Behavioral and neural alterations of the ventral tegmental area by Cafeteria diet exposure in rats

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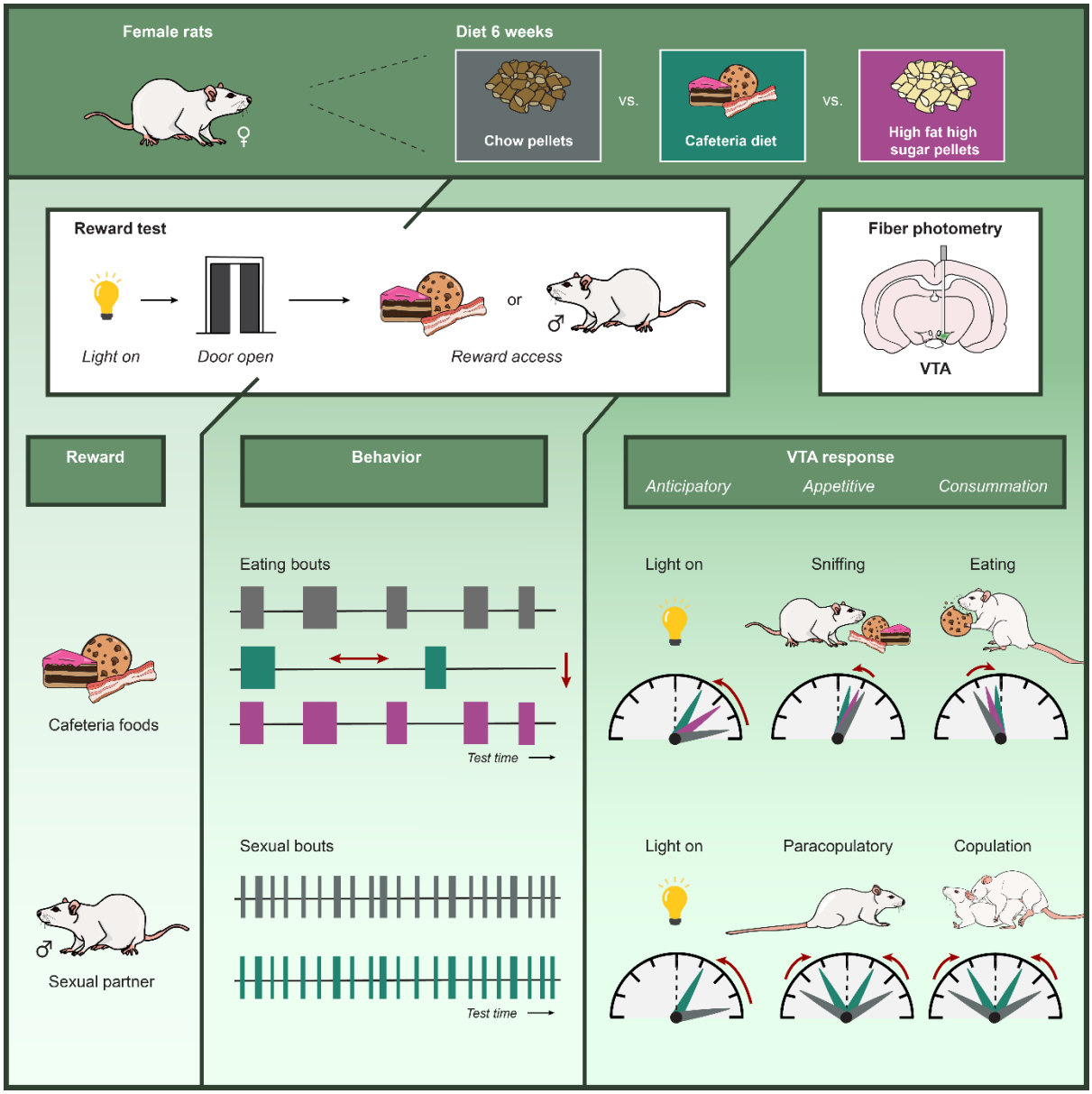

Summary

The brain reward system is essential for regulating appetitive and consummatory behaviors in response to various incentive stimuli. Junk food, known for its high palatability, is particularly associated with the potential for excessive consumption. While previous studies have shown that excessive junk food intake can impact reward circuitry, the precise mechanisms remain unclear. Additionally, it is unknown whether the functionality of this brain system is similarly altered in response to other natural rewards. In this study, we used fiber photometry combined with a behavioral reward test to investigate how six weeks of excessive cafeteria (CAF) diet consumption affects VTA neural activity and behavioral responses to food and sexual rewards in sexually experienced female rats. For the first time, we demonstrate that prolonged exposure to a CAF diet decreases interest in a food reward, resulting in reduced consumption. These behavioral changes were accompanied by diminished neural activity in the VTA. Similarly, but to a lesser extent, reductions in VTA activity responses were observed in response to a sexual partner, although no significant behavioral differences were detected during sexual interactions. Furthermore, a two-week reversal diet of standard chow was insufficient to restore VTA neural activity in CAF-exposed animals, which continued to show decreased VTA responses to both food rewards and sexual partners. Our findings suggest that prolonged junk food exposure leads to desensitization of the VTA, a key node in the brain's reward circuitry, resulting in reduced responsiveness to natural rewards.

Keywords: cafeteria diet, food, sexual behavior, reward, ventral tegmental area, high fat high sugar diet, rat

Introduction

The widespread availability and excessive consumption of processed foods rich in fat and sugar, also called junk food, are a potential cause of the increased prevalence of obesity and overweight around the world[1-3]. The highly palatable characteristics of these foods activate hedonic and motivational mechanisms to promote food-seeking behavior and overeating[1], and it has been shown that modern energy-rich diets are consumed not solely for their nutritional content but also for their hedonically pleasant traits[4,5]. Besides the risk for obesity, the consumption of these foods is associated with a high risk for other diseases such as cardiovascular disease, type 2 diabetes, infertility, hypertension and some cancers[6-11]. Moreover, multiple studies have found intricate links between the consumption of junk food, and cognitive and neurological changes[12-15]. Impairments in aspects of short-term memory, executive function and reinforcement learning have been found in humans and rodents[16-18].

Excessive junk food consumption can also disrupt neural reward mechanisms. In normal circumstances, evaluation of the palatability and consumption of food are in part regulated by dopamine transmission from the ventral tegmental area (VTA) to the nucleus accumbens (NAc)[19,20]. VTA dopamine neurons regulate many aspects of reward-related behavior including reinforcement, decision making, attention, and impulsivity[21-24]. These cells are involved in predicting the value of the reward, and in integrating past experiences with the effort required to obtain a reward before approach to that reward is made[25-30]. Acute exposure to palatable foods increases dopamine release in the NAc [31-34] and increases NAc neural activity[35,36], while chronic exposure to food rich in fat and sugar leads to an attenuated NAc response [35,37] and a lower dopamine turnover in the NAc and VTA at baseline or after a palatable food reward[31,38-45]. In addition, down-regulation of D2, but up-regulation of D1 receptors in the NAc were reported after chronic junk food exposure[46-48]. Similarly, it has been found that junk food can alter the opioid signaling in the VTA[48-52].

If palatable food can affect the reward system in such a way, it seems plausible that longer-term junk food consumption can also lead to *behavioral* changes. Interestingly, previous studies have shown conflicting results. While some studies reported increased levels of motivation to work for food rewards in obesity-prone rats or rats exposed to junk food diets[48,53-57], others have found no changes or reported reduced interest in cues for reward or attenuated operant responses for palatable food rewards[37,38,58-62]. Variations in diet types, durations, and models used, along with differences in food rewards, might contribute to these inconsistencies. Another interesting element to consider when interpreting these previous studies is the use of food rewards in the behavioral tests that are different to what was used in the original palatable diet. This suggests that the way junk food consumption can alter neural reward mechanisms is not only limited to a familiar

palatable food reward. As such, it has also been shown that long-term junk food exposure could alter the behavioral responses toward other kind of unnatural drug rewards. For example, reduced levels of ethanol consumption[63-65], amphetamine cue preference[38], conditioned place preference for cocaine[66], or elevated brain stimulation reward thresholds [47] have been found.

Although a significant amount of evidence suggests that prolonged consumption of junk food can impact neural reward mechanisms, numerous unresolved questions persist. For example, many of the studies mentioned above used operant conditioning designs that are highly dependent on learning and motor skills. How micropatterns of rewarding behavior change upon junk food consumption when free access to and interaction with a food reward is provided remains unknown. In addition, if different rewarding behaviors make use of the same neural pathways, and prolonged exposure to junk food not only affects food-related behavior, but also the neural substrate behind that behavior, it can be hypothesized that prolonged junk food might also affect other rewarding behaviors. Sexual behavior is one of these rewarding behaviors in which VTA [67-69] and nucleus accumbens [70-72] are involved during different phases and/or aspects. By combining behavioral analysis with real-time VTA neural activity recording, we were able to study the effects of excessive junk food consumption on the different phases involved in rewarding behaviors (anticipatory, appetitive and consummatory behavior), including the VTA response upon a reward-predictive light cue.

Finally, rodents in junk food exposure studies are often fed with pellets rich in fat and/or sugar. The advantage of such diets is the control over the intake of received nutrients and energy. However, an important disadvantage of these pellet diets is that they do not engage many dietary behaviors that are important to humans in the context of junk food. Humans typically eat unhealthy products due to their taste, texture, novelty, and variety[73]. In addition, the element of choice of what to consume has been shown to play an important role in overeating. For example, studies have shown that the body weight of rats on a high-fat, high-sugar diet with free choice increased more than that of rats on a forced high-fat, high-sugar diet[74], and that the presentation of a wide range of foods induces over-eating[75], meaning that variety in a meal and greater dietary variation also plays a significant role. *Cafeteria diet* (CAF), as used in this study, gives rats *a choice* of the same unhealthy, but tasty, products that humans consume (e.g., bacon, muffins, and cookies)[76,77]. This increases the hedonic properties of the 'wanted' food and the motivation to consume this food[78,79].

Our study aimed to investigate whether excessive consumption of junk food, in the form of CAF, could alter neural activity patterns of the VTA in response to food and sexual rewards in rats. We hypothesized that long-term CAF exposure could disrupt VTA function not only in response to food

rewards but also by impairing processing of other types of reward, such as the pursuit of a sexual partner. Moreover, we expected that CAF diet would alter the functioning of brain reward circuitry more severely than a high-fat, high-sugar (HFHS) pellet diet that is commonly used in palatable food research. Finally, we investigated whether reversal to a healthy diet could restore the behavioral and neural changes caused by long-term CAF exposure.

Results

To examine the impact of long-term CAF diet exposure on the behavioral response to food or sexual rewards, we subjected three groups of adult, female Wistar rats to 6-week dietary regimens (Fig 1A). Each group received either a control diet (CTR, standard chow), a high-fat, high-sugar diet (HFHS, RD Western Diet pellets), or a cafeteria diet (CAF, offering a *choice* of palatable, albeit unhealthy, human-like food choices, See Tables S2-S5 for more detail about the diets, food intake and nutritional values). Subsequently, we conducted a sequence of behavior tests that involved a food reward (consisting of a selection of the favorite CAF diet items) first, and a sexual reward (a sexually trained male rat) second (Fig 1B and Fig S1A). Our experimental design facilitated the examination of distinct phases of reward-related behavior. These phases included a baseline phase (without any reward), a pre-reward phase (introducing the reward, but without access to the reward), and a reward phase allowing free interaction with the reward (Fig 1B).

Long-term CAF diet exposure reduces VTA activity in response to food rewards

To investigate the impact of long-term CAF diet exposure on the neural activity patterns of the VTA, we conducted stereotaxic surgery to induce GCaMP6s expression in VTA neurons (Fig 1C). Following a one-week recovery period, the rats were placed on their respective diets for six weeks, after which behavioral testing commenced.

During the first food reward test, when the rats were not yet familiar with the test setup, all rats exhibited an increase in VTA neuronal activity in response to a light cue signaling the imminent opening of the doors after a 10-second delay (Fig 1D). Interestingly, the actual opening of the doors (allowing access to the CAF food reward) did not elicit an activity change in the VTA. However, heatmap analysis of these trials per rat showed that this increased VTA activity upon the light cue was only observed in a selection of rats, and across dietary groups (Fig 1D). As the rats became more familiar with the behavioral reward test and associated the light cue with gaining access to the food reward (on the 5th test day with a food reward), a surge in neural activity in the VTA was observed in most CTR rats (Fig 1E), whereas only a subset of HFHS and CAF rats displayed a similar response. Further analysis revealed that CAF rats, but not HFHS rats, displayed an attenuated response compared to CTR rats (Fig 1F).

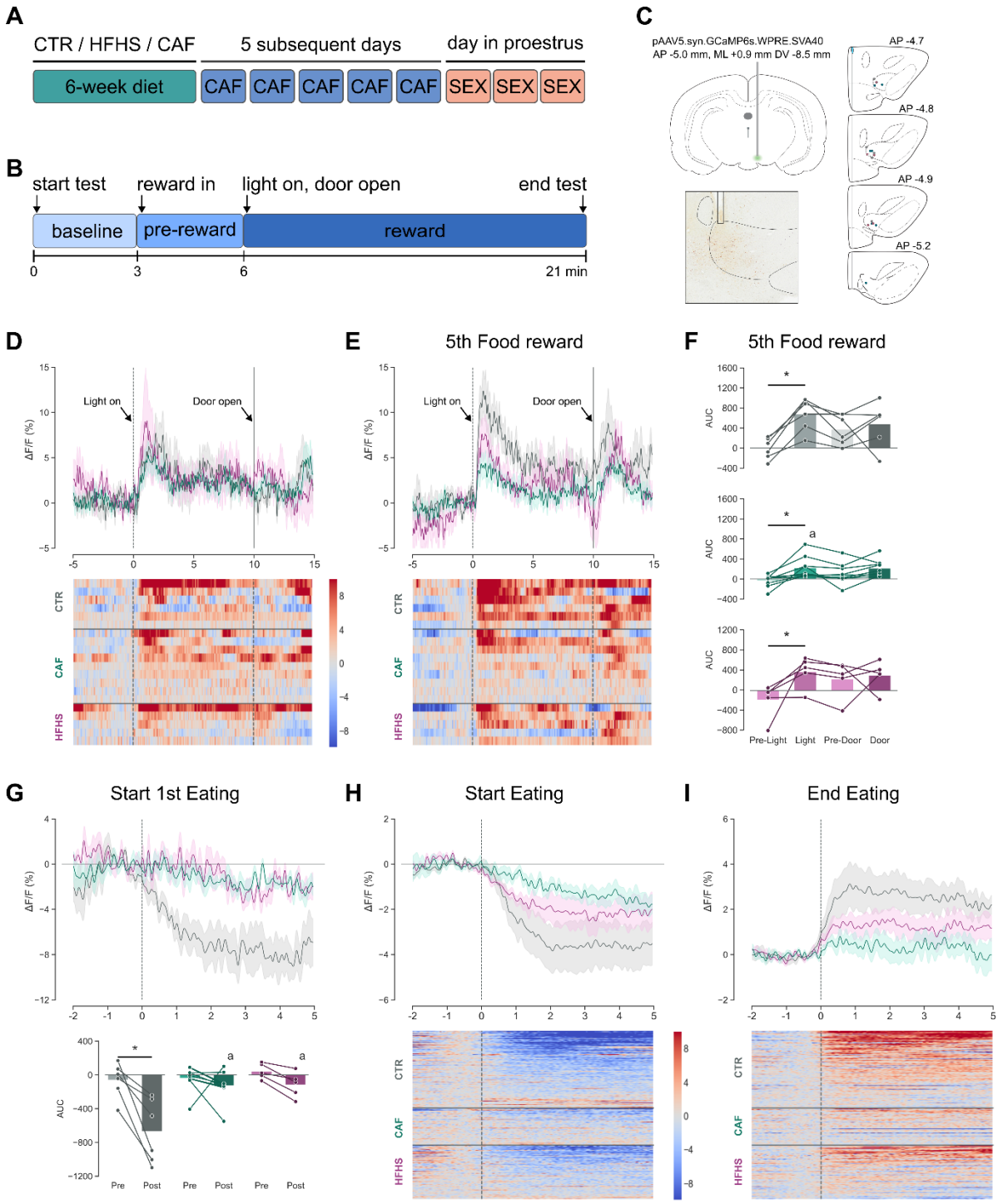

Figure 1. *The effects of Cafeteria, High fat high sugar, and control diet on the ventral tegmental area during a food reward test.* ***A)*** *Schematic representation of the experimental design. The female rats received 6 weeks of either a control (CTR), cafeteria (CAF), or high-fat, high-sugar (HFHS) diet, after which they were exposed to 5 sessions with a food reward (CAF reward) and 3 sessions with a sexual reward (male rat).* ***B)*** *Schematic representation of the behavioral reward test. Female rats are introduced into the left chamber with doors closed. After a baseline period of 3 minutes, the reward is placed into the right chamber while the doors remain closed (pre-reward phase). 3 minutes later, the light will turn on for 10 seconds before the door opens. Then, the subject has 15 minutes of free access to the reward and both chambers (reward phase).* ***C)*** *Schematic representation of surgical details and histologically determined fiber locations and AAV vector spread in the VTA.* ***D)*** *Upper panel: Group data from CTR, CAF and HFHS rats of the 1st food reward test showing mean GCaMP signals (ΔF/F in percentages, calculated from fluorescent changes aligned to the light cue at t=0). At*

t=10s, the door opened allowing access to the reward. Shaded areas represent SEM. Lower panel: Heat maps for the 1st food reward test of all CTR, CAF and HFHS rats showing mean GCaMP signals (ΔF/F in percentages, calculated from fluorescent changes aligned the light cue at t=0). At t=10s, the door opened allowing access to the reward. Trials are sorted by largest responses. ***E)*** *Upper panel: Group data from CTR, CAF and HFHS rats of the 5th food reward test showing mean GCaMP signals (ΔF/F in percentages, calculated from fluorescent changes aligned to the light cue at t=0). At t=10s, the door opened allowing access to the reward. Shaded areas represent SEM. Lower panel: Heat maps for the 5th food reward test of all CTR, CAF and HFHS rats showing mean GCaMP signals (ΔF/F in percentages, calculated from fluorescent changes aligned to the light cue at t=0). At t=10s, the door opened allowing access to the reward. Trials are sorted by largest responses.* ***F)*** *AUC for the 5th food reward test of CTR, CAF and HFHS rats before (pre-Light) and after (Light) the light cue, and before (Pre-door) and after (Door) the door opening. t-test 5th food reward: light: CTR: t=-4.304, p=0.002; CAF: t=-3.098, p=0.007; HFHS: t=-2.755, p=0.025; door: CTR: t=-0.508, p=0.623; CAF: t=-1.209, p=0.244; HFHS: t=-0.356, p=0.731; One-Way ANOVA 5th food reward: before light: F=0.96, p=0.403; CAF vs CTR: t=0.323, p=2.254, CAF vs HFHS: t=1.189, p=0.772, HFHS vs CTR: t=1.037, p=0.981, after light: F=5.422, p=0.015; post hoc: CAF vs CTR: t=3.231, p=0.02, CAF vs HFHS: t=-0.742, p=1.417, HFHS vs CTR: t=1.954, p=0.247, before door: F=1.803, p=0.195; CAF vs CTR: t=2.034, p=0.189, CAF vs HFHS: t=-0.629, p=1.622, HFHS vs CTR: t=0.952, p=1.098, after door: F=1.604, p=0.23; CAF vs CTR: t=1.678, p=0.352, CAF vs HFHS: t=-0.409, p=2.07, HFHS vs CTR: t=0.996, p=1.036.* ***G)*** *Upper panel: Group data from CTR, CAF and HFHS rats showing mean GCaMP signals (ΔF/F in percentages, calculated from fluorescent changes aligned to the start of eating episodes of the 1st eating in the 5th food reward test). Shaded areas represent SEM. Lower panel: AUC of CTR, CAF and HFHS rats before (pre) and after (post) the start of the very first eating episodes in the 5th reward test with a food reward. One-Way ANOVA 5th food reward: pre: F=0.684, p=0.518; post: F=9.932, p=0.001; post hoc: CAF vs CTR: t=-3.741, p=0.007, CAF vs HFHS: t=-0.131, p=2.693, HFHS vs CTR: t=-3.047, p=0.042, t-test pre vs post: CTR: t=3.449, p=0.006; HFHS: t=2.056, p=0.074; CAF: t=1.185, p=0.253.* ***H)*** *Upper panel: Group data from CTR, CAF and HFHS rats showing mean GCaMP signals (ΔF/F in percentages, calculated from fluorescent changes aligned to the start of eating episodes of all food reward tests). Shaded areas represent SEM. Lower panel: Heat maps for each eating episode of all CTR, CAF and HFHS rats showing mean GCaMP signals (ΔF/F in percentages, calculated from fluorescent changes aligned to the start of eating episodes of all food reward tests). Trials are sorted by largest responses.* ***I)*** *Upper panel: Group data from CTR, CAF and HFHS rats showing mean GCaMP signals (ΔF/F in percentages, calculated from fluorescent changes aligned to the end of eating episodes of all food reward tests). Shaded areas represent SEM. Lower panel: Heat maps for each eating episode of all CTR, CAF and HFHS rats showing mean GCaMP signals (ΔF/F in percentages, calculated from fluorescent changes aligned to the end of eating episodes of all food reward tests). Trials are sorted by largest responses.* ***All)*** *Figures show rats on control (CTR, grey color bars and lines), cafeteria (CAF, green color bars and lines), and high-fat, high-sugar pellet (HFHS, purple color bars and lines) diet. CTR n=6, CAF n=9, HFHS n=5. * p<0.05 compared to by line connected group. [a] p<0.05 compared to same bar of CTR.*

A comprehensive behavioral assessment allowed us to examine neural responses in the VTA during specific behaviors during the time the rats had unrestricted access to the food reward (Table S1). Analysis revealed a significant reduction in VTA activity, relative to baseline, upon initial consumption of the reward by CTR rats (Fig 1G). In contrast, long-term exposure to HFHS was associated with a smaller activity change than CTR, while long-term exposure to CAF was not associated with any change in activity. Furthermore, when assessing VTA responses to all eating episodes across all tests, rats exposed to the CAF diet displayed a pronounced attenuation of ΔF/F event-related transients in the VTA compared to HFHS rats and CTR rats (Fig 1H and S1B).

Moreover, the decrease in VTA activity appears to be sustained for the duration of eating episodes. When aligning the fiber photometry signal to the end of each episode of eating, a return to baseline

was evident in CTR rats (Fig 1I, shown as an increase due to ΔF/F normalization to baseline at t = 0). This effect was confirmed by analysis of the AUC, comparing the increase in ΔF/F before and after the end of the behavior (Fig S1C). Interestingly, the alterations in VTA neural activity upon eating persisted throughout the entirety of the behavioral reward test. CTR rats consistently displayed activity decreases in the VTA upon eating during the initial, middle, and final five-minute intervals of the test (Fig S1D), whereas CAF rats demonstrated an attenuated response in all intervals. This trend was also reflected in the AUC analysis, with the most pronounced effect observed during the middle interval of the reward period (Fig S1E).

We also analyzed changes in neural activity during appetitive behaviors such as sniffing. While CTR- and HFHS-rats demonstrated an elevation in ΔF/F at the onset of a sniffing episode, in comparison, the CAF-exposed rats showed attenuated activity (Fig S1F/G), resulting in a lack of effect on the AUC post versus pre sniffing.

Hence, our findings suggest that prolonged exposure to HFHS and CAF diets significantly impairs the VTA's reactivity to food rewards and predictive cues associated with food. Typically, an elevation in VTA activity reflects the perceived value of a cue associated with a reward. However, this response is compromised in rats consuming HFHS or CAF diets, suggesting that the value of food rewards might be diminished in these groups. Moreover, long-term CAF diet intake diminishes VTA responsiveness to both the anticipation and consumption of food rewards more profoundly compared to exposure to a HFHS diet. This underscores that the *choice of palatable food*, in combination with fat and sugar content (see Table S3-S5), is a major contributor to disrupting the neural mechanisms governing rewarding behaviors.

Long-term CAF diet exposure, in contrast to HFHS, reduces interest in a food reward.

Next, we sought to investigate whether the reduced VTA responses to the food (CAF) reward would also be connected to altered behavioral responses. A detailed behavioral assessment during the various phases of the reward test unveiled significant behavioral differences in rats subjected to long-term CAF diets compared to CTR and HFHS diets. During the reward phase, CAF-exposed rats exhibited a reduced frequency of eating (Fig S2A) and spent notably less time consuming the reward compared to both CTR and HFHS rats (Fig 2A). This disparity was evident from the initial exposure to the food reward and intensified over subsequent days, as HFHS and CTR rats increased their eating duration on the 3rd and 5th test days compared to the 1st. While one might attribute this observation to a novelty effect, given that CAF rats, in contrast to CTR and HFHS rats, were already

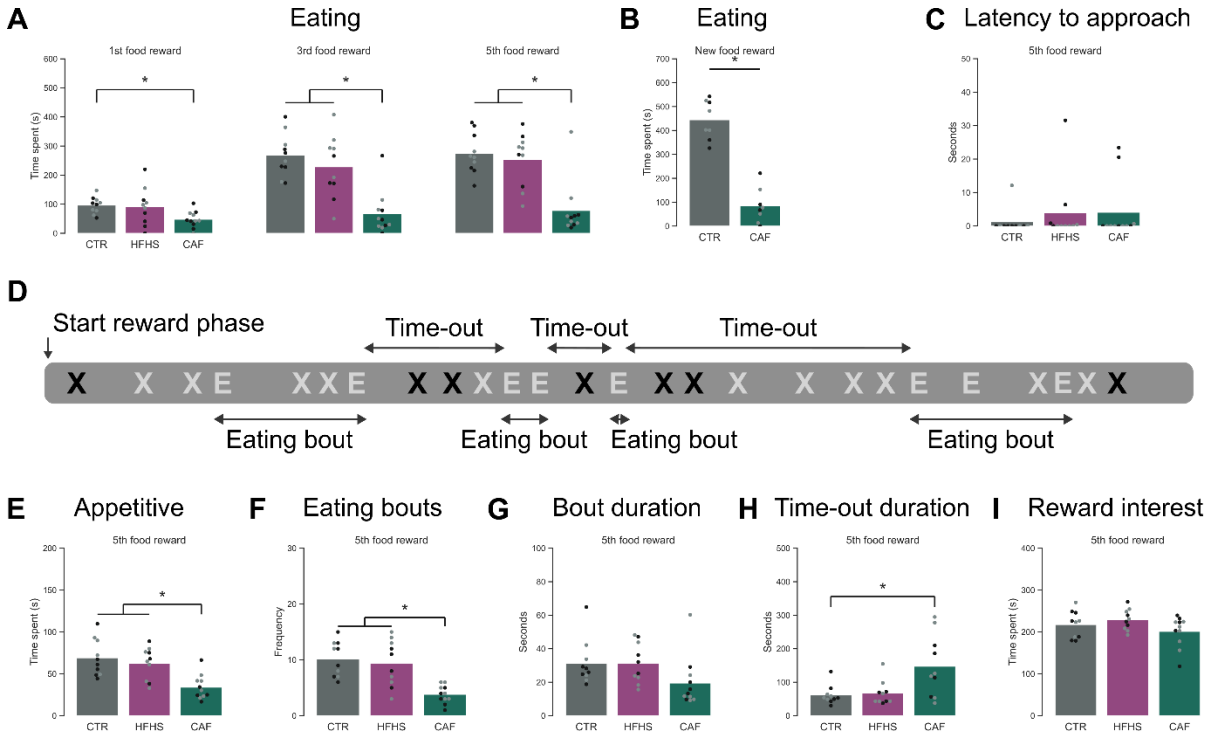

Figure 2. *The effects of Cafeteria, High fat high sugar, and control diet on behavioral responses during a food reward test.* ***A)*** *Time spent eating during the reward phase of the 1st, 3rd and 5th behavioral reward test with a food reward. One-Way ANOVA 1st food reward: F=4.459, p=0.022; post hoc: CAF vs CTR: t=-3.641, p=0.006, CAF vs HFHS: t=-2.39, p=0.086, HFHS vs CTR: t=0.245, p=2.429, 3rd food reward: F=14.172, p<0.001; post hoc: CAF vs CTR: t=-5.868, p<0.001, CAF vs HFHS: t=-3.754, p=0.004, HFHS vs CTR: t=-0.991, p=1.004, 5th food reward: F=16.647, p<0.001; post hoc: CAF vs CTR: t=-5.388, p<0.001, CAF vs HFHS: t=-4.326, p=0.001, HFHS vs CTR: t=-0.553, p=1.76, One-Way ANOVA CTR: F=14.973, p<0.001; post hoc: 1st vs 3rd food reward test: t=-6.863, p<0.001, 1st vs 5th food reward test: t=-7.464, p<0.001, 3rd vs 5th food reward test: t=-0.167, p=2.608, HFHS: F=9.599, p<0.001; post hoc: 1st vs 3rd food reward test: t=-3.152, p=0.017, 1st vs 5th food reward test: t=-4.235, p=0.002, 3rd vs 5th food reward test: t=-0.58, p=1.708, CAF: F=8.245, p<0.001; post hoc: 1st vs 3rd food reward test: t=-0.857, p=1.209, 1st vs 5th food reward test: t=-0.822, p=1.264, 3rd vs 5th food reward test: t=-0.108, p=2.746.* ***B)*** *Time spent eating a novel CAF reward during the reward phase; t-test: t=-8.78, p<0.001; CTR: n=8, CAF: n=8.* ***C)*** *Latency to interact with the CAF reward after door opening; One-Way ANOVA 5th food reward: F=0.405, p=0.671. CAF vs CTR: t=0.934, p=1.086, CAF vs HFHS: t=0.036, p=2.915, HFHS vs CTR: t=0.803, p=1.298.* ***D)*** *Schematic overview of eating bouts during the reward phase of the behavioral reward test with a food reward. An eating bout is defined as a sequence of eating episodes (E), with or without reward-related behaviors (X in white) in between, that are not interrupted by non-reward related behaviors (X in black). As soon as the attention is withdrawn from the food, an eating bout was ended with the last eating episode, and the next eating bout started with the next eating episode, with time-outs representing the breaks between eating bouts.* ***E)*** *Number of appetitive behaviors towards the food reward during the reward phase of the 5th food reward test. Appetitive behaviors include approaching and sniffing the reward. One-Way ANOVA 5th food reward: F=20.933, p<0.001; post hoc: CAF vs CTR: t=-7.872, p<0.001, CAF vs HFHS: t=-3.761, p=0.004, HFHS vs CTR: t=-2.157, p=0.134.* ***F)*** *Number of eating bouts during the reward phase of the 5th food test. An eating bout is defined as a row of eating episodes that are not interrupted by non-reward related behaviors. One-Way ANOVA 5th food reward: F=13.351, p<0.001; post hoc: CAF vs CTR: t=-5.931, p<0.001, CAF vs HFHS: t=-4.15, p=0.002, HFHS vs CTR: t=-0.486, p=1.899.* ***G)*** *Mean duration of eating bouts during the reward phase of the 5th food test. One-Way ANOVA 5th food reward: F=2.679, p=0.086. CAF vs CTR: t=-1.889, p=0.223, CAF vs HFHS: t=-1.984, p=0.186, HFHS vs CTR: t=0.004, p=2.991.* ***H)*** *Mean duration of time-outs between eating bouts during the reward phase of the 5th food test. One-Way ANOVA 5th food reward: F=6.569, p=0.005; post hoc: CAF vs CTR: t=2.828, p=0.033, CAF vs HFHS: t=2.569, p=0.058, HFHS vs CTR: t=0.392, p=2.099.* ***I)*** *Time spent on behaviors that indicate interest in the reward in the other chamber during the pre-reward phase of the 5th behavioral reward test with a food reward. Reward interest behaviors include sniffing and rearing the door, head oriented at the door, and sitting close to the door. One-Way ANOVA 5th food reward: F=2.029, p=0.15.*

CAF vs CTR: t=-1.079, p=0.882, CAF vs HFHS: t=-2.011, p=0.176, HFHS vs CTR: t=0.89, p=1.156. ***All)*** *Figures show rats on control (CTR, grey bar), high-fat, high-sugar pellet (HFHS, purple bar), and cafeteria (CAF, green bar) diet, with their dot colors representing their body weight category (grey: lighter than average, black: heavier than average). CTR n=10, HFHS n=10, CAF n=11. * p<0.05 compared to by line connected group.*

familiar with the CAF reward after 6 weeks of diet, it is noteworthy that longer latencies to consume novel foods or a so-called taste neophobia have been reported before[80]. Still, to investigate the potential influence of novelty on reward consumption, we repeated the study with CTR and CAF rats and added one food reward test to the test sequence and exposed them to a novel CAF reward (with different food products) after they had been tested in the behavioral reward test with the familiar CAF reward for 3 subsequent days. As in the previous tests, we found that CAF rats spent less time eating a novel CAF reward compared to CTR rats (Fig 2B). This, along with the fact that HFHS and CTR rats did not reduce their CAF consumption over the course of the week, argues against the suggestion that these results are purely dependent on novelty of food items and implies that short-term CAF exposure elicits distinct effects compared to long-term exposure.

When the behavior was explored in more detail, we found that the lower food consumption of CAF rats was not due to a lack of interest in gaining access to the reward. In fact, CAF rats interacted just as quickly with the food reward as CTR and HFHS rats in all tests (Fig 2C, 5th test). However, once the reward was explored, the CAF rats seemed to lose interest in it. Both the number of appetitive (sniffing and approaching) and consummatory (eating) behaviors toward the food were reduced (Fig 2E, 5th test), indicating a general decline in interest toward the CAF reward. This interest even reduced from the 1st to the 5th food reward test in CAF rats, while it increased in CTR females (Fig S2B).

Continuous free access to the reward facilitated a more advanced behavioral assessment than more standardly used conditioning reward tests. We adopted a method similar to our previous sexual behavior analyses with copulatory behavior divided into mount bouts [81-83], and segmented eating episodes into distinct eating bouts. This enabled us to look more closely into temporal patterning of the behavior. Here, we defined an eating bout as a sequence of eating episodes that are not interrupted by non-reward related behaviors (Fig 2D). As soon as attention was withdrawn from the food reward (e.g., exploring the environment, self-grooming, or turning the head away from reward), an eating bout would end at the last eating behavior. The next eating bout would start with the next eating behavior. Our findings revealed that CAF rats partitioned their eating episodes into fewer eating bouts (Fig 2F and Fig S2C), which were either slightly shorter or comparable in duration to those of CTR and HFHS rats (Fig 2G and Fig S2D). Instead, the time-outs between the eating bouts

were longer (Fig 2H and Fig S2E). Together, these results suggest that CAF rats exhibit reduced interest in the food reward, as evidenced by a decreased frequency of eating episodes. The reduced time spent eating thus appears to stem from the decreased number of eating bouts rather than shorter durations of individual bouts. Interestingly, no such effects were found in rats exposed to HFHS diet, suggesting that long-term CAF diet is a stronger manipulation than HFHS diet.

The observed changes in behaviors during the reward phase were not attributable to differences in baseline activity, as all rats spent comparable amounts of time exploring the chamber and engaging in grooming activities (Fig S2F/G). Similarly, no differences in behavior were found during the pre-reward phase in which the reward was presented in the opposite chamber while the door remained closed. CAF rats did not spend more or less time on behaviors indicating interest in the reward compared to HFHS and CTR rats (Fig 2I), such as exploring the door or orienting themselves toward the reward chamber. However, as anticipated, all rats spent approximately twice as much time engaging in these behaviors during the pre-reward phase compared to the baseline phase when no reward was present behind the doors (Fig S2H).

The combination of these findings suggests that in addition to its impact on VTA neural activity, long-term exposure to a CAF diet alters both appetitive and consummatory behavioral responses to a food reward, while leaving anticipatory behaviors and other reward-unrelated behaviors unaffected. Interestingly, long-term exposure to an HFHS diet did not produce the same effects and resulted in comparable levels of behavioral interest in the food reward as observed in CTR rats. These behavioral findings align with the effects observed in VTA neural activity.

Long-term CAF diet also affects VTA neural responses to other natural rewards

Next, we aimed to investigate whether long-term CAF diet consumption would influence both neural and behavioral responses to a natural reward other than food. To address this, we conducted three additional behavioral reward tests, this time with a sexual partner (a sexually experienced male rat) as reward. One limitation of using naturally cycling females in the study is the potential occurrence of pseudopregnancies following copulation sessions. In the event of pseudopregnancy, female rats are unable to copulate again for approximately two weeks. To mitigate this issue, we conducted all three sexual reward tests on a single day. Regrettably, not all females reached the proestrus phase and these females were excluded from the analysis. Notably, only six out of ten females of the HFHS group reached proestrus, and two of these females did not engage in copulation with the stimulus male. Due to these insufficient copulation occurrences in the HFHS group, in addition to the observation that HFHS did not differ much from CTR in food reward responses, we proceeded to analyze only the CTR and CAF rats. In the CAF group, ten females reached the proestrus phase, but

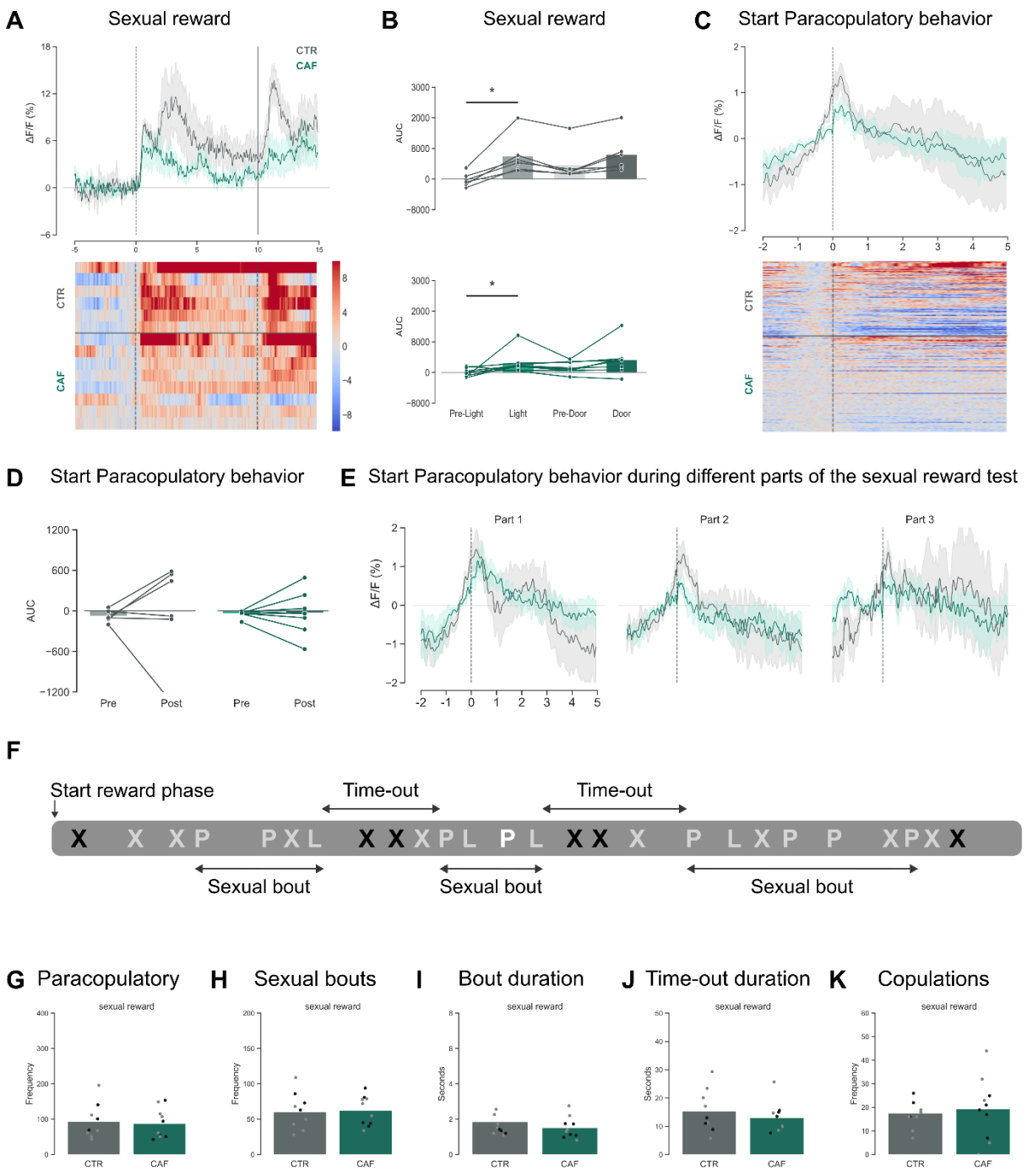

Figure 3. *The effects of Cafeteria diet on the ventral tegmental area and behavioral responses during a sexual reward test.* ***A)*** *Upper panel: Group data from CTR and CAF rats of the best sexual reward test showing ΔF/F percentages calculated from fluorescent changes aligned to the light cue at t=0. At t=10, the door opened allowing access to the reward. Solid lines indicate mean. Shaded area represents SEM. The best test was defined by the reward test (1st, 2nd or 3rd) in which the female had most paracopulatory behaviors. Lower panel: Heat maps for best sexual reward test of CTR and CAF rats showing ΔF/F percentages calculated from fluorescent changes aligned the light cue at t=0. At t=10, the door opened allowing access to the reward. Trials are sorted by largest responses.* ***B)*** *AUC for the best sexual reward test of CTR (upper) and CAF (lower) rats before (pre-Light) and after (Light) the light cue, and before (Pre-door) and after (Door) the door opening. t-test: light: CTR: t= -2,816, p= 0.018; CAF: t=-2.226, p=0.043; door: CTR: t=-0.98, p=0.35; CAF: t=-1.261, p=0.228. CTR vs CAF: pre-light: t=-0.246, p=0.81; post-light: t=1.624, p=0.13; pre-door: t=0.211, p=0.632; post-door: t=1.289, p=0.222.* ***C)*** *Upper panel: Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the start of paracopulatory behaviors in the best*

sexual reward test). Shaded areas represent SEM. Lower panel: Heat maps for each paracopulatory behavior episode of all CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the start of paracopulatory behaviors of best sexual reward tests). Trials are sorted by largest responses. ***D)*** *AUC of CTR and CAF rats before (pre) and after (post) the start of paracopulatory behaviors of best sexual reward tests. t-test CTR vs CAF pre: t=-0.941, p=0.365; post: t=0.118, p=0.908; t-test pre vs post: CTR: t=-0.269, p=0.793; CAF: t=-0.102, p=0.92.* ***E)*** *Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the start of the start of paracopulatory behaviors of the best sexual reward tests during the 1st, the 2nd and last part of the reward phase). Shaded areas represent SEM.* ***F)*** *Schematic overview of sexual bouts, with a sexual bout defined as a sequence of paracopulatory behaviors (P) and lordosis responses (L), with or without reward-related behaviors (X in white) in between, that are uninterrupted by non-reward related behaviors (X in black). As soon as the attention is withdrawn from the male rat, a sexual bout was ended with the last paracopulatory or lordosis behavior and the next bout started with the next paracopulatory or lordosis behavior.* ***G)*** *Total number of paracopulatory behaviors (darts and hops) during the reward phase of the best sexual reward test. t-test CAF vs CTR: t=-0.272, p=2.367.* ***H)*** *The total number of sexual bouts during the reward phase of the best sexual reward test. t-test: CAF vs CTR: t=0.176, p=2.588.* ***I)*** *Mean duration of a sexual bout during the reward phase of the best sexual reward test. t-test: CAF vs CTR: t=-0.951, p=1.063.* ***J)*** *Mean duration of time-outs during the reward phase of the best sexual reward test. t-test: CAF vs CTR: t=-0.809, p=1.287.* ***K)*** *Total number of received copulations (mounts, intromissions and ejaculations) during the reward phase of the best sexual reward test. t-test CAF vs CTR: t=0.897, p=1.147.* ***ALL)*** *Figures show rats on control (CTR, grey color bars and lines) and cafeteria (CAF, green color bars and lines) diet. CTR n=6, CAF n=8 for fiber photometry data and CTR n= 10, CAF n=9 for behavioral data. The dots in the behavioral data have colors representing their body weight category (grey: lighter than average, black: heavier than average) * p<0.05 compared to by line connected group. [a] p<0.05 compared to same bar of CTR.*

three of them did not exhibit any paracopulatory behaviors during the first sexual reward test. This observation suggests a slight delay in the onset of the receptive phase compared to CTR females. To be able to make proper inter-rat comparisons, we analyzed the photometry and behavioral data from the most successful sexual reward test. The *best sexual reward test* was defined as the test (either the first, second, or third) in which the female displayed the highest number of paracopulatory behaviors.

First, we looked at the VTA response evoked by the light cue that preceded the opening of the door. Interestingly, in the same way as in the test with food reward, the cue indicating access to a sexual reward elicited a more pronounced neural activity increase in CTR rats compared to CAF rats (Fig 3A). While there was a significant increase in activity after the light cue in CAF rats (Fig 3B), from the heatmap it becomes apparent that this increase was far smaller and more transient than it was for CTR rats (Fig 3A). In contrast, all CTR rats displayed heightened VTA activity following the cue. Therefore, we conclude that rats exposed to a long-term CAF diet show an attenuated VTA cue-response compared to CTR rats.

In addition, we explored activity patterns of the VTA during sexual behaviors, with particularly intriguing findings regarding paracopulatory behaviors. Notably, while Fig 3C illustrates only a marginal 1% increase in ΔF/F levels during paracopulatory behavior in CTR rats, CAF rats exhibited

diminished VTA responses. Despite the absence of significant changes in the AUC (Fig 3D) after the onset of paracopulatory behaviors in both CTR and CAF rats, heatmap visualization revealed notable differences (Fig 3C). Specifically, there was a greater variation in VTA responses among CTR rats, compared to the CAF rats. Interestingly, while half of the paracopulatory behavior trials in CTR rats coincided with increased VTA activity, the remaining trials resulted in a decrease.

One potential factor causing a lack of clear and consistent response during paracopulatory behaviors could be the time these behaviors took place within the reward test. However, considerable variation in activity levels was seen across the first, second, and third five-minute intervals of the reward phase (Fig 3E). This suggests that timing alone does not fully account for the variability in VTA responses to paracopulatory behavior. This was supported by an analysis of sexual bouts (done in a similar way as eating bouts, Fig 3F), which showed a lack of distinct VTA responses to paracopulatory behaviors that start or end a sexual bout, even though the last dart of a sexual bout appears to trigger a more pronounced distinction between rats with increased and decreased neural activity (Fig S3A/B). Overall, these findings suggest that the VTA likely encodes paracopulatory behaviors, and that this function is diminished by long-term exposure to CAF diet

This impact of CAF diet on VTA responses is particularly intriguing, given that no differences in behavioral expressions were observed during the sexual reward test. CAF rats exhibited a comparable number of paracopulatory behaviors to CTR rats during the reward phase (Fig 3G). Moreover, when the micropatterns of sexual bouts were assessed, no differences were found in the number of sexual bouts (Fig 3H), the mean duration of sexual bouts (Fig 3I), or the mean duration of the time-outs (Fig 3J). Consequently, CAF females received a similar number of copulations from male partners as CTR females (Fig 3K), even when the number of mounts, intromissions, and ejaculations are considered separately (data not shown).

While paracopulatory behavior is often defined as appetitive or proceptive behavior, lordosis is considered a consummatory or receptive behavior. Our data revealed that long-term exposure to CAF diet does not alter the receptive responses toward a sexual reward, resulting in similar levels of lordosis quotient (Fig 4A) and lordosis score (Fig 4B) in CTR and CAF rats. Similarly, when we explored the role of the VTA during these behaviors we found no change in VTA activity after the display of a lordosis behavior (Fig 4C). However, lordosis behavior is usually reflexive and triggered by a touch from the male. It is displayed throughout a copulation behavior, with considerable variability in both duration and quality (also quantified as a lordosis score[84]). Because these factors could be relevant for analysis of activation patterns of the VTA, we classified the lordosis behaviors based on intensity and type of stimulation when linking these behavioral epochs with the fiber

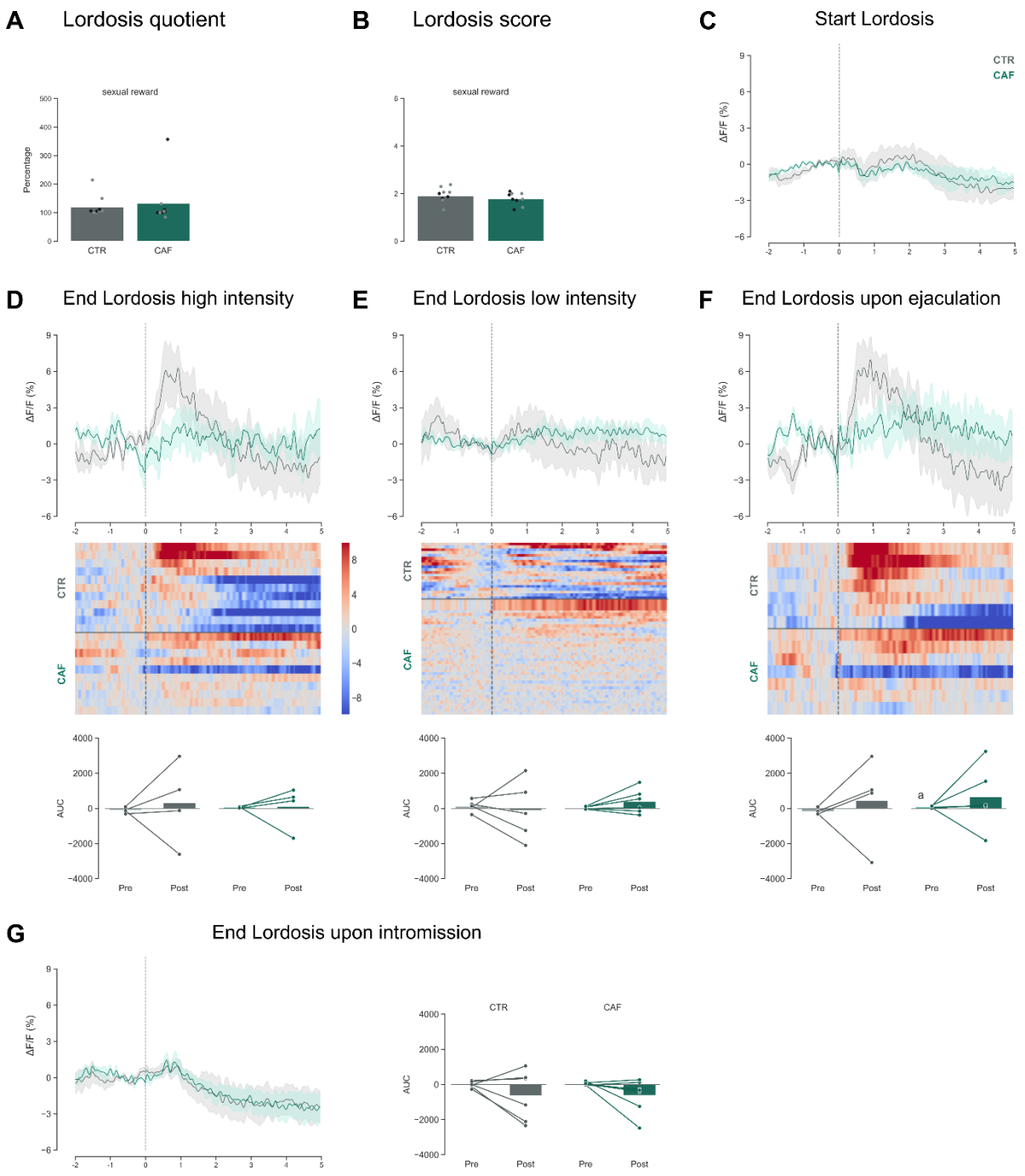

Figure 4. *The effects of Cafeteria diet on the ventral tegmental area and lordosis responses during a sexual reward test.* ***A)*** *Lordosis quotient during the reward phase of the best sexual reward test. The lordosis quotient was calculated by the sum of lordosis responses (score 1-3) divided by the number of received copulations (mounts, intromissions and ejaculations) multiplied by 100%. t-test CAF vs CTR: t=0.425, p=2.029.* ***B)*** *Lordosis score during the reward phase of the best sexual reward test. The lordosis score was calculated by multiplying the quality of the score (0-3 with 0 for no lordosis and 3 for the most intense lordosis) by the number of times it was performed, divided by the total number of lordosis responses. t-test CAF vs CTR: t=-0.778, p=1.341.* ***C)*** *Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the start of lordosis behaviors in the best sexual reward test). Shaded areas represent SEM.* ***D)*** *Upper panel: Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the end of lordosis behaviors of high intensity in the best sexual reward test). Shaded areas represent SEM. Middle panel: Heat maps for each lordosis behavior episode of all CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent*

changes aligned to the lordosis behaviors of high intensity of best sexual reward tests). Trials are sorted by largest responses. Lower panel: AUC of CTR and CAF rats before (pre) and after (post) the end of lordosis behaviors of high intensity of best sexual reward tests. t-test CTR vs CAF pre: t=-1.905, p=0.105; post: t=0.165, p=0.875; t-test pre vs post: CTR: t=-0.367, p=0.726; CAF: t=-0.071, p=0.945. ***E)*** *Upper panel: Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the end of lordosis behaviors of low intensity in the best sexual reward test). Shaded areas represent SEM. Middle panel: Heat maps for each lordosis behavior episode of all CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the lordosis behaviors of low intensity of best sexual reward tests). Trials are sorted by largest responses. Lower panel: AUC of CTR and CAF rats before (pre) and after (post) the end of lordosis behaviors of low intensity of best sexual reward tests. t-test CTR vs CAF pre: t=0.69, p=0.508; post: t=-0.673, p=0.518; t-test pre vs post: CTR: t=0.316, p=0.76; CAF: t=-1.257, p=0.237.* ***F)*** *Upper panel: Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the end of lordosis behaviors upon ejaculation in the best sexual reward test). Shaded areas represent SEM. Middle panel: Heat maps for each lordosis behavior episode of all CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the lordosis behaviors upon ejaculations of best sexual reward tests). Trials are sorted by largest responses. Lower panel: AUC of CTR and CAF rats before (pre) and after (post) the end of lordosis behaviors upon ejaculation of best sexual reward tests. t-test CTR vs CAF pre: t=-2.824, p=0.026; post: t=-0.137, p=0.895; t-test pre vs post: CTR: t=-0.488, p=0.643; CAF: t=-0.674, p=0.519.* ***G)*** *Left panel: Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the end of lordosis behaviors upon intromission in the best sexual reward test). Shaded areas represent SEM. Right panel: AUC of CTR and CAF rats before (pre) and after (post) the end of lordosis behaviors upon intromission of best sexual reward tests. t-test CTR vs CAF pre: t=-0.672, p=0.515; post: t=-0.03, p=0.976; t-test pre vs post: CTR: t=1.07, p=0.31; CAF: t=1.828, p=0.093.* ***All)*** *Figures show rats on control (CTR, grey color bars and lines) and cafeteria (CAF, green color bars and lines) diet. CTR n=6, CAF n=8. * p<0.05 compared to by line connected group. [a] p<0.05 compared to same bar of CTR.*

photometry data. It is important to mention that the fiber photometry signals are normalized for baseline at t = 0. As a consequence, signals that appear as an activity increase, may be a rebound from a period of decreased activity. In any case, we found that changes in neural activity in the VTA corresponded best with the end of lordosis responses and that only high intensity lordoses result in increases in VTA activity (Fig 4D), although no significant effects were detected in the AUC due to substantial response variability (Fig 4D). Low intensity lordosis responses, on the other hand, fail to elicit changes in VTA activity (Fig 4E). Overall, this suggests that the VTA does not encode the onset of a lordosis behavior, but rather responds to the received stimulation.

When we analyzed the photometry data based on the type of copulation the female received, we found an increase in VTA activity after receiving an ejaculation in CTR females (Fig 4F). However, this increase failed to materialize as a significant change in the AUC metric, due to substantial variability of the responses, even when the AUC analysis was limited to the first two seconds post-lordosis (Fig 4F). Interestingly, CAF rats did not show this activity increase, once again demonstrating an attenuated VTA response. Additionally, no changes in activity levels were observed following a lordosis response to an intromission (Fig 4G). Instead, both CTR and CAF rats exhibited a decrease in neural activity, although this was not reflected in the AUC.

In summary, our findings indicate a role for the VTA in responses to both food and sexual rewards. While the effect of junk food consumption has a profound effect on the response to food rewards, a modest impact extends to sexual rewarding behaviors as well. Long-term exposure to CAF diet, however, affects the VTA in such a way that the behavior can still be normally displayed despite an attenuated VTA response.

Two weeks of standard chow diet is not enough to reverse the CAF-induced changes in response to food rewards.

To assess the reversibility of neural and behavioral alterations induced by long-term exposure to CAF diet, all rats were, after their last behavioral test sequence, transitioned to a standard chow diet for a period of two weeks. This was followed by three iterations of the behavioral reward test with a food stimulus (CAF) and one instance with a sexual stimulus (Fig 5A). Initially, we observed that the two-week period of dietary reversal was insufficient to restore the attenuated VTA responses elicited by the light cue associated with a food reward in CAF-exposed rats (Fig 5B/C). A similar lack of recovery was evident in the VTA neural responses during the behavioral interactions with the food reward. Specifically, CAF-exposed rats continued to exhibit attenuated neural activity responses during eating both in the 1st (Fig 5D) and 3rd food reward test (Fig 5E) post-reversal diet. The attenuated response persisted in CAF rats during the initial food reward test after dietary reversal, as demonstrated by a significant difference in AUC (Fig 5D), but this effect diminished in magnitude by the third food reward test (Fig 5E), even though it remained visible in the heatmaps. These results suggest a persistent effect of long-term CAF diet on the VTA, which a two-week reversal diet cannot readily undo.

Interestingly, even though two weeks of healthy diet was not sufficient to restore VTA function, a partial behavioral recovery was observed. Analysis revealed that CAF rats still ate less frequently than the CTR in both the 1st and 3rd food reward test following the dietary reversal period (Fig 5G). However, regarding time spent eating (Fig S5A), number of eating bouts (Fig S5B), or mean duration of time-outs (Fig S5C), CAF rats displayed diminished levels compared to CTR rats only in the 1st food reward test, with no significant differences observed in the third test. Furthermore, consistent with pre-reversal observations, all rats spent an equal amount of time within an eating bout in all tests (the mean duration of eating bouts (Fig S5D). Likewise, although behavioral indications suggested a recovery in appetitive behavior (approaching and sniffing the food reward) among CAF rats in the 3rd food reward test post-reversal (although not in the 1st, Fig 5H), neural responses within the VTA remained attenuated compared to CTR rats at both the 1st (Fig S5E) and 3rd food reward test (Fig 5F).

Altogether, our findings suggest that a two-week period of healthy dietary intervention is insufficient to fully restore the immediate behavioral or neural responses to a CAF food reward. However, it does partially improve behavioral alterations after repeated exposures, *despite* persistent reduced responsiveness in the VTA.

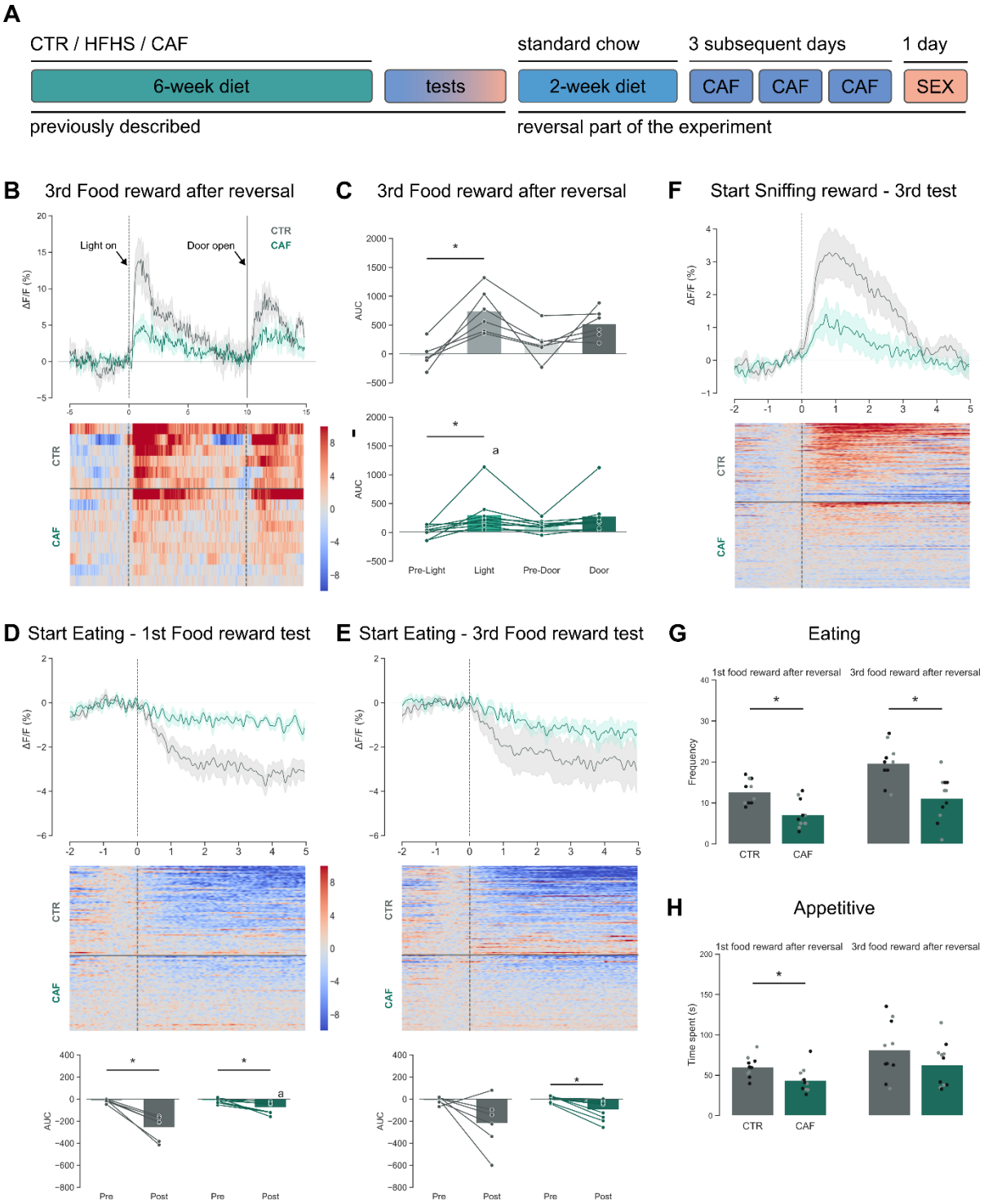

Figure 5. *The effects of reversal diet after Cafeteria diet exposure on the ventral tegmental area and behavioral responses during a food reward test* ***A)*** *Schematic representation of the experimental design. After the female rats received 6 weeks of either a control (CTR), or cafeteria (CAF) diet, they were tested five times with a food*

reward (CAF reward) and 3 times with a sexual reward (male rat), all rats were placed on a reversal diet of standard chow for 2 weeks. Next, they were tested three more time with a food reward and once with a sexual reward in the behavioral reward test. ***B)*** *Upper panel: Group data from CTR and CAF rats of the 3rd food reward test post-reversal diet showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the light cue at t=0). At t=10s, the door opened allowing access to the reward. Shaded areas represent SEM. Lower panel: Heat maps for the 3rd food reward test post-reversal diet of all CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned the light cue at t=0). At t=10s, the door opened allowing access to the reward. Trials are sorted by largest responses.* ***C)*** *AUC from CTR (upper) and CAF (lower) for the 3rd food reward test post-reversal diet before (pre-Light) and after (Light) the light cue, and before (Pre-door) and after (Door) the door opening. t-test pre vs post light: CTR: t=-4.308, p=0.002; CAF: t=-2.52, p=0.023; pre vs post door: CTR: t=-2.161, p=0.056; CAF: t=-1.469, p=0.161; t-test CAF vs CTR: pre-light: t=-0.416, p=0.684; post-light: t=2.451, p=0.029; pre-door: t=0.429, p=1.287; post-door: t=1.609, p=0.132.* ***D)*** *Upper panel: Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the start of eating in the 1st food reward test post-reversal diet). Shaded areas represent SEM. Middle panel: Heat maps for each eating behavior episode of all CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the start of eating in the 1st food reward test post-reversal diet). Trials are sorted by largest responses. Lower panel: AUC of CTR and CAF rats before (pre) and after (post) the start of eating in the 1st food reward test post-reversal diet. t-test CTR vs CAF pre: t=-0.405, p=0.692; post: t=-4.207, p=0.001; t-test pre vs post: CTR: t=5.16, p<0.001; CAF: t=3.255, p=0.005.* ***E)*** *Upper panel: Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the start of eating in the 3rd food reward test post-reversal diet). Shaded areas represent SEM. Middle panel: Heat maps for each eating behavior episode of all CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the start of eating in the 3rd food reward test post-reversal diet). Trials are sorted by largest responses. Lower panel: AUC of CTR and CAF rats before (pre) and after (post) the start of eating in the 1st food reward test post-reversal diet. t-test CTR vs CAF pre: t=-0.815, p=0.43; post: t=-1.457, p=0.169; t-test pre vs post: CTR: t=2.2, p=0.052; CAF: t=3.1, p=0.007.* ***F)*** *Upper panel: Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the start of sniffing the reward in the 3rd food reward test post-reversal diet). Shaded areas represent SEM. Lower panel: Heat maps for each sniffing episode of all CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the start of sniffing in the 3rd food reward test post-reversal diet). Trials are sorted by largest responses.* ***G)*** *Total number of eating episodes of the food reward during the reward phase of the 3rd food reward test post-reversal diet. 1st food reward test after reversal: t-test CAF vs CTR: t=-3.987, p=0.002; 3rd food reward after reversal: t-test CAF vs CTR: t=-3.786, p=0.004; t-test 1st vs 3rd food reward test after reversal: CTR: t=-3.885, p=0.001, CAF: t=-2.123, p=0.046.* ***H)*** *Time spent on appetitive behaviors towards the food reward during the reward phase of the 3rd food reward test post-reversal diet. Appetitive behaviors include approaching and sniffing the reward. 1st food reward test after reversal: t-test CAF vs CTR: t=-2.864, p=0.03; 3rd food reward after reversal: t-test CAF vs CTR: t=-1.334, p=0.594; t-test 1st vs 3rd food reward test after reversal: CTR: t=-1.715, p=0.104, CAF: t=-2.11, p=0.048.* ***All)*** *Figures show rats on control (CTR, grey color bars and lines) and cafeteria (CAF, green color bars and lines) diet. CTR n=6, CAF n=9 for fiber photometry data and CTR n= 10, CAF n=11 for behavioral data. The dots in the behavioral data have colors representing their body weight category (grey: lighter than average, black: heavier than average) * p<0.05 compared to by line connected group. [a] p<0.05 compared to same bar of CTR.*

Two weeks of standard chow diet inadequate in restoring the CAF-induced changes to sexual rewards.

Lastly, following a two-week exposure to a healthy diet, the rats underwent another sexual reward test. Once more, CAF-exposed rats exhibited no recovery in VTA responsiveness to the light-cue, maintaining an attenuated response compared to control rats (Fig 6A). Unfortunately, technical issues prevented recording from some CTR animals resulting in insufficient power for analysis of AUC

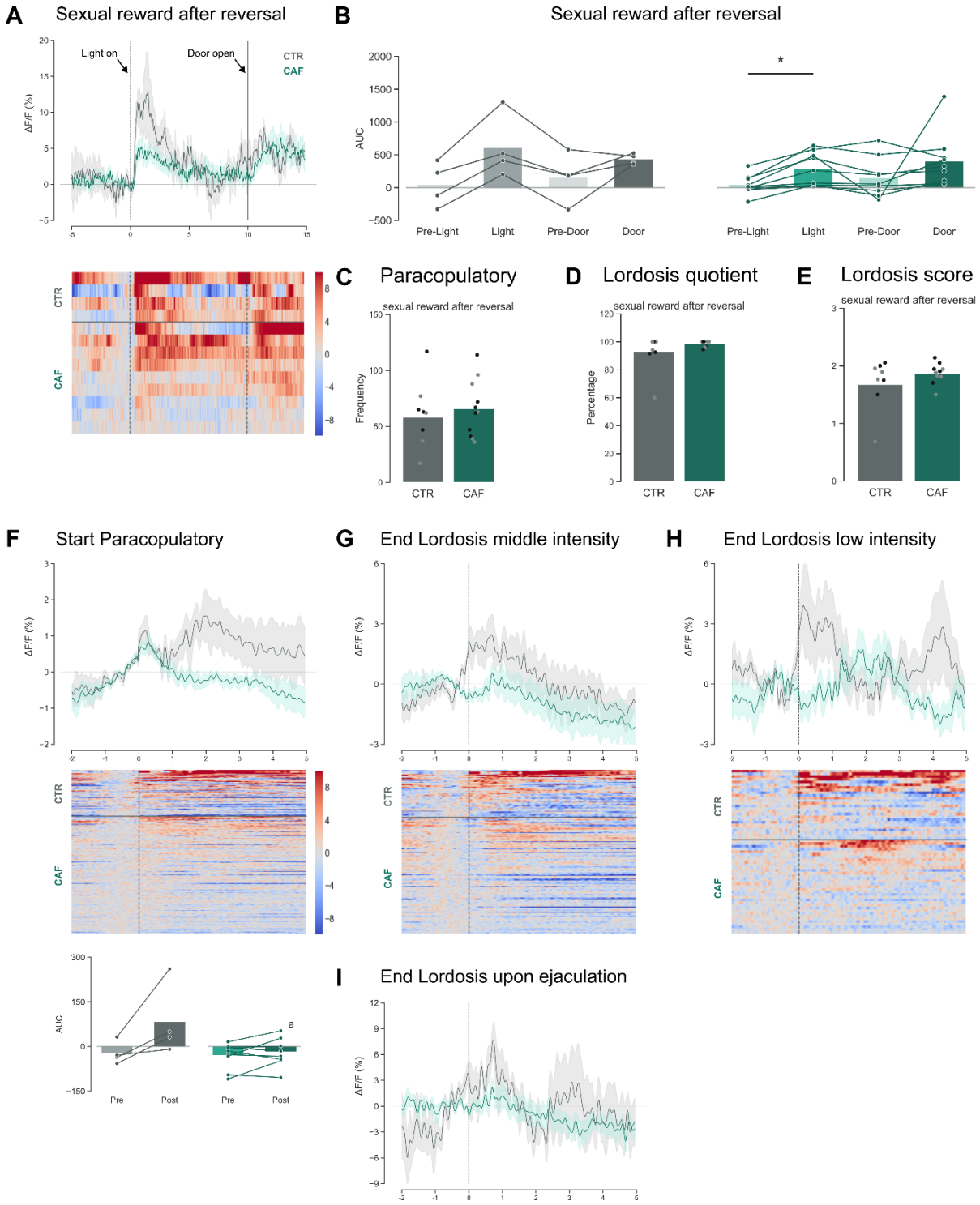

Figure 6. *The effects of reversal diet after Cafeteria diet exposure on the ventral tegmental area and behavioral responses during a sexual reward test.* ***A)*** *Upper panel: Group data from CTR and CAF rats of the sexual reward test post-reversal diet showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the light cue at t=0). At t=10s, the door opened allowing access to the reward. Solid lines indicate mean. Shaded area represents SEM. Lower panel: Heat maps for the sexual reward test post-reversal diet of all CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned the light cue at t=0). At t=10s, the door opened allowing access to the reward. Trials are sorted by largest responses.* ***B)*** *AUC from CTR (left) and CAF (right) for the sexual reward test post-reversal diet before (pre-Light) and after (Light) the light cue, and before (Pre-door) and after (Door) the door opening. t-test pre vs post light: CTR: t=-1.909, p=0.105; CAF: t=-2.435, p=0.027; pre vs post door: t=-1.457, p=0.195; CAF: t=-1.508,*

p=0.151; t-test CAF vs CTR: pre-light: t=-0.026, p=0.98; post-light: t=1.614, p=0.135; pre-door: t=0.02, p=0.984; post-door: t=0.133, p=0.896. ***C)*** *Total number of paracopulatory behaviors during the reward phase of the sexual reward test after reversal diet. t-test CAF vs CTR: t=0.647, p=1.577.* ***D)*** *Lordosis quotient during the reward phase of during the sexual reward test after reversal diet. The lordosis quotient was calculated by the sum of lordosis responses (score 1-3) divided by the number of received copulations (mounts, intromissions and ejaculations) multiplied by 100%. t-test CAF vs CTR: t=1.078, p=0.888.* ***E)*** *Lordosis score during the reward phase of the sexual reward test after reversal diet. The lordosis score was calculated by multiplying the quality of the score (0-3 with 0 for no lordosis and 3 for the most intense lordosis) by the number of times it was performed, divided by the total number of lordosis responses. t-test CAF vs CTR: t=1.321, p=0.612.* ***F)*** *Upper panel: Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the start of paracopulatory behaviors in the sexual reward test after reversal diet). Shaded areas represent SEM. Middle panel: Heat maps for each paracopulatory behavior episode of all CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the paracopulatory behaviors in the sexual reward test after reversal diet). Trials are sorted by largest responses. Lower panel: AUC of CTR and CAF rats before (pre) and after (post) the start of paracopulatory behaviors in the sexual reward test after reversal diet. t-test CTR vs CAF pre: t=0.3, p=0.77; post: t=2.253, p=0.046; t-test pre vs post: CTR: t=-1.668, p=0.146; CAF: t=-0.596, p=0.559.* ***G)*** *Upper panel: Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the end of lordosis behavior with middle intensity in the sexual reward test after reversal diet). Shaded areas represent SEM. Lower panel: Heat maps for each lordosis behavior episode of all CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the end of lordosis behavior with middle intensity in the sexual reward test after reversal diet). Trials are sorted by largest responses.* ***H)*** *Upper panel: Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the end of lordosis behavior with low intensity in the sexual reward test after reversal diet). Shaded areas represent SEM. Lower panel: Heat maps for each lordosis behavior episode of all CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the end of lordosis behavior with low intensity in the sexual reward test after reversal diet). Trials are sorted by largest responses.* ***I)*** *Group data from CTR and CAF rats showing mean GCaMP signals (ΔF/F in percentages calculated from fluorescent changes aligned to the end of lordosis behavior upon ejaculation in the sexual reward test after reversal diet). Shaded area represents SEM.* ***All)*** *Figures show rats on control (CTR, grey color bars and lines) and cafeteria (CAF, green color bars and lines) diet. CTR n=4, CAF n=9 for fiber photometry data and CTR n= 10, CAF n=9 for behavioral data. The dots in the behavioral data have colors representing their body weight category (grey: lighter than average, black: heavier than average) * p<0.05 compared to by line connected group. [a] p<0.05 compared to same bar of CTR.*

(Fig 6B). Nevertheless, the heatmap illustrates that some CAF rats displayed increased VTA activity, while others exhibited a diminished response to the light cue and door opening (Fig 6A).

Furthermore, the two weeks of healthy diet did not restore the VTA responsiveness in relation to sexual behavior, despite the absence of differences in sexual behavior between CTR and CAF rats (Fig 6C-E). Interestingly, certain effects only appeared to have intensified. Specifically, while the VTA response to paracopulatory behaviors was unclear in the initial test, a distinct attenuating effect in VTA neural activity among CAF rats compared to CTR rats was evident after reversal of diets (Fig 6F). Although CTR rats did not exhibit a significant increase in AUC after the onset of paracopulatory behaviors, a difference with CAF rats was found (Fig 6F). Again, a notable divergence in VTA responses (increased vs decreased activity levels) to paracopulatory behaviors was apparent among CTR rats, whereas CAF rats displayed a lack of responsiveness.

Moreover, the data revealed that CAF rats continued to exhibit an attenuated neural response in the VTA during lordosis episodes. While there were an insufficient trial number of the highest lordosis intensity in CTR rats for a robust comparison, the second-highest intensity (Fig 6G) and now also the low intensity of lordosis (Fig 6H) still evoked an increase in activity in CTR rats immediately following the end of a lordosis. However, these increases were too subtle to elicit significant effects in the AUC (data not shown). Likewise, the dietary reversal failed to restore the attenuated responses of CAF rats regarding received ejaculations (Fig 6I).

Overall, we conclude that two weeks of healthy diet is not yet sufficient to reverse the effects that long-term exposure to CAF diet had on the VTA. In contrast, behavioral responses toward food rewards were partially restored. Interestingly, as the VTA begins to exhibit more discrete responses to sexual behavior, it becomes increasingly apparent that rats previously exposed to long-term CAF diets displayed attenuated VTA responses.

Discussion

Our study reveals that rats on a long-term CAF diet, which consists of a *free choice* of highly palatable and unhealthy products, exhibited reduced interest in the CAF reward and consumed it less compared to rats on a control diet. These behavioral changes were accompanied by diminished neural activity in the VTA in response to a food-predictive cue and when the rats interacted with the food reward directly. Similarly, but to a lesser extent, reductions in VTA activity were observed in relation to a sexual reward. However, no significant behavioral differences were observed between the control and CAF rats in terms of appetitive or consummatory behaviors during sexual interactions. Furthermore, the study confirmed our hypothesis that CAF diet alters the functioning of the reward circuitry more severely than a high-fat, high-sugar diet. This finding coincided with the marked difference that CAF and HFHS diets had on food reward-related behavior: while long-term exposure to CAF seemed to limit interactions with the food reward, exposure to HFHS did not. Finally, we found that a reversal diet intervention of two weeks with healthy food was not sufficient to restore the behavioral and neural alterations seen after CAF diet consumption. Overall, these results suggest that prolonged exposure to junk food leads to diminished activity of VTA neurons, corresponding with behavioral changes specifically in response to food rewards but not sexual rewards.

Our data supports our hypothesis that CAF diet more severely affects the VTA than HFHS diet does. This effect is most likely due to the element of *choice* of unhealthy products with different taste, texture, novelty and variety in CAF diet in contrast to HFHS pellets[76,77]. Consistent with other studies, we observed that the free choice consumption and variation of food items led to greater body

weight gain [74] and increased overeating [75] (See Table S4). Notably, each cage showed different consumption patterns, with preferences for different foods (shown in Table S5). This overeating may contribute to more pronounced alterations in the brain, as indicated by stronger attenuation of VTA responses in CAF-fed rats compared to those on the HFHS diet. Since HFHS rats did not show differences in behavior toward a food reward, one could argue that the alterations in the brain (which were less pronounced than with CAF exposure) were simply not substantial enough to trigger behavioral responses. Overall, CAF diet seems to be a more robust model to study excessive junk food consumption than HFHS pellets, even though implementing it is more laborious and provides less control over the nutritional value of the consumed food.

Furthermore, our study was the first to show that long-term CAF diet can change event-related neural responses in the VTA toward a food and sexual reward, as well as reward-predictive cues. So far, most studies have shown effects of junk food on the hippocampus or nucleus accumbens, but only a few studies have reported potential changes in the VTA[85-88]. Interestingly, all these studies report reduced levels of receptors [85,87,89] or tyrosine hydroxylase[88], supporting our findings of reduced VTA functioning after junk food exposure. In addition, it was recently found that the ways dopamine neurons in the VTA encode food and opposite-sex social motivation show overlap[90]. This study supports our hypothesis that desensitization of the VTA by excessive consumption of CAF diet can disrupt the neural responses toward other natural rewards as well, as shown in our study.

Our findings are in support of the reward hyporesponsivity theory claiming that long-term exposure to foods rich in fat and sugar can cause a blunted behavioral reward response[47,60,91]. While some studies have shown an increase in motivation to work for a food reward[48,53-56], or no effect at all[59], more studies found that rats on a junk food diet show reduced interest in cues for a food reward and show attenuated operant responses[38,57,58,60,92-96], or longer latencies to collect a reward[57,62]. In contrast to most of these studies, our experimental design permitted free access to, and interaction with the reward and is therefore considered a 'low effort' paradigm. In this regard, our findings are in line with the other studies in which little or no effort was required to obtain the rewards[49,53,60,61,97].

Since our behavioral reward test included a light cue indicating free access to the reward, our set-up enabled us to study the effects of excessive junk food consumption on the VTA both during classical conditioning and during reward approach and consumption. Initially, in the first test in all groups, the VTA responded to the light cue in a way previously described for novel and neutral cues[98]. By the fifth and final test however, VTA activity in the control group was higher in reaction to the cue light, relative to the first test, suggesting that the cue light had become a conditioned stimulus. In contrast, light-evoked activity in the VTA for both the CAF and HFHS groups did not differ from the

first food reward test. Furthermore, all groups had elevated activity levels following opening of the door, which provided access to the reward, during the fifth food reward test, but not the first. We interpreted this response as a part of the cue-reward sequence that was not yet fully predicted by the cue light, and this might suggest that the VTA is mainly affected in its role to encode the predictive and incentive properties of a reward rather than in its role to signal novel stimuli that could lead to potential rewards[98-100]. After experiencing decreased pleasure and desire for the reward, the adjusted reward prediction after Pavlovian conditioning should lead to diminished VTA responses to the light-cue and reward[29,101,102]. We hypothesize that this is what happens in the rats exposed to CAF and HFHS diets.

Another benefit of our experimental approach was the possibility to assess micropatterns of behavior by looking at the pattern of eating bouts. We found that the reduced reward interest of the CAF exposed group was a result of fewer eating bouts and longer breaks between them. The latency to approach the reward or duration of the eating bouts, however, remained the same as for CTR rats. This suggests that the lack of interest in the reward did not result from a decrease of initial value of the offered reward, but rather the willingness to consume it. This idea is in line with previous studies that investigated the micropattern of licking behavior of rodents when exposed to a sweet solution and found a reduction in total licks after being maintained on an obesogenic diet, but no alterations in lick cluster size (a reflection of palatability and thus 'liking'[37,60,103]). Ducrocq et al. previously suggested that the decrease in behavioral responses could correspond to a combination of decreased baseline hunger and enhanced post-ingestive regulation of food intake rather than an alteration of reward processing such as incentive or hedonic processes[60]. Although this sounds like a plausible explanation, it should be mentioned that our rats underwent a short-term food deprivation before the reward tests and that our test only lasted for 15 minutes. It is therefore unlikely that these factors are underlying the change behavioral responses in our study. The fact that in our study the event-related activity of the VTA of CAF exposed animals appeared to be narrowed across different behaviors also makes it more likely that the reduced interest in the food reward is caused by changes in neural reward mechanisms.

The lack of behavioral changes in responses toward a sexual reward, on the other hand, was slightly surprising. The observation that the VTA activity was attenuated despite the absence of explicit changes in behavioral responses to a sexual reward, does support the notion that while long-term CAF exposure may exert effects on some of the same anatomical substrates of different natural rewarding behaviors, redundancy by means of other pathways might contribute to the robustness of sexual behavior. We have previously shown how different natural rewards can recruit similar brain networks to regulate the display of motivated behaviors[83]. Still, the actual display of either feeding

or sexual behavior is also regulated by two different and specialized circuitries. While the lateral hypothalamus plays a key role in feeding behavior[104,105], the ventromedial nucleus of the hypothalamus is the main neural center for female sexual behavior[106-110]. With the dense projections between the VTA and the feeding and sexual behavior brain networks[111], it is possible that CAF diet exposure also affects other regions than the VTA related to feeding behavior, while having less effect on the sexual behavior related regions. Alternatively, it can be argued that changes in sexual motivation might be found using more subtle behavioral tests, in which consummatory behaviors are not displayed, such as the sexual incentive motivation test[112,113], or a test in which the rats have to work to gain access to the sexual reward. It is also plausible that prolonged testing durations can unveil behavioral disparities between CTR and CAF rats. For example, if the motivation to continue copulation is affected, it would likely become more apparent with longer test durations. Another explanation for the lack of effects on sexual behavior can be the fact that our female rats were sexually experienced at the start of testing. Beloate et al. have shown that inhibition of dopaminergic neurons in the VTA does not change the initiation and performance of sexual behavior in male rats, but that it does prevent sexual experience to affect neuronal plasticity and cross-sensitization of amphetamine-induced conditioned place preference[114]. Following this line of thought, it might be possible that the diminished CAF-induced VTA responses might lead to altered sexual behavior in sexually naïve female rats as well.

The lack of effects on sexual behavior was partly in line with another study that investigated the effects of HFHS diet on social behavior in rats. Like our findings, they reported no changes in the time spent on social interaction 1 hour after the last HFHS pellet access after a two-week HFHS diet exposure[115]. Surprisingly though, 23 hours after the last HFHS pellet exposure, reduced duration of social interaction was reported[115]. Since this study was conducted during adolescence, our study is the first to investigate the effects of long-term junk food diet exposure on other natural rewarding behaviors during adulthood. We hope that future research will provide a better understanding of the lack of behavioral effects on sexual functioning despite the attenuated responses in the VTA.

Finally, we found that a 2-weeks reversal to a healthy diet did not fully restore the behavioral and neural responses caused by long-term CAF diet. This finding is in line with another study that reported remaining behavioral changes caused by CAF diet exposure after two weeks of diet withdrawal[47]. Interestingly though, other studies have reported binge-like eating behavior after withdrawal from junk food diets[86,88]. Others found a restoration of sucrose preference after 4 weeks of chow feeding[86]. More research is needed to determine whether the behavioral changes will return to normal after longer exposure to healthy chow diet. The findings of unchanged, blunted VTA responses, on the other hand, seem to be rather consistent with other studies that evaluated

the persistence of neural changes caused by junk food exposure. It was found that D1 receptor expression and TH levels remained lower after abstinence from CAF diet[52,88]. Interestingly though, a sex difference was present in these findings. While female rats maintained reduced D1 receptor expression levels, males seem to return to baseline after 72 hours[52].

Sex differences are not uncommon when it comes to research on feeding behavior. Many studies have shown different responses between male and female rodents, especially in relation to the dopaminergic system[52,86,116]. Generally, it appears that females exhibit more extreme effects than males, and that binge eating is more prevalent in females than in males[117]. Besides the fact that the estrous cycle impacts the spine plasticity in the nucleus accumbens[118], and that it plays a role in reward prediction error and reinforcement learning[119], not much is known about the impact of the estrous cycle on the reward system when it comes to feeding behavior. Still, it has been hypothesized that gonadal hormones could play an important role in regulating eating patterns[120]. Fluctuations in hormonal levels are reported to affect food intake [121] and they can modify the preference for macronutrients[122]. As mentioned previously, we also observed potentially interesting effects of HFHS and CAF diets on the onset of proestrus. While 40% of the HFHS diet group never reached proestrus, approximately 33% of the remaining HFHS rats did not copulate during the sexual reward tests. In our CAF diet group, approximately one third of the rats in proestrus showed delayed receptivity. Even though we must remain cautious with conclusions, this could suggest that foods high in fats and sugar affect the estrous cycle[123]. These data strengthen the need for more research into sex differences and the use of female subjects in general research so that the underlying mechanisms of these effects can be explored.

On an additional note, we would like to stress that this is the first study to investigate how the VTA is activated during female sexual behavior. In our study, we found that an increase in neural activity in the VTA was linked to the start of paracopulatory events, but not lordosis behavior. However, lordosis behavior is a response toward a copulatory behavior by the male and when this behavior has ended, an increase in VTA activity was seen exclusively after an ejaculation. This suggests that the VTA has a stimulation-specific response and that it might encode the rewarding elements perceived from such a stimulation.

In conclusion, the results indicate that long-term junk food exposure severely affects the reward system by altering behavioral responses to a CAF food reward and desensitizing the VTA to a food and sexual reward. Exposure to a CAF diet in which rats are exposed to a choice of foods rich in fat and sugar has more severe consequences than a HFHS diet. Finally, two weeks of healthy diet is not

sufficient to restore the behavioral and neural changes. Our findings support the notion that excessive junk food consumption can have undesirable long-lasting consequences.

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Author contributions

JFL: Experimental design, data gathering, behavioral annotation, data curation, analysis, writing – review and editing, funding acquisition

JCO: Data gathering, behavioral annotation, analysis, writing – review and editing

PTH: Methodology, writing – review and editing

JEM: Methodology, writing - review and editing

RH: Experimental design, methodology, data gathering, data curation, analysis, supervision, writing – original draft, funding acquisition

EMSS: Experimental design, methodology, data gathering , data curation, Programming/software, analysis, supervision, writing – original draft, funding acquisition

Declaration of interests

The authors declare no competing interests.

Methods

Rats

A total of 36 Wistar female rats (200-250 g upon arrival) and 10 male rats (250 g upon arrival) were purchased from Janvier labs (France). All rats were pair-housed in Macrolon IV ® cages on a reversed 12 h light/dark cycle (lights on between 23:00 and 11:00) in a room with controlled temperature (21 ± 1 °C) and humidity (55 ± 10 %), with ad libitum access to standard rodent food pellets (low phytoestrogen maintenance diet, #V1554, Ssniff, Germany) and tap water throughout the

experiment (unless otherwise indicated). All animal care and experimental procedures employed in this study were conducted in agreement with the European Union council directive 2010/63/EU and in accordance with the Norwegian Food Safety Authority.

Experimental design

After sexual training, the females underwent stereotaxic brain surgery in two batches of 18 rats with two weeks in between. Once the females recovered from surgery, they were randomly divided into three experimental groups of control (CTR), high-fat, high-sugar (HFHS), or cafeteria (CAF) diet for six weeks.

After six weeks of diet exposure, the females were tested in the behavioral reward box (Fig S1A), during which they were tethered to the fiber photometry system (RZ10x from Tucker-Davis Technologies, USA). First, five tests (once per day on five consecutive days) with a food reward were conducted, followed by three tests with a sexual reward (on the same day of their proestrus to prevent losing the test subject for data analysis due to pseudopregnancy; 2 hour break between tests). The food reward consisted of small portions of the favorite products selected from the CAF diet (Table S2). A male rat was used as sexual reward.

To rule out the potential effects of pseudopregnancies the female rats remained on their experimental diet for another two weeks after the behavioral reward tests. Subsequently, all females rats were switched to a diet of standard chow for another two weeks (reversal diet), followed by a final round of behavioral reward testing. They were tested three times (on consecutive days) with the food (CAF) reward, and one time with the sexual reward (on a proestrus day).

After completion of the experiment, all rats were perfused and their brains were collected to assess viral infusion location and expression and verification of fiber location.

Surgeries

Brain surgery. After sexual training, the females underwent brain surgery for AAV vector infusion and fiber implantation. The subjects were anesthetized with isoflurane, placed in a stereotaxic apparatus (Stoelting Europe, Ireland), and received 0.05 mg/kg buprenorphine and 2 mg/kg meloxicam subcutaneously. The scalp was shaved, disinfected with povidone-iodine and locally anaesthetized with bupivacaine (0.15 ml, s.c.). Core body temperature, oxygen saturation and heart rate were monitored throughout the surgery. A hole was drilled above the VTA of the right hemisphere (coordinates: AP -5.0 mm, ML +0.9 mm, DV -8.5 mm relative to bregma[124]) and a 2 µl Hamilton neuros syringe with a 30G blunt needle mounted in a Hamilton injector (Stoelting) on the stereotaxic apparatus was slowly inserted. N=11 of each group was infused with 750 nl 10^{13} GC/mL

pAAV5.syn.GCaMP6s.WPRE.SVA40 (a gift from Douglas Kim & GENIE Project (Addgene plasmid # 100843 ; http://n2t.net/addgene:100843; RRID:Addgene_100843) and N=1 of each group with 750 nl 10^{13} GC/mL pAAV5.hSyn.eGFP.WPRE.bGH (a gift from James M. Wilson (Addgene plasmid # 105539 ; http://n2t.net/addgene:105539 ; RRID:Addgene_105539)) as control, both at a rate of 125 nl/min. Following infusion, the needle was left in place for 10 minutes before withdrawal. 4 anchor screws (1 x 3 mm, Agnthos, Sweden) were placed in the skull, after which the fiber-optic cannula (diameter 400 µm, NA 0.66, Doric Lenses, USA) was inserted in the right hemisphere at AP -5.0 mm, ML +0.9 mm, DV -8.4 mm relative to bregma. It was fixed to the skull with super bond (C&B Super bond, Technomedics, Norway) followed by regular dental acrylic (Meliodent, Kulzer GmbH, Germany). Post-operative analgesic treatment consisted of 0.05 mg/kg buprenorphine and 2 mg/kg meloxicam after 24 and 48 hours. Immediately after surgery the rats were pair-housed and recovered for minimum one week before the diet manipulation was started.

Vasectomy. To prevent pregnancies in experimental female rats while maintaining their reproductive integrity, the stimulus male rats were subjected to vasectomy by ligation of a small portion of the vas deferens of both testes, as previously described[125]. On each side, a small incision was made in the scrotal skin covering the testes and the tissue layer covering the testicle was opened. The testicle, epididymis and vas deferens were exposed and the vas deferens was thermally cauterized. The organs were returned inside the incision and the wound was closed with stitches. Immediately after surgery the male rats were pair-housed and were allowed to recover for two weeks before undergoing sexual training.

Diet manipulation

After at least one week of surgery recovery, experimental rats were randomly assigned one of the three experimental diets which they received for a period of six weeks. Standard Chow (CTR): standard laboratory chow. High-Fat, High-Sugar (HFHS): laboratory chow with high content of fat and sugar that simulates the nutritional values of junk food, but in the shape of regular pellets (Western Diet #D12079B, Research Diets Inc., USA). Cafeteria diet (CAF): composed of standard chow and 16 different food items obtained in a supermarket including biscuits, cookies, processed meat and salty snacks (Table S2 and S3), based on a proposed protocol for cafeteria diet (Lalanza & Snoeren, 2021). The food items were divided into 4-daily-rotated plates balanced by type of food, texture, taste, and nutritional composition. All three experimental diets were provided *ad libitum* to the rats The rats had access to tap water ad libitum.

Behavioral testing

Sexual training. The natural estrous cycle of the females was monitored using an impedance meter (Muromachi MK-12, Japan). When in proestrus, the females were sexually trained in a copulation box (60 x 40 x 40 cm, with a Plexiglas front) in a room with dimmed lighting conditions. A receptive female was first habituated to the copulation box for five minutes, followed by the introduction of a male until ejaculation was achieved or for a maximum of 30 minutes. During training, all females were exposed to one ejaculation, except for one rat that received 20 intromissions without ejaculation. Stimulus males achieved at least three ejaculations in different sessions with different females.

Behavioral reward test. All rats were habituated to the behavioral reward test box for 10 minutes on the day before testing began. On a behavior test day, the food was removed from the cage at 9 am, while the animals continued to have access to water. Between 12:00 and 19:00, the behavioral testing was conducted, after which fresh food was placed back in the cage.

The behavioral reward test was conducted in a box (75x30x70 cm, Fig S1A) in which two chambers (30x30x70 cm and 45x30x70 cm) were divided with a wall containing 2 sliding doors. The side of the dividing wall facing the left chamber (starting chamber, not containing the reward) had a light between the two sliding doors. Small apertures in these doors enabled the subject rat to see, hear and smell the reward in the right chamber. The floors were covered with fresh wood chip bedding. The front wall of the box facing the camera was made of transparent methacrylate.

First, a subject rat was connected to the fiber photometry system with a fiber-optic cable and placed in a small cage for approximately 2 minutes to allow the GCaMP and isosbestic signals to stabilize. The reward test started when the female subject was placed in the left chamber, with the doors in the dividing wall closed (Baseline phase, Fig 1B). After 3 minutes, a reward (either food or a male rat) was placed in the right chamber, while the doors remained closed for another 3 minutes (Pre-reward phase). Subsequently, the light turned on for 10 seconds, after which the doors opened. The light turned off again, and the rat was allowed to interact freely with the reward for a total of 15 minutes (Reward phase). After the test, the female was returned to the home cage and the reward box was cleaned with ethanol. Fresh bedding was provided for the next rat.

All behavioral reward tests were recorded on video with a digital video camera (Sony HDR-CX240E handycam) for behavioral assessment.

Fiber photometry

To assess the activity of VTA neurons during the behavioral reward tests, the bulk fluorescence signal generated by GCaMP6s expressing cells was recorded using fiber photometry[126,127]. Signal processing and acquisition hardware (RZ10x; Tucker-Davis Technologies, USA) was used to control a 465 nm Lux RZ10x integrated LED for fluorophore excitation, and a 405 nm Lux RZ10x integrated LED for isosbestic excitation. The excitation lights were modulated at 210 Hz (465 nm) and 330 Hz (405 nm), filtered and combined by a fluorescence mini cube (Doric Lenses, USA), and delivered through a 400 µm core, 0.48 NA, low-autofluorescence fiber optic patch cable (Doric, MFP_400/430/1100-0.57_5m_FCM-MF2.5_LAF) to an implanted fiber (Doric, MFC_400/430-0.66_9mm_MF2.5_FLT). LED power was set at a level which resulted in a ca 15 and 30 mV signal for 405 and 465 nm LED, respectively. All signals were acquired using Synapse Essentials software (Tucker-Davis Technologies). Fluorescence was sampled at 1017 Hz and demodulated by the processor. The start of the behavioral reward test and the moment the light was turned on were time stamped by registering TTL signals generated by the USB-I/O box 12 system (Noldus, The Netherlands). With the help of The Observer XT 12.5 (most behavioral data) and XT 16 (Novel food reward data, Noldus), the behavioral events were time stamped and aligned with the fiber photometry data.

Data were exported to Python from Synapse using a script provided by Tucker-Davis Technology. Custom Python scripts were used to independently normalize the demodulated 465 nm and 405 nm signals. To calculate ΔF/F, the 405 nm signal was linearly regressed to the 470 nm signal, and then subtracted to create ΔF/F (465 nm signal – fitted 405 nm signal)/ fitted 405 nm signal. Fractions of the signal recording in which noisy signals were recorded (e.g., when the cable detached or hit the wall or door) were removed from the recordings when an interquartile range of more than 2 was found. We then aligned the ΔF/F to baseline zero by subtracting the average of a ΔF/F signal collected 1 second before each of the events. The area under the curve (AUC) was calculated from this corrected signal. To be able to compare the AUC before and after the event, the AUC was corrected per second.

Behavioral assessment

From the videos, which were recorded at a frequency of 25 fps, the frequency and duration of the multiple behaviors (Table S1) were annotated and the start of the behaviors was time-locked on the exact video frames using The Observer XT12.5 and Observer XT16 (Noldus).

Then, some additional parameters were calculated, such as the lordosis score ((number of lordosis with score 1 times 1 + number of lordosis with score 2 times 2 + number of lordosis with score 3 times 3) / number of received copulations)[84], lordosis quotient (number of lordosis responses (1-3) / number of received copulations * 100%) , and the mean contact-return latency after a received

mount, intromission or ejaculation (latency to 1st paracopulatory behavior after receiving a copulation) were calculated per rat.

Moreover, a more detailed behavioral assessment paradigm was used to explore the micropatterns in reward-related behaviors. For this, the behavior was divided into eating bouts (Fig 2D, food reward tests) and sexual bouts (Fig 3F, sexual reward test). An eating bout consisted of all eating episodes that were not interrupted by any behavior that was not focused on the food reward. As soon as a behavior undirected at the food reward was displayed, the last eating episode was considered the ending of the eating bout. The break until the next eating episode is called a time-out. A similar approach was used for the sexual bout, but now both a lordosis and paracopulatory behavior could start and/or end a sexual bout. The number of bouts, the mean duration of bouts, and the mean time-out duration were determined.

To mitigate the effects of potential pseudopregnancies, all three sexual reward tests were conducted on a single day and with the same male for each female rat. Regrettably, not all females reached the proestrus phase and these were consequently excluded from the analysis. Notably, only six out of ten females on the HFHS diet reached proestrus, and two of these females never engaged in copulation with the male. Furthermore, although ten of our CAF-exposed females reached the proestrus phase, three of them did not exhibit any paracopulatory behaviors during the initial sexual reward test. This observation suggests a potential slight delay in the onset of the receptive phase compared to CTR females as the first reward test was conducted right after the lights went off. To address these challenges in conducting a reliable behavioral reward test with a sexual reward, we analyzed the neural and behavioral data from the most successful sexual reward test. The *best sexual reward test* was defined as the test (either the first, second, or third) in which the female displayed the highest number of paracopulatory behaviors. In practice this meant that two 1st, four 2nd and four 3rd tests were included in the analysis for the CTR rats; one 1st, two 2nd and three 3rd tests for the HFHS rats; and two 1st, four 2nd, and three 3rd tests for the CAF rats. The sex reward test after reversal was conducted 2.5 hours after the lights went off to avoid the delay in the onset of the receptive phase.

Brain processing, immunostaining and imaging

At the end of the experiments, rats were injected intraperitoneally with a lethal dose of pentobarbital (100 mg/kg), and transcardially perfused with 0.01 M phosphate buffered saline (PBS; pH 7.4) followed by 4% formaldehyde in 0.01 M PBS. Brains were removed and post-fixed in 4% formaldehyde for 48 hours. Then, the brains were transferred to 20% sucrose in 0.01 M PBS for one day and placed in 30% sucrose in 0.01 M PBS. When sunken, the brains were snap frozen in

isopentane cooled by dry ice and stored in -80 °C until sectioning on a sliding microtome (SM2010R, Leica Biosystems, Germany) into 40 µm coronal sections. These sections were stored in cryoprotectant solution (30% sucrose w/v, 30% ethylene glycol v/v in 0.01 M PBS, pH 7.4) until further use.

To assess the expression of GCaMP throughout and surrounding the VTA, immunohistochemistry was performed on selected sections. These were washed in 0.1M Tris-buffered-saline (TBS), blocked for 30 minutes in 0.5% BSA in TBS, and incubated with polyclonal chicken anti-EYFP (1:100 000), Abcam, cat. ab13970) antibody solution containing 0.1% Triton-X and 0.1% BSA in TBS on an orbital shaker for 24 hours at room temperature + 24 hours at 4 °C. The sections were then washed four times in TBS and subsequently incubated in biotinylated goat anti-rabbit (1:400, Abcam, cat. ab6720) or biotinylated goat anti-chicken (1:400, Abcam, cat. ab6876) antibody solution containing 0.1 % BSA in TBS for 30 min, followed by incubation with avidin-biotin-peroxidase complex (VECTASTAIN ABC-HRP kit, Vector laboratories, cat. PK-6100, dilution: 1 drop A + 1 drop B in 10 mL TBS) solution for 30 min, and 3,3'-diaminobenzidine solution (DAB substrate kit (HRP), Vector laboratories, cat. SK-4100, dilution: 1 drop R1 (buffer solution) + 2 drops R2 (3,3'-diaminobenzidine solution) + 1 drop R3 (hydrogen peroxide solution) in 5 mL water) for 5-10 min, with TBS washes between all steps. The slides were left until dry, after which the sections were dehydrated with 50-99.8% ethanol baths, cleared with xylene, and coverslipped using Entellan mounting medium (Sigma, St. Louis, USA).

Finally, the location of the fiber and GCaMP expression were visually verified on the sections with a microscope (Primo Star, Carl Zeiss, Germany). Only the rats that had both GCaMP expression and a fiber within the VTA were included in the fiber photometry data analysis.

Statistical analysis

All rats were included in the behavioral analysis, except for three rats that had lost their fiber caps at an early stage of the experiment. For the fiber photometry analysis, however, seven rats were excluded from analysis as they did not express GCaMP or had a wrong fiber placement, resulting in 6 CTR-, 7 HFHS- and 9- CAF included rats. In addition, a small number of sessions (2 x first sexual reward tests; 3 x second sexual reward tests; 2 x third sexual reward tests; and 1 x sexual reward test after the reversal diet, coming from different rats) had to be excluded from the analysis due to insufficient quality of the fiber photometry recording.

Both the behavior and fiber photometry data were analyzed with customized Python 3.9 scripts (available upon request). Statistical analysis of the behavioral data and area under the curve was also performed with the same customized Python 3.9 script. When CTR rats were compared to CAF

rats, an independent sample t-test was used. However, when HFHS rats were included in the data analysis, a One-way ANOVA test was used to determine the level of significance. In case of significance ($p<0.05$), several independent sample t-tests with p-value correction for multiple testing were conducted as post-hoc analysis.

Supplemental information

Document S1. Supplementary Table S1-S5.

Document S2. Supplementary Figure S1, S2, S3, S5.

References

1. Morris, M.J., Beilharz, J.E., Maniam, J., Reichelt, A.C., and Westbrook, R.F. (2015). Why is obesity such a problem in the 21st century? The intersection of palatable food, cues and reward pathways, stress, and cognition. Neurosci Biobehav Rev *58*, 36-45. 10.1016/j.neubiorev.2014.12.002.
2. Malik, V.S., Willett, W.C., and Hu, F.B. (2013). Global obesity: trends, risk factors and policy implications. Nat Rev Endocrinol *9*, 13-27. 10.1038/nrendo.2012.199.
3. WHO (2024). http://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight.
4. Berridge, K.C., Ho, C.Y., Richard, J.M., and DiFeliceantonio, A.G. (2010). The tempted brain eats: pleasure and desire circuits in obesity and eating disorders. Brain Res *1350*, 43-64. 10.1016/j.brainres.2010.04.003.
5. Zheng, H., and Berthoud, H.R. (2007). Eating for pleasure or calories. Curr Opin Pharmacol *7*, 607-612. 10.1016/j.coph.2007.10.011.
6. Oikonomou, E., Psaltopoulou, T., Georgiopoulos, G., Siasos, G., Kokkou, E., Antonopoulos, A., Vogiatzi, G., Tsalamandris, S., Gennimata, V., Papanikolaou, A., and Tousoulis, D. (2018). Western Dietary Pattern Is Associated With Severe Coronary Artery Disease. Angiology *69*, 339-346. 10.1177/0003319717721603.
7. Guh, D.P., Zhang, W., Bansback, N., Amarsi, Z., Birmingham, C.L., and Anis, A.H. (2009). The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. BMC Public Health *9*, 88. 10.1186/1471-2458-9-88.
8. Briggs, M.A., Petersen, K.S., and Kris-Etherton, P.M. (2017). Saturated Fatty Acids and Cardiovascular Disease: Replacements for Saturated Fat to Reduce Cardiovascular Risk. Healthcare (Basel) *5*. 10.3390/healthcare5020029.

9. Elias, M.F., Elias, P.K., Sullivan, L.M., Wolf, P.A., and D'Agostino, R.B. (2005). Obesity, diabetes and cognitive deficit: The Framingham Heart Study. Neurobiol Aging *26 Suppl 1*, 11-16. 10.1016/j.neurobiolaging.2005.08.019.

10. Leggio, M., Lombardi, M., Caldarone, E., Severi, P., D'Emidio, S., Armeni, M., Bravi, V., Bendini, M.G., and Mazza, A. (2017). The relationship between obesity and hypertension: an updated comprehensive overview on vicious twins. Hypertens Res *40*, 947-963. 10.1038/hr.2017.75.

11. Lane, M.M., Gamage, E., Du, S., Ashtree, D.N., McGuinness, A.J., Gauci, S., Baker, P., Lawrence, M., Rebholz, C.M., Srour, B., et al. (2024). Ultra-processed food exposure and adverse health outcomes: umbrella review of epidemiological meta-analyses. BMJ *384*, e077310. 10.1136/bmj-2023-077310.

12. O'Brien, P.D., Hinder, L.M., Callaghan, B.C., and Feldman, E.L. (2017). Neurological consequences of obesity. Lancet Neurol *16*, 465-477. 10.1016/S1474-4422(17)30084-4.

13. Raji, C.A., Ho, A.J., Parikshak, N.N., Becker, J.T., Lopez, O.L., Kuller, L.H., Hua, X., Leow, A.D., Toga, A.W., and Thompson, P.M. (2010). Brain structure and obesity. Hum Brain Mapp *31*, 353-364. 10.1002/hbm.20870.

14. Davidson, T.L., Hargrave, S.L., Swithers, S.E., Sample, C.H., Fu, X., Kinzig, K.P., and Zheng, W. (2013). Inter-relationships among diet, obesity and hippocampal-dependent cognitive function. Neuroscience *253*, 110-122. 10.1016/j.neuroscience.2013.08.044.

15. Noble, E.E., Hsu, T.M., and Kanoski, S.E. (2017). Gut to Brain Dysbiosis: Mechanisms Linking Western Diet Consumption, the Microbiome, and Cognitive Impairment. Frontiers in behavioral neuroscience *11*, 9. 10.3389/fnbeh.2017.00009.

16. Prickett, C., Brennan, L., and Stolwyk, R. (2015). Examining the relationship between obesity and cognitive function: A systematic literature review. Obes Res Clin Pract *9*, 93-113. 10.1016/j.orcp.2014.05.001.

17. Kanoski, S.E., and Davidson, T.L. (2011). Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. Physiol Behav *103*, 59-68. 10.1016/j.physbeh.2010.12.003.

18. Jones, S., Hyde, A., and Davidson, T.L. (2020). Reframing appetitive reinforcement learning and reward valuation as effects mediated by hippocampal-dependent behavioral inhibition. Nutr Res *79*, 1-12. 10.1016/j.nutres.2020.05.001.

19. Berridge, K.C., and Robinson, T.E. (2003). Parsing reward. Trends Neurosci *26*, 507-513. 10.1016/S0166-2236(03)00233-9.

20. Yoshida, M., Yokoo, H., Mizoguchi, K., Kawahara, H., Tsuda, A., Nishikawa, T., and Tanaka, M. (1992). Eating and drinking cause increased dopamine release in the nucleus accumbens and ventral tegmental area in the rat: measurement by in vivo microdialysis. Neurosci Lett *139*, 73-76. 10.1016/0304-3940(92)90861-z.

21. Fields, H.L., Hjelmstad, G.O., Margolis, E.B., and Nicola, S.M. (2007). Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. Annual review of neuroscience *30*, 289-316. 10.1146/annurev.neuro.30.051606.094341.

22. Flores-Dourojeanni, J.P., van Rijt, C., van den Munkhof, M.H., Boekhoudt, L., Luijendijk, M.C.M., Vanderschuren, L., and Adan, R.A.H. (2021). Temporally Specific Roles of Ventral Tegmental Area Projections to the Nucleus Accumbens and Prefrontal Cortex in Attention and Impulse Control. J Neurosci *41*, 4293-4304. 10.1523/JNEUROSCI.0477-20.2020.

23. Morales, M., and Margolis, E.B. (2017). Ventral tegmental area: cellular heterogeneity, connectivity and behaviour. Nat Rev Neurosci *18*, 73-85. 10.1038/nrn.2016.165.

24. Solie, C., Girard, B., Righetti, B., Tapparel, M., and Bellone, C. (2022). VTA dopamine neuron activity encodes social interaction and promotes reinforcement learning through social prediction error. Nature Neuroscience *25*, 86-+. 10.1038/s41593-021-00972-9.

25. Berke, J.D. (2018). What does dopamine mean? Nature Neuroscience *21*, 787-793. 10.1038/s41593-018-0152-y.

26. Hamid, A.A., Pettibone, J.R., Mabrouk, O.S., Hetrick, V.L., Schmidt, R., Vander Weele, C.M., Kennedy, R.T., Aragona, B.J., and Berke, J.D. (2016). Mesolimbic dopamine signals the value of work. Nat Neurosci *19*, 117-126. 10.1038/nn.4173.

27. Syed, E.C., Grima, L.L., Magill, P.J., Bogacz, R., Brown, P., and Walton, M.E. (2016). Action initiation shapes mesolimbic dopamine encoding of future rewards. Nat Neurosci *19*, 34-36. 10.1038/nn.4187.

28. Eshel, N., Tian, J., Bukwich, M., and Uchida, N. (2016). Dopamine neurons share common response function for reward prediction error. Nat Neurosci *19*, 479-486. 10.1038/nn.4239.

29. Schultz, W. (2016). Dopamine reward prediction-error signalling: a two-component response. Nat Rev Neurosci *17*, 183-195. 10.1038/nrn.2015.26.

30. Schultz, W. (2007). Multiple dopamine functions at different time courses. Annual review of neuroscience *30*, 259-288. 10.1146/annurev.neuro.28.061604.135722.

31. Martel, P., and Fantino, M. (1996). Influence of the amount of food ingested on mesolimbic dopaminergic system activity: a microdialysis study. Pharmacol Biochem Behav *55*, 297-302. 10.1016/s0091-3057(96)00087-1.

32. Hajnal, A., Smith, G.P., and Norgren, R. (2004). Oral sucrose stimulation increases accumbens dopamine in the rat. Am J Physiol Regul Integr Comp Physiol *286*, R31-37. 10.1152/ajpregu.00282.2003.

33. Roitman, M.F., Wheeler, R.A., Wightman, R.M., and Carelli, R.M. (2008). Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. Nat Neurosci *11*, 1376-1377. 10.1038/nn.2219.

34. Bassareo, V., and Di Chiara, G. (1999). Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. Eur J Neurosci *11*, 4389-4397. 10.1046/j.1460-9568.1999.00843.x.

35. Heijkoop, R., Lalanza, J.F., Solanas, M., Alvarez-Monell, A., Subias-Gusils, A., Escorihuela, R.M., and Snoeren, E.M.S. (2024). Changes in reward-induced neural activity upon Cafeteria Diet consumption. Physiol Behav *276*, 114478. 10.1016/j.physbeh.2024.114478.

36. Delaere, F., Akaoka, H., De Vadder, F., Duchampt, A., and Mithieux, G. (2013). Portal glucose influences the sensory, cortical and reward systems in rats. Eur J Neurosci *38*, 3476-3486. 10.1111/ejn.12354.

37. Fam, J., Clemens, K.J., Westbrook, R.F., Morris, M.J., and Kendig, M.D. (2022). Chronic exposure to cafeteria-style diet in rats alters sweet taste preference and reduces motivation for, but not 'liking' of sucrose. Appetite *168*, 105742. 10.1016/j.appet.2021.105742.

38. Davis, J.F., Tracy, A.L., Schurdak, J.D., Tschop, M.H., Lipton, J.W., Clegg, D.J., and Benoit, S.C. (2008). Exposure to elevated levels of dietary fat attenuates psychostimulant reward and mesolimbic dopamine turnover in the rat. Behav Neurosci *122*, 1257-1263. 10.1037/a0013111.

39. Geiger, B.M., Haburcak, M., Avena, N.M., Moyer, M.C., Hoebel, B.G., and Pothos, E.N. (2009). Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. Neuroscience *159*, 1193-1199. 10.1016/j.neuroscience.2009.02.007.

40. Geiger, B.M., Behr, G.G., Frank, L.E., Caldera-Siu, A.D., Beinfeld, M.C., Kokkotou, E.G., and Pothos, E.N. (2008). Evidence for defective mesolimbic dopamine exocytosis in obesity-prone rats. FASEB J *22*, 2740-2746. 10.1096/fj.08-110759.

41. Martel, P., and Fantino, M. (1996). Mesolimbic dopaminergic system activity as a function of food reward: a microdialysis study. Pharmacol Biochem Behav *53*, 221-226. 10.1016/0091-3057(95)00187-5.

42. Nguyen, J.C., Ali, S.F., Kosari, S., Woodman, O.L., Spencer, S.J., Killcross, A.S., and Jenkins, T.A. (2017). Western Diet Chow Consumption in Rats Induces Striatal Neuronal Activation While Reducing Dopamine Levels without Affecting Spatial Memory in the Radial Arm Maze. Frontiers in behavioral neuroscience *11*, 22. 10.3389/fnbeh.2017.00022.

43. Rada, P., Bocarsly, M.E., Barson, J.R., Hoebel, B.G., and Leibowitz, S.F. (2010). Reduced accumbens dopamine in Sprague-Dawley rats prone to overeating a fat-rich diet. Physiol Behav *101*, 394-400. 10.1016/j.physbeh.2010.07.005.

44. South, T., Westbrook, F., and Morris, M.J. (2012). Neurological and stress related effects of shifting obese rats from a palatable diet to chow and lean rats from chow to a palatable diet. Physiol Behav *105*, 1052-1057. 10.1016/j.physbeh.2011.11.019.

45. Totten, M.S., Wallace, C.W., Pierce, D.M., Fordahl, S.C., and Erikson, K.M. (2022). The impact of a high-fat diet on physical activity and dopamine neurochemistry in the striatum is sex and strain dependent in C57BL/6J and DBA/2J mice. Nutr Neurosci *25*, 2601-2615. 10.1080/1028415X.2021.1992082.

46. Burke, M.V., and Small, D.M. (2016). Effects of the modern food environment on striatal function, cognition and regulation of ingestive behavior. Curr Opin Behav Sci *9*, 97-105. 10.1016/j.cobeha.2016.02.036.

47. Johnson, P.M., and Kenny, P.J. (2010). Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. Nat Neurosci *13*, 635-641. 10.1038/nn.2519.

48. Robinson, M.J., Burghardt, P.R., Patterson, C.M., Nobile, C.W., Akil, H., Watson, S.J., Berridge, K.C., and Ferrario, C.R. (2015). Individual Differences in Cue-Induced Motivation and Striatal Systems in Rats Susceptible to Diet-Induced Obesity. Neuropsychopharmacology *40*, 2113-2123. 10.1038/npp.2015.71.

49. Vucetic, Z., Kimmel, J., and Reyes, T.M. (2011). Chronic high-fat diet drives postnatal epigenetic regulation of mu-opioid receptor in the brain. Neuropsychopharmacology *36*, 1199-1206. 10.1038/npp.2011.4.

50. Blendy, J.A., Strasser, A., Walters, C.L., Perkins, K.A., Patterson, F., Berkowitz, R., and Lerman, C. (2005). Reduced nicotine reward in obesity: cross-comparison in human and mouse. Psychopharmacology (Berl) *180*, 306-315. 10.1007/s00213-005-2167-9.

51. Smith, S.L., Harrold, J.A., and Williams, G. (2002). Diet-induced obesity increases mu opioid receptor binding in specific regions of the rat brain. Brain Res *953*, 215-222. 10.1016/s0006-8993(02)03291-2.

52. Ong, Z.Y., Wanasuria, A.F., Lin, M.Z., Hiscock, J., and Muhlhausler, B.S. (2013). Chronic intake of a cafeteria diet and subsequent abstinence. Sex-specific effects on gene expression in the mesolimbic reward system. Appetite *65*, 189-199. 10.1016/j.appet.2013.01.014.

53. la Fleur, S.E., Vanderschuren, L.J.M.J., Luijendijk, M.C., Kloeze, B.M., Tiesjema, B., and Adan, R.A.H. (2007). A reciprocal interaction between food-motivated behavior and diet-induced obesity. Int J Obesity *31*, 1286-1294. 10.1038/sj.ijo.0803570.

54. Vasselli, J.R., Cleary, M.P., Jen, K.L., and Greenwood, M.R. (1980). Development of food motivated behavior in free feeding and food restricted Zucker fatty (fa/fa) rats. Physiol Behav *25*, 565-573. 10.1016/0031-9384(80)90123-7.

55. Greenwood, M.R., Quartermain, D., Johnson, P.R., Cruce, J.A., and Hirsch, J. (1974). Food motivated behavior in genetically obese and hypothalamic-hyperphagic rats and mice. Physiol Behav *13*, 687-692. 10.1016/0031-9384(74)90241-8.

56. Narayanaswami, V., Thompson, A.C., Cassis, L.A., Bardo, M.T., and Dwoskin, L.P. (2013). Diet-induced obesity: dopamine transporter function, impulsivity and motivation. Int J Obes (Lond) *37*, 1095-1103. 10.1038/ijo.2012.178.

57. Harb, M.R., and Almeida, O.F. (2014). Altered motivation masks appetitive learning potential of obese mice. Frontiers in behavioral neuroscience *8*, 377. 10.3389/fnbeh.2014.00377.

58. Steele, C.C., Pirkle, J.R.A., Davis, I.R., and Kirkpatrick, K. (2019). Dietary effects on the determinants of food choice: Impulsive choice, discrimination, incentive motivation, preference, and liking in male rats. Appetite *136*, 160-172. 10.1016/j.appet.2019.01.023.

59. Spaulding, M.O., Hoffman, J.R., Madu, G.C., Lord, M.N., Iizuka, C.S., Myers, K.P., and Noble, E.E. (2024). Adolescent food insecurity in female rodents and susceptibility to diet-induced obesity. Physiol Behav *273*, 114416. 10.1016/j.physbeh.2023.114416.

60. Ducrocq, F., Hyde, A., Fanet, H., Oummadi, A., Walle, R., De Smedt-Peyrusse, V., Laye, S., Ferreira, G., Trifilieff, P., and Vancassel, S. (2019). Decrease in Operant Responding Under Obesogenic Diet Exposure is not Related to Deficits in Incentive or Hedonic Processes. Obesity (Silver Spring) *27*, 255-263. 10.1002/oby.22358.

61. Duca, F.A., Swartz, T.D., and Covasa, M. (2014). Effect of diet on preference and intake of sucrose in obese prone and resistant rats. PLoS One *9*, e111232. 10.1371/journal.pone.0111232.

62. Shin, A.C., Townsend, R.L., Patterson, L.M., and Berthoud, H.R. (2011). "Liking" and "wanting" of sweet and oily food stimuli as affected by high-fat diet-induced obesity, weight loss, leptin, and genetic predisposition. Am J Physiol-Reg I *301*, R1267-R1280. 10.1152/ajpregu.00314.2011.

63. Cook, J.B., Hendrickson, L.M., Garwood, G.M., Toungate, K.M., Nania, C.V., and Morikawa, H. (2017). Junk food diet-induced obesity increases D2 receptor autoinhibition in the ventral tegmental area and reduces ethanol drinking. PLoS One *12*, e0183685. 10.1371/journal.pone.0183685.

64. Takase, K., Tsuneoka, Y., Oda, S., Kuroda, M., and Funato, H. (2016). High-fat diet feeding alters olfactory-, social-, and reward-related behaviors of mice independent of obesity. Obesity (Silver Spring) *24*, 886-894. 10.1002/oby.21441.

65. Gac, L., Kanaly, V., Ramirez, V., Teske, J.A., Pinto, M.P., and Perez-Leighton, C.E. (2015). Behavioral characterization of a model of differential susceptibility to obesity induced by standard and personalized cafeteria diet feeding. Physiol Behav *152*, 315-322. 10.1016/j.physbeh.2015.10.001.

66. Blanco-Gandia, M.C., Aracil-Fernandez, A., Montagud-Romero, S., Aguilar, M.A., Manzanares, J., Minarro, J., and Rodriguez-Arias, M. (2017). Changes in gene expression and sensitivity of cocaine reward produced by a continuous fat diet. Psychopharmacology (Berl) *234*, 2337-2352. 10.1007/s00213-017-4630-9.

67. Caggiula, A.R., Herndon, J.G., Jr., Scanlon, R., Greenstone, D., Bradshaw, W., and Sharp, D. (1979). Dissociation of active from immobility components of sexual behavior in female rats by central 6-hydroxydopamine: implications for CA involvement in sexual behavior and sensorimotor responsiveness. Brain Res *172*, 505-520.

68. Hasegawa, T., Takeo, T., Akitsu, H., Hoshina, Y., and Sakuma, Y. (1991). Interruption of the lordosis reflex of female rats by ventral midbrain stimulation. Physiol Behav *50*, 1033-1038. 10.1016/0031-9384(91)90433-o.

69. Sirinathsinghji, D.J., Whittington, P.E., and Audsley, A.R. (1986). Regulation of mating behaviour in the female rat by gonadotropin-releasing hormone in the ventral tegmental area: effects of selective destruction of the A10 dopamine neurones. Brain Res *374*, 167-173. 10.1016/0006-8993(86)90406-3.

70. Pfaus, J.G., Damsma, G., Wenkstern, D., and Fibiger, H.C. (1995). Sexual activity increases dopamine transmission in the nucleus accumbens and striatum of female rats. Brain Res *693*, 21-30.

71. Guarraci, F.A., Megroz, A.B., and Clark, A.S. (2002). Effects of ibotenic acid lesions of the nucleus accumbens on paced mating behavior in the female rat. Behav Neurosci *116*, 568-576. 10.1037//0735-7044.116.4.568.

72. Rivas, F.J., and Mir, D. (1990). Effects of nucleus accumbens lesion on female rat sexual receptivity and proceptivity in a partner preference paradigm. Behav Brain Res *41*, 239-249.

73. McCrickerd, K., and Forde, C.G. (2016). Sensory influences on food intake control: moving beyond palatability. Obes Rev *17*, 18-29. 10.1111/obr.12340.

74. la Fleur, S.E., Luijendijk, M.C., van der Zwaal, E.M., Brans, M.A., and Adan, R.A. (2014). The snacking rat as model of human obesity: effects of a free-choice high-fat high-sugar diet on meal patterns. Int J Obes (Lond) *38*, 643-649. 10.1038/ijo.2013.159.

75. Rolls, B.J., Rowe, E.A., Rolls, E.T., Kingston, B., Megson, A., and Gunary, R. (1981). Variety in a meal enhances food intake in man. Physiol Behav *26*, 215-221. 10.1016/0031-9384(81)90014-7.

76. Sampey, B.P., Vanhoose, A.M., Winfield, H.M., Freemerman, A.J., Muehlbauer, M.J., Fueger, P.T., Newgard, C.B., and Makowski, L. (2011). Cafeteria Diet Is a Robust Model of Human Metabolic Syndrome With Liver and Adipose Inflammation: Comparison to High-Fat Diet. Obesity *19*, 1109-1117. 10.1038/oby.2011.18.

77. Lalanza, J.F., and Snoeren, E.M.S. (2021). The cafeteria diet: A standardized protocol and its effects on behavior. Neurosci Biobehav Rev *122*, 92-119. 10.1016/j.neubiorev.2020.11.003.

78. Martire, S.I., Holmes, N., Westbrook, R.F., and Morris, M.J. (2013). Altered feeding patterns in rats exposed to a palatable cafeteria diet: increased snacking and its implications for development of obesity. PLoS One *8*, e60407. 10.1371/journal.pone.0060407.

79. Scheggi, S., Secci, M.E., Marchese, G., De Montis, M.G., and Gambarana, C. (2013). Influence of palatability on motivation to operate for caloric and non-caloric food in non food-deprived and food-deprived rats. Neuroscience *236*, 320-331. 10.1016/j.neuroscience.2013.01.027.

80. Lin, J.Y., Roman, C., Arthurs, J., and Reilly, S. (2012). Taste neophobia and c-Fos expression in the rat brain. Brain Res *1448*, 82-88. 10.1016/j.brainres.2012.02.013.

81. Huijgens, P.T., Guarraci, F.A., Olivier, J.D.A., and Snoeren, E.M.S. (2021). Male rat sexual behavior: Insights from inter-copulatory intervals. Behav Process *190*, 104458. 10.1016/j.beproc.2021.104458.

82. Huijgens, P.T., Heijkoop, R., and Snoeren, E.M.S. (2021). Silencing and stimulating the medial amygdala impairs ejaculation but not sexual incentive motivation in male rats. Behav Brain Res *405*, 113206. 10.1016/j.bbr.2021.113206.

83. Huijgens, P.T., Heijkoop, R., Vanderschuren, L., Lesscher, H.M.B., and Snoeren, E.M.S. (2024). CaMKIIa+ neurons in the bed nucleus of the stria terminalis modulate pace of natural reward

seeking depending on internal state. Psychopharmacology (Berl). 10.1007/s00213-024-06561-5.

84. Hardy, D.F., and Debold, J.F. (1971). Effects of mounts without intromission upon the behavior of female rats during the onset of estrogen-induced heat. Physiol Behav *7*, 643-645.

85. Bourdy, R., Hertz, A., Filliol, D., Andry, V., Goumon, Y., Mendoza, J., Olmstead, M.C., and Befort, K. (2021). The endocannabinoid system is modulated in reward and homeostatic brain regions following diet-induced obesity in rats: a cluster analysis approach. Eur J Nutr *60*, 4621-4633. 10.1007/s00394-021-02613-0.

86. Carlin, J.L., McKee, S.E., Hill-Smith, T., Grissom, N.M., George, R., Lucki, I., and Reyes, T.M. (2016). Removal of high-fat diet after chronic exposure drives binge behavior and dopaminergic dysregulation in female mice. Neuroscience *326*, 170-179. 10.1016/j.neuroscience.2016.04.002.

87. Martire, S.I., Maniam, J., South, T., Holmes, N., Westbrook, R.F., and Morris, M.J. (2014). Extended exposure to a palatable cafeteria diet alters gene expression in brain regions implicated in reward, and withdrawal from this diet alters gene expression in brain regions associated with stress. Behav Brain Res *265*, 132-141. 10.1016/j.bbr.2014.02.027.

88. Sharma, S., Fernandes, M.F., and Fulton, S. (2013). Adaptations in brain reward circuitry underlie palatable food cravings and anxiety induced by high-fat diet withdrawal. Int J Obes (Lond) *37*, 1183-1191. 10.1038/ijo.2012.197.

89. Carlin, J., Hill-Smith, T.E., Lucki, I., and Reyes, T.M. (2013). Reversal of dopamine system dysfunction in response to high-fat diet. Obesity (Silver Spring) *21*, 2513-2521. 10.1002/oby.20374.

90. Willmore, L., Minerva, A.R., Engelhard, B., Murugan, M., McMannon, B., Oak, N., Thiberge, S.Y., Pena, C.J., and Witten, I.B. (2023). Overlapping representations of food and social

stimuli in mouse VTA dopamine neurons. Neuron *111*, 3541-3553 e3548. 10.1016/j.neuron.2023.08.003.

91. Stice, E., Spoor, S., Bohon, C., and Small, D.M. (2008). Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. Science *322*, 449-452. 10.1126/science.1161550.

92. Ibias, J., Miguens, M., Del Rio, D., Valladolid-Acebes, I., Stucchi, P., Ambrosio, E., Martin, M., Morales, L., Ruiz-Gayo, M., and Del Olmo, N. (2016). Decreased rates of operant food self-administration are associated with reward deficits in high-fat feeding mice. Eur J Nutr *55*, 1615-1622. 10.1007/s00394-015-0980-4.

93. Tantot, F., Parkes, S.L., Marchand, A.R., Boitard, C., Naneix, F., Laye, S., Trifilieff, P., Coutureau, E., and Ferreira, G. (2017). The effect of high-fat diet consumption on appetitive instrumental behavior in rats. Appetite *108*, 203-211. 10.1016/j.appet.2016.10.001.

94. Rossi, H.L., Luu, A.K., Kothari, S.D., Kuburas, A., Neubert, J.K., Caudle, R.M., and Recober, A. (2013). Effects of diet-induced obesity on motivation and pain behavior in an operant assay. Neuroscience *235*, 87-95. 10.1016/j.neuroscience.2013.01.019.

95. Blaisdell, A.P., Lau, Y.L., Telminova, E., Lim, H.C., Fan, B., Fast, C.D., Garlick, D., and Pendergrass, D.C. (2014). Food quality and motivation: a refined low-fat diet induces obesity and impairs performance on a progressive ratio schedule of instrumental lever pressing in rats. Physiol Behav *128*, 220-225. 10.1016/j.physbeh.2014.02.025.

96. Tracy, A.L., Wee, C.J., Hazeltine, G.E., and Carter, R.A. (2015). Characterization of attenuated food motivation in high-fat diet-induced obesity: Critical roles for time on diet and reinforcer familiarity. Physiol Behav *141*, 69-77. 10.1016/j.physbeh.2015.01.008.

97. Johnson, A.W. (2012). Dietary manipulations influence sucrose acceptance in diet induced obese mice. Appetite *58*, 215-221. 10.1016/j.appet.2011.09.015.

98. Horvitz, J.C., Stewart, T., and Jacobs, B.L. (1997). Burst activity of ventral tegmental dopamine neurons is elicited by sensory stimuli in the awake cat. Brain Res *759*, 251-258. 10.1016/s0006-8993(97)00265-5.

99. Ljungberg, T., Apicella, P., and Schultz, W. (1992). Responses of monkey dopamine neurons during learning of behavioral reactions. J Neurophysiol *67*, 145-163. 10.1152/jn.1992.67.1.145.

100. Day, J.J., Roitman, M.F., Wightman, R.M., and Carelli, R.M. (2007). Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nat Neurosci *10*, 1020-1028. 10.1038/nn1923.

101. Schultz, W., Dayan, P., and Montague, P.R. (1997). A neural substrate of prediction and reward. Science *275*, 1593-1599. 10.1126/science.275.5306.1593.

102. Schultz, W. (2015). Neuronal Reward and Decision Signals: From Theories to Data. Physiol Rev *95*, 853-951. 10.1152/physrev.00023.2014.

103. Dwyer, D.M. (2012). EPS Prize Lecture. Licking and liking: the assessment of hedonic responses in rodents. Q J Exp Psychol (Hove) *65*, 371-394. 10.1080/17470218.2011.652969.

104. Stuber, G.D., and Wise, R.A. (2016). Lateral hypothalamic circuits for feeding and reward. Nat Neurosci *19*, 198-205. 10.1038/nn.4220.

105. Anand, B.K., and Brobeck, J.R. (1951). Localization of a "feeding center" in the hypothalamus of the rat. Proc Soc Exp Biol Med *77*, 323-324. 10.3181/00379727-77-18766.

106. Snoeren, E.M.S. (2019). Female Reproductive Behavior. Curr Top Behav Neurosci *43*, 1-44. 10.1007/7854_2018_68.

107. Snoeren, E.M., Antonio-Cabrera, E., Spiteri, T., Musatov, S., Ogawa, S., Pfaff, D.W., and Agmo, A. (2015). Role of Oestrogen alpha Receptors in Sociosexual Behaviour in Female Rats Housed in a Seminatural Environment. J Neuroendocrinol *27*, 803-818. 10.1111/jne.12321.

108. Matthews, T.J., Grigore, M., Tang, L., Doat, M., Kow, L.M., and Pfaff, D.W. (1997). Sexual reinforcement in the female rat. J Exp Anal Behav *68*, 399-410.

109. Pfaff, D.W., and Sakuma, Y. (1979). Facilitation of the lordosis reflex of female rats from the ventromedial nucleus of the hypothalamus. J Physiol *288*, 189-202.

110. Yin, L., and Lin, D. (2023). Neural control of female sexual behaviors. Horm Behav *151*, 105339. 10.1016/j.yhbeh.2023.105339.

111. Swanson, L.W. (1982). The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull *9*, 321-353. 10.1016/0361-9230(82)90145-9.

112. Heijkoop, R., Huijgens, P.T., and Snoeren, E.M.S. (2018). Assessment of sexual behavior in rats: The potentials and pitfalls. Behav Brain Res *352*, 70-80. 10.1016/j.bbr.2017.10.029.

113. Huijgens, P.T., Heijkoop, R., and Snoeren, E.M.S. (2023). Sexual Incentive Motivation. In Animal Models of Reproductive Behavior, R.G. Paredes, W. Portillo, and M. Bedos, eds. (Springer US), pp. 197-210. 10.1007/978-1-0716-3234-5_9.

114. Beloate, L.N., Omrani, A., Adan, R.A., Webb, I.C., and Coolen, L.M. (2016). Ventral Tegmental Area Dopamine Cell Activation during Male Rat Sexual Behavior Regulates Neuroplasticity and d-Amphetamine Cross-Sensitization following Sex Abstinence. J Neurosci *36*, 9949-9961. 10.1523/JNEUROSCI.0937-16.2016.

115. Reichelt, A.C., Loughman, A., Bernard, A., Raipuria, M., Abbott, K.N., Dachtler, J., Van, T.T.H., and Moore, R.J. (2020). An intermittent hypercaloric diet alters gut microbiota, prefrontal cortical gene expression and social behaviours in rats. Nutr Neurosci *23*, 613-627. 10.1080/1028415X.2018.1537169.

116. Ramos, J., Hardin, E.J., Grant, A.H., Flores-Robles, G., Gonzalez, A.T., Cruz, B., Martinez, A.K., Beltran, N.M., and Serafine, K.M. (2020). The Effects of Eating a High Fat Diet on Sensitivity of Male and Female Rats to Methamphetamine and Dopamine D(1) Receptor Agonist SKF 82958. J Pharmacol Exp Ther *374*, 6-15. 10.1124/jpet.119.263293.

117. Kessler, R.C., Berglund, P.A., Chiu, W.T., Deitz, A.C., Hudson, J.I., Shahly, V., Aguilar-Gaxiola, S., Alonso, J., Angermeyer, M.C., Benjet, C., et al. (2013). The prevalence and correlates of

binge eating disorder in the World Health Organization World Mental Health Surveys. Biol Psychiatry *73*, 904-914. 10.1016/j.biopsych.2012.11.020.

118. Beeson, A.L.S., and Meitzen, J. (2023). Estrous cycle impacts on dendritic spine plasticity in rat nucleus accumbens core and shell and caudate-putamen. J Comp Neurol *531*, 759-774. 10.1002/cne.25460.

119. Golden, C.E.M., Kaur, D., Mah, A., Martin, A.C., Levy, D.H., Yamaguchi, T., Lin, D., Aoki, C., and Constantinople, C.M. (2023). Estrogenic control of reward prediction errors and reinforcement learning. bioRxiv. 10.1101/2023.12.09.570945.

120. Babbs, R.K., Unger, E.L., and Corwin, R.L. (2013). 2-Hydroxyestradiol enhances binge onset in female rats and reduces prefrontal cortical dopamine in male rats. Horm Behav *63*, 88-96. 10.1016/j.yhbeh.2012.10.010.

121. Eckel, L.A., Houpt, T.A., and Geary, N. (2000). Spontaneous meal patterns in female rats with and without access to running wheels. Physiol Behav *70*, 397-405. 10.1016/s0031-9384(00)00278-x.

122. Leibowitz, S.F., Akabayashi, A., Alexander, J.T., and Wang, J. (1998). Gonadal steroids and hypothalamic galanin and neuropeptide Y: role in eating behavior and body weight control in female rats. Endocrinology *139*, 1771-1780. 10.1210/endo.139.4.5867.

123. Liu, C., Dou, Y., Zhang, M., Han, S., Hu, S., Li, Y., Yu, Z., Liu, Y., Liang, X., Chen, Z.J., et al. (2024). High-fat and high-sucrose diet impairs female reproduction by altering ovarian transcriptomic and metabolic signatures. J Transl Med *22*, 145. 10.1186/s12967-024-04952-y.

124. Paxinos, G., and Watson, C. (1998). The rat brain in stereotaxic coordinates, 4th Edition (Academic Press).

125. Phang, C.-H. (1993). Vasectomy of Male Rat. In Transgenesis Techniques. Principles and Protocols, D. Murphy, and D.A. Carter, eds. (Humana Press).

126. Gunaydin, L.A., Grosenick, L., Finkelstein, J.C., Kauvar, I.V., Fenno, L.E., Adhikari, A., Lammel, S., Mirzabekov, J.J., Airan, R.D., Zalocusky, K.A., et al. (2014). Natural neural projection dynamics underlying social behavior. Cell *157*, 1535-1551. 10.1016/j.cell.2014.05.017.

127. Lerner, T.N., Shilyansky, C., Davidson, T.J., Evans, K.E., Beier, K.T., Zalocusky, K.A., Crow, A.K., Malenka, R.C., Luo, L., Tomer, R., and Deisseroth, K. (2015). Intact-Brain Analyses Reveal Distinct Information Carried by SNc Dopamine Subcircuits. Cell *162*, 635-647. 10.1016/j.cell.2015.07.014.