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

Running Title: Transient Effects of Junk-Food in Females

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Study Importance Questions

What is already known about this subject?

- 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 MSN intrinsic excitability and increases in NAc core CP-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 are the new findings in your manuscript?

- Junk-food reduces intrinsic excitability and increases glutamatergic transmission after a short (24-72 hour) junk-food deprivation period in females.
- These effects do not persist after a long (14-16 day) 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 your results change the direction of research or the focus of clinical practice?

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

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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. Here we examine 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 lib junk-food for 10 days and recordings were made from MSNs in the NAc core immediately or after a short (27-72 hour) or long (14-16 day) junk-food deprivation period in which rats were returned to ad lib 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: We found that intrinsic excitability is reduced while glutamatergic transmission is enhanced after the short, but not long, junk-food deprivation period. A brief junk-food deprivation period was necessary for increases in NAc CP-AMPAR transmission and sEPSC frequency.

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.

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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 non-obese rats and 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]. Thus, 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 or obesity-resistant, to best model human obesity suseptibility[7,8]. However, the majority of these studies have used males, despite established roles of ovarian hormones in feeding and energy expenditure[9] and mounting evidence that neural mechanisms underlying seemingly similar behaviors differ by sex[10].

The NAc is comprised 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, while fast transient potassium currents influence action potential firing following depolarization[11]. Dopamine receptor activation bi-directionally modulates these intrinsic properties[12,13], and 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 non-obese mice[17] as does pharmacological AMPAR or calcium-permeable AMPARs (CP-AMPAR) blockade within the NAc core[18,19]. Thus, 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) alters NAc core function, and these effects are more

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

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

Materials and Methods:

Subjects:

Adult female selectively-bred obesity-prone (OP) rats[23] bred in house were used for all studies. Rats were ~55 days old at the start of the experiment, housed on a reverse 12-h light/dark cycle (lights off at 0800), had free access to water and food, and were group housed unless otherwise noted. Procedures were approved by The University of Michigan Committee on the Use and Care of Animals in accordance with AAALAC and AVMA guidelines.

Diet Manipulation:

The junk-food diet consisted of a mash of Ruffles™ potato chips (40 g), Chips Ahoy!™ chocolate chip cookies (130 g), Nesquik™ chocolate powder (130 g), Jiff™ 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) and was made in house[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 junk-food was removed and replaced with standard lab chow (i.e. junk-food deprivation; Lab Diet 5001: 4 kcal/g; 4.5% fat, 23% protein, 48.7% carbohydrates; % of caloric content) for either 14-16 days or 24-72 hours. Thus only one type of food was available during each phase. Controls remained on standard chow throughout. This timing was chosen to determine persistence of junk-food effects, and to establish whether removal of junk-food is needed for NAc plasticity. To maintain feasibility of whole-cell recordings, separate cohorts were used for each time point after junk-food exposure and groups were

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counterbalanced for starting weight.

Cycle Monitoring:

Estrous cycle phase was determined by daily observations of vaginal epithelial cell cytology, 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. 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.), brains were removed and placed in ice-cold oxygenated (95% O2–5% CO2) aCSF containing (in mM): 125 NaCl, 25 NaHCO3, 12.5 glucose, 1.25 NaH2PO4, 3.5 KCl, 1 L-ascorbic acid, 0.5 CaCl2, 3 MgCl2, pH 7.45, 300-305 mOsm. Coronal slices (300 µm) containing the NAc were made on a vibratome (Leica Biosystems, Buffalo Grove, IL, USA). Slices were allowed to recover in oxygenated aCSF (30 min, 37 °C), and then maintained at room temperature (30 min) prior to recording. For the recording aCSF, CaCl2 was 2.5 mM and MgCl2 was 1 mM. All recordings were conducted in the presence of the $GABA_A$ receptor antagonist, picrotoxin (50 µM). For recordings of intrinsic properties and sEPSCs pipettes were filled with a solution containing (in mM): 130 K-gluconate, 10 KCl, 1 EGTA, 2 Mg2+-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 +400pA, 500ms). Current/voltage (I/V) relationships were determined by calculating the difference between the baseline voltage and the voltage 200ms after initial current injections. Input resistance was determined by the change in voltage from -50pA to +50pA current injections. The number of action potentials elicited by each depolarizing current injection were used to determine neuronal excitability. Rheobase was defined as the minimum amount of current required to elicit an action potential. Spontaneous excitatory post-synaptic currents (sEPSCs) were recorded at a holding potential of -70mV (5 min). For recordings of CP-AMPARs, pipettes were filled with (in mM): 140 CsCl, 10 HEPES, 2 MgCl2, 5 Na+-ATP, 0.6 Na+-GTP, 2 QX314, pH 7.3, 285 mOsm for evoked responses. Evoked EPSCs (eEPSCs) were elicited by local stimulation (0.02 to 0.30 mA square pulses, 0.1 ms, delivered every 20s) 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 junk-food group each day. Intrinsic membrane

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properties and action potentials, and sEPSC were measured on day 14-16 of junk-food deprivation. Intrinsic membrane properties and action potentials, sEPSCs and CP-AMPAR-mediated transmission were measured after 24-72 hours of junk-food deprivation, and sEPSCs and CP-AMPAR-mediated transmission were measured without junk-food deprivation.

Analysis and Statistics:

Evoked responses and intrinsic excitability data were analyzed using Clampfit 10.7 (Molecular Devices). sEPSCs were analyzed using MiniAnalysis (Synaptosoft V.6.0.7) and verified by hand. Comparisons were made between data collected within the same cohort of animals (i.e., given chow or junk-food side by side). Two-tailed t-tests, Mann-Whitney U Tests, Mixed-effects models, and one- or two-way ANOVAs with Sidak's post-hoc comparisons were used (Prism 9, GraphPad, San Diego, CA). 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 junk-food and subsequent deprivation on MSN intrinsic excitability and glutamatergic transmission. Rats were given junk-food for 10 days followed by a 14-16-day return to standard lab chow (i.e., "deprivation"), or standard chow throughout prior to whole cell patch clamp recordings (see timeline, Fig. 1A).

During junk-food exposure, obesity-prone females gained more weight than chow controls (Fig. 1B: two-way RM ANOVA, time x diet interaction $F_{(10,100)}$=5.13, p<0.0001, Sidak's multiple comparison's test: days 6-10: p<0.05). However, by the recording day, the weights of chow- and junk-food groups were comparable (Mann-Whitney U Test: U=8, p=0.35; data not shown). This occurred because although rats in the junk-food group ate more when junk-food was available, they ate significantly less chow during the deprivation period than controls (Fig. 1C,D; C: two-way RM ANOVA group x period interaction: $F_{(19,190)}$=4.88, p<0.0001; Sidak's multiple comparison's test: day 11: p<0.01; D: two-way RM ANOVA group x period interaction: $F_{(1,10)}$=71.37, p<0.0001; Sidak's multiple comparison's test Chow vs JF: during diet exposure, p=0.07; after deprivation period: p=0.02).

When recordings were made on deprivation day 14-16 (Chow: 14 cells from 3 rats, JF: 15 cells from 4 rats), there were no differences in membrane response to current injections (Fig. 1E: two-way RM ANOVA: $F_{(16,432)}$=0.55, p=0.92), input resistance (Fig. 1F: two-tailed unpaired t-test: $t_{(27)}$=0.97, p=0.34), or rheobase (Fig. 1G: two-tailed unpaired t-test: $t_{(27)}$=0.08, p=0.93) between cells from chow and junk-food rats. While 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 junk-food vs chow (Fig. 1H: two-way RM ANOVA: $F_{(16,432)}$=2.02, p=0.01).

When sEPSC were recorded (Chow: 9 cells from 3 rats, JF:17 cells from 9 rats), no differences were observed in average frequency (Fig. 2A: Mann-Whitney U Test: U=100.5, p=0.96) or amplitude (Fig. 2B: Mann-Whitney U Test: U=75, p=0.24) between groups. Similarly, the distributions for frequency and amplitude were

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unchanged. Thus overall, junk-food and deprivation did not produce long-lasting changes in MSN intrinsic excitability or glutamatergic transmission in females.

It is possible that junk-food 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 junk-food deprivation (24-72 hours; see timeline, Fig. 3A). As above, obesity-prone female rats given junk-food (N=22) gained more weight (Fig. 3B:Mixed-effects analysis time x diet interaction: $F_{(10,359)}$=76.88, p<0.0001; Sidak's multiple comparison's test: days 5-10: p<0.01), ate more when junk-food was available, but ate significantly less chow during the first 24-hours of the deprivation period than their chow counterparts (N=18; Fig. 3C: diet exposure: two-tailed unpaired t-test: $t_{(28)}$=2.10, p=0.04; deprivation period: two-tailed unpaired t-test: $t_{(17)}$=5.30, p<0.0001). Note that separate t-tests were conducted for Fig. 3C because food intake during the deprivation period was only measured 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 M/D: 9 cells from 5 rats; Chow 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 (Fig.3 D-G). We found that junk-food followed by this short deprivation shifted I/V relationships at positive current injections compared to cells from chow controls (Fig. 3D: two-way RM ANOVA group x current injection interaction: $F_{(16,544)}$= 5.5, p<0.0001; Sidak's multiple comparison p<0.01). In addition, input resistance was decreased (Fig. 3E: two-tailed unpaired t-test, $t_{(34)}$=2.4, p=0.02), while rheobase was increased (Fig. 3F: two-tailed unpaired t-test, $t_{(34)}$=2.1, p=0.04) in cells from junk-food vs chow groups. Consistent with these effects, we also observed reduced action potential firing in cells from junk-food vs chow groups (Fig. 3G: two-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, two-way RM ANOVA group x current interaction: $F_{(16, 224)}$=1.99, p<0.02; P/E, two-way RM ANOVA group x current interaction: $F_{(16, 288)}$=3.38, p<0.001); number of action potentials: M/D, two-way RM ANOVA main effect of group: $F_{(16, 224)}$=3.03, p=0.001; P/E, two-way RM ANOVA main effect of group: $F_{(16, 288)}$=1.89, p=0.02; data not shown). Thus, junk-food 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 junk-food vs chow controls, indicative of a greater contribution from CP-AMPARs (Fig. 4A: Mann-Whitney U Test: U=12, p=0.02). Furthermore, both sEPSC frequency (Fig. 4C: Mann-Whitney U Test: U=48, p=0.04) and amplitude (Fig. 4D: Mann-Whitney U Test: U=43, p=0.02) were significantly increased in cells from junk-food vs chow groups (Chow: 10 cells from 5 rats; JF: 18 cells from 11 rats). Thus, junk-food followed by a short deprivation period results in enhancements in NAc core excitatory transmission in females.

Lastly, we evaluated CP-AMPAR mediated transmission and sEPSC amplitude and frequency following junk-food consumption without any deprivation period (see timeline, Fig. 5A). As before, obesity-prone females given junk-food gained more weight (Fig. 5B: Mixed Effect Model; main effect of diet, $F_{(1,9)}$=4.17, p=0.07; diet x

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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, junk-food with no deprivation failed to alter CP-AMPAR transmission (Chow: 5 cells from 4 rats; JF: 4 cells from 3 rats; Fig. 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; Fig. 5D; Mann-Whitney U Test: U=14, p=0.30) compared to chow controls. However, sEPSC amplitude was increased in junk-food vs. chow groups (Fig. 5E; Mann-Whitney U Test: U=5, p=0.02). Thus, a short deprivation period following junk-food consumption is necessary for enhancements in NAc CP-AMPAR transmission and sEPSC frequency, but not sEPSC amplitude.

Discussion

Effects of junk-food and subsequent deprivation on MSN intrinsic excitability in females.

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

In outbred Sprague Dawley female rats, and the selectively bred obesity-prone model used here, MSN excitability is greater in the metestrus/diestrus phase of the cycle compared to the proestrus/estrus phase[25,28]. Given these small, but consistent shifts in intrinsic properties of MSNs with the cycle, we compared effects of junk-food on excitability when recordings were made in the proestrus/estrus vs metestrus/diestrus phases following short junk-food deprivation. A reduction in MSN excitability was present regardless of cycle phase (see results). Thus, effects following junk-food 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 are found in obesity-prone males shortly after this same junk-food diet exposure[20]. Thus, although there is evidence for basal sex differences in MSN excitability[15], reductions in excitability following junk-food and subsequent deprivation do not appear to be sex-specific. In the previous study using males, all food was removed from the home cage 14-16 hours prior to recording, whereas in the current study all females were given free access to chow for 24-72 hrs prior to recording. However, when junk-food is removed and rats are returned to *ad lib* chow, they voluntarily reduce their food intake, largely refusing to eat standard lab chow for a period of 1-3 days before gradually resuming levels of chow consumption comparable to controls; this is seen in females here (Fig. 1C, 3C) and in males from previous studies[29,30]. Thus, while 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 junk-food followed by deprivation. Further, it is

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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 below). However, to our knowledge there are no data that examine effects of voluntary food restriction on HPA axis activation, or other measures of stress. Thus, this remains an outstanding question.

Effects of junk-food on glutamatergic transmission in females.

The one previous study of NAc excitatory transmission conducted in females focused on relatively long-lasting effects of junk-food consumption. In that study, 10 days of eating junk-food followed by 14 days of junk-food 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 at that time. Consistent with previous results, we found no group differences in sEPSC amplitude or frequency following long deprivation (Fig 2). In addition, the recording conditions used here biased measures towards AMPAR-mediated EPSCs. Thus, the absence of effects on sEPSC amplitude suggest 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 vs. evoked responses.

Following the short junk-food deprivation, we found increases in sEPSC frequency and amplitude and in CP-AMPAR transmission in junk-food vs. chow groups (Fig. 4). This suggests that NAc excitatory transmission following junk-food consumption is indeed enhanced in females, but that this effect is transient and returns to baseline when rats are returned to *ad lib* 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 up-regulation, 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 mEPSC 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 suggested that increases in frequency were not due to increased glutamate release, but rather to increases in MSN spine density. Self-administration of high-fat food pellets increases 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 post-synaptic transmission, we found increases in CP-AMPAR-mediated transmission in addition to increases in sEPSC amplitude following the short junk-food 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 junk-food consumption (Fig. 4). Transient trafficking of CP-AMPARs is a normal part of synaptic plasticity thought to contribute to learning and memory, while 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 obesity-prone male rats and blunts the incubation of cocaine craving in

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males[19,33]. However, the transience in CP-AMPAR upregulation in females is in contrast to what we have previously seen in obesity-prone males, where increases in both CP-AMPAR surface expression and transmission are rapid and persistent[21,22]. Therefore, although junk-food 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 junk-food 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 junk-food deprivation period in females. The same occurs in males, where removal of junk-food is also required for increases in NAc core CP-AMPAR transmission[22]. As mentioned above, whether voluntary reductions in food intake contribute to this effect is unknown. Indeed, what triggers the recruitment of CP-AMPARs, vs "standard" GluA1/2 containing AMPARs is also unknown (see[35] for review). However, it's 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 results 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 increases 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 are 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]. Thus, the presence of ovarian hormones in the absence of continued junk-food consumption may help reverse the effects and return the system to baseline. This would be consistent with the ability of ovarian hormones to suppresses 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 to other phases in naturally cycling females[28]. In addition, acute estradiol treatment of striatal slices from adult females produces rapid, but small reductions in mEPSC frequency and amplitude in the NAc core[45]. Thus, it's possible that effects are transient in females, but not in males, due to ongoing fluctuations in ovarian hormones across the junk-food deprivation period. However, additional studies addressing fundamental physiological effects of ovarian hormones on NAc glutamatergic transmission and MSN

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excitability are needed.

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

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Figure 1

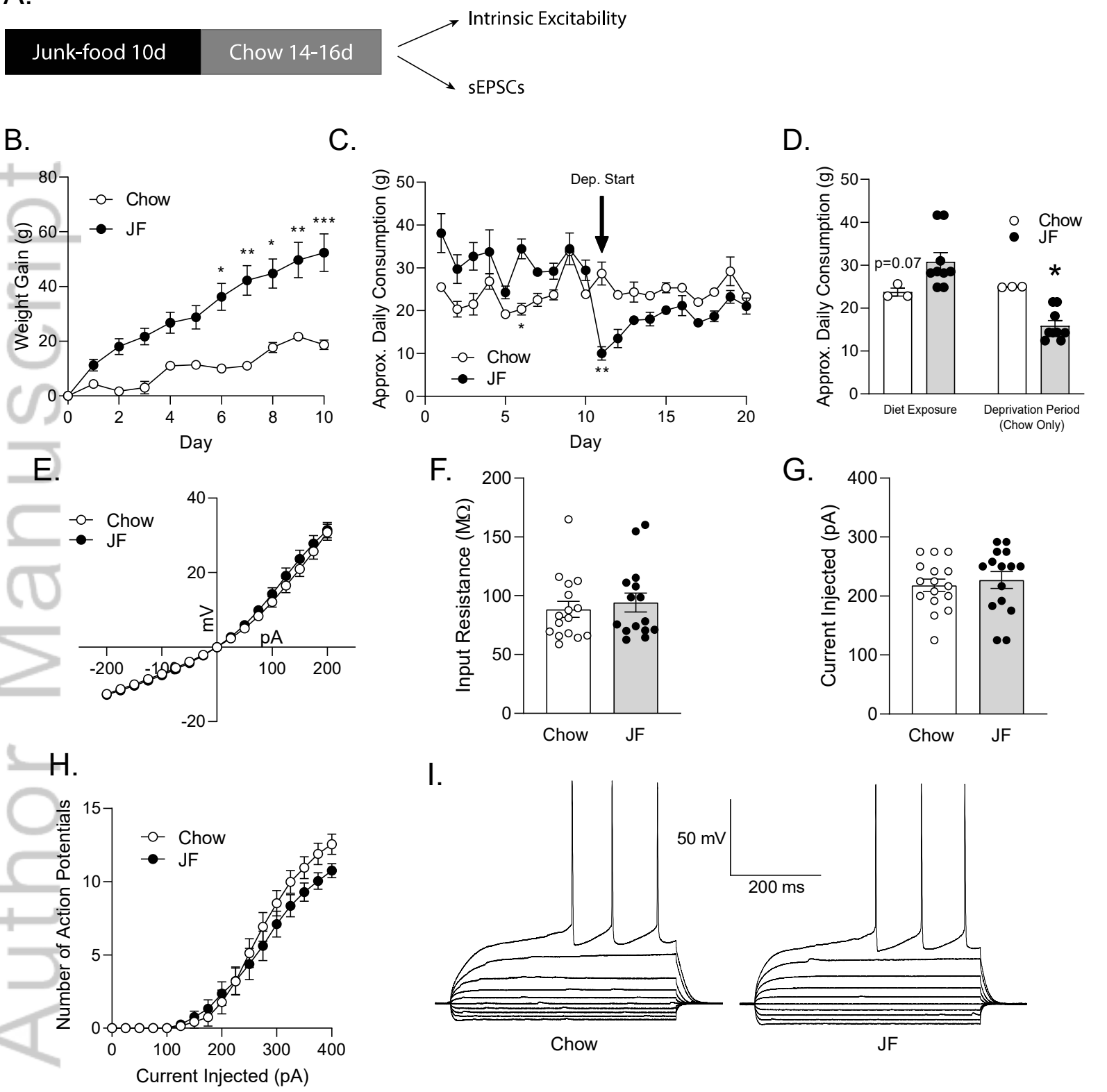

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Figure 2

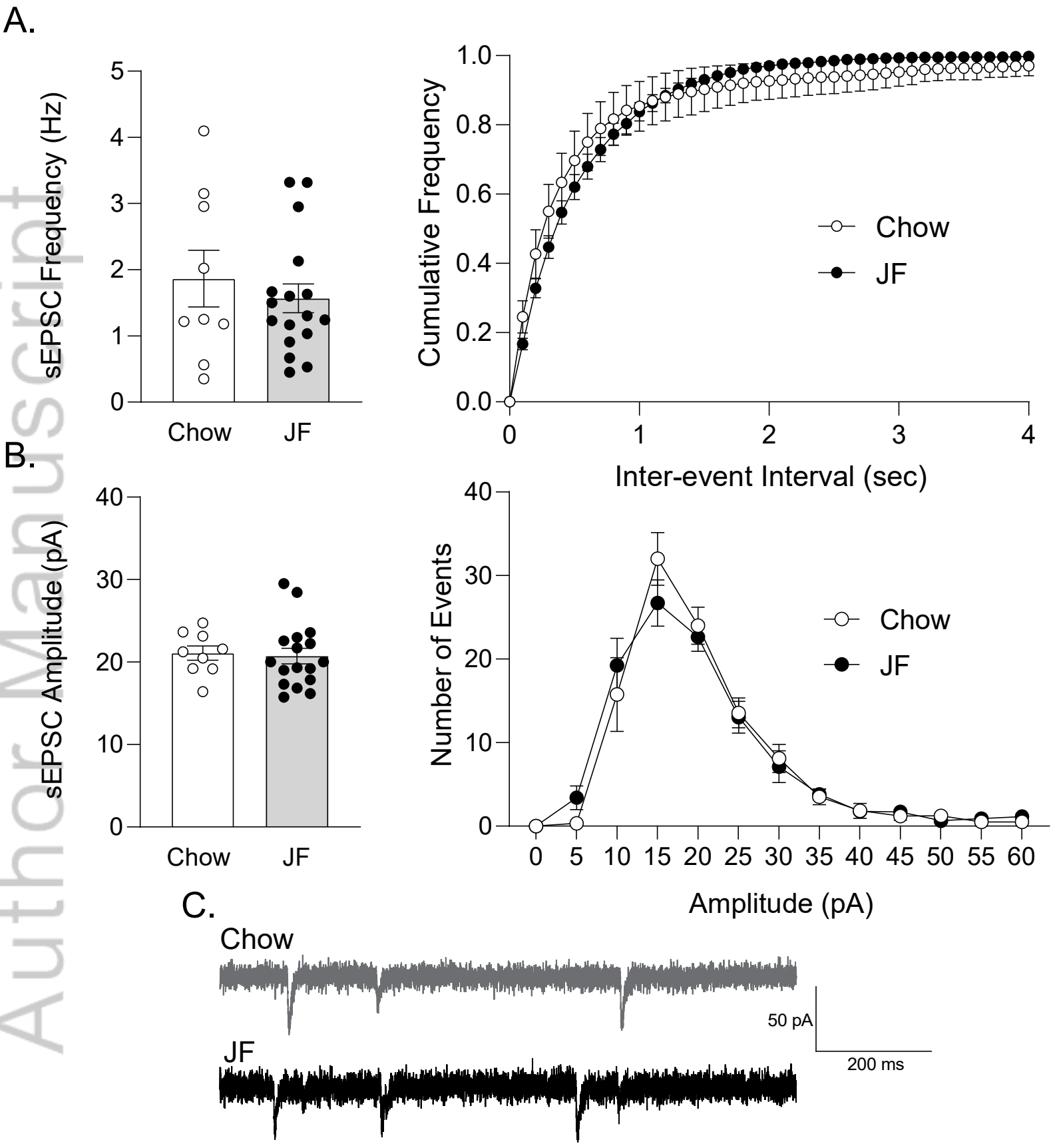

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Figure 3

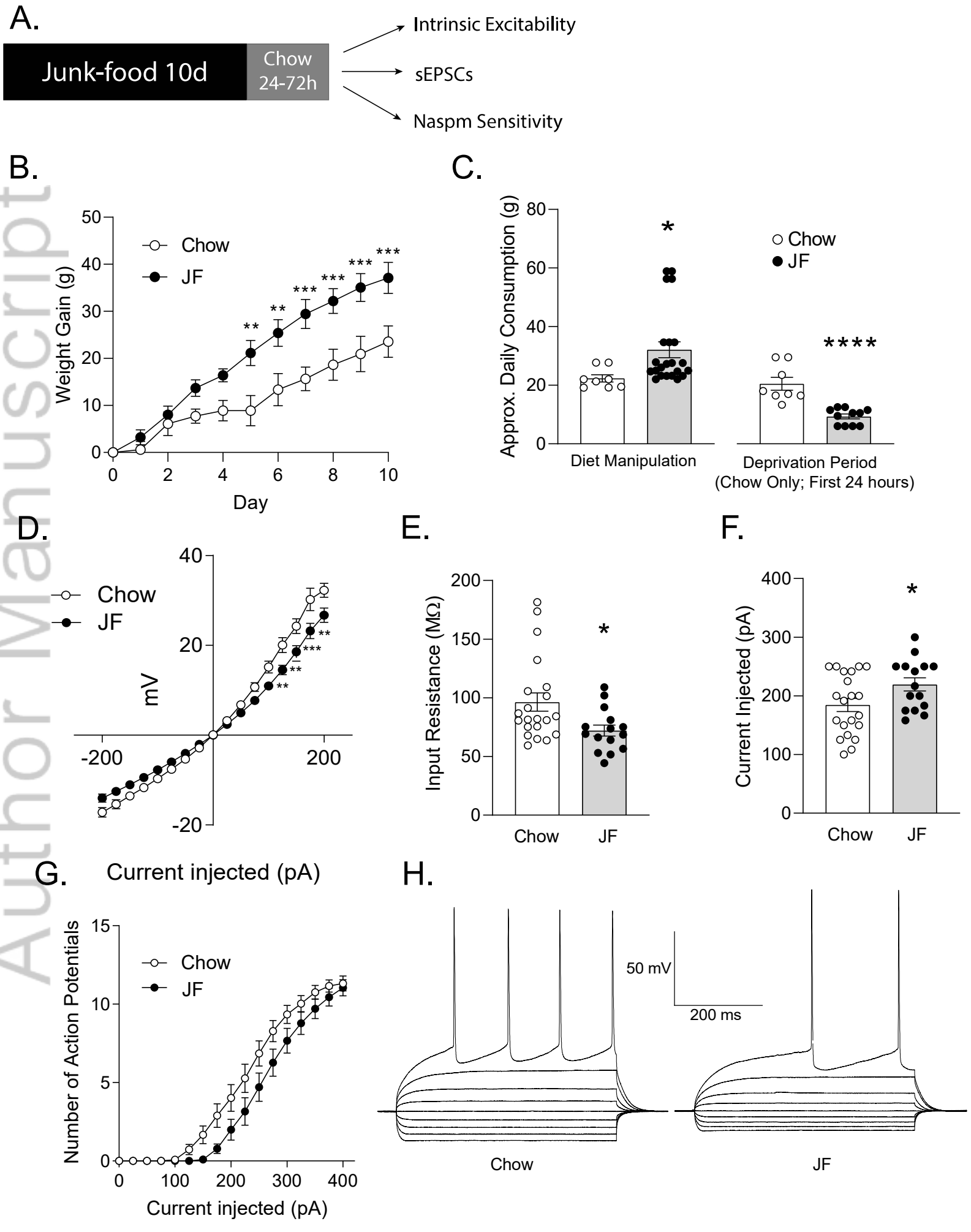

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Figure 4

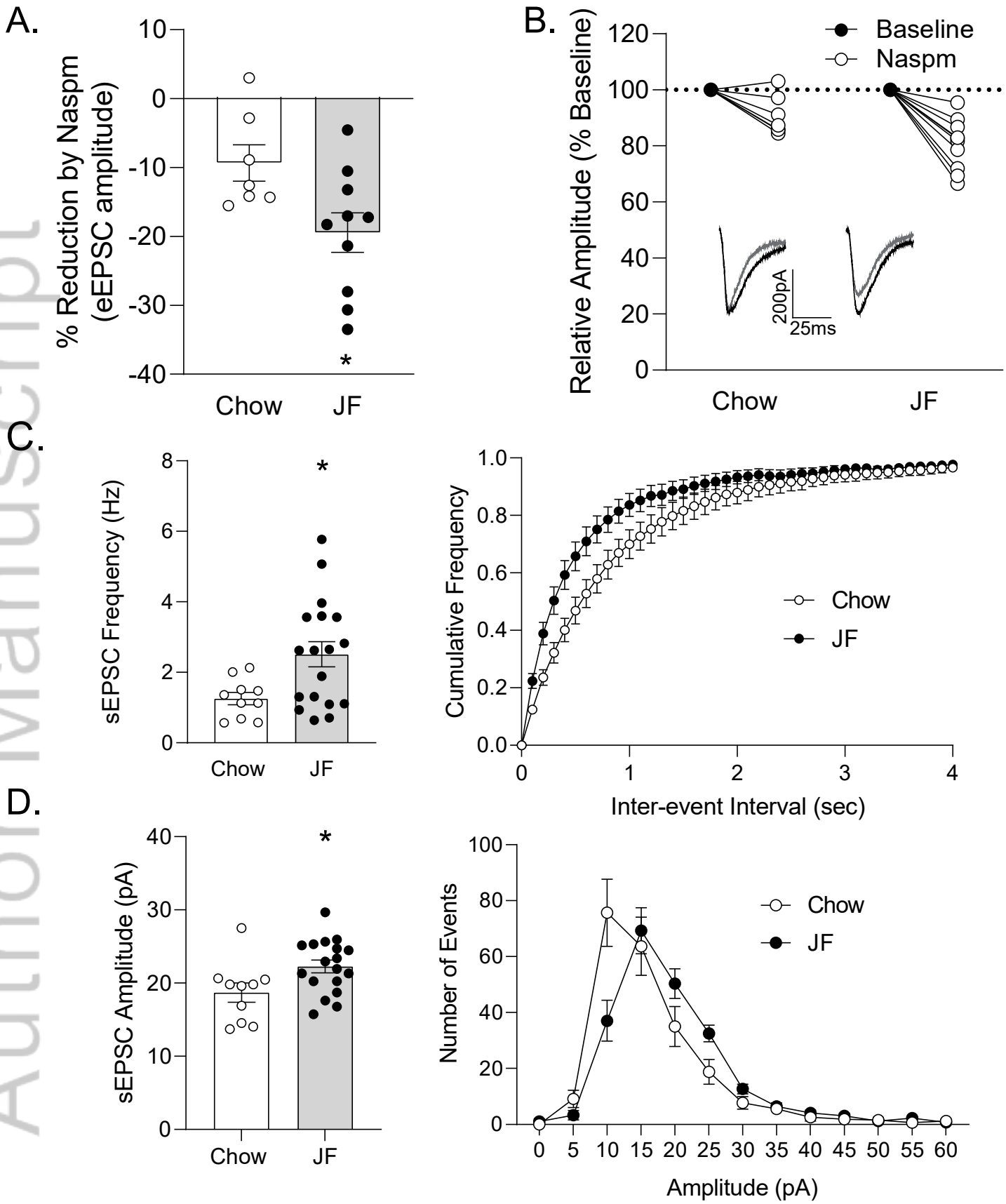

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Figure 5

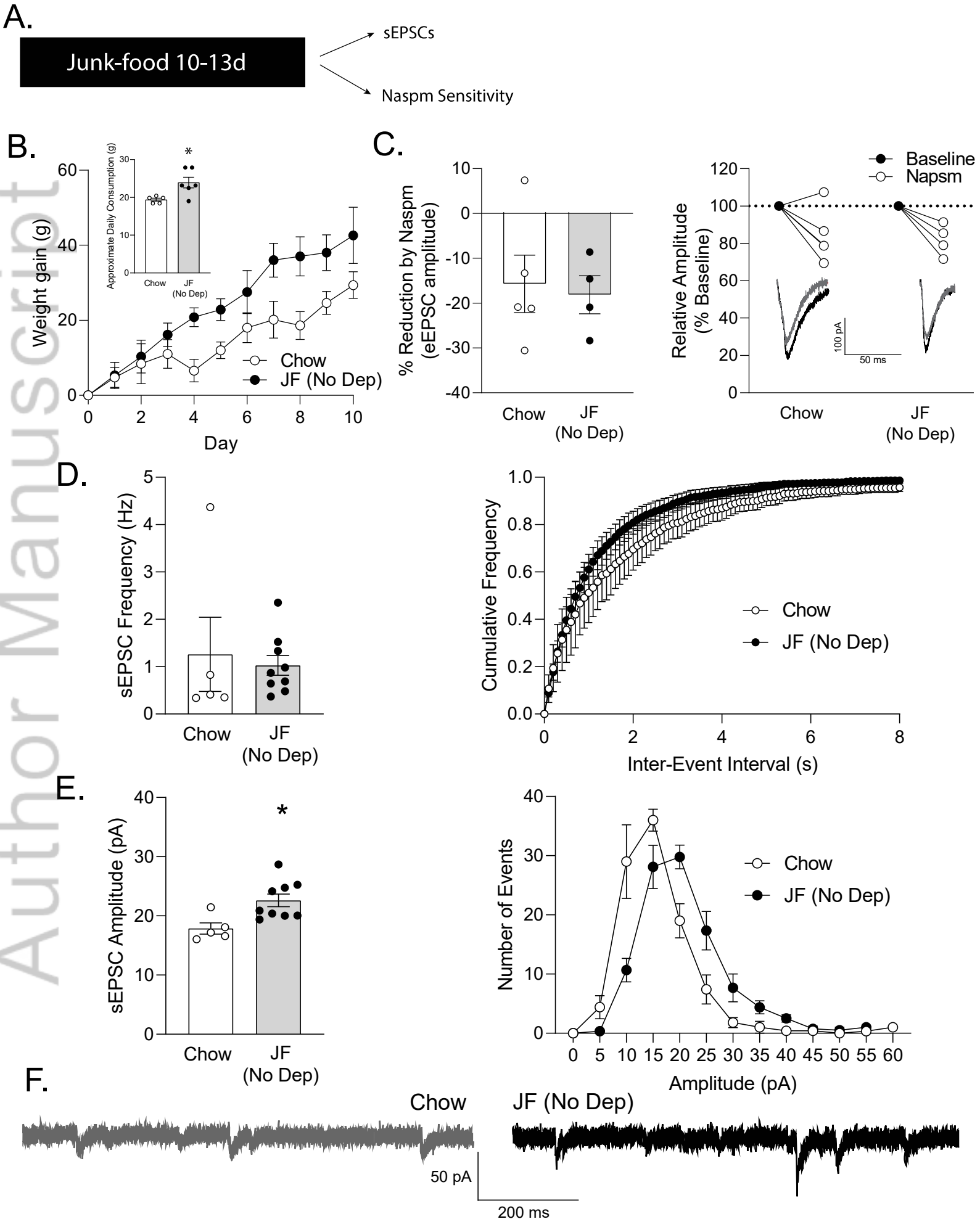

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Figure 1. Effect of junk-food followed by long deprivation on MSN intrinsic excitability. A) Experimental timeline. B) Weight gain across time. Rats in the junk-food group (JF) gained more weight than chow controls. C, D) Average daily food intake. Compared to chow controls, obesity-prone female rats given junk-food ate more during the diet manipulation, but 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) Example recordings from chow (left) and junk-food (right) cells. All data shown as average ±SEM, *=p<0.05, **=p<0.01, ***=p<0.001.

Figure 2. Effect of junk-food followed by long deprivation on 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 junk-food (JF) groups.

Figure 3. Effect of junk-food followed by short deprivation on MSN intrinsic excitability. A) Experimental timeline. B) Weight gain across time. Obesity-prone female rats given junk-food (JF) gained more weight across the diet manipulation than chow controls. C) Average daily food consumption in junk-food and chow groups. Compared to chow controls, obesity-prone female rats on junk-food 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 obesity-prone females given junk-food followed by a brief deprivation compared to controls. E) Average input resistance. Input resistance is decreased following junk-food consumption and a brief deprivation. F) Average rheobase. Rheobase is increased following junk-food consumption and a brief deprivation. G) Number of action potentials elicited by each current injection. Junk-food consumption followed by brief deprivation reduced in the number of action potentials fired at intermediate current injections compared to chow controls. H) Example traces from chow (left) and junk-food (right) cells. *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

Figure 4. Effect of junk-food followed by short deprivation on glutamatergic transmission. A,B) Reduction in eEPSC amplitude following bath application of the CP-AMPAR antagonist Naspm. CP-AMPAR transmission was enhanced following junk-food consumption and a brief deprivation period compared to controls. Inset in B: black trace=before Naspm; gray trace=after Naspm. C) Average frequency (left) and cumulative frequency distribution (right) of sEPSCs. sEPSC frequency is enhanced following junk-food consumption and a brief deprivation period in obesity-prone female rats. D Average amplitude (left) and amplitude distribution (right) of sEPSCs. sEPSC amplitude is increased following junk-food consumption and a brief deprivation period. E) Representative traces of sEPSCs from both groups. *=p<0.05.

Figure 5. Effect of junk-food (no deprivation) on glutamatergic transmission. A) Experimental timeline. B) Weight gain across time. Obesity-prone female rats given junk-food (JF) gained more weight across time than chow controls. Inset shows average daily food

consumption in junk-food and chow groups. Rats in the junk-food group ate more than rats in the chow group. C) Reduction in eEPSC amplitude following bath application of the CP-AMPAR antagonist Naspm (left); percent change from baseline (right). Junk-food without deprivation (No Dep) did not alter CP-AMPAR transmission compared to chow controls; black trace=before Naspm; gray trace=after Naspm. D) Average frequency (left) and cumulative frequency distribution (right) of sEPSCs. No group differences were found. E) Average amplitude (left) and amplitude distribution (right) of sEPSCs. sEPSC amplitude is increased after junk-food consumption compared to chow controls. F) Representative traces of sEPSCs in both groups. All data shown as average ±SEM. *=$p<0.05$.

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