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Transient effects of junk food on NAc core MSN excitability and glutamatergic transmission in obesity-prone female rats

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Abstract

Objective: The nucleus accumbens (NAc) plays critical roles in eating and food seeking in rodents and humans. Diets high in fats and sugars ("junk food") produce persistent increases in NAc function in male obesity-prone rats. This study examines effects of junk food and junk food deprivation on NAc core medium spiny neuron (MSN) excitability and glutamate transmission in females.

Methods: Obesity-prone female rats were given access to ad libitum junk food for 10 days, and recordings were made from MSNs in the NAc core immediately or after a short (27-72 hours) or long (14-16 days) junk food deprivation period in which rats were returned to ad libitum standard chow. Controls remained on chow throughout. Whole-cell slice electrophysiology was used to examine MSN intrinsic membrane and firing properties and glutamatergic transmission.

Results: The study found that intrinsic excitability was reduced, whereas glutamatergic transmission was enhanced, after the short, but not long, junk food deprivation period. A brief junk food deprivation period was necessary for increases in NAc calcium-permeable-AMPA receptor transmission and spontaneous excitatory postsynaptic current (sEPSC) frequency, but not for increases in sEPSC amplitude.

Conclusions: This study reveals that females are protected from long-lasting effects of sugary fatty foods on MSN neuronal function and provides evidence for sex-specific effects on plasticity in brain centers that influence food-seeking and feeding behavior.

INTRODUCTION

The nucleus accumbens (NAc) plays critical roles in eating and food seeking. For example, NAc activity is required for cue-triggered food seeking in nonobese rats and it involves both dopamine and glutamate transmission [1–3]. In men and women, the magnitude of NAc

activation in response to food cues corresponds to future weight gain [4], and this activation is stronger in individuals with obesity [5, 6]. Therefore, recent preclinical studies examining the neurobiology of obesity and over eating have focused on diet-induced alterations in NAc function within populations that are obesity-prone (OP) or obesity-resistant (OR) to best model human obesity susceptibility [7, 8]. However, the majority of these studies have used males, despite established roles of ovarian hormones in feeding and energy

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expenditure [9] and mounting evidence that neural mechanisms underlying seemingly similar behaviors differ by sex [10].

The NAc is composed predominantly of medium spiny projection neurons (MSNs). The activity of these cells is influenced by their intrinsic properties and by ongoing neurotransmission. Within MSNs, inwardly rectifying potassium currents help maintain a hyperpolarized state, whereas fast transient potassium currents influence action potential firing following depolarization [11]. Dopamine receptor activation bidirectionally modulates these intrinsic properties [12, 13], and it can indirectly influence glutamatergic transmission [14–16]. AMPA type glutamate receptors (AMPARs) provide the main source of excitation to the NAc. Disruption of AMPAR synaptic trafficking blocks cue-triggered motivation for sucrose in nonobese mice [17], as does pharmacological AMPAR or calcium-permeable AMPAR (CP-AMPAR) blockade within the NAc core [18, 19]. Therefore, food-seeking behaviors are influenced by MSN intrinsic properties and excitatory drive to the NAc.

Eating diets high in fats and sugars (i.e., junk food [JF]) alters NAc core function, and these effects are more pronounced in OP rats, which model at-risk human populations [7, 8]. For example, eating a JF diet reduces MSN intrinsic excitability in OP, but not OR, males [20]. In addition, JF increases NAc CP-AMPAR transmission in OP, but not OR, males [21, 22]. This increase requires a JF-free period (24 hours) and persists for at least 14 days after JF removal [22]. In contrast, the same diet regimen and 2-week JF-deprivation period does not alter NAc core CP-AMPAR transmission in OP females [22]. However, shorter time points following JF deprivation were not examined in females, and prior studies did not examine effects of JF alone versus JF followed by deprivation in females.

In the present study, females were given free access to JF or chow for 10 days. Recordings were then made with and without a period of JF deprivation (24-48 hours or 14-16 days). This was done to determine persistence of JF effects and to establish whether removal of JF is needed for NAc plasticity. We found reductions in intrinsic excitability and increases in NAc glutamatergic transmission following the short JF-deprivation period (24-48 hours), but these effects did not persist following the long deprivation period (14-16 days). In addition, JF deprivation was required for increased CP-AMPAR transmission and spontaneous excitatory postsynaptic current (sEPSC) frequency, but not for enhancements in sEPSC amplitude. As a whole, the data suggest that although the general pattern of JF and JF-deprivation effects are somewhat similar across sex, in females these effects are transient and return to levels comparable to chow after long-term JF removal.

METHODS

Subjects

Adult female selectively bred OP rats [23] bred in house were used for all studies. Rats were ~55 days old at the start of the experiment, were housed on a reverse 12-hour light/dark cycle (lights off at 8:00 a.m.), had free access to water and food, and were group housed unless otherwise noted. Procedures were approved by The University

Study Importance

What is already known?

- The nucleus accumbens (NAc) plays critical roles in eating and food seeking.
- Diets high in fats and sugars (i.e., junk food) produce long-lasting reductions in medium spiny neuron (MSN) intrinsic excitability and increases in NAc core calcium permeable (CP)-AMPA receptor (AMPAR) transmission in obesity-prone male rats.
- Junk-food does not alter NAc core CP-AMPARs in obesity-prone female rats following the same exposure and 2-week deprivation period.

What does this study add?

- Junk food reduces intrinsic excitability and increases glutamatergic transmission after a short (24-72 hours) junk food deprivation period in females.
- These effects do not persist after a long (14-16 days) junk food deprivation period in females.
- A junk food deprivation period is needed for CP-AMPAR increases, but not for enhancements in general excitatory transmission, in females.

How might these results change the direction of research?

- These results provide evidence for sex-specific effects of eating sugary fatty junk food on NAc function.

of Michigan Committee on the Use and Care of Animals in accordance with AAALAC and AVMA guidelines.

Diet manipulation

The JF diet, made in house, consisted of a mash of Ruffles potato chips (40 g), Chips Ahoy! chocolate chip cookies (130 g), Nesquik chocolate powder (130 g), Jif peanut butter (130 g), powdered Lab Diet 5001 (200 g), and 180 mL of water (19.6% fat, 14% protein, and 58% carbohydrates; 4.5 kcal/g) [21]. Body weight and food intake were measured daily. Rats were maintained on this diet for 10 days, after which recordings were made immediately, or JF was removed and replaced with standard lab chow (i.e., JF deprivation; Lab Diet 5001: 4 kcal/g; 4.5% fat, 23% protein, 48.7% carbohydrates; percentage of caloric content) for either 14 to 16 days or 24 to 72 hours. Therefore, only one type of food was available during each phase. Controls remained on standard chow throughout. This timing was chosen to determine persistence of JF effects and to establish

whether removal of JF is needed for NAc plasticity. To maintain feasibility of whole-cell recordings, separate cohorts were used for each time point after JF exposure and groups were counterbalanced for starting weight.

Cycle monitoring

Estrous cycle phase was determined by daily observations of vaginal epithelial cell cytology and precopulatory and copulatory behaviors [24, 25]. Epithelial cells were collected by vaginal lavage (1-2 hours after the start of the dark phase) and visualized using an inverted light microscope (Olympus CKX53) under bright-field conditions. Recordings were made during the metestrus/diestrus phase of the cycle unless otherwise noted. These phases were chosen because this is when motivation for food, food intake, and cue-triggered food seeking are highest in females [9, 25, 26].

Whole-cell patch clamp recordings

Established whole-cell patch clamping approaches were used [20, 22, 25]. Briefly, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and brains were removed and placed in ice-cold oxygenated (95% O_2–5% CO_2) aCSF containing (in mM): 125 NaCl, 25 NaHCO$_3$, 12.5 glucose, 1.25 NaH$_2$PO$_4$, 3.5 KCl, 1 L-ascorbic acid, 0.5 CaCl$_2$, 3 MgCl$_2$, pH 7.45, 300 to 305 mOsm. Coronal slices (300 μm) containing the NAc were made on a vibratome (Leica Biosystems). Slices were allowed to recover in oxygenated aCSF (30 minutes, 37 °C) and then maintained at room temperature (30 minutes) prior to recording. For the recording aCSF, CaCl$_2$ was 2.5 mM and MgCl$_2$ was 1 mM. All recordings were conducted in the presence of the γ-aminobutyric acid (GABA)$_A$ receptor antagonist picrotoxin (50 μM). For recordings of intrinsic properties and sEPSCs pipettes were filled with a solution containing the following (in mM): 130 K-gluconate, 10 KCl, 1 EGTA, 2 Mg$_2^+$-ATP, 0.6 Na$^+$-GTP, and 10 HEPES, pH 7.45, 285 mOsm. MSNs were identified based on their hyperpolarized resting membrane potential and distinct firing pattern in response to square pulse current injections (−200 to +400 pA, 500 milliseconds). Current/voltage (I/V) relationships were determined by calculating the difference between the baseline voltage and the voltage 200 milliseconds after initial current injections. Input resistance was determined by the change in voltage from −50 pA to +50 pA current injections. The number of action potentials elicited by each depolarizing current injection was used to determine neuronal excitability. Rheobase was defined as the minimum amount of current required to elicit an action potential. sEPSCs were recorded at a holding potential of −70 mV (5 minutes). For recordings of CP-AMPARs, pipettes were filled with the following (in mM): 140 CsCl, 10 HEPES, 2 MgCl$_2$, 5 Na$^+$-ATP, 0.6 Na$^+$-GTP, 2 QX314, pH 7.3, 285 mOsm. Evoked EPSCs (eEPSCs) were elicited by local stimulation (0.02-0.30 mA square pulses, 0.1 milliseconds, delivered every 20 seconds) using a bipolar electrode placed ~300 μm lateral to recorded neurons. The

minimum amount of current needed to elicit a synaptic response with <20% variability in amplitude was used. If >0.30 mA was required, the neuron was discarded. eEPSCs were recorded at −70 mV before and after application of the CP-AMPAR selective antagonist Naspm (200 μM) [21, 22]. For all data analysis, only cells with an access resistance of less than 30 MΩ were used. Cell parameters (capacitance and membrane resistance) were recorded at the start and end of data collection, and only cells with less than a 20% change across time were included in analyses.

Recordings alternated between slices from rats in the chow and JF group each day. Intrinsic membrane properties and action potentials and sEPSC were measured on day 14 to 16 of JF deprivation. Intrinsic membrane properties and action potentials, sEPSCs, and CP-AMPAR-mediated transmission were measured after 24 to 72 hours of JF deprivation, and sEPSCs and CP-AMPAR-mediated transmission were measured without JF deprivation.

Analysis and statistics

Evoked responses and intrinsic excitability data were analyzed using Clampfit 10.7 (Molecular Devices). sEPSCs were analyzed using MiniAnalysis (Synaptosoft version 6.0.7) and verified by hand. Comparisons were made between data collected within the same cohort of animals (i.e., given chow or JF side by side). Two-tailed t tests, Mann–Whitney U tests, mixed-effects models, and 1- or 2-way ANOVA with Sidak's post hoc comparisons were used (Prism 9, GraphPad). Interpretation of p values is based on guidelines set forth by the American Statistical Association [27]. Experimenters were not blind to grouping during data acquisition but were during analysis. All final ns are reported in the results and based on expected effect size and variance of our primary measures.

RESULTS

We first assessed persistent effects of JF and subsequent deprivation on MSN intrinsic excitability and glutamatergic transmission. Rats were given JF for 10 days followed by a 14- to 16-day return to standard lab chow (i.e., "deprivation") or standard chow throughout prior to whole-cell patch clamp recordings (timeline provided in Figure 1A).

During JF exposure, OP females gained more weight than chow controls (Figure 1B: 2-way repeated-measures [RM] ANOVA, time-× diet interaction: $F_{[10,100]}$ = 5.13, $p < 0.0001$, Sidak's multiple comparison test: days 6-10: $p < 0.05$). However, by the recording day, the weights of chow and JF groups were comparable (Mann–Whitney U test: U = 8, p = 0.35; data not shown). This occurred because, although rats in the JF group ate more when JF was available, they ate significantly less chow during the deprivation period than controls (Figure 1C,D; C: 2-way RM ANOVA, group × period interaction: $F_{[19,190]}$ = 4.88, $p < 0.0001$; Sidak's multiple comparison test: day 11:
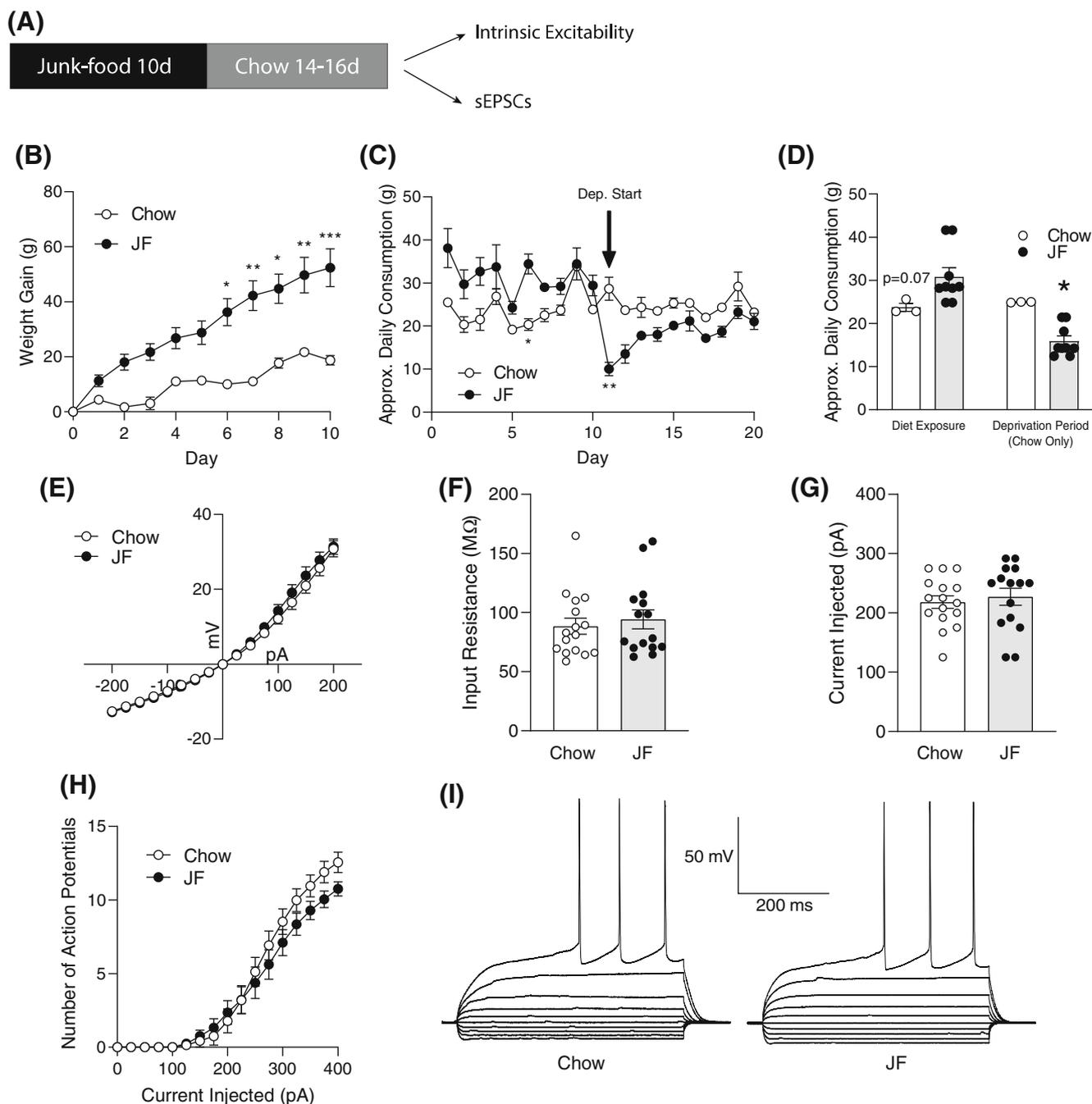
FIGURE 1 Effect of junk food (JF) followed by long deprivation on medium spiny neuron intrinsic excitability. (A) Experimental timeline. (B) Weight gain across time. Rats in the JF group gained more weight than chow controls. (C,D) Average daily food intake. Compared with chow controls, obesity-prone female rats given JF ate more during the diet manipulation but ate less chow during the deprivation period. (E) Change in membrane potential across current injection. (F) Average input resistance. (G) Average rheobase. (H) Number of action potentials elicited by each current injection. (I) Sample recordings from chow (left) and JF (right) cells. All data shown as average ± SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. sEPSC, spontaneous excitatory postsynaptic current

$p < 0.01$; D: 2-way RM ANOVA, group × period interaction: $F_{[1,10]} = 71.37$, $p < 0.0001$; Sidak's multiple comparison test chow vs. JF: during diet exposure, $p = 0.07$; after deprivation period: $p = 0.02$).

When recordings were made on deprivation day 14 to 16 (chow: 14 cells from 3 rats, JF: 15 cells from 4 rats), there were no differences in membrane response to current injections (Figure 1E: 2-way RM ANOVA: $F_{[16,432]} = 0.55$, $p = 0.92$), input resistance (Figure 1F:

2-tailed unpaired t test: $t_{[27]} = 0.97$, $p = 0.34$), or rheobase (Figure 1G: 2-tailed unpaired t test: $t_{[27]} = 0.08$, $p = 0.93$) between cells from chow and JF rats. Although there were not strong effects on MSN firing, there was a small but significant reduction in the number of action potentials fired in response to higher current injections in cells from rats given JF versus chow (Figure 1H: 2-way RM ANOVA: $F_{[16,432]} = 2.02$, $p = 0.01$).
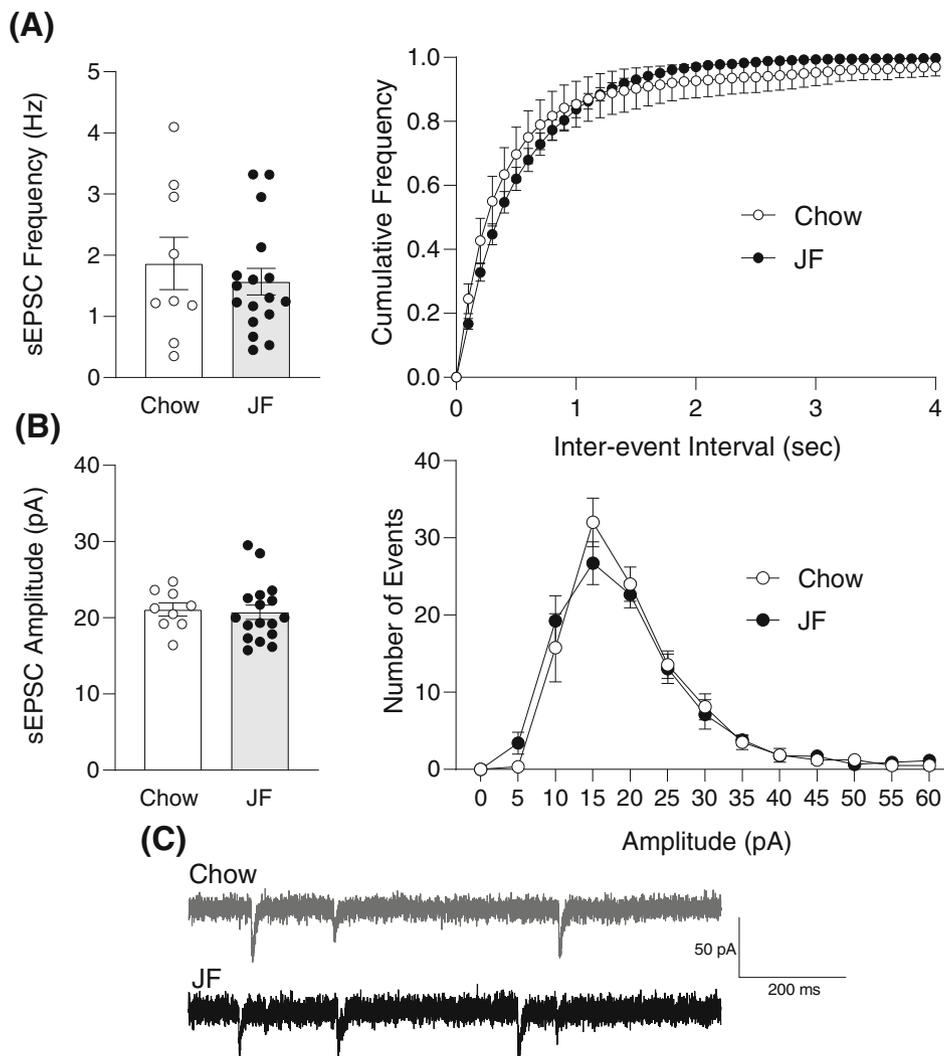
FIGURE 2 Effect of junk food (JF) followed by long deprivation on spontaneous excitatory postsynaptic current (sEPSC) frequency and amplitude. (A) Average frequency (left) and cumulative frequency distribution (right) of sEPSCs. (B) Average amplitude (left) and amplitude distribution (right) of sEPSCs. (C) Representative traces of sEPSCs from chow and JF groups

When sEPSCs were recorded (Chow: 9 cells from 3 rats, JF:17 cells from 9 rats), no differences were observed in average frequency (Figure 2A: Mann–Whitney U test: $U = 100.5$, $p = 0.96$) or amplitude (Figure 2B: Mann–Whitney U test: $U = 75$, $p = 0.24$) between groups. Similarly, the distributions for frequency and amplitude were unchanged. Therefore, overall, JF and deprivation did not produce long-lasting changes in MSN intrinsic excitability or glutamatergic transmission in females.

It is possible that JF produces rapid changes in MSN physiology that return to baseline during the deprivation period. Therefore, we next determined whether effects on NAc MSN function are present following a shorter period of JF deprivation (24–72 hours; timeline in Figure 3A). As described earlier, OP female rats given JF ($n = 22$) gained more weight (Figure 3B: mixed-effects analysis, time × diet interaction: $F_{[10,359]} = 76.88$, $p < 0.0001$; Sidak's multiple comparison test: days 5-10: $p < 0.01$) and ate more when JF was available, but they ate significantly less chow during the first 24 hours of the

deprivation period than their chow counterparts ($n = 18$; Figure 3C: diet exposure: 2-tailed unpaired t test: $t_{[28]} = 2.10$, $p = 0.04$; deprivation period: 2-tailed unpaired t test: $t_{[17]} = 5.30$, $p < 0.0001$). Note that separate t tests were conducted for Figure 3C because food intake during the deprivation period was measured only in a subset of rats (chow $n = 8$; JF $n = 11$).

To ensure that we were not missing any effects, the cycle was monitored, and recordings were made in all phases (chow metestrus/diestrus [M/D]: 9 cells from 5 rats; chow proestrus/estrus [P/E]: 12 cells from 6 rats; JF M/D: 7 cells from 6 rats; JF P/E: 8 cells from 4 rats). No cycle effects were found, and thus data were collapsed across phase (Figure 3D–G). We found that JF followed by this short deprivation shifted I/V relationships at positive current injections compared with cells from chow controls (Figure 3D: 2-way RM ANOVA, group × current injection interaction: $F_{[16,544]} = 5.5$, $p < 0.0001$; Sidak's multiple comparison: $p < 0.01$). In addition, input resistance was decreased (Figure 3E: 2-tailed unpaired t test,
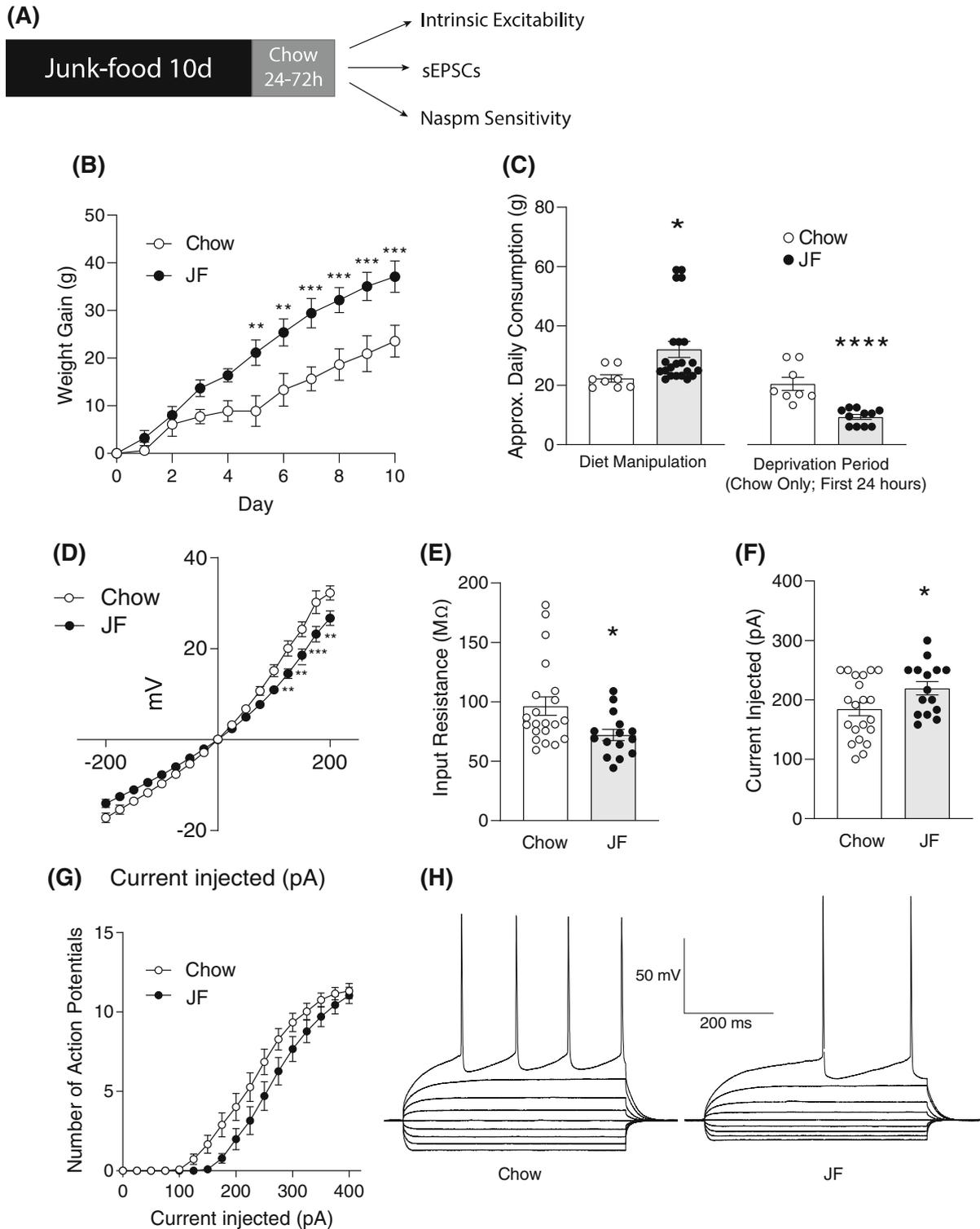
FIGURE 3 Effect of junk food (JF) followed by short deprivation on medium spiny neuron intrinsic excitability. (A) Experimental timeline. (B) Weight gain across time. Obesity-prone (OP) female rats given JF gained more weight across the diet manipulation than chow controls. (C) Average daily food consumption in JF and chow groups. Compared with chow controls, OP female rats on JF ate more during the diet manipulation but ate less chow during the deprivation period. (D) Change in membrane potential across current injection. Changes in membrane potential in response to positive current injection were reduced in OP females given JF followed by a brief deprivation compared with controls. (E) Average input resistance. Input resistance was decreased following JF consumption and a brief deprivation. (F) Average rheobase. Rheobase was increased following JF consumption and a brief deprivation. (G) Number of action potentials elicited by each current injection. JF consumption followed by brief deprivation reduced the number of action potentials fired at intermediate current injections compared with chow controls. (H) Sample traces from chow (left) and JF (right) cells. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. sEPSC, spontaneous excitatory postsynaptic current
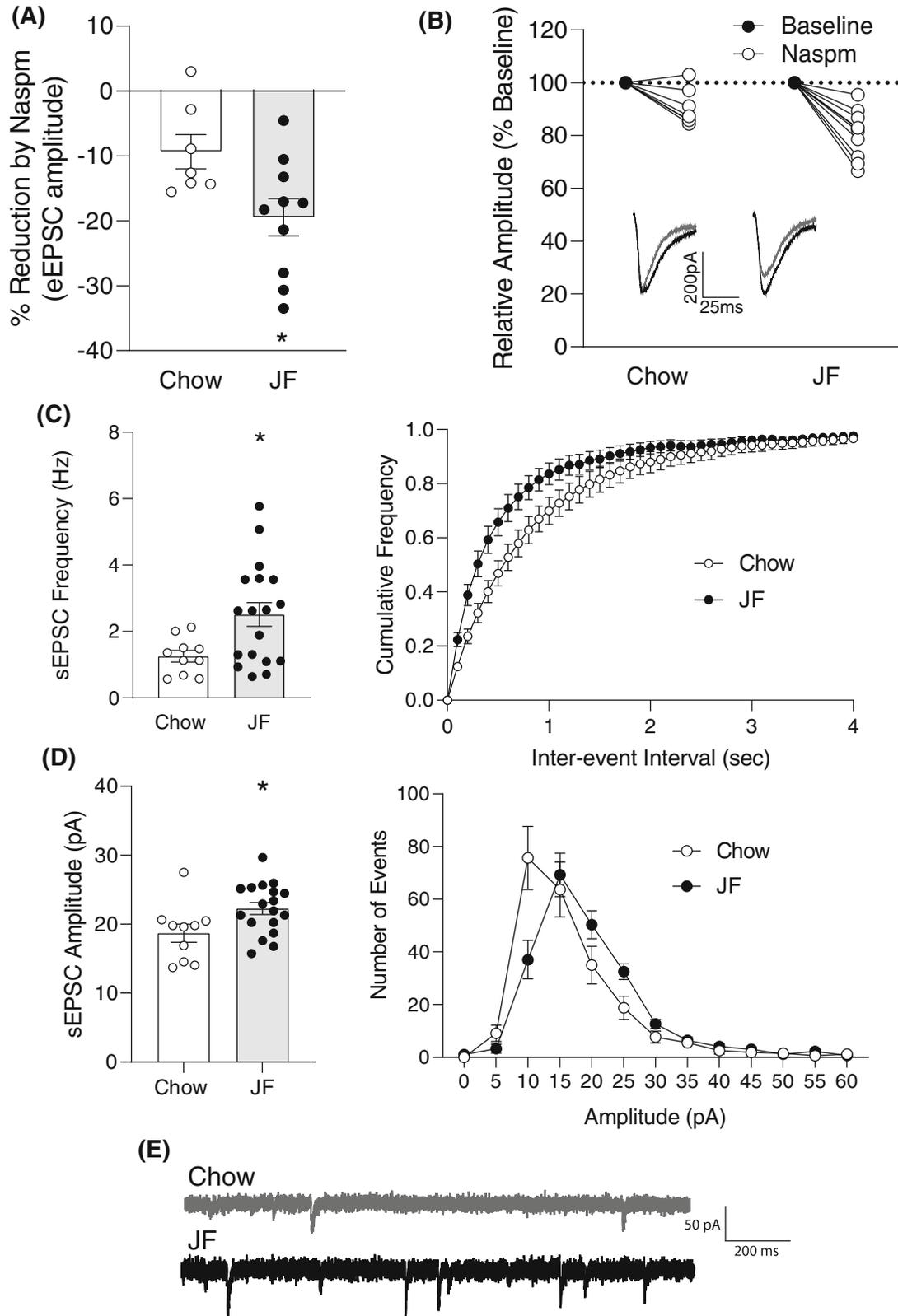
FIGURE 4 Effect of junk food (JF) followed by short deprivation on glutamatergic transmission. (A,B) Reduction in eEPSC amplitude following bath application of the calcium-permeable-AMPAR (C-PAMPAR) antagonist Naspm. CP-AMPAR transmission was enhanced following JF consumption and a brief deprivation period compared with controls. Inset in panel B: black trace = before Naspm; gray trace = after Naspm. (C) Average frequency (left) and cumulative frequency distribution (right) of sEPSCs. sEPSC frequency was enhanced following JF consumption and a brief deprivation period in obesity-prone female rats. (D) Average amplitude (left) and amplitude distribution (right) of sEPSCs. sEPSC amplitude was increased following JF consumption and a brief deprivation period. (E) Representative traces of sEPSCs from both groups. *$p < 0.05$. eESPC, evoked excitatory postsynaptic current; sEPSC, spontaneous excitatory postsynaptic current
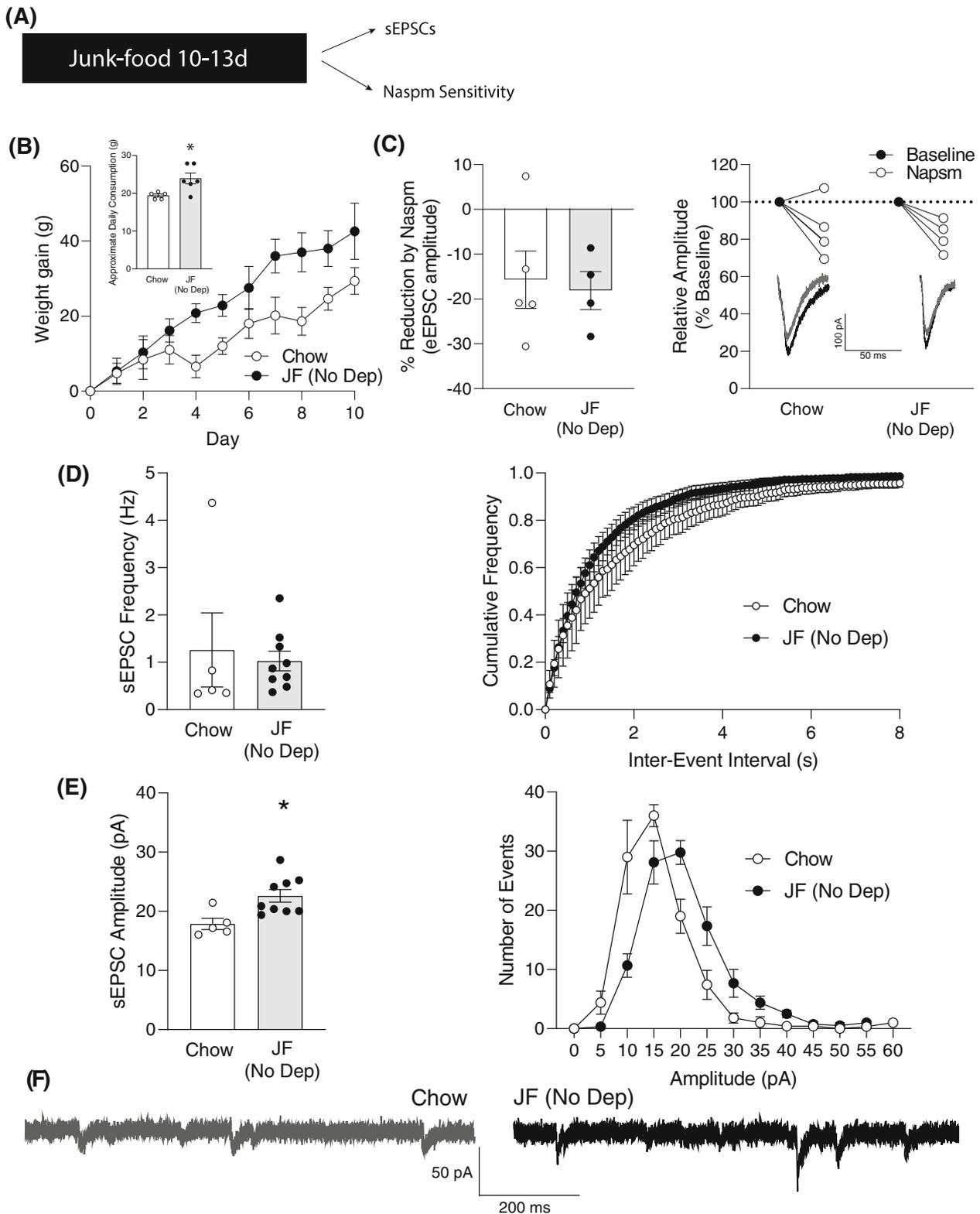
FIGURE 5 Effect of junk food (JF) with no deprivation on glutamatergic transmission. (A) Experimental timeline. (B) Weight gain across time. Obesity-prone female rats given JF gained more weight across time than chow controls. Inset shows average daily food consumption in JF and chow groups. Rats in the JF group ate more than rats in the chow group. (C) Reduction in evoked excitatory postsynaptic current (eEPSC) amplitude following bath application of the calcium-permeable AMPAR (CP-AMPAR) antagonist Naspm (left); percentage change from baseline (right). JF without deprivation (No Dep) did not alter CP-AMPAR transmission compared with chow controls; black trace = before Naspm; gray trace = after Naspm. (D) Average frequency (left) and cumulative frequency distribution (right) of spontaneous excitatory postsynaptic currents (sEPSCs). No group differences were found. (E) Average amplitude (left) and amplitude distribution (right) of sEPSCs. sEPSC amplitude was increased after JF consumption compared with chow controls. (F) Representative traces of sEPSCs in both groups. All data shown as average ± SEM. *$p < 0.05$

$t_{[34]} = 2.4$, $p = 0.02$), whereas rheobase was increased (Figure 3F: 2-tailed unpaired t test, $t_{[34]} = 2.1$, $p = 0.04$) in cells from JF versus chow groups. Consistent with these effects, we also observed reduced action potential firing in cells from JF versus chow groups (Figure 3G: 2-way RM ANOVA, main effect of group: $F_{[16,544]} = 2.56$, $p = 0.08$). Furthermore, these effects held when data were separated out by estrous cycle phase (I/V: M/D, 2-way RM ANOVA, group- $\times$ current interaction: $F_{[16,224]} = 1.99$, $p < 0.02$; P/E, 2-way RM ANOVA, group $\times$ current interaction: $F_{[16,288]} = 3.38$, $p < 0.001$; number of action potentials: M/D, 2-way RM ANOVA, main effect of group: $F_{[16,224]} = 3.03$, $p = 0.001$; P/E, 2-way RM ANOVA, main effect of group: $F_{[16,288]} = 1.89$, $p = 0.02$; data not shown). Therefore, JF followed by a brief deprivation resulted in a reduction in MSN intrinsic excitability across the cycle.

We also examined effects of this same manipulation on NAc glutamatergic transmission. Bath application of the selective CP-AMPAR antagonist Naspm (chow: 7 cells from 7 rats; JF: 10 cells from 9 rats) resulted in a larger reduction in eEPSC amplitude in cells from JF versus chow controls, indicative of a greater contribution from CP-AMPARs (Figure 4A: Mann–Whitney U test: $U = 12$, $p = 0.02$). Furthermore, both sEPSC frequency (Figure 4C: Mann–Whitney U test: $U = 48$, $p = 0.04$) and amplitude (Figure 4D: Mann–Whitney U test: $U = 43$, $p = 0.02$) were significantly increased in cells from the JF versus chow groups (chow: 10 cells from 5 rats; JF: 18 cells from 11 rats). Therefore, JF followed by a short deprivation period resulted in enhancements in NAc core excitatory transmission in females.

Last, we evaluated CP-AMPAR-mediated transmission and sEPSC amplitude and frequency following JF consumption without any deprivation period (timeline in Figure 5A). As before, OP females given JF gained more weight (Figure 5B: mixed-effect model, main effect of diet: $F_{[1,9]} = 4.17$, $p = 0.07$; diet $\times$ time interaction: $F_{[12,100]} = 4.02$, $p < 0.001$) and ate significantly more (5B Inset: Mann–Whitney U test: $U = 3$, $p = 0.03$) than chow controls. Interestingly, JF with no deprivation failed to alter CP-AMPAR transmission (chow: 5 cells from 4 rats; JF: 4 cells from 3 rats; Figure 5C; Mann–Whitney U test: $U = 10$, $p > 0.99$) or sEPSC frequency (chow: 5 cells from 3 rats; JF: 9 cells from 5 rats; Figure 5D; Mann–Whitney U test: $U = 14$, $p = 0.30$) compared with chow controls. However, sEPSC amplitude was increased in the JF versus chow groups (Figure 5E; Mann–Whitney U test: $U = 5$, $p = 0.02$). Therefore, a short deprivation period following JF consumption was necessary for enhancements in NAc CP-AMPAR transmission and sEPSC frequency, but not sEPSC amplitude.

DISCUSSION

Effects of JF and subsequent deprivation on MSN intrinsic excitability in females

We began by evaluating the effects of a JF diet on MSN intrinsic excitability in OP females. We found that JF (10 days) followed by a 14- to 16-day deprivation period had no effect on MSN intrinsic excitability (Figure 1). However, after a brief period of deprivation (24-72 hours), MSN excitability and firing were reduced compared with chow controls (Figure 3). Together, these data suggest that JF has transient effects on MSN intrinsic excitability in OP females that return to baseline in the absence of continued JF consumption.

In outbred Sprague Dawley female rats, and the selectively bred OP model used here, MSN excitability is greater in the metestrus/ diestrus phase of the cycle compared with the proestrus/estrus phase [25, 28]. Given these small but consistent shifts in intrinsic properties of MSNs with the cycle, we compared effects of JF on excitability when recordings were made in the proestrus/estrus versus metestrus/diestrus phases following short JF deprivation. A reduction in MSN excitability was present regardless of cycle phase. Therefore, effects following JF and deprivation were not strongly affected by cycle phase. Recordings after the longer deprivation were made only from animals in metestrus/diestrus. Although we cannot rule out potential cycle effects, we think it is unlikely that effects of the cycle impeded our ability to detect group differences at the longer deprivation time point, given that effects were detectable across the cycle following the short deprivation period.

Similar reductions in MSN excitability were found in OP males shortly after this same JF diet exposure [20]. Therefore, although there is evidence for basal sex differences in MSN excitability [15], reductions in excitability following JF and subsequent deprivation do not appear to be sex specific. In the previous study using males, all food was removed from the home cage 14 to 16 hours prior to recording, whereas in the current study all females were given free access to chow for 24 to 72 hours prior to recording. However, when JF was removed and rats were returned to ad libitum chow, they voluntarily reduced their food intake, eating very little standard lab chow for a period of 1 to 3 days before gradually resuming levels of chow consumption comparable to controls; this was seen in females here (Figures 1C and 3C) and in males from previous studies [29, 30]. Therefore, although complete fasting is not likely to be necessary for reductions in MSN excitability, we cannot rule out possible contributions of reduced food intake to effects of JF followed by deprivation. Furthermore, it is possible that voluntary reductions in food intake during the deprivation period may produce a "stress" response that contributes to effects on excitability and glutamate transmission (discussed later). However, to our knowledge, there are no data that examine effects of voluntary food restriction on hypothalamic-pituitary axis activation or other measures of stress. Therefore, this remains an outstanding question.

Effects of JF on glutamatergic transmission in females

The one previous study of NAc excitatory transmission conducted in females focused on relatively long-lasting effects of JF consumption. In that study, 10 days of eating JF followed by 14 days of JF deprivation did not alter NAc core CP-AMPAR transmission but produced slight increases in the AMPA/NMDA ratio [22]. Shorter deprivation periods were not examined. Consistent with previous results, we found no group differences in sEPSC amplitude or frequency

following long deprivation (Figure 2). In addition, the recording conditions used here biased measures toward AMPAR-mediated EPSCs. Therefore, the absence of effects on sEPSC amplitude after long JF deprivation suggests that previously reported shifts in the AMPA/NMDA ratio after this same regimen may be due to alterations in NMDAR transmission [22], although caution should be used when integrating results from spontaneous versus evoked responses.

Following the short JF deprivation, we found increases in sEPSC frequency and amplitude and in CP-AMPAR transmission in JF versus chow groups (Figure 4). This suggests that NAc excitatory transmission following JF consumption is indeed enhanced in females, but that this effect is transient and that it returns to baseline when rats are returned to ad libitum chow for an extended period. In regard to effects on glutamate transmission, increases in sEPSC frequency are often indicative of increases in glutamate release, whereas increases in sEPSC amplitude are indicative of enhancements in postsynaptic receptor expression. The latter is consistent with CP-AMPAR upregulation, and both are consistent with enhancements in excitatory transmission. However, changes in sEPSC frequency can also occur in the absence of changes in glutamate release. For example, Wissman et al. found increases in miniature EPSC frequency with no differences in paired pulse ratio (a common measure of the probability of glutamate release) in MSNs of male and female rats following repeated experimenter-administered cocaine injections [31]. This suggests that increases in frequency are not due to increased glutamate release but rather to increases in MSN spine density. Self-administration of high-fat food pellets increased the number of mushroom-type spines on MSNs in the NAc core of male rats [32]. Therefore, the increases in sEPSC frequency here could be due to increases in presynaptic glutamate release or increases in synaptic contacts. These possibilities can be examined in future studies.

Pertaining to postsynaptic transmission, we found increases in CP-AMPAR-mediated transmission in addition to increases in sEPSC amplitude following the short JF deprivation. Similar to reports in males [21, 33], CP-AMPARs mediated ∼10% of the AMPA current in chow-fed female controls and a little over 20% of the current following JF consumption (Figure 4). Transient trafficking of CP-AMPARs is a normal part of synaptic plasticity thought to contribute to learning and memory, whereas persistent upregulation of CP-AMPAR expression and transmission is thought to induce nonconventional forms of synaptic remodeling that lead to pathological states, including addiction [34, 35]. For example, blockade of CP-AMPARs in the NAc core prevents the expression of cue-triggered food seeking in OP male rats and blunts the incubation of cocaine craving in males [19, 33]. However, the transience in CP-AMPAR upregulation in females is in contrast to what we have previously seen in OP males, in which increases in both CP-AMPAR surface expression and transmission are rapid and persistent [21, 22]. Therefore, although JF increases CP-AMPARs in both sexes, females appear to be protected from long-lasting diet-induced alterations in NAc function. This could suggest that potential behavioral effects of JF diet exposure, such as enhanced cue-triggered food seeking found in males [30], may also be transient in females.

Finally, we found that increases in CP-AMPAR transmission required a JF-deprivation period in females. The same occured in males, in which removal of JF was also required for increases in NAc core CP-AMPAR transmission [22]. As mentioned previously, whether voluntary reductions in food intake contribute to this effect is unknown. Indeed, what triggers the recruitment of CP-AMPARs versus "standard" GluA1/2 containing AMPARs is also unknown; see [35] for review. However, it is worth noting that increases in CP-AMPARs following cocaine consumption require a drug-free period, albeit longer (at least 30 days following cessation of cocaine self-administration) [36]. Overall, data to date suggest that CP-AMPARs may be recruited in response to the absence of continued consumption of reinforcing stimuli, be they food or drug.

Although regulation of intrinsic excitability and glutamate transmission can be independent [37, 38], alterations in synaptic transmission often result in opposing changes in membrane excitability, and vice versa [28, 39]. Therefore, it is possible that reductions in intrinsic excitability are a compensatory response to enhancements in glutamatergic drive onto MSNs. This hypothesis is supported by data showing that reducing excitatory input increased membrane excitability in MSNs of the NAc [39]. However, it is also possible that increased excitatory transmission is instead a compensatory response to initial experience-induced reductions in MSN excitability, for which there is also evidence [40, 41]. Nonetheless, the pattern of effects found here in females is consistent with overall enhancements in excitatory drive to the NAc.

What might be causing these transient effects in females?

When considering what might be driving sex-specific effects, one starting point is the potential role of gonadal hormones. Naturally circulating ovarian hormones (estradiol and progesterone) influence food-seeking and feeding behaviors and modulate neuroplasticity associated with alterations in motivation [25, 28, 31, 42, 43]. Therefore, the presence of ovarian hormones in the absence of continued JF consumption may help reverse the effects and return the system to baseline. This would be consistent with the ability of ovarian hormones to suppress food intake and reduce food-seeking behaviors [25, 26]. However, there are strikingly few studies of the effects of ovarian hormones on MSN synaptic transmission on which to build strong mechanistic hypotheses. Evidence suggests that circulating levels of progesterone and estradiol correlate with mEPSC frequency and amplitude measures [44], and that NAc glutamatergic transmission increases during proestrus and estrus compared with other phases in naturally cycling females [28]. In addition, acute estradiol treatment of striatal slices from adult females produced rapid, but small, reductions in mEPSC frequency and amplitude in the NAc core [45]. Therefore, it is possible that effects are transient in females, but not in males, owing to ongoing fluctuations in ovarian hormones across the JF-deprivation period. However, additional studies addressing fundamental physiological effects of ovarian hormones on NAc glutamatergic transmission and MSN excitability are needed.

In summary, JF consumption reduces MSN intrinsic excitability, increases NAc core glutamate transmission, and enhances CP-AMPAR-mediated transmission when followed by a brief period of deprivation. Although this brief deprivation is required, these effects are absent after a longer deprivation period. Therefore, this study reveals that females are protected from long-lasting effects of sugary fatty food consumption on NAc core function.⃝

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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