ***p < 0.001. 24-hour fast, n=5, Not fasted, n=10.

Tukey: $t_{(365)}$ = 3.19, p < .05) but not in hour 2 or 3 (Figure 5J, top insets, hr 2:Tukey: $t_{(365)}$ = -1.19, p = 0.840; hr 3: $t_{(365)}$ = 0.44, p = 0.998). This enhanced DA peak at approach in fasted mice during the first hour only is consistent with the increased consumption in the first hour and, moreover, suggest that this increased dopamine specifically corresponds to prolonged meals, or conversely diminished satiety and meal termination. In contrast, increased frequency of meal initiation occurs in hours 2 and 3 in the absence of any detectable difference in dopamine between the fasted and non-fasted mice.

We evaluated whether a 24-hour fast impacts the progressive attenuation of DA as individual pellets are consumed within a meal. Comparing DA release between the first and last pellet of a meal, we observe as before, a decrease in peak dopamine on pellet approach for both fasted (Figure 6A, Tukey: $t_{(493)}$ = 5.06, p < .001) and non-fasted mice (Figure 6A, Tukey: $t_{(493)}$ = 4.15, p < 0.001). Similarly, as above, both the fasted mice (Figure 6B, right, Tukey: $t_{(437)}$ = 4.08, p < .001) and non-fasted mice (Figure 6B, right, Tukey: $t_{(437)}$ = -363, p < .01) showed significant recovery of DA peak on pellet approach-retrieval between meals.

Surprisingly, we did not observe a difference between fasted and non-fasted mice in this within meal decline in DA peaks (last pellet DA - first pellet DA, Figure 6C), though we did observe that the magnitude of the first DA peak of a meal was higher in fasted mice (Figure 6A, Tukey: $t_{(67)} = -3.17$, $p < .05$; also 5J hour 1). Since the first pellet DA peak in a meal is larger in fasted mice, but the last pellet peak is not significantly different (Figure 6A, Tukey: $t_{(67)} = -1.79$, $p = .63$), and since there are more pellets in the fasted mice's meals, at least in hour 1 (Figure 5G), this suggests the magnitude of the stepwise decline in DA peaks within a meal may be smaller. If the decrement is smaller, then the DA peaks at approach within a meal are on average larger in fasted compared to non-fasted mice (Figure 5I, hour 1), where higher DA increases the likelihood of a next pellet, extending the meal size as observed.

This tentative interpretation of the data suggests that, as observed above, there is a relationship between the magnitude of any given dopamine peak during approach and the likelihood of the next pellet. When we examine this for the entire period comparing fasted and non-fasted mice, greater DA release increased the probability of the following food pellet similarly for both the fasted (Figure 6E, MLR: Conditional $R^2 = 0.22$, 95% CI [0.25, 0.67], $\beta = 0.46$, $p < 0.001$) and non-fasted mice (Figure 6E, MLR: Conditional $R^2 = 0.20$, 95% CI [0.30, 0.83], $\beta = 0.57$, $p < 0.001$). However, when we examine only fasted mice breaking out the first hour, we see a change in the relationship between the magnitude of a dopamine transient on pellet approach and the likelihood of a next pellet; specifically, in the first hour all magnitudes of DA transients were associated with a greater than 50% chance of a next pellet (Figure 6F, MLR: Conditional $R^2 = 0.20$, 95% CI [0.30, 1.17], $\beta = 0.74$, $p < 0.001$) whereas in hours 2-3 the likelihood of a next pellet was decreased across most DA peak magnitudes (Figure 6F, MLR: Conditional $R^2 = 0.10$, 95% CI [-0.009, 0.43], $\beta = 0.21$, $p = 0.06$).

Finally, we examined the relationship between the amount of time elapsed from the end of the previous meal and the magnitude of the dopamine transient on approach to the first pellet of the next meal (Figure 6G). As mentioned above, we observe a linear relationship in the non-fasted mice (Figure 6G, left, $F_{(1, 110)} = 6.57$, $p = 0.012$), such that the longer the time elapsed since the last pellet, the higher the dopamine peak on the first pellet when the mouse resumes eating. This relationship, however, seems to be lost in fasted mice where more frequent meals occur with smaller intermeal intervals and when there are longer intervals, these do not clearly increase with elapsed time from the prior meal (Figure 6G, right, $F_{(1, 127)} = 0.12$, $p = 0.74$). These data suggest the recovery of hunger over a shorter period and possibly a decoupling of dopamine peaks from this recovery process.

Pharmacological satiety blunts DA's attenuation in starting and stopping meals.

We induced pharmacological satiety by administering a GLP-1 agonist, exendin-4 (Ex-4). Based on prior literature (Kjaergaard et al., 2019), we administered Ex-4 45 minutes prior to the onset of the dark cycle to achieve 1.5-2 hours of pharmacological satiety during the initial onset of feeding (Figure 7A). Ex-4 administration did not significantly change the distribution of the interpellet intervals (Figure 7B). The initial rate of consumption remained unchanged between Ex-4 and Veh, with the effect of Ex-4 observable in the second hour from the onset of the dark cycle (Figure 7C-D, $t_{(1, 15.6)} = 5.11$, $p < .001$). Ex-4 administration robustly decreased pellet consumption (Figure 7E, Tukey: $t_{(10)} = 15.29$, $p < .01$). Ex-4 decreased meal size (Figure 7E, $F_{(1, 9)} = 7.27$, $p < .05$), though the apparent decrease in the number of meals (Figure 7E, $F_{(1, 10)} = 4.00$, $p = 0.25$), and increase in intermeal intervals did not reach statistical significance (Figure 7E, $F_{(1, 10)} = 1.53$, $p = 0.24$).

Notably, we observed no appreciable difference in the DA release between Ex-4 and Veh during baseline or pellet approach in either the first or second hour (Figure 8A & 8B, LMM: dose x hour x feeding stage: $F_{(1, 280.4)} = 0.55$, $p = 0.46$). This is inconsistent with the behavioral

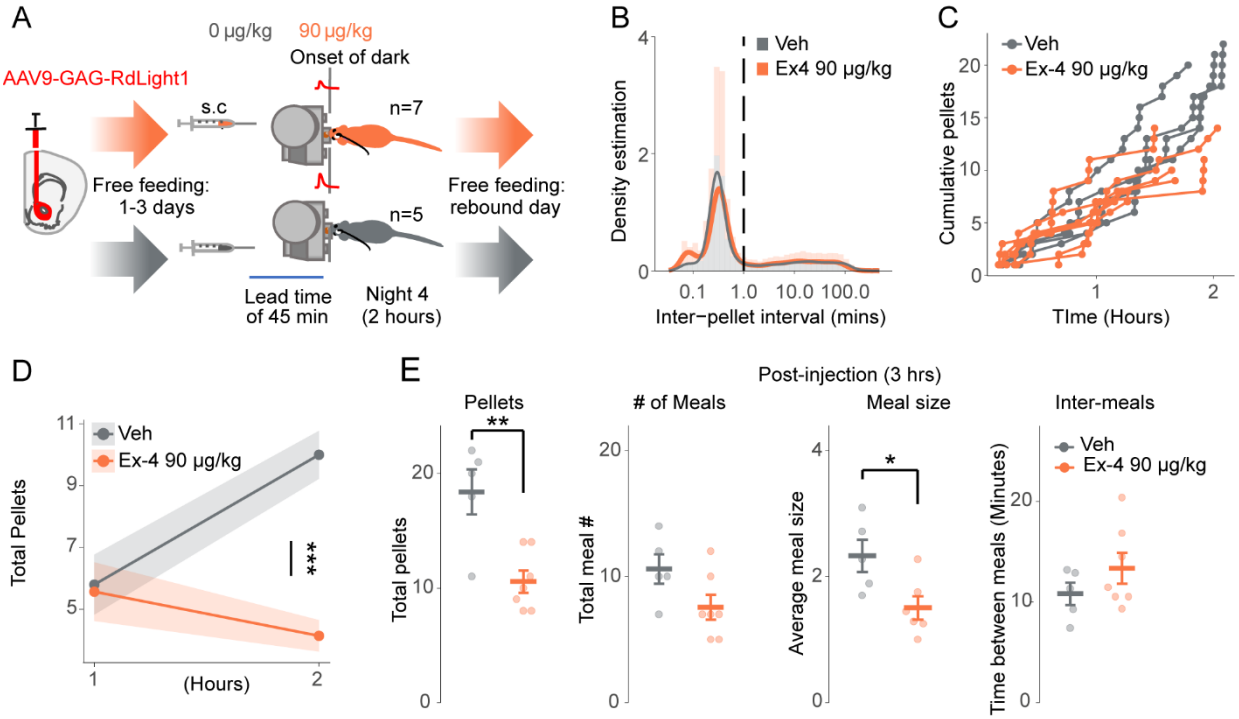

Figure 7. Ex-4 decreases natural food consumption and meal size. **A)** (left) Surgery for expressing RdLight1 in WTB6 mice in the NAcc, (right) Meal patterns assessed using FED3 for 24 hours across 3 days. On night 4, Ex-4 or Veh was injected 45 minutes before the onset of the dark cycle, and DA release was measured for a total time of 2 hours. **B)** Inter-pellet interval density during recordings (2 hours). **C)** The cumulative pellets eaten across time for each mouse, with colored lines indicating Veh or Ex-4 at 90 µg/kg. **D)** The average number of pellets, meals, and meal size 3 hours post-injection on night four. **E)** The total number of pellets, meals, average meal size, and inter-meal interval post i.p. injections. Error bars: ± s.e.m.; *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

data that shows a decrease in consumption during the second hour, with the behavioral change not reflected in the co-occurring DA signal. We examined whether Ex-4 changes DA dynamics from the first pellet to the last pellet of a meal. In Veh mice, DA levels attenuate from the first to the last pellet of a meal (Figure 8C, left; Tukey: $t_{(115)} = -4.27$, $p < .001$), as observed above. In contrast, this attenuation is diminished in mice administered Ex-4 (Figure 8C, left, Tukey: $t_{(115)} = -2.14$, $p = 0.40$). Also as above, peak DA at pellet approach-retrieval significantly recovered between meals in Veh mice (Figure 8C, within meals, Tukey: $t_{(87.8)} = 3.27$, $p < 0.05$) but not Ex-4 mice (Figure 8C, between meals, Tukey: $t_{(87.8)} = 0.152$, $p = .99$). These data suggest that

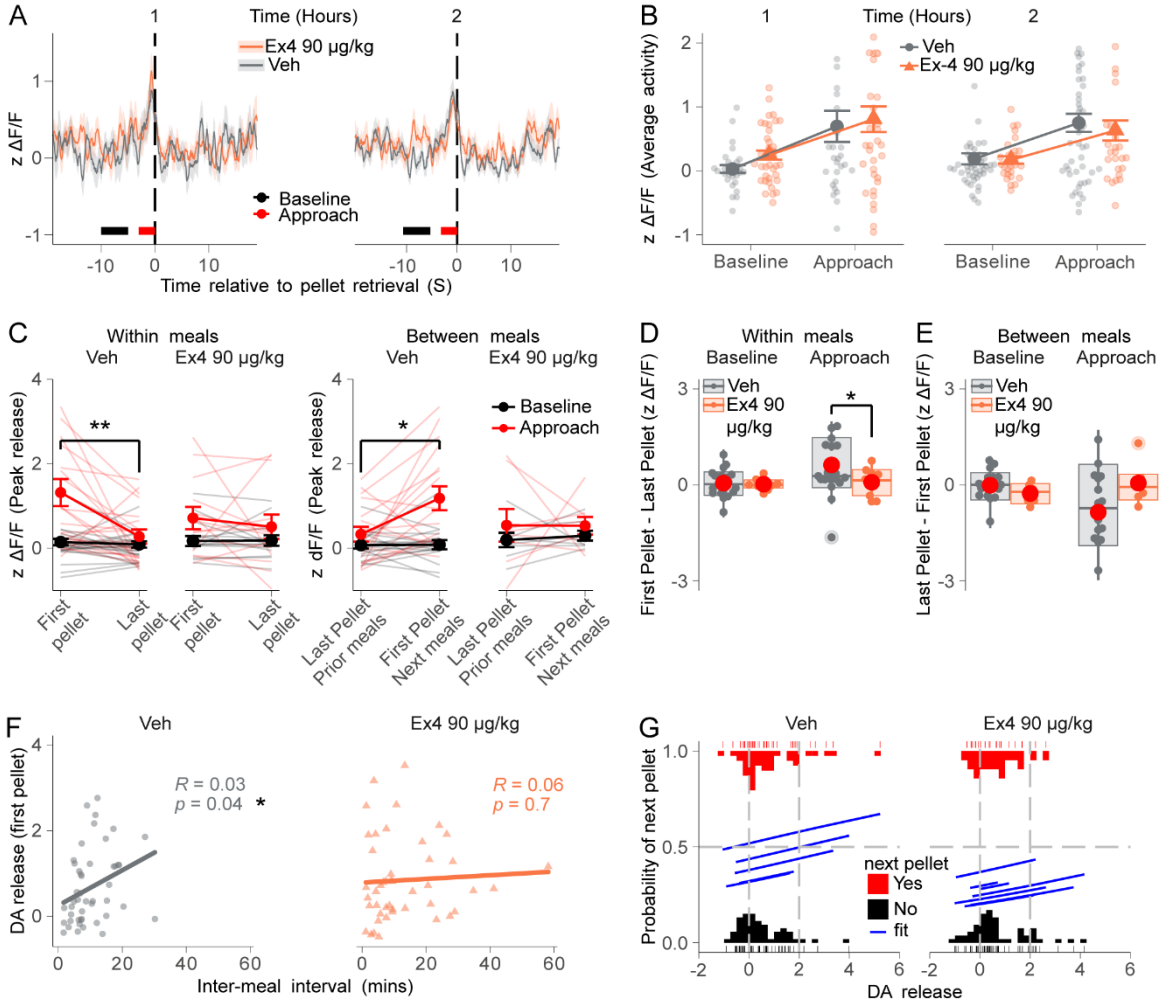

Figure 8. Ex-4 blunts DA attenuation as mice reach satiation. **A)** The average DA release per hour for Veh and Ex-4 90 µg/kg. **B)** The average DA release per hour for each dose, for both the baseline and approach. **C)** The average DA release from the first and last pellets of a meal is grouped by the Veh and Ex-4 at 90 µg/kg. **D)** The difference in peak DA at the start and end of a meal for baseline and approach, the red dot indicates the mean. **E)** The difference in peak DA from the end of a meal and the start of the next meal for baseline and approach. **F)** Linear regression of the average DA release for the first pellet of a meal during food approach, predicted by the inter-meal interval for Veh and Ex-4 at 90 µg/kg. **G**) A mixed logistic regression model showing the relationship between DA and the probability of the next food pellet in the Ex-4 group. Error bars: ± s.e.m.; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

although Ex-4 does not alter the average peak DA on pellet approach (Figure 8A-B), it flattens out within and between meal modulation of these peaks (Figure C). In vehicle treated mice, we observe greater variability in approach peak dopamine compared to baseline, but this variability is lost in Ex-4 mice (Figure 8D-E). We performed a linear regression to assess if Ex-4 disturbs the relationship between the elapsed time since the previous meal and the peak DA observed at the first pellet of the next meal. As above, the intermeal interval predicts the magnitude of DA release for the first pellet of a meal for mice administered Veh (Figure 8F, left, $F_{(1, 44)} = 4.43$, $p < .05$). This relationship is lost in mice administered Ex-4 (Figure 8F, right, Ex-4: $F_{(1, 43)} = 0.16$, $p = 0.69$).

Finally, as above, we tested whether DA release increases the probability of taking a next pellet, comparing Ex-4 and Veh. In Veh treated mice (not combined with prior data), there was a trend toward higher DA release increasing the probability of taking a next food pellet (Figure 8G, LR: Conditional $R^2 = 0.04$, 95% CI, [-0.07, 1.29], $\beta = 0.61$, $p = 0.080$), but in Ex-4 mice the probability of a next pellet was less than 50% for all magnitudes of dopamine release with no clear relationship between the magnitude of DA and probability of a next pellet (Figure 8G, LR: Conditional $R^2 = 0.002$, 95% CI [-0.19, 0.43], $\beta = 0.12$, $p = 0.43$).

In summary, Ex-4 reduced consumption, an effect observed primarily in the second hour of consumption, suggesting enhanced satiety. Surprisingly, DA release associated with pellet approach showed no significant differences between Ex-4 and Veh groups, though the dynamic regulation of the magnitude of these DA transients across meals was substantially diminished, as reflected in several measures. In short, the GLP-1 agonist appears to decouple dopamine activity from fluctuating signals or changing states presumably associated with on-going feeding activity, including short-term satiety during consumption and recovery of hunger between bouts of eating. Moreover, the relationship between the magnitude of dopamine transient activity and likelihood of continuing to eat is diminished, suggesting a decoupling of DA from modulating eating behavior. Together, these two decouplings seem to suggest that GLP-1 disengages the dopamine system from its normal modulatory role in feeding, where what is lost is a modulatory push that normally facilitates greater feeding.

Discussion

Here we examined DA as well as direct and indirect striatal SPN activity during naturalistic feeding (no food restriction, no work requirement, no structured task or cues) in the nucleus accumbens. As expected, we observe DA transients during food approach, consistent with the idea that DA facilitates appetitive approach (Berridge, 2007; da Silva et al., 2018; Hamid, 2021; Ko and Wanat, 2016; Roitman, 2004). The magnitude of these approach-associated transients,

however, decreases with successive pellets within a meal prior to meal termination and then recovers at resumption of feeding after an intermeal interval, suggesting that DA also mediates *reduced* appetitive motivation associated with short-term satiation. Together, these data suggest that dopamine contributes to meal patterning, i.e., stopping and starting meals, by tracking short-term fluctuations in hunger and satiety.

Similarly, we found that both dSPN and iSPN activity increased during food approach, consistent with a complementary rather than an opponent model of direct and indirect pathway function. Our data is consistent with (London et al., 2018) and a model in which dSPNs facilitate movement while iSPNs inhibit competing actions (Klaus et al., 2019). As with DA, both d- and i-SPN activity at food approach decreases across successive pellets within a meal, but only in the nucleus accumbens core. dSPN activity recovered between meals, while this effect was less robust in iSPNs and did not reach statistical significance. Our study does not speak to the mechanisms underlying the similarities between DA and d/iSPN activity. The increase in dSPN activity could be facilitated by dopamine transients via activation of the dopamine D1 receptor; however, the same DA increase would be expected to inhibit iSPNs through the dopamine D2 receptor, though iSPN D2 receptors are largely saturated at basal extracellular DA and less responsive to phasic transient activity (Dreyer et al., 2010). The co-occurring increase in dopamine and SPN activity in both pathways likely reflects common afferent input driving all three populations of neurons, such as cortical structures projecting to both the striatum and midbrain.

While earlier microdialysis studies demonstrate that extracellular dopamine increases during feeding (Hernandez and Hoebel, 1988a, 1988b) and later FSCV studies demonstrate dopamine transients associated with appetitive approach, our data demonstrate not only that this dopaminergic modulation occurs through subsecond transients (i.e., phasic dopamine) but that it is sensitive to short-term fluctuations in hunger and satiety that correspond to starting and stopping meals and meal patterning.

Fasted mice: dissociation between meal initiation and termination

We examined 24-hour fasting to evaluate how increased hunger altered these patterns of DA signaling. Consistent with previous findings, an overnight fast induced compensatory eating. Here, this reflected both altered meal size (meal termination) and meal frequency (meal initiation), effects that were time-dependent across feeding. In the first hour, meal initiation was increased, and meal termination was delayed, resulting in more frequent, larger meals that substantially increased consumption. In the second and third hours, we still observe an increased frequency in meal initiation (more meals), but the meals are actually *shorter* than the

non-fasted mice, resulting in comparable consumption in hours 2-3. This suggests a dissociation between the mechanisms that regulate starting (initiation) and stopping (termination) meals, where the former reflects a persistent incentive-salience process that orients behavior toward food pursuit while the latter reflects a more variable satiation-feedback process that terminates food pursuit.

DA was only increased in fasted mice during the first hour when we observed increased meal sizes, i.e., delayed meal termination, and not in hours 2-3 when we observed only more frequent meal initiation combined with shorter, truncated meals. This suggests that dopamine modulation *during* feeding regulates whether the animal continues to eat or terminates the meal. Fasting increases the magnitude of DA transients associated with food approach and likely diminishes the progressive decrease observed with successive pellets, amplifying motivation to feed and diminishing feedback from satiety. In contrast, fasting induced changes in subsecond phasic dopamine does not appear to mediate the increased frequency of meal initiation as this persists even when DA signaling normalizes in fasted mice compared to non-fasted mice.

One interpretation of these data is that the energy deficit generated by fasting induces a persistent motivation to seek food, i.e., enhanced incentive-salience of food-related stimuli, which leads to increased initiation of food pursuit and more frequent meals. However, this elevated food seeking is tempered by feedback from satiation. More specifically, as an enhanced energy deficit facilitates compensatory eating during the first hour, this compensatory eating generates proportionally larger post-ingestive signals. As a result, in hours 2-3 the hunger level of fasted mice may be similar to non-fasted mice, reflected in similar dopamine transients on approach. However, for the fasted mice, their continued consumption occurs in the context of greater post-ingestive satiation signals from greater consumption during preceding compensatory feeding, thus truncating their meals. Nevertheless, because meal frequency and interest in food (incentive-salience) remains elevated-- in hours 2-3 fasted mice eat shorter but more frequent meals-- and consumption is maintained at levels comparable to non-fasted mice. Thus, increased initiation might serve to maintain appetitive pursuit despite increased satiety signals from compensatory eating.

Pharmacological satiety decouples DA modulation from fluctuating hunger and satiety

Ex-4 significantly decreased meal size. This effect was consistent with previous studies showing that Ex-4 reduces food intake (Kjaergaard et al., 2019; Talsania et al., 2005). Despite the robust reduction in consumption, we observe no discernible effect of Ex-4 on DA transients at food approach; that is, average DA peaks preceding pellet retrieval are unchanged. However, the modulation of these transients within and between meals, reflecting fluctuations in hunger

and satiety, is blunted. Specifically, while vehicle mice exhibit greater amplitude DA transients at food approach at the start of a meal that decline with progressive pellet consumption, Ex-4 treated mice do not display this change. Similarly, in vehicle treated mice, longer intermeal intervals result in larger DA transients at the first pellet of the next meal, a modulation again lost in Ex-4 treated mice. These data suggest that the modulation of dopamine that tracks short-term fluctuations in hunger and satiety are flattened out in Ex-4 treated mice. This suggests that the GLP-1 agonist suppresses the sensitizing effect of hunger on DA release (Cassidy and Tong, 2017): it is as if the GLP-1 treated mice, in terms of DA release at food approach, are always in a state analogous to the end of a meal.

Prior investigations of how GLP-1 agonists impact mesolimbic dopamine have yielded inconsistent results. Using FSCV, Fortin and Roitman (2017) did not observe alterations in DA release in the NAc induced by Ex-4. However, studies in the VTA show that GLP-1 agonists decrease excitatory synaptic input in mesolimbic DA neurons (Wang et al., 2015) and inhibit their burst firing activity (Konanur et al., 2020). These and other data introduce the possibility of diverging effects of GLP-1 on dopamine cell activity versus dopamine release in target regions (Kanoski et al., 2016). Regardless of GLP-1 effect on the midbrain, our data, consistent with Fortin and Roitman (2017), suggest at the level of phasic dopamine release in the accumbens, dopamine is not diminished but instead decoupled from short-term fluctuations in hunger and satiety, an effect that alters meal patterns decreasing consumption.

The timescales of dopamine, hunger and satiety

In this study, we observe minute-to-minute fluctuations in the magnitude of dopamine transients associated with food approach that correspond to bouts of starting and stopping feeding, i.e., 'meals,' that correspond to short-term satiation and recovery of hunger between these short meals. These findings suggest that dopamine plays a role in the microstructure of feeding patterns, reflecting both the drive to pursue food due to hunger as well as a diminution of that drive through satiation. A detailed consideration of the possible mechanisms underlying these dopamine dynamics responsive to short-term fluctuations in hunger and satiety is beyond the scope of this discussion. That said, it might seem evident that post-ingestive signals that are known to modulate midbrain dopamine cells, such as insulin, ghrelin, glucose, leptin (this latter, for example, may be altered with short-term fasting, modulating the other signals) likely play a central role. However, the changes we observe-- for example, the decline in DA transients on food approach within a meal-- occur at a timescale of less than a minute (i.e., 10s of seconds between pellets). It seems unlikely that a pellet can be ingested, travel the alimentary canal and be digested and absorbed providing a post-ingestive signal rapidly enough to modulate the

dopamine peak at the next pellet approach occurring 10s of seconds later. Nevertheless, dopamine does seem to fluctuate on this timescale during active eating. One possibility that could act at such a rapid timescale is vagal transmission. Fernandes et al (2020) demonstrated that gastric infusion of sucrose can rapidly increase burst activity in dopamine neurons, necessary to facilitate food pursuit. However, gastric infusion eliminates travel time down the esophagus and the breakdown of the pellets into glucose. Moreover, sucrose infusion *increased* dopamine activity to *facilitate* feeding, suggesting this mechanism would increase, not decrease DA activity.

Satiation has multiple components that operate at different timescales, including initial, rapid sensory and cognitive components and more delayed post-ingestive and post-absorptive signals (Blundell, 2010; Smeets et al., 2010; Zimmerman and Knight, 2020). Sensory and cognitive components are essentially learned expectations that associate sensory information (chewing, taste and smell of pellet) with anticipated nutritional content based on prior experience. Sensory information during consumption may activate these learned associations and modulate dopamine signaling based on *expected* post-ingestive signals, providing a mechanism of fast satiation consistent with changes in dopamine from pellet to pellet within a meal. When the signaling that mediates this anticipatory satiety during consumption is terminated after the animal stops eating, this allows an 'accounting' as post-ingestive and post-absorptive signals come on-line that establish a new state of hunger-satiety. If this new state is less than full-satiety, then 'hunger is recovered' and eating resumes, repeating the process. As this cycle repeats, the slower timecourse post-ingestive/absorptive signals accumulate until they reflect a state of full satiety and eating stops for an extended period. This process is analogous to the behavior of Agouti-related peptide (AgRP) neurons in the hypothalamus whose activity signals hunger (Chen et al., 2019, 2016; Deem et al., 2022; Reed et al., 2023). AgRP neurons reduce their activity when food is or is expected to be available, *before* food is actually consumed, indicating that these neurons drive food *pursuit* but not consumption itself. If, however, the animal does not consume the encountered food, then AgRP activity returns to its previous higher level (Su et al., 2017).

It is worth additionally noting that though we see an *overall* pattern of decrease in DA transients associated with approach across meals, this is not a cleanly monotonically decreasing function. Instead, variability is high-- transient amplitude only decreases *on average across a meal*, but within a meal individual DA transients associated with pellet approach may go up or down. This variability suggests the confluence of multiple processes. Three primary modulators might include: vagal stimulation and other signals that *increase* DA activity during consumption (Fernandes et al., 2020) to maintain on-going feeding, sensory-cognitive satiation

as discussed above that constrains and pauses feeding and the slower timecourse energy signals, including rising post-ingestive/absorptive signaling as well as pro-feeding signals, such as ghrelin that reflect newly updated energy states. All of these change at different timecourses— moreover, presumably each with its own variability— such that the net effect on DA at any given moment is highly variable. Despite this complexity and variability, we nevertheless can clearly discern a pattern of changing DA transients related to food approach that correspond to short-term fluctuations in hunger and satiety.

Noisy, stochastic character of dopamine signal

Finally, two aspects of our study highlight the fundamental *neuromodulatory* aspect of dopamine and merit comment. First, although we see consistent spikes in DA associated with food approach, zoomed out these approach-aligned DA spikes are merely a few waves in a sea of dopamine transients. Even within meals there are dopamine transients *not* associated with food approach. That none of these other DA transients, even within an active bout of eating, provoke food approach indicates that a dopamine transient cannot be directly linked to activating a specific behavior, including eating. Second, though we see a robust increase in DA associated with each pellet approach, this is only statistically robust in the aggregate. As noted above, there is substantial variability across pellet approach events, with DA actually *decreasing* in some instances-- and yet the animal ate. This highlights that dopamine signaling is noisy and its actions on behavior stochastic.

It is not clear how we reconcile this kind of noisy, stochastic neuromodulatory signaling with dopamine's amply demonstrated, substantial role in feeding, where often it *appears* as if dopamine is *determining* a behavior. Indeed, mice without dopamine will not eat and simply die (Palmiter, 2009). How such a noisy neuro*modulator* can be so critical to mediating behavior that it appears to be *effectively* driving behavior in aggregate is not clear but in our estimation a central question. Our data clearly demonstrates the probabilistic nature of the relationship between DA transients and specific behaviors, which only in aggregate and on average correspond to short-term fluctuations in hunger, satiety and meal patterning.

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Author Contributions

JAB and DM developed the conceptual framework, designed the study, and wrote the manuscript. DM, CEM, EH, and KC collected the data. DM analyzed the data. FG mapped and analyzed histological data and did sensor validation. All the authors approved the final manuscript as submitted.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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