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1 **Title: Separate orexigenic hippocampal ensembles shape dietary choice by**
2 **enhancing contextual memory and motivation**

3 Mingxin Yang[1,2], Arashdeep Singh[1,2], Molly McDougle[1,2], Léa Décarie-Spain[3], Scott Kanoski[3],
4 and Guillaume de Lartigue[1,2]

5 1. Monell Chemical Senses Center, Philadelphia, Pennsylvania, United States
6 2. Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania,
7 Philadelphia, PA Pennsylvania, United States
8 3. Human & Evolutionary Biology Section, Department of Biological Sciences, University of
9 Southern California, Los Angeles, California, United States

10
11 ***Correspondence:*** Guillaume de Lartigue, Monell Chemical Senses Center, 3500 Market Street,
12 Philadelphia, PA 19104, USA.
13
14 Email: gdelartigue@monell.org
15

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16 ## Abstract

17 The hippocampus (HPC), traditionally known for its role in learning and memory, has emerged
18 as a controller of food intake. While prior studies primarily associated the HPC with food intake
19 inhibition, recent research suggests a critical role in appetitive processes. We hypothesized that
20 orexigenic HPC neurons differentially respond to fats and/or sugars, potent natural reinforcers
21 that contribute to obesity development. Results uncover previously-unrecognized, spatially-
22 distinct neuronal ensembles within the dorsal HPC (dHPC) that are responsive to separate
23 nutrient signals originating from the gut. Using activity-dependent genetic capture of nutrient-
24 responsive HPC neurons, we demonstrate a causal role of both populations in promoting
25 nutrient-specific preference through different mechanisms. Sugar-responsive neurons encode
26 an appetitive spatial memory engram for meal location, whereas fat-responsive neurons
27 selectively enhance the preference and motivation for fat intake. Collectively, these findings
28 uncover a neural basis for the exquisite specificity in processing macronutrient signals from a
29 meal that shape dietary choices.

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31 **Introduction**

32 Survival hinges upon the acquisition of sufficient food to meet metabolic demands.
33 Therefore, possessing the capacity to construct a cognitive map and navigate accurately to a
34 known food source within the environment confers a distinct competitive advantage. Animals
35 learn to utilize contextual cues linked to the nutritional value of the food,[1] and forming episodic
36 memories of the spatial location of the cues enables efficient return to previously encountered
37 food sources. Repeatedly associating discrete or contextual cues with food in a manner that
38 predicts food intake induces a motivational state that amplifies the desire to eat - a phenomenon
39 termed cue-potentiated eating.[2] This adaptive behavior becomes overwhelmed in our current
40 food environment characterized by an inundation of food-associated cues and readily-available
41 foods rich in fats and sugars. Associative learning mechanisms linking food cues with intake of
42 calorie-dense diets amplifies susceptibility to obesity development. Supporting this notion, brain
43 reactivity to food cues predicts current weight status[3], the inclination to gain weight in future[4,5],
44 and food choice[6,7] Hence, unraveling mechanisms governing memory formation regarding
45 contextual cues linked to fat and sugar intake holds potential for combating obesity.
46
47 The hippocampus (HPC) is a neural substrate critical for cognitive mapping[8] and the
48 formation of episodic memories related to autobiographical experiences and their contextual
49 details[9,10]. Given the pivotal role of navigational and contextual memory in acquiring food, it is
50 not surprising that recent evidence suggests the HPC also plays a role in the control of food
51 intake[11,12]. Specifically, the HPC becomes activated by post-ingestive signals following a mixed
52 meal,[13] hormones released from the gut in response to eating,[13] and sensory cues associated
53 with meals, including odors,[14,15] taste,[16,17] texture,[18] tones,[19] and visual cues.[20] HPC lesioning
54 studies in rats have demonstrated an increase in food intake[21] and body weight in both
55 females[22] and males.[11] Conversely, chemogenetic stimulation of glutamatergic HPC neurons
56 inhibits 24-hour food intake.[23] Patients with retrograde amnesia resulting from brain lesions that
57 encompass the HPC consume multiple successive meals,[24,25] which can be interpreted as
58 impaired memory or impaired sensing of internal metabolic needs, an outcome also observed in
59 rodents with HPC lesions.[26,27] Disruption of HPC function has also been associated with obesity.
60 In a human fMRI study, hippocampal blood flow was lower after a meal in individuals that were
61 obese compared to those of a healthy weight.[28] Feeding rats a high-fat high-sugar diet impairs
62 performance on hippocampal-dependent spatial learning and episodic memory tasks.[29] Taken
63 together, these data highlight the HPC as having an anorexigenic role in energy metabolism,
64 with mechanisms involving episodic memory,[30,31] spatial memory,[32] and appetitive reward.[26,33]

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65 The HPC has also been found to be activated in conditions associated with increased
66 food intake. Ghrelin, an orexigenic hormone released from the stomach under fasting
67 conditions,[34] increases food intake and motivation to work for sugar reward when administered
68 into the HPC of rats.[35] In human fMRI studies, HPC activity is enhanced in response to images
69 of food and tastants,[36,37] shown to promote arousal and motivation to eat,[38,39] and these effects
70 are strongest in individuals with obesity. Recent findings identified an HPC subregion in humans
71 as a key hub for encoding the appetitive value of sugar and fat, with a compromised HPC
72 appetitive subnetwork in individuals with obesity.[40] These data suggest a potential role for the
73 HPC in increasing food intake, although the existence of a specific orexigenic population of HPC
74 neurons remains unproven. This knowledge gap may partly stem from limitations in the
75 temporal and spatial resolution of previous studies employing lesions and pharmacological
76 approaches. Recent advances in transcriptomic analyses have unveiled extensive molecular
77 diversity in HPC neurons[41-44] and efforts continue to functionally characterize subpopulations of
78 HPC neurons based on their projection patterns and/or genetic markers.[45] Notably, screening of
79 meal-responsive neurons revealed that a substantial number of neurons in the dHPC are
80 activated by eating.[23] Among these, a subset of neurons in the hilar region of the dHPC has
81 been identified as expressing DRD2, and using molecular and genetic tools were demonstrated
82 to inhibit food intake.[23] These types of approaches provide an opportunity to identify orexigenic
83 populations within the HPC. We hypothesized that fat and sugar may activate a subset of HPC
84 neurons with orexigenic function. Our results find subsets of HPC neurons that are recruited in
85 response to fats or sugars, and leverage Fos^{TRAP} mice as an unbiased approach to manipulate
86 the activity of these HPC neurons to test their role in appetitive behavior.

87

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88 **Results**

89 **Dorsal hippocampal neurons are responsive to different post-ingestive nutrients**

90 Previous studies demonstrate that the HPC is activated in response to mixed nutrient
91 chow[23] and following intragastric infusion of a mixed meal[13]. To test if the HPC is activated in
92 response to individual reinforcing nutrients, we measured Fos immunofluorescence, a marker of
93 neuronal activity, in mildly-fasted wildtype mice in response to intragastric infusions (500 µl, 100
94 µl/min) of sugar (sucrose, 15% w/v), equicaloric fat (microlipid, 6.8% v/v) or isosmotic saline
95 (0.9% w/v) (Fig 1A). Fos was increased in discrete neuronal populations within the dorsal
96 hippocampus (dHPC) in mice receiving infusions of sucrose or fat compared to saline (Fig 1B).
97 Similar Fos density was found in response to both nutrients (Fig 1C), and the highest density of
98 neurons were proportionally enriched in the dentate gyrus (DG) in response to both sucrose (Fig
99 1D) or fat (Fig 1E). Notably, intragastric infusions of sucrose and fat also resulted in similar
100 density of Fos labeling in the ventral HPC (vHPC) (S1A-E), but in this region responsive
101 neurons were particularly enriched in the CA1 (S1F-G). Together these data highlight that
102 separate dHPC neurons are responsive to different post-ingestive nutrient signals from the gut.

103 The vagus nerve is a key neural pathway that connects the gut and the brain. Subsets of
104 vagal sensory neurons in the nodose ganglia (NG) are activated in response to intestinal
105 nutrients, and these NG neurons are necessary to mediate the reinforcing value of fat and
106 sugar.[46] Although vagal sensory fibers terminate in the nucleus tractus solitarius (NTS) of the
107 hindbrain, there is evidence of a polysynaptic circuit connecting the gut via the NTS to the
108 HPC.[47] Furthermore, vagal stimulation increases HPC activity in mice[48] and in humans[49] while
109 deletion of gut-innervating vagal sensory neurons impairs HPC-dependent contextual episodic
110 memory.[47 50] To test whether post-ingestive fats and/or sugars require the vagus nerve to recruit
111 dHPC neurons, we quantified Fos expression in the dHPC in response to intragastric nutrient
112 infusions in mice following subdiaphragmatic vagotomy (SDV) or sham surgery (Fig 1F).
113 Nutrient-induced dHPC Fos expression was impaired in SDV animals, significantly reducing
114 response to intragastric sucrose (Fig 1G-H) and fat (Fig 1I-J). These data demonstrate the
115 vagus nerve acts as an important neural relay connecting nutrient signals in the gut to the
116 dHPC.

117 We recently reported that separate NG populations sense fat or sugar and that these
118 nutrients activate separable downstream central circuits.[46] Thus, we inquired whether separate
119 populations of dHPC neurons are recruited in response to fat and sugar. We used a Fos^{TRAP}
120 mouse[51] with a previously validated approach[46] to compare neuronal activity in response to two

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121 separate nutrient infusions in the same mouse (Fig 1K). These mice express an inducible Cre
122 recombinase, iCreER^{T2}, under the control of an activity-dependent Fos promoter (*Fos*^{TRAP} mice),
123 enabling permanent genetic access to neuronal populations based on their activation to a
124 specific, time-restricted stimulus.[52-54] To validate the approach in the HPC, we compared the
125 number of Fos^{TRAP} positive neurons and Fos immunofluorescence and found that the density of
126 responsive neurons within the dHPC was similar (S1I-J). When analyzing the overlap between
127 the repeated infusion of the same stimulus within the same animal, we found a high level of
128 overlap in the dHPC between Fat^{TRAP} tdTomato labeling and Fat Fos immunofluorescence (Fig
129 1L-M). However, when comparing the response to different stimuli, there was low overlap in the
130 dHPC neurons between tdTomato labeling of the Sucrose^{TRAP} neurons and Fos labeling of fat
131 responsive neurons (Fig 1L, N). The difference between overlap in Fat^{TRAP}/Fat^{cFos} mice and
132 Sugar^{TRAP}/Fat^{cFos} was particularly large in the dDG (S1H), consistent with the role of the DG as a
133 pattern segregator[50-53] and suggesting that dDG neurons may play a role in differentiating
134 responses to different types of interoceptive information from the gut. Interestingly, we also find
135 that IG infusion of starch (cornstarch, 15% w/v) colocalizes with dHPC neurons that are trapped
136 with equicaloric solution of sucrose (15% w/v, S1K-L), suggesting that these neurons are
137 broadly tuned to carbohydrates. Importantly, neither fat nor sucrose infusion resulted in
138 activation of neurons of the hilar region of the dHPC (Fig 1B), suggesting that these populations
139 are distinct from the previously described DRD2-expressing hilar neurons of the dHPC known to
140 inhibit food intake.[23] Thus, we identify two new populations of dHPC neurons that are
141 responsive to separate post-ingestive nutrients that both involve vagally-dependent signaling
142 mechanisms.

143

144 **Fat- and sugar-responsive dHPC neurons control nutrient-specific preference and intake**

145 Next, we wanted to determine the role of nutrient-responsive dHPC populations in the
146 control of food intake, and reasoned that these spatially segregated populations recruited by
147 separate post-ingestive nutrients may differentially resolve food intake at the macronutrient
148 level.[55,56] To genetically access dHPC neurons that were active in response to intragastric
149 infusion of fat or sugar, we used TRAP2 mice (Fig 2A).[57,58] To assess necessity of these
150 neurons in the control of feeding behavior, we injected a cre-dependent virus expressing
151 caspase in the construct AAV-flex-taCasp3-TEVp.[59] This approach allowed selective lesioning of
152 dHPC neurons that respond to either fat (6.8%) or sugar (15%) compared to a control mouse
153 that received dHPC viral injection that did not cause lesioning. Caspase treatment ablated

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154 sugar-responsive and fat-responsive dHPC neurons, as demonstrated by the greater than 50%
155 loss in tdTomato-positive neurons in the caspase-treated mice compared to controls (Fig2B-D).
156 To behaviorally assess the role of dHPC neurons in nutrient preference, we presented the mice
157 with a choice between two bottles containing equicaloric solutions of either fat (6.8%) or sugar
158 (15%) and quantified intake using lickometers (Fig 2A). Over three test days the control mice
159 exhibited a preference for the fat solution over the sucrose solution (Fig 2E-H). The mice with
160 ablated sugar-responsive dHPC neurons significantly decreased sucrose consumption
161 compared to controls (Fig 2E), with no effect on fat intake (Fig 2F). Deletion of fat-responsive
162 dHPC neurons resulted in no change in sucrose intake (Fig 2G), but reduced fat consumption
163 by 40% compared to control mice (Fig 2H). The reduction in nutrient intake does not appear to
164 be in response to reduced taste[60] since there was no group differences in lick numbers over the
165 first 10 seconds for either fat or sucrose between groups (S2A-B). Furthermore, deletion of
166 sucrose-responsive dHPC neurons had no effect on sucrose or fat intake in a one bottle task,
167 suggesting a primary role of $dHPC^{Sugar}$ neurons in sucrose preference (S2C-D). Ablation of
168 $dHPC^{Fat}$ neurons had no effect on sucrose intake in a one bottle task (S2E), but reduced the
169 number of licks for fat (S2F). In summary, these data demonstrate that separate populations of
170 dHPC neurons are necessary for nutrient-specific preference.

171 To assess the sufficiency of dHPC neurons in macronutrient preference, we performed
172 chemogenetic stimulation of fat- or sugar-responsive dHPC neurons. A Cre-inducible viral Gq-
173 coupled designer receptor encoded in the construct AAV-EF1a-DIO-hM3Dq-mCherry[61], were
174 bilaterally injected into the dHPC of Fos^{TRAP} mice (Fig 2I). CNO injection increased Fos
175 expression in dHPC neurons in both $Sugar^{TRAP}$ and Fat^{TRAP} mice expressing hM3Dq (Fig 2J-K),
176 confirming our ability to chemogenetically activate Fos^{TRAP} HPC neurons in a nutrient-specific
177 manner. In the one bottle task described above, chemogenetic activation of $dHPC^{Sugar}$ neurons
178 increased sucrose intake compared to vehicle treatment (Fig 2L), but had no effect on fat intake
179 (Fig 2M). Stimulation of $dHPC^{Fat}$ neurons exclusively increased fat consumption (Fig 2N-O).
180 Importantly, none of these effects were observed when CNO was injected in mice not carrying
181 the chemogenetic construct (S2G-H). These data suggest that the dHPC is attuned to specific
182 macronutrients allowing for highly-refined feeding decisions.

183 **Fat- and sugar- responsive dHPC neurons control nutrient-specific episodic spatial**
184 **memory**

185 Next, we wanted to address the mechanisms by which dHPC neurons control nutrient-
186 specific intake. The HPC forms context-specific neural representations that provide a

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187 physiological substrate of spatial memory[62], and HPC activity is altered by contextual features of
188 rewarding stimuli.[63-65] To address whether dHPC^{Sugar} and dHPC^{Fat} neurons retain contextual
189 information about the location of natural reinforcers, such as post-ingestive fats and sugars, we
190 adapted a previously described food cup task.[23] Mice were habituated to a novel context with
191 two empty petri dishes, and during the training phase, one petri dish contained droplets of water
192 while the other contained droplets of fat (6.8% v/v) or sucrose (15% w/v) solutions (Fig 3A).
193 After training to learn the location of a nutrient-containing dish, we tested the mice with empty
194 petri dishes in the same context to determine if they could remember the location of the nutrient-
195 paired quadrant (Fig 3A). Control mice discriminated the sugar-paired quadrant above chance in
196 tests 1h and 24h after the final training session (Fig 3B), suggesting that they were able to learn
197 and remember the location of sucrose. Mice with ablated dHPC^{Sugar} neurons failed to
198 discriminate the location of the sugar dish in the 1h and 24h tests (Fig 2C). However, when
199 these mice repeated the task with fat solution using different contextual cues, both the control
200 and sugar-ablated mice spent more time exploring the fat location at both timepoints (Fig 3D-E).
201 In a separate group of mice trapped with fat, we found that controls and dHPC^{Fat} ablated mice
202 were able to discriminate the sugar location in both 1 and 24h tests compared to the pretest (Fig
203 3F-G). Although the control mice exhibited fat location memory (Fig 3H), the ablation of fat-
204 responsive dHPC neurons abolished the ability to discriminate the fat-paired location in both 1h
205 and 24h tests (Fig 3I). Importantly, the order in which the nutrients were presented was
206 counterbalanced, and there was no residual preference for the previous nutrient location
207 following a 7-day washout period (S3A), favoring exploration of the new petri dish locations.

208 To confirm that generalized spatial memory is not impaired, we performed a
209 hippocampal-dependent[66] novel object in context (NOIC) task (S3B). As expected, control mice
210 spent more time exploring the object that is novel to the context, and similarly the ablation of
211 either fat- or sugar-responsive dHPC neurons had no effect on the time spent exploring the
212 novel object (S3C-F). These data indicate that the loss of nutrient-responsive dHPC neurons
213 influences contextual memory of nutrient location, but that these neurons are specific to food
214 and do not impair contextual memory for non-food related objects.

215 Increasing evidence suggests that the HPC is involved in working memory,[67,68] that
216 allows retention of a small amount of information for a short period of time. To assess whether
217 the nutrient-responsive dHPC neurons influence working memory related to food location, we
218 performed a modified Barnes maze task.[47] Mice were positioned in the center of a circular table
219 and 8 petri dishes containing water solution and one containing either sucrose (15% w/v) or

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220 equicaloric fat (6.8% v/v) were evenly distributed around the edge (S3G). The location of the
221 nutrient-containing dish remained the same across two consecutive trials per day, but changed
222 each subsequent day. The index of working memory on this task is the difference in the number
223 of errors (exploration of water dishes) between trials on 3 individual experimental days. We
224 observed no difference in the number of errors between any of the groups in response to sugar
225 (S3H-K) or fat (S3L-O), suggesting that ablation of nutrient-responsive dHPC neurons does not
226 play a role in working memory. Altogether, these data suggest that both fat- and sugar-
227 responsive dHPC neurons are necessary for episodic spatial memory for the location of
228 individual nutrients.

229 Next, we assessed whether activation of nutrient-responsive dHPC neurons can improve
230 context-dependent spatial memory for individual nutrients. Mice expressing hM3Dq or control
231 virus in dHPC neurons trapped with intragastric infusion of either sucrose (15%) or fat (6.8%)
232 were habituated to two novel contexts. During a 3-day training phase the mice receive saline
233 injections 20 minutes prior to being placed in context A in the morning to learn to associate the
234 location of a nutrient-containing dish for 10 min, and then received another saline injection
235 before being placed in context B in the afternoon to learn a different location for the second
236 nutrient-containing dish. Twenty-four hours later the mice were tested to determine if they could
237 discriminate the correct context-specific nutrient-paired quadrant (Fig 3J). The mice failed to
238 discriminate context-specific locations of sucrose or fat when treated with saline (Fig 3K-L). After
239 7 days the same test was repeated but the mice received CNO (3 mg/kg, IP) during training and
240 test days before they were reintroduced into the context that had been previously paired with
241 the specific nutrient with which they were initially trapped. To avoid desensitization, CNO was
242 not injected on the third training day. We found that CNO had no impact on the performance of
243 control mice (Fig 3K-L); however, chemogenetic stimulation improved the discrimination of
244 hM3Dq-expressing $dHPC^{Sugar}$ mice in response to CNO compared to vehicle treatment (Fig 3K).
245 No improvement was observed in response to chemogenetic stimulation of $dHPC^{Fat}$ neurons
246 (Fig 3L). These data indicate that sugar-responsive dHPC neurons encode an engram of spatial
247 and context-dependent memory for sugar.

248 Fat-responsive HPC neurons encode motivation for fat

249 Dietary preferences are largely learned[69] and this process involves reward-based
250 associations.[46,52,69,70] In light of the increased preference for nutrients caused by dHPC neurons
251 (Fig 2), we hypothesize that these neurons are involved in reinforcement learning. To address
252 this, we assessed the role of nutrient-responsive dHPC neurons in a flavor-nutrient conditioning

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253 task, in which animals are trained to prefer a novel non-nutritive flavor that has been
254 experimentally paired to an intragastric infusion of nutrient (Fig 4A).[71] During conditioning, both
255 control and caspase-treated mice with ablated dHPC^{Sugar} neurons received the same number of
256 sucrose (15%) infusions (S4A), suggesting that sucrose resulted in similar levels of appetition in
257 both groups.[72] After conditioning, both control mice and caspase-treated mice formed increased
258 preferences for the flavor paired with intragastric infusion of sucrose compared to their initial
259 flavor preference (Fig 4B). Notably, control mice retain the flavor preference (Fig 4C), while the
260 caspase-treated mice forget the conditioned preference by day 3 (Fig 4D). In mice lacking
261 dHPC^{Fat} neurons the number of conditioning infusions of intragastric fat (6.8%) were severely
262 reduced compared to control mice (S4B). After conditioning, control mice increased preference
263 for the flavor paired with fat, while loss of dHPC^{Fat} neurons prevented fat reinforcement learning
264 (Fig 4E). These data highlight different functions of the separate populations of nutrient-
265 responsive dHPC neurons between formation and retention of conditioned preferences.

266 There is evidence that the dHPC is involved in motivation,[73] thus we next assessed if
267 nutrient-responsive dHPC neurons will increase the motivation for food. Effort-related motivation
268 can be assessed by testing behavior using progressive ratio (PR) schedule reinforcement.[74] We
269 used an exponential PR task to probe the willingness of mice to lick for a dry sipper that
270 requires an increasing number of licks for a small nutrient reward (Fig 4F). We quantified the
271 number of licks required before an animal ceases to be willing to expend effort for a single
272 reward, known as the breakpoint.[75] Deletion of dHPC^{Sugar} neurons had no impact on the
273 willingness to work for sucrose compared to control mice (Fig 4G). However, deletion of dHPC^{Fat}
274 neurons reduced the breakpoint for fat compared to control mice, suggesting an important role
275 of dHPC^{Fat} neurons in motivation (Fig 4H). During the training phase, we observed no group
276 differences in the discrimination for the active nose hole or the number of licks the animals
277 performed under FR1 or FR5 ratio (S4C-D). Next, we tested whether stimulation of dHPC
278 neurons could increase the motivation to work for nutrients using a similar PR task as above
279 (Fig 4I). All mice rapidly learned to discriminate the active nose hole to receive a small nutrient
280 droplet triggered by a pump under FR1 schedule and FR5 schedule (S4E-F). After training, the
281 mice were tested on an exponential PR schedule in response to saline or CNO on subsequent
282 days. CNO (3 mg/kg, IP) had no effect compared to saline on sucrose breakpoint in dHPC^{Sugar}
283 control mice or hM3Dq mice (Fig 4J). CNO significantly increased the willingness to nose poke
284 for a small fat reward in the mice expressing hM3Dq, but had no effect in mice that did not
285 express the chemogenetic construct (Fig 4K). Together these data identify a novel population of
286 neurons in the dHPC that are necessary and sufficient for the motivation to consume fat.

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287 **Nutrient-sensing dHPC neurons guide food intake based on diet composition**

288 Having demonstrated the importance of dHPC neurons in memory, preference and motivation
289 for individual macronutrients, we next wanted to assess the necessity of these neurons in the
290 control of consumption of complex diets with mixed nutrient composition. We monitored
291 continuous and uninterrupted ad libitum chow intake. Caspase-treated mice with ablated
292 $dHPC^{Sugar}$ neurons ate significantly less over 24 hours compared to control mice (Fig 5A). The
293 hypophagia in the caspase-treated mice was caused by smaller meal size compared to controls,
294 with no effect on meal duration or frequency (Fig 5B). We also compared food intake within
295 animals before and after caspase-mediated neuron ablation, and found that the caspase-treated
296 mice ate significantly less post- compared to pre- ablation (S5A), but no difference in food intake
297 was observed in the control mice before and after TRAP (S5B). There were also no group
298 differences in food intake pre-ablation (S5C). Deletion of $dHPC^{Fat}$ neurons had no effect on
299 chow intake (Fig 5C) or meal patterning (Fig 5D) compared to controls. There were no within
300 animal differences before or after undergoing the fat TRAP protocol (S5D-F). These data
301 indicate subpopulations of dHPC neurons increase daily cumulative food intake. The fact that
302 only $dHPC^{Sugar}$ neurons increased intake of chow, a carbohydrate-rich diet, suggests that dHPC
303 neurons are attuned to select nutrients in a mixed meal and selectively increase food intake
304 according to the composition of the diet.

305

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306 **Discussion**

307 In the present study we identify novel populations of neurons in the HPC that influence
308 decisions about where to locate food, what to eat, and how much to consume. We present
309 multiple lines of evidence revealing distinct neural populations within the dHPC that respond to
310 fat and sugar stimuli. Notably, these neurons respond to either fat or sugar infused into the gut,
311 which allowed precise control over the volume and caloric content that each animal received,
312 and isolated the post-ingestive effects from sensory cues like sight, smell and taste. These
313 neuronal subsets not only exhibit spatial segregation in dHPC, but also exert distinct control of
314 nutrient-specific preference and intake. Furthermore, they play a pivotal role in shaping feeding
315 behavior through separate mechanisms involving memory, motivation, and preference.

316 **Identification of orexigenic neurons in the HPC**

317 Prior studies have firmly established the crucial role of an intact HPC in the control of
318 normal eating behavior. Notably, individuals with lesions that encompass the HPC diagnosed
319 with anterograde amnesia have shown deficits in the regulation of satiety.[25,76,77] In rodent
320 models, pharmacological lesioning studies that remove the entire HPC have demonstrated an
321 increase in food approach behavior,[26] meal size[78] and in some cases increased daily food intake
322 and body weight.[11,21,22] Furthermore, transient inhibition of neurons in either the ventral or dorsal
323 HPC has been found to augment food intake,[23,30,31,79,80] while stimulation of glutamatergic
324 neurons decreases food intake.[23,80] Additionally, the administration of satiety hormones directly
325 into the vHPC has been shown to decrease food intake, whereas the deletion of the receptors
326 for these hormones increases food intake.[81,82] Together these data support the idea that the
327 HPC plays a causal role in the inhibition of food intake. Yet the HPC is activated by ghrelin[34] and
328 food cues that both promote food intake,[36,37] and an orexigenic hippocampal circuit was recently
329 discovered in humans and is strengthened in obesity.[40] Circuits have been identified to connect
330 HPC neurons with brain regions associated with motivated behavior,[23,45,83-89] but these have for
331 the most part not been linked with an increase in food intake. An exception is a vHPC to lateral
332 hypothalamus circuit necessary to mediate endogenous ghrelin's orexigenic effect in meal
333 entrainment.[81] Whether ghrelin receptor expressing vHPC neurons are an orexigenic population
334 or if ghrelin inhibits an anorexigenic population remains to be determined. This study identifies
335 two novel orexigenic populations in the dHPC.

336 We demonstrate a pivotal role of both dHPC neuronal populations in regulating the
337 preference and intake of orally consumed solutions of isolated nutrients. Notably, deletion of
338 nutrient-responsive dHPC neurons decreases intake and stimulation increases intake in a

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339 nutrient-specific manner. Specifically, dHPC^{Fat} neurons influence the quantity of fat solutions that
340 animals consume, with no discernable impact on sugar intake. Conversely, dHPC^{Sugar} neurons
341 exclusively govern sugar preference. Remarkably, our findings extend to complex diets. When
342 exposed to chow, deletion of dHPC^{Sugar} neurons resulted in reduced food intake, whereas
343 deletion of dHPC^{Fat} neurons had no effect. We found that dHPC^{Sugar} neurons are activated in
344 response to both sucrose and starch, which suggests broad sensitivity to various carbohydrates.
345 This could explain why chow - a grain-based diet composed of high levels of complex
346 carbohydrate levels (63%, w/w), and low-fat content (<7%, w/w) – is impacted by dHPC^{Sugar}, but
347 not dHPC^{Fat} neurons. Collectively, our data suggest that the dHPC is highly attuned to the
348 composition of the meal and separate recruitment of fat- or sugar-responsive dHPC neurons
349 based on the nutrient composition can differentially influence food choice.

350 While previous studies have recognized a role for the HPC in responding to internal
351 states of hunger and satiety,[12,24-26] the interoceptive HPC neurons responsible for this process
352 had not previously been identified. We demonstrate that the vagus nerve is necessary for
353 internal nutrient sensing in the gut to activate nutrient-responsive dHPC neurons that control
354 food intake. Our findings build upon established evidence that connects the vagus nerve and
355 the dHPC, including vagal-mediated HPC neurogenesis,[90-92] synaptic function,[93,94] and the
356 requirement of vagal sensory neurons that innervate the gut for optimal performance in
357 hippocampal-dependent behavioral tasks.[47,50] Therefore, although the existence of a functional
358 circuit was previously known, our data fills a gap in knowledge by pinpointing fats and sugars as
359 physiological stimuli that activate this gut-hippocampal circuit to shape food preference.

360 **Explaining separate fat and sugar signaling mechanisms**

361 One intriguing question that emerges from these findings is why distinct HPC neurons
362 respond separately when activated by different post-ingestive stimuli? In the natural world,
363 foods are rarely composed of a combination of both fat and sugar, potentially exerting selective
364 evolutionary pressures that favored the development of separate biological systems for
365 encoding distinct memories for either fat-rich or sugar-rich foods. Several studies have
366 demonstrated that individuals exhibit more accurate spatial memory for the locations of high-
367 calorie foods,[95-97] hinting at the presence of memory systems finely tuned for efficiently locating
368 and recalling nutritionally valuable food sources. These separate memory systems likely
369 necessitate the ability to remember specific contextual cues associated with these different food
370 types. We suggest that the formation of separate HPC neurons dedicated to either fat and sugar

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371 preference and appetitive memory may occur through one of two non-mutually exclusive
372 mechanisms.

373 Firstly, ingested fats and sugars may activate separate parallel gut-brain circuits. Prior
374 work from our lab provides evidence that fats and sugars are sensed by two separate
375 populations of vagal sensory neurons.[46] Notably, the deletion of these separate vagal
376 populations was shown to impair learned preferences in a nutrient-specific manner.[46]
377 Furthermore, segregated cellular responses to fats or sugars in central reward circuits
378 downstream of vagal sensory neurons were observed,[46] suggesting the existence of separate
379 hardwired signaling mechanisms for different nutrient reward. In support of this possible
380 mechanism, we find that an intact vagus nerve is necessary for the response of dHPC neurons
381 to either nutrient.

382 A second mechanism that would enable fat and sugar to activate separate dHPC
383 populations is pattern separation. The DG in the HPC plays a pivotal role in the process of
384 pattern separation,[98-101] a fundamental computation that allows neural circuits to distinguish
385 between similar input activity patterns and transform them into distinct output patterns.[102,103] This
386 mechanism is crucial for avoiding the confusion of memories associated with similar
387 experiences. Pattern separation has been well-established in rodent studies[104-109] and is
388 supported by human studies,[110,111] where the DG's large number of neurons and sparse coding
389 contribute to the decorrelation of input signals before reaching CA3.[103,112] Lesions to the DG
390 result in novelty detection impairments following exposure to new spatial environments,[113]
391 highlighting its importance in reducing interference from previous experiences. We observe
392 enrichment of DG activity in the dHPC in response to post-ingestive fat or sugar, which aligns
393 with the possible role of the DG for discriminating contexts associated with appetitive compared
394 to non-food related stimuli, but also encoding post-ingestive fat and sugar as dissimilar, non-
395 overlapping memory representations.

396 **Identification of an appetitive engram for sugar**

397 Neurons in the HPC play a pivotal role in transforming novel experiences into lasting
398 memories that shape future behaviors. Immediate early genes (IEG), like Fos, are transiently
399 expressed in specific HPC neuron populations following learned experiences.[114-116] Reactivation
400 of neurons based on IEG activity is essential for memory retrieval[117] while inhibition of these
401 ensembles impairs memory recall,[118] underscoring the critical role of IEG in consolidating and
402 recalling specific memories. The use of activity-dependent expression of reporters, therefore

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403 provides a framework for exploring engram ensemble. We utilize the Fos^{TRAP} mouse model to
404 permanently tag activated neurons expressing Fos to target ensembles of appetitive stimuli. We
405 find a network of Fos-expressing neurons in the dHPC responsive to the natural reinforcers, fat
406 and sugar, that encode appetitive memory.

407 The term "engram" was originally introduced by Richard Semon to describe a memory
408 representation.[119] Since then, there have been ongoing efforts to locate the physical memory
409 trace within the brain based on the ability to observe, erase and artificially express it as defining
410 criteria.[120] During the learning process, specific neuronal populations that constitute engram
411 ensembles become activated and undergo cellular changes.[121,122] Inhibiting these changes
412 impairs memory,[118] while reactivation of these ensembles enable memory retrieval.[117] Thus,
413 significant progress has been made in understanding memory and engrams, particularly in the
414 context of aversive and social interactions,[117,123,124] but an engram associated with appetitive
415 memory has not been defined, despite evidence that memory for food is highly conserved
416 across species from insects to humans.[125-129]

417 We observe activation of a sparse population of neurons in response to post-ingestive
418 nutrients, the first criteria of a memory trace. Selectively deleting sugar-responsive or fat-
419 responsive neurons in the dHPC reduced nutrient-specific memory expression, satisfying the
420 second criteria. Deleting the neurons tagged in response to sugar impaired the contextual
421 memory for sugar, but had no effect on the subsequent expression of the contextual fat memory,
422 and vice versa for fat responsive neurons. These findings support the idea that inhibiting
423 components of one hippocampal engram does not affect expression of another separate
424 engram[130]. Therefore, the sugar engram does not broadly disrupt memory retrieval, even of
425 other appetitive memories.

426 Crucially, when we chemogenetically stimulated this specific ensemble of sugar-
427 responsive dHPC neurons during training or testing, the mice exhibited improved performance
428 in a highly-complex, contextually-dependent spatial memory task related to sugar. These
429 findings strongly suggest that the dHPC^{Sugar} neurons contribute to the formation of a memory
430 engram for sugar location and are sufficient for memory recall. Interestingly, activation of fat-
431 responsive neurons did not improve performance in locating fat. Furthermore, ablation of the
432 sugar-responsive neurons did not affect the learning a preference for a flavor associated with
433 post-ingestive sugar, but led to rapid decline in the memory for the conditioned preference,
434 highlighting the crucial role of these neurons in memory expression during the days following

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435 appetitive conditioning. Taken together, these data provide evidence of a sugar engram, and
436 demonstrate that dHPC populations for fat and sugar are distinct.

437 In a separate set of experiments, we attempt to address the nature of the
438 interrelationship between short-term memory (STM) and long-term memory (LTM). The debate
439 revolves around whether these processes are distinct or part of a single memory system. Some
440 argue that the STM system must be able to store complex representational structures that have
441 never been encountered before,[131,132] while others propose a unified memory system[133-136] or
442 suggest that STM is an active component of LTM.[137-141] Recent neuroimaging research has
443 leaned towards the idea of a unified memory system,[134,142-148] although it may be difficult to
444 parse out the overlapping features of STM and LTM that include encoding, retention, and recall.
445 Our findings suggest that separate neural populations are involved in short-term working
446 memory and long-term episodic memory. Specifically, deletion of sugar-responsive neurons in
447 the dHPC impaired episodic memory, without impacting performance in a task of working
448 memory.

449 **The hedonic hippocampus**

450 The role of the hippocampus in motivated behavior remains unclear with mixed results
451 from loss of function studies [33,149-151],[152]. In our study, we reveal that fat-responsive dHPC
452 neurons are involved in both motivation and Pavlovian conditioning. When we deleted these
453 neurons, mice displayed reduced effort to obtain fat reward, while stimulation increased their
454 motivation to work for fat. These mice exhibited normal response during fixed ratio training,
455 suggesting no impairment in learning. Furthermore, fat-responsive dHPC neurons are
456 necessary and sufficient in flavor nutrient conditioning, providing clear evidence for a causal role
457 for this small population of dHPC neurons in classical Pavlovian conditioning. Intriguingly, sugar-
458 responsive neurons had no effect on motivation to work for sugar reward or formation of
459 conditioned preference associated with sugar, aligning with previous studies indicating
460 hippocampal lesions do not impact sugar conditioning.[73] These results further underscore the
461 different functions of fat and sugar dHPC neurons.

462 Gauthier and Tank (2018) identified a small but reliable population of hippocampal
463 neurons that code for reward location across contingencies and environments.[64] These neurons
464 are thought to be involved in the process of encoding and retrieving memories related to
465 rewards, regardless of the context in which the reward was experienced. The exact function of
466 these reward anchored neurons has not yet been determined. Our data suggest a possible role

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467 for the reward location neurons for mapping the site of a reward and/or increasing the
468 motivation to access the reward.

469 **Conclusion**

470 The HPC is a brain region that is well known for its role in learning and memory, making
471 it a candidate for supporting higher order decisions that underpin motivated behaviors, including
472 feeding. Here we identify two novel populations of interoceptive dHPC neurons that are attuned
473 to specific nutrients and allow highly-refined control over feeding behavior. We demonstrate that
474 sugar-responsive dHPC neurons are part of an appetitive engram that encodes sugar location
475 memory that can be erased or artificially activated. Conversely, fat-responsive dHPC neurons
476 promote motivation and strengthen cue associations for post-ingestive fat. These neurons
477 therefore have separate functions in creating an internal model that maps the environmental
478 availability and locations of high-calorie foods (Sugar neurons) and modulates the internal drive
479 to obtain them (Fat neurons). The role of these neurons in the pathophysiology of binge eating
480 or obesity is yet to be explored. However, in our current food environment, there is the potential
481 for devastating impact of these orexigenic neurons to exacerbate cue-induced consumption of
482 obesogenic foods rich in fat and sugar.

483

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492 **Methods**

493 **Animals and Housing**

494 All animal procedures followed the ethical guidelines, and all protocols were approved by
495 the Institutional Animal Care and Use Committee (IACUC) at the University of Florida (Protocol
496 # 202110305) and Monell Chemical Senses Center (Protocol # 1187 and 1190). Adult mice (6-
497 20 weeks of age of both males and females on a C57BL/6J background) were used and
498 maintained on a reverse 12-h light/dark circle. Strain details and number of animals in each
499 group are as follows: C57BL/6J wild type: n=48: 24 male, 24 female; bred in house by UF
500 breeding core, Fos Cre Tomato: n=68: 34 male, 34 female; bred in-house from Jackson
501 Laboratory B6.129(Cg)- Fos$^{tm1.1(cre/ERT2)Luo}$/J (JAX stock no.021882) and Ai14 (B6.Cg-
502 Gt(ROSA)26Sor$^{tm14(CAG-tdTomato)Hze}$/J, JAX stock no.007914). Animals were single housed at 22°C
503 with ad libitum access to standard rodent chow (3.1 kcal/g, Teklad 2018, Envigo, Sommerset,
504 NJ) unless otherwise stated. We did not observe significant sex differences between male and
505 female mice in our experiments. Prior to experiments, animals were habituated for 2-3 days to
506 experimental conditions, including handling, injections, behavior chambers and attachment of
507 gastric catheters for nutrient infusion.

508 **Surgeries**

509 **Vagotomy**

510 Surgeries were performed aseptically following the IACUC Guidelines for Rodent
511 survival surgery. Mice were anesthetized by inhalation of a continuous flow of 1.5-2% isoflurane.
512 The pedal reflex test was performed prior to surgery to ensure that each mouse had reached an
513 appropriate level of anesthesia. Mice were placed on a sterile drape warmed by a heating pad.
514 Fur was shaved from the abdomen before cleansing with three exchanges of EtOH and
515 Betadine. Sterile surgical equipment was used to create a 2-4cm midline laparotomy. The small
516 intestine and colon were externalized and placed on sterile gauze moistened with sterile 0.9%
517 NaCl saline. The subdiaphragmatic vagus nerve was visualized by gentle retraction of the liver
518 and stomach. Complete vagotomy was performed by cutting the left and right cervical branches
519 of the vagus directly caudal of the diaphragm using spring scissors. Sham animals had their
520 subdiaphragmatic vagus nerve visualized, but not tampered with. The internal organs were
521 repositioned and the incision site was covered with sterile gauze moistened with 0.9% NaCl
522 saline until intestinal infusions.

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523 Following the vagotomy, a silicone tubing was inserted via a small opening in the
524 stomach wall, into the proximal section of the duodenal lumen. The duodenum received a 5-
525 minute infusion of either sucrose (15%, w/v or fat (6.8%, v/v) solution (500 μL, 100 μL/min).
526 Post-stimulation, incisions were sutured, and the mice were allowed to recover on a heating pad
527 until they voluntarily moved to the unheated section of the cage. After 90 minutes, the mice
528 were perfused and brains harvested, post-fixed in 4% PFA for 24 hours, and kept at 4 °C in a 30%
529 sucrose in PBS solution until processing.
530

531 **Stereotaxic viral injections**

532 Mice were anaesthetized with 1.5-2 % isoflurane and were injected with carprofen
533 analgesia (5 mg/kg, s.c.) prior to bilateral injection in the dorsal hippocampus (dHPC). Core
534 temperature was maintained using a homeothermic monitoring system and the absence of
535 pedal reflex was utilized as a standard for appropriate depth of anesthesia. Animals were
536 restrained in a stereotaxic frame (World Precision Instruments, Sarasota, FL) and their skulls
537 were secured by positioning the bilateral ear crossbars into auditory meatus. A 2-3 mm incision
538 was made in the midline of the scalp using a scalpel and the sagittal suture, bregma, and
539 lambda of the skull were then exposed. With the bregma serving as an anatomical landmark, a
540 dental drill was utilized to penetrate the skull above the target brain area. For dHPC viral
541 injections, a Hamilton neuros syringe (Hamilton, Reno, NV) filled with a viral construct was
542 lowered to the injection site in the dHPC (anteroposterior (AP): - 1.8 mm, mediolateral (ML): ±
543 0.4 mm, dorsoventral (DV): - 2.1 mm). The viral construct (0.2 μL/side, 0.1 μL/min) was injected
544 via stereotaxic injector pump (Harvard Apparatus, Holliston, MA) and the needle remained in
545 place for an additional 10 minutes to minimize the backflow of solution out of the injection site.
546 The needle was removed slowly after the injection and 5-0 absorbable suture was used to close
547 the skin. pAAV5-flex-taCasp3-TEVp was a gift from Nirao Shah and Jim Wells (Addgene viral
548 prep # 45580-AAV5; http://n2t.net/addgene:45580; RRID:Addgene_45580),[59] pAAV9-EF1a-DIO-
549 hM3D(Gq)-mCherry was a gift from Bryan Roth (Addgene plasmid # 50460;
550 http://n2t.net/addgene:50460; RRID:Addgene_50460), and pAAV9-EF1a-DIO-EYFP was a gift
551 from Bryan Roth (Addgene viral prep # 44361-AAV9; http://n2t.net/addgene:44361;
552 RRID:Addgene_44361).[153]

553 **Intragastric (IG) catheter implantation**

554 IG catheters were made from 6 cm silicon tubing (.047” OD x .024” ID, SIL047, Braintree
555 Scientific, MA) composed of 6 beads of silicon glue (#31003, Marineland, Blacksburg, VA) and a

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556 Pinport (Instech Labs, Plymouth Meeting, PA) for infusions. Analgesics buprenorphine XR (1
557 mg/kg) and carprofen (5 mg/kg) were injected (s.c.) 20 minutes prior to the surgery. Once
558 animals had been anesthetized, a midline incision was made with a scalpel into the abdomen
559 and hemostats were used to blunt dissect the skin layer away from the muscle layer to allow the
560 catheter to be pulled between the abdominal incision site and the back of neck incision site. The
561 stomach was exteriorized using a blunt forcep and a 4-mm purse suture was then placed at the
562 junction of the greater curvature and fundus. Fine tip forceps were used to puncture the center
563 of the purse suture and the end of the IG catheter was inserted into the stomach. The purse
564 suture was then tightened and tied around the catheter. Next, a puncture hole was made in the
565 left lateral abdominal wall using fine tip forceps and the catheter was pulled through and
566 secured using 5-0 absorbable suture. The muscle layer of the abdominal incision site was then
567 sutured closed and the open end of the catheter was pulled through to the back of the neck via
568 a hole made in the middle of the shoulder blade. A 22-gauge Pinport was anchored in the tubing
569 using superglue and once the patency of the catheter was confirmed via flushing with sterile
570 saline, the catheter was secured with a purse suture around the hole in the back. Finally, the
571 skin of the abdomen was closed with sterilized suture clips. For recovery, animals were fed with
572 moistened chow in their home cage and were administrated Carprofen for 2 days after the
573 surgery.

574 **Behavioral Tests**

575 **Food restriction**

576 For all memory and motivation tasks involving food animals were maintained at 85-90%
577 of their original body weight by food restriction. Briefly, for weight maintenance, the animals'
578 body weight was recorded every 24 hours and they were fed with a set amount of food
579 calculated based on the loss of their original body weight. Animals were food restricted 6 hours
580 before the task and not refed until 2h after the end of the task to prevent interference from food
581 consumed outside of the task. If any mouse weighed less than 85% of their starting body
582 weight, they were fed 2.5 g plus the excess weight loss until they reached 85% of starting body
583 weight again. *Ad libitum* water access was provided in home cage.

584 **Food intake measurement**

585 Food intake measurement and meal pattern analysis were performed using the BioDAQ
586 episodic Food Intake Monitor (BioDAQ, Research Diets, Inc., New Brunswick, NJ). Previously
587 validated meal criteria were used for food intake analysis (minimal meal size = 0.02 g, maximum

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588 inter-meal interval = 300 s).[154] Animals were single housed and acclimated to the BioDAQ cages
589 and fed ad libitum with chow for at least 3 days. Baseline food intake was then recorded for 3-5
590 days prior to performing the TRAP protocol, permitting within animal comparisons. Once the
591 TRAP protocol was completed, animals were placed back in the BioDAQ cages, and their food
592 intake was monitored for an additional 7 days. Meal parameters included meal size, the number
593 of meals (meal frequency), meal duration and inter-meal interval were calculated by the BioDAQ
594 Monitoring Software.

595 **Behavioral apparatus**

596 Measurement of nutrient solution consumption and flavor-nutrient conditioning tests
597 were conducted in mouse behavioral chambers enclosed in a ventilated and sound attenuating
598 cubicle (Med Associates Inc., St. Albans, VT). Each chamber was equipped with slots for sipper
599 tubing equipped with contact lickometers with 10 ms resolution (Med Associates Inc.) used for
600 licking detection. All memory tests, except for the nutrient-driven Barnes Maze task, were
601 conducted in open field apparatus (41x41 cm; 30 cm height). The foraging-related Barnes maze
602 task[47] involved an elevated white circular Barnes maze (Diameter: 92 cm, Height: 95 cm) with
603 16 holes (Diameter: 5 cm) evenly spaced around the outer edge of the table's circumference.
604 The holes were covered with petri dishes and visuospatial cues were placed on each of the
605 walls surrounding the table. All memory tests were monitored and analyzed by tracking an
606 animal's head or body using the EthoVision XT Behavior Tracking Software.

607 **Nutrient solution consumption measurement**

608 Food restricted mice were habituated and trained in these operant chambers with
609 saccharin (0.2%, w/v) for 1 h/day for at least 3 days or until their total licking number reached at
610 least 1,000 times/h. The bottle containing saccharin was placed in a different slot each day to
611 avoid side preference. For caspase ablation studies, once animals were fully trained, they
612 underwent consumption tests for either sucrose solution (15%, w/v) or isocaloric fat solution
613 (6.8%, v/v) in a randomized order to minimize the influence of systematic contrast effects. Next,
614 during consumption preference tests; one bottle with sucrose solution was placed on one side
615 and another bottle with isocaloric fat solution placed on the other side. All the tests were
616 conducted for 1 h/day for 3 days and the number of licks were recorded. For chemogenetic
617 manipulation, baseline sucrose or fat consumption was assessed 20 min after saline injection.
618 On the experimental day, sucrose or fat consumption was measured 20 min following the
619 administration of clozapine-N-oxide (CNO; diluted in saline, 3 mg/kg, Enzo Life Sciences, NY).

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620 **Two-bottle choice flavor nutrient conditioning test**

621 To test whether ablation of nutrient-responsive HPC neurons affects specific nutrient-
622 flavor association, a two-bottle preference test was performed. Once animals were considered
623 trained to saccharin licking as described previously, a 'pre'-test was performed in which they
624 were given 10-min access to two novel Kool-Aid flavored solutions (cherry or grape, 0.05%, w/v)
625 in saccharin (0.025%, w/v). To avoid side preference formation, sipper bottle positions were
626 switched after 5 minutes. Subsequently, animals underwent a 1-hour conditioning session each
627 day for 6 days where the lesser preferred flavor defined in the 'pre'-test was paired with IG
628 infusions of nutrients (CS+; 6.8% fat or 15% sucrose) and the preferred flavor was paired with
629 IG infusions of saline (CS-). Specifically, during conditioning sessions, IG infusions of either
630 nutrients or saline delivered by a syringe pump (20 µL/lick, 600 µL/min) were triggered by
631 detection of the first lick and additional licks detected within 6 seconds had no programmed
632 consequences. Upon completion of these conditioning sessions, mice underwent a 'post'-test
633 identical to the 'pre'-test. The number of licks for the nutrient-paired flavor during 'pre' and 'post'
634 tests was used to calculate flavor preference ratios (CS+ licks / total licks) before and after
635 conditioning. For Sugar^{TRAP} mice, an additional 'post'-test was also performed two days after
636 the initial 'post'-test to assess post-ingestive sucrose-conditioned flavor memory.

637 **Progressive ratio licking test**

638 To assess whether nutrient-responsive dHPC neurons are important for nutrient-specific
639 motivation, a progressive ratio (PR) operant licking test[71] was performed. Food-restricted mice
640 were initially trained to lick an active sipper spout to receive 15% sucrose or isocaloric fat
641 solution via tubing mounted in a syringe pump (1 µL/lick, 600 µL/min) under fixed ratio (FR) 1
642 schedule (one hour/day for three days). After reaching >80% discrimination for the active sipper
643 over the inactive sipper, the schedule was increased to FR5 for an additional two days. Tests
644 under the PR schedule were then performed and failure to lick the active sipper in any 10 min
645 period resulted in termination of the session (one hour/session). For chemogenetic experiments,
646 on PR test days mice received either saline or CNO (i.p; 3 mg/kg) 20 minutes prior to entering
647 the operant chamber followed by the opposite drug injection on the subsequent day. The
648 number of licks was recorded and the breakpoint of reinforcement was calculated to quantify an
649 animal's willingness to work for a nutrient solution.

650 **Nutrient-driven food location memory test**

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651 To assess whether nutrient-responsive dHPC neurons are necessary for food location
652 reference memory, a modified nutrient-driven food cup task[23,155] was conducted. Food restricted
653 mice were habituated in open field apparatus, as described previously, for 5 minutes. The next
654 day, a 'pre'-test was performed where animals were allowed to explore the same arena
655 containing two empty petri dishes placed in opposite corners for 5 minutes and the baseline
656 preference for two quadrants was determined. Twenty-four hours later, animals underwent
657 conditioning sessions (3 x 5 minute sessions) where the lesser preferred quadrant was paired
658 with a petri dish containing drops of nutrient solution (CS+, 20 x 10 μL drops/session) and the
659 preferred quadrant was paired with a petri dish containing drops of water (CS-, 20 x 10 μL
660 drops/session). One or 24 hours after the last conditioning session, a 'post'-test identical to the
661 'pre'-test was conducted, i.e., both petri dishes were available with no stimuli. The time spent
662 exploring each petri dish was recorded across the whole experiment and the discrimination
663 index was calculated as the time spent exploring CS+ / total exploration time to assess animals'
664 memory performance.

665 **Nutrient-driven food location memory test with chemogenetic manipulation**

666 To assess whether activation of nutrient-responsive dHPC neurons can improve context-
667 dependent spatial memory for individual nutrients, a modified nutrient-driven food location
668 memory test with chemogenetic manipulation with CNO was performed. Mice expressing
669 hM3Dq or control virus in dHPC neurons trapped with intragastric infusion of sucrose (15% w/v)
670 or fat (6.8% v/v) were habituated to two novel contexts (context A and context B). Two clean
671 petri dishes were placed in opposing quadrants for each context. During a 3-day training phase,
672 drops of either nutrient or water were added to the petri dishes (CS+, 20 x 10 μL drops/petri
673 dish). Mice received saline injections 20 min prior to being placed in one context in the morning
674 to learn to associate the location of a nutrient-containing dish for 10 min, and then received
675 another saline injection 20 min before being placed in context B in the afternoon to learn a
676 different location for the second nutrient-containing dish. Twenty-four hours after the last
677 conditioning session, mice were tested with empty petri dishes in context A or context B to
678 determine whether they could discriminate the correct context-specific nutrient-paired quadrant.
679 After 7 days, the same test was repeated but CNO (3 mg/kg) was injected 20 min before mice
680 were placed in the context which was paired with the nutrient with which the mice were trapped.
681 To avoid desensitization, CNO was not injected on the third training day. The time spent
682 exploring each petri dish was recorded across the whole experiment and the discrimination
683 index was used to assess animals' memory performance.

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684 **Nutrient-driven Barnes maze test[47]**

685 To evaluate the effect of nutrient-responsive dHPC neurons on food-related spatial
686 working memory, a nutrient-driven Barnes maze test was performed. Food-restricted mice were
687 first allowed to explore the Barnes maze apparatus for 5 min. The next day, animals were
688 trained to utilize spatial cues to locate the correct petri dish containing sucrose (15% w/v) or
689 isocaloric fat (6.8% v/v). All other petri dishes contained water. Each animal received two trials
690 per day for three training days with 2-min inter-trial interval (during which time the maze is
691 cleaned using 70% ethanol to avoid any confounding odor effect). Importantly, the target hole
692 remains in a consistent position in both trials conducted on each training day but is relocated to
693 a new position at the beginning of the initial trial on each subsequent training day. The number
694 of incorrect investigations were recorded and the difference in the number of errors between
695 trail 2 and trail 1 on an individual training day was calculated to determine whether animals
696 improved their appetitive spatial working memory performance.

697 **Novel object in context (NOIC) test**

698 To examine whether nutrient-responsive dHPC neurons only affect food-related memory,
699 an HPC-dependent NOIC test[156] was performed. Animals underwent 2 days of habituation (day
700 1 and day 2): half of the animals were allowed to freely explore context A, an opaque box with
701 black stripes, for 10 minutes, whereas the other half were habituated to context B, an opaque
702 box with no cues on the wall. The following day, groups were switched and habituated to the
703 other context under the same environment. Training sessions were performed 24 hours after the
704 last habituation session. On the training day (day 3), half of the animals were placed in context A
705 for 10 minutes containing two identical blocks of Lego (object 1) placed in opposite corners,
706 whereas the other half were first placed in context B for 10 minutes containing another two
707 identical blocks of Lego (object 2) placed in opposite corners. Animals were placed back to their
708 home cage between exposure to context A and context B and the inter-trial interval was 1-3
709 minutes. Animals were then switched and trained in the other context for another 10 minutes.
710 On day 4 (test day), the NOIC recognition memory was tested by placing animals for 10 minutes
711 in their last trained context (familiar context) containing one object (familiar object) which
712 belonged to the familiar context on day 3 and one object (novel object) which belonged to the
713 other context on day 3. The amount of time spent exploring each object was recorded and the
714 discrimination index (DI) was calculated as $[t_{novel}/(t_{novel}+t_{familiar})]$ in order to assess NOIC
715 recognition memory. Seventy percent ethanol was used to clean all objects and contexts
716 between tests.

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717 **Histology**

718 **TRAP protocol**

719 As previously described,[46] animals were fasted for 6 hours prior to IG infusion. Thirty
720 minutes before onset of the dark phase, mice received an IG infusion of either sugar solution
721 (15%, w/v) or fat solution (6.8%, v/v, Microlipid, Nestle, Vevey, Switzerland) (500 μL, 100
722 μL/min) in their home cage based on their assigned group. 4-hydroxytamoxifen (4-OHT, 30
723 mg/kg, i.p., MilliporeSigma, Burlington, MA) was injected 3 hours after the stimulus and
724 standard chow was returned to animals' home cage 3 hours after 4-OHT injection.

725 **Perfusions**

726 Transcardial perfusion was performed in deeply anesthetized animals with phosphate
727 buffer saline (PBS), followed by 4% paraformaldehyde (PFA). Following perfusion, brains were
728 harvested and left in 4% paraformaldehyde for 24 hours and then transferred to a 30% sucrose
729 solution containing 0.1% sodium azide for at least 72 hours before further processing.

730 **Tissue processing & storage**

731 **Slicing.** Whole brains were frozen and embedded in OCT. A Leica frozen microtome
732 (CM 3050 S, Leica Biosystems) was utilized to slice the frozen brains into 3 series at a
733 thickness of 35 μm per section and slices were stored in cryoprotectant at -80°C until further
734 staining or imaging.

735 **Immunohistochemistry – Fos.** The tissue was removed from cryoprotectant and rinsed
736 in PBS 3 times (10 minutes/time) at room temperature. Subsequently, tissue was incubated for
737 30 minutes in a blocking buffer consisting of permeabilizing agent (244.5 mL of PBS, 5 mL of
738 serum, 0.5 mL of Triton-X100, 0.25 g of Bovine Serum Albumin) and 20% normal donkey serum
739 at 37 °C to prevent non-specific antibody binding. Tissue was then incubated overnight in PA
740 containing a rabbit anti-cFos primary antibody (1:1000, Cell Signaling) at 4°C. On the following
741 day, the tissue was rinsed in PBS 3 times for 20 each at room temperature followed by
742 incubation in PA containing a donkey anti-rabbit IgG-AlexaFlour 647 secondary antibody (1:500,
743 Abcam). Tissue was then rinsed in PBS 3 times for 1 hour each, mounted on slides,
744 coverslipped with Prolong Diamond Antifade Mountant (Invitrogen, Waltham, MA), and stored at
745 -20°C until imaging and analysis.

746 **Imaging**

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747 The HPC was identified using a mouse brain atlas (Paxinos and Franklin, 2001) and
748 images of each region of interest were acquired with a Keyence BZ-X800 microscope using 10x
749 objective. The number of positive cells, including trapped cells, $cFos^+$ cells, and colocalization
750 was counted automatically using NIS Element software with manual correction.

751 **Data analysis**

752 Statistical analyses are described for each figure and were performed using GraphPad
753 Prism 9 software. Two-tailed unpaired Student's t tests were used for comparing two groups;
754 Two-tailed paired Student's t tests were used for comparing two treatments or tests in the same
755 animal. One-way ANOVA, with or without repeated-measures, was used for comparing three
756 groups; two-way ANOVA, with or without repeated-measures, was used for comparing more
757 than one factor between groups. Data are presented as mean ± SEM and statistical significance
758 is declared at $p < 0.05$.

759

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760 **Figure Legends**

761 **Figure 1. Dorsal hippocampal neurons are responsive to different post-ingestive**
762 **nutrients.** A Schematic of Fos staining approach to assess dHPC neuronal responsiveness to
763 intragastric saline, sucrose (15%), or fat (6.8%). **B** Representative images of Fos expression in
764 the dHPC. **C** Quantification of (B) showing increased Fos expression in dHPC neurons in
765 response to sucrose or fat infusions compared to saline (N=3-5/group, one-way ANOVA with
766 Turkey post hoc analysis). **D-E** Quantification of Fos expression in DG, CA3 and CA1 of the
767 dHPC following intragastric sucrose (**D**) or fat (**E**) infusions (N=4-5/group, one-way ANOVA with
768 Tukey post hoc analysis). **F** Schematic illustration of SDV for evaluating the role of the vagus
769 nerve in dHPC neuronal nutrient sensing. **G** Representative images of Fos expression in the
770 dHPC following intragastric sucrose infusion in mice with or without VGX. **H** Quantification
771 demonstrating dHPC Fos expression in response to sucrose is blunted by VGX (N=4/group,
772 unpaired t test). **I** Representative images of Fos expression in the dHPC following intragastric
773 fat infusion in mice with or without VGX. **J** Quantification demonstrating dHPC Fos expression in
774 response to fat is blunted by VGX (N=5/group, unpaired t test). **K** Schematic of Fos^{TRAP}
775 approach comparing tdTomato labeling to Fos labeling in response to intragastric nutrient
776 infusions. **L** Quantification showing higher overlap between repeated infusions of fat compared
777 to separate macronutrients in the dHPC (N=5/group, unpaired t test). **M-N** Representative
778 images of the dHPC in response to Fat (TRAP, magenta), and (**M**) colocalization after infusion
779 two weeks later of Fat (Fos, cyan; top) or (**N**) fat (Fos, cyan; bottom) in the same animal. Data
780 are presented as mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, NS, not significant. Scale
781 bars 100 µm.

782 **Figure 2. Fat- and sucrose-responsive dHPC neurons control nutrient-specific**
783 **preference.** **A** Schematic of the Fos^{TRAP} approach to selectively ablate nutrient-responsive HPC
784 neurons that respond to intragastric infusion of sucrose or fat. **B** Representative images of
785 nutrient-responsive HPC neurons from Fos^{TRAP} mice following intragastric infusion of sucrose or
786 fat with or without viral-mediated caspase ablation. **C-D** Quantification demonstrating caspase
787 deletion of tdTomato neurons (N=5-7/group, unpaired t test) in response to (**C**) sucrose or (**D**)
788 fat. **E** Intake in a two-bottle choice test in mice with ablation of $dHPC^{Sugar}$ neurons reduces
789 sucrose solution consumption, **F** without affecting fat solution intake when sucrose and fat
790 solution are both presented (N= 8-9/group, two-way ANOVA with Holm-Sidak post hoc analysis).
791 **G** Intake in a two-bottle choice test in mice with ablation of $dHPC^{Fat}$ neurons has no effect on
792 sucrose intake, but **H** reduces fat solution consumption without affecting sugar solution intake

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793 (N= 8/group, two-way ANOVA with Holm-Sidak post hoc analysis). **I** Schematic of the Fos^{TRAP}
794 approach to selectively stimulate nutrient-responsive HPC neurons that respond to intragastric
795 infusion of sucrose or fat. **J** Quantification injection to demonstrated increased Fos labeling in
796 dHPC after CNO in dHPC^{Fat} and dHPC^{Fat} mice expressing Gq-DREADD compared to control
797 mice (N=4-5/group, one-way ANOVA with Two-stage linear step-up procedure of Benjamini,
798 Krieger and Yekutieli post hoc analysis). **K** Representative images of Fos labeling in the dHPC
799 after CNO. **L** Stimulation of sucrose-responsive dHPC neurons increases sucrose consumption
800 **M** without affecting fat consumption (N=4-5/group, two-way ANOVA with Holm-Sidak post hoc
801 analysis). **N** Stimulation of fat-responsive dHPC neurons has no effect on sucrose consumption,
802 but **O** increases fat consumption without affecting sucrose consumption (N=4/group, two-way
803 ANOVA with Holm-Sidak post hoc analysis). Data are presented as mean ± s.e.m. *P < 0.05,
804 **P < 0.01, ***P < 0.001, NS, not significant. Scale bars 100 μm.

805 **Figure 3. Fat- and sucrose-responsive dHPC neurons control nutrient-specific episodic**
806 **spatial memory. A** Schematic of nutrient-driven location memory task to assess the necessity
807 of nutrient-responsive dHPC neurons in food-related reference memory. **B** Control mice
808 showing increased discrimination for sucrose-paired location. **C** Ablation of dHPC^{Sugar} neurons
809 prevents sugar location memory. **D** Control mice and **E** caspase-treated dHPC^{Sugar} mice form
810 fat-driven memory (N=8-10/group, one-way ANOVA with Holm-Sidak post hoc analysis). **F** Fat
811 control mice, and **G** mice with ablated dHPC^{Fat} neurons form sucrose memory. **H** Control mice
812 spend more time exploring fat location after training, while **I** fat-ablated mice do not form
813 location memory for fat. (N=7-10/group, one-way ANOVA with Holm-Sidak post hoc analysis). **J**
814 Schematic of nutrient-driven location memory task to assess whether stimulation of nutrient-
815 responsive dHPC neurons can improve nutrient-related memory. **K** Stimulation of dHPC^{Sugar}
816 neurons improves sucrose-related memory recall (N=4-5/group, paired Student's t test). **L**
817 Stimulation of dHPC^{Fat} neurons does not improve fat-related memory recall (N=4/group, paired
818 Student's test). Data are presented as mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, NS,
819 not significant.

820 **Figure 4. Fat-responsive dHPC neurons promote fat reinforcement. A** Diagram
821 demonstrating the flavor-nutrient conditioning paradigm. **B** Conditioning increases preference
822 for the flavor paired with intragastric sucrose in Sugar^{TRAP} mice (N=7-8/group, two-way ANOVA
823 with Holm-Sidak post hoc analysis). **C** Control mice remember the flavor preference 3 days
824 post-training, while **D** caspase-treated dHPC^{Sugar} mice reduce preference by day 3. (N=7-
825 8/group, paired Student's t test). **E** Ablation of fat-responsive dHPC neurons prevents fat

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826 reinforcement (N=7-9/group, two-way ANOVA with Holm Sidak post hoc analysis). **F** Schematic
827 illustration of progressive ratio licking test to assess necessity of nutrient-responsive dHPC in
828 motivation. **G** Ablation of sucrose-responsive dHPC neurons has no effect on sucrose
829 motivation (N=6-7/group, unpaired t test). **H** Ablation of fat-responsive dHPC neurons reduces
830 fat motivation, indicated by the decrease in breakpoint (N=4-5/group, unpaired t test). **I**
831 Schematic illustration of progressive ratio licking test assessing sufficiency of nutrient-
832 responsive dHPC neurons in motivation. **J** Stimulation of $dHPC^{Sugar}$ neurons has no effect on
833 sucrose motivation (N=5-7/group, unpaired t test). **K** Stimulation of Gq DREADD expressing
834 $dHPC^{Fat}$ neurons promotes fat motivation in response to CNO compared to the baseline (N=5-
835 7/group, unpaired t test). Data are presented as mean ± s.e.m. *P < 0.05, **P < 0.01, ***P <
836 0.001, NS, not significant.

837 **Figure 5. Sucrose-responsive dHPC neurons promote food intake. A** Ablation of sucrose-
838 responsive dHPC neurons reduces average daily chow intake, starting at dark onset
839 (N=5/group, two-way ANOVA with Holm-Sidak post hoc analysis). **B** Ablation of sucrose-
840 responsive dHPC neurons reduces meal size without affecting meal frequency, meal duration
841 and inter-meal interval. (N=5/group, unpaired t test). **C** Ablation of fat-responsive dHPC neurons
842 has no effect on chow intake (N=7-8/group, two-way ANOVA with post hoc analysis). **D** Ablation
843 of fat-responsive dHPC neurons has no effect on meal pattern (N=7=8/group, unpaired t test).
844 Data are presented as mean ± s.e.m. *P < 0.05, NS, not significant.

845 **Supplemental Figures**

846 **Supplemental Figure 1. vHPC neuronal response to intragastric nutrient infusions. A-B**
847 Representative images of Fos expression in vHPC following intragastric infusion of saline,
848 sucrose or fat. Scale Bar: (A) 100 μm, (B) 50 μm. **C-E** Quantification of Fos expression in dHPC
849 and vHPC following intragastric infusion of saline, sucrose or fat. (N=3-5/group, paired Student's
850 t test). **F-G** Quantification of Fos expression in DG, CA3 and CA1 of the vHPC following
851 intragastric sucrose or fat infusions (N=4-5/group, one-way ANOVA with Tukey post hoc
852 analysis). **H** Quantification showing higher overlap between repeated infusions of fat compared
853 to separate macronutrients in the dDG (N=5/group, unpaired t test). **I-J** Quantification of Fos^{TRAP}
854 positive neurons and Fos immunofluorescence in the dHPC following intragastric sucrose or fat
855 infusions, revealing a comparable density of responsive neurons (N=6/group, paired Student's t
856 test). **K** Representative images of tdTomato in the dHPC in response to sucrose (TRAP,
857 magenta) compared with Fos-immunoreactivity following intragastric infusion of isocaloric starch
858 (Fos, cyan) in the same animal. Scale Bar: 100 μm. **L** Quantification of (K) demonstrating that

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859 60% of tdTomato labeled sucrose-responsive dHPC neurons co-express Fos activated by starch
860 infusion (n=2/group). Data are presented as mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001,
861 **** P < 0.0001, NS, not significant.

862 **Supplemental Figure 2. In the absence of choice, fat-responsive dHPC neurons increase**
863 **fat intake independently of taste. A-B** Ablation of sucrose or fat-responsive dHPC neurons
864 has no effect on taste of sucrose or fat (N=8-9/group, unpaired t test). **C-D** Ablation of sucrose-
865 responsive dHPC neurons has no effect on sucrose or fat consumption when individual solution
866 is presented (N=8-9/group, two-way ANOVA with Holm-Sidak post hoc analysis). **E-F** Ablation of
867 fat-responsive dHPC neurons decreases fat consumption without affecting sucrose
868 consumption (N=8/group, two-way ANOVA with Holm-Sidak post hoc analysis). **G-H** CNO
869 injection has no effect on sucrose or fat consumption in control virus-injected mice (N=5/group,
870 two-way ANOVA with Holm-Sidak post hoc analysis). Data are presented as mean ± s.e.m.
871 **P < 0.01, NS, not significant.

872 **Supplementary Figure 3. Nutrient-responsive dHPC neurons have no effect on nutrient-**
873 **related working memory and non-food related memory. A** Animals exhibit a diminished
874 ability to recall the nutrient-paired quadrant in the first trial, indicated by a reduced discrimination
875 index (N=8-10/group, unpaired t test). **B** Schematic of novel object in context test. **C-F** Ablation
876 of sucrose or fat-responsive dHPC neurons has no effect on non-food related memory test
877 using novel object in context test (N=4-9/group, two-way ANOVA with Holm-Sidak post hoc
878 analysis for C and E, unpaired t test for D and F). **G** Schematic of nutrient-related Barnes maze
879 test. **H-O** Ablation of sucrose or fat-responsive dHPC neurons has no effect on sucrose-driven
880 Barnes maze (H-K) and fat-driven Barnes maze test (L-O) (N=6-9/group, paired Student's t
881 test). Data are presented as mean ± s.e.m. *P < 0.05, **P < 0.01, NS, not significant.

882 **Supplemental Figure 4. Fat- and sucrose-responsive dHPC neurons are not required for**
883 **learning. A** Ablation of sucrose-responsive dHPC neurons has no effect on the number of
884 sucrose infusions acquired by animals during the sucrose conditioning sessions (N=4-6/group,
885 two-way ANOVA with Holm-Sidak post hoc analysis). **B** Ablation of fat-responsive dHPC
886 neurons reduces the number of fat infusions during the fat conditioning sessions (N=3-7/group,
887 two-way ANOVA with Holm-Sidak post hoc analysis). **C-F** $Sugar^{TRAP}$ and Fat^{TRAP} mice learned to
888 lick in the active side to obtain infusion of sucrose or fat solution (N=3-7/group, two-way ANOVA
889 with Holm-Sidak post hoc analysis). Data are presented as mean ± s.e.m. *P < 0.05.

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890 **Supplemental Figure 5. Sucrose-responsive dHPC neurons control food intake. A** Ablation
891 of sucrose-responsive dHPC neurons reduces chow intake compared to the baseline chow
892 consumption within the same animal (N=5/group, two-way ANOVA with Holm-Sidak post hoc
893 analysis). **B** TRAP protocol has no effect on chow intake in sucrose^{TRAP} mice expressing control
894 virus (N=5/group, two-way ANOVA with Holm-Sidak post hoc analysis). **C** No group difference in
895 baseline chow intake between Sucrose^{Con} and Sucrose^{Casp3} mice (N=5/group, two-way ANOVA
896 with Holm-Sidak post hoc analysis). **D** Ablation of fat-responsive dHPC neurons has no effect
897 on chow intake compared to baseline chow consumption within the same animal (N=3/group,
898 two-way ANOVA with Holm-Sidak post hoc analysis). **E** TRAP protocol has no effect on chow
899 intake in fat^{TRAP} mice expressing control virus (N=3/group, two-way ANOVA with Holm-Sidak
900 post hoc analysis). **F** No group difference in baseline chow intake between Fat^{Con} and Fat^{Casp3}
901 mice (N=3/group, two-way ANOVA with Holm-Sidak post hoc analysis).

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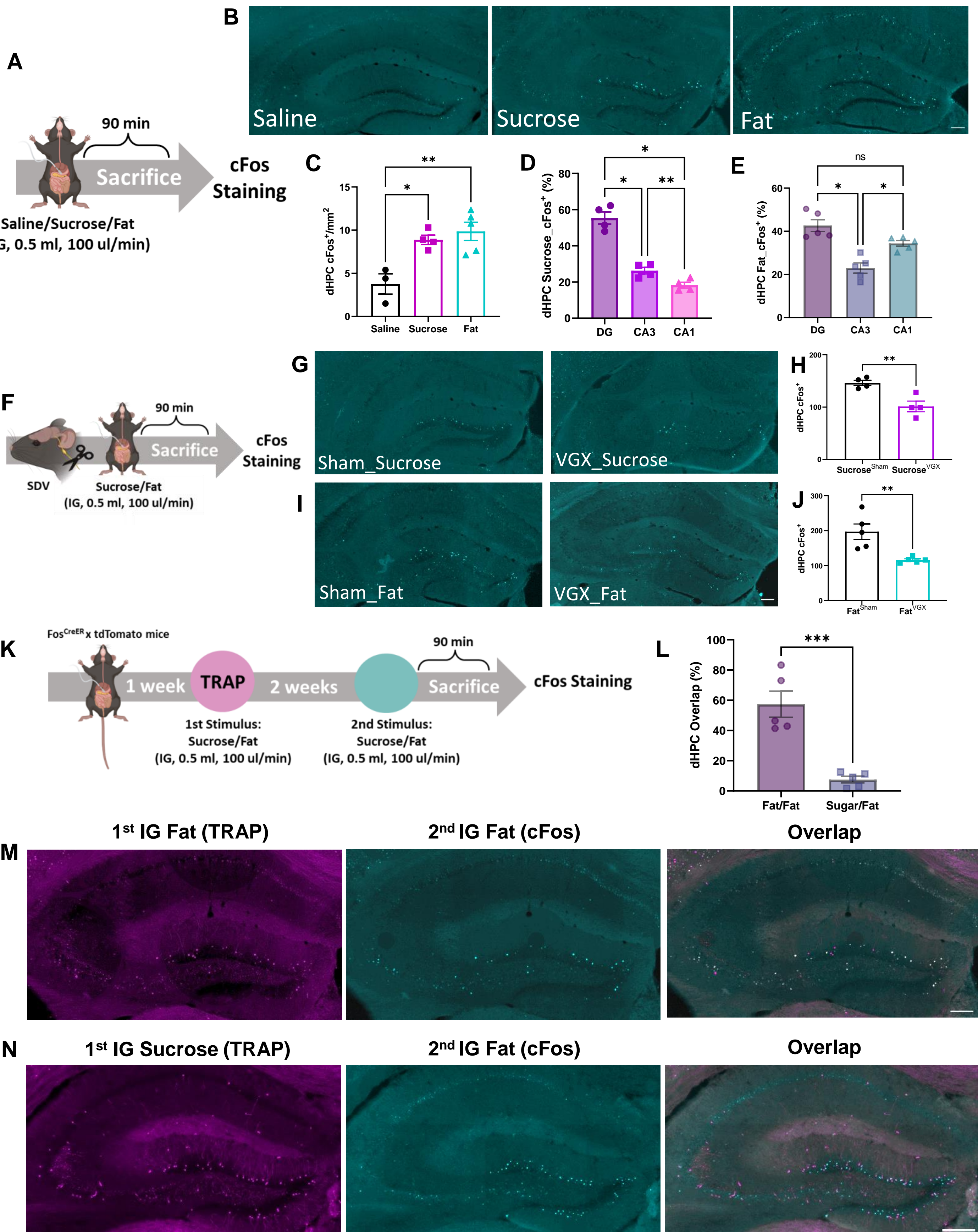
1296

Figure 1



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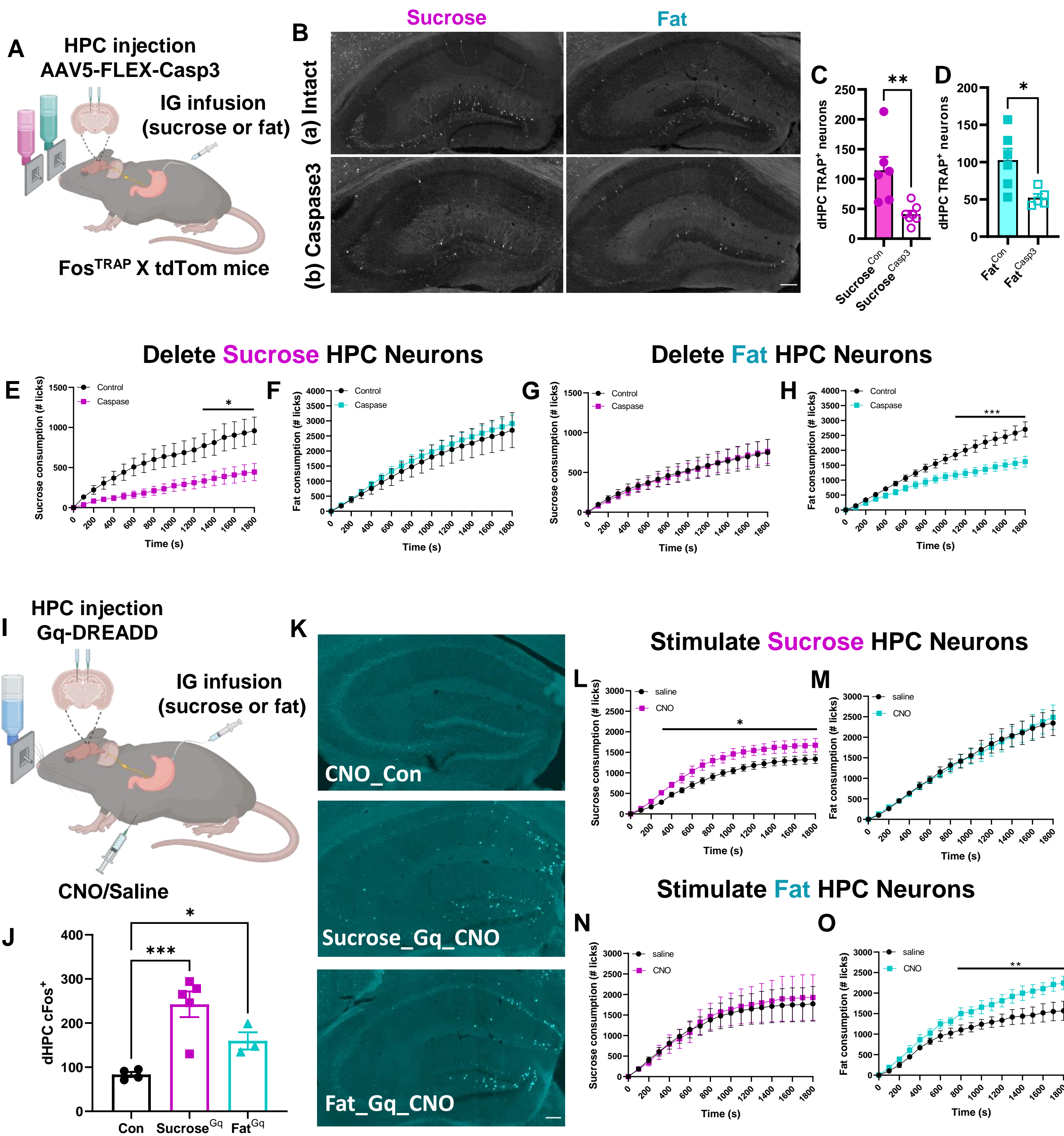

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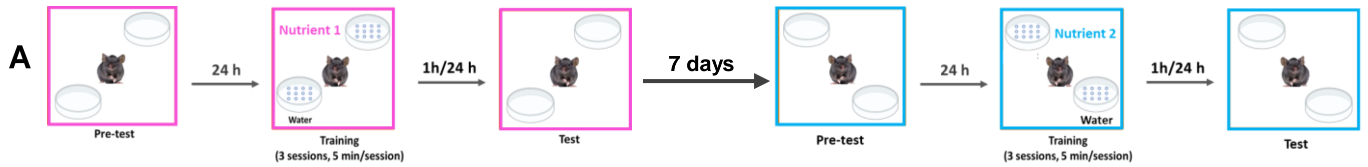

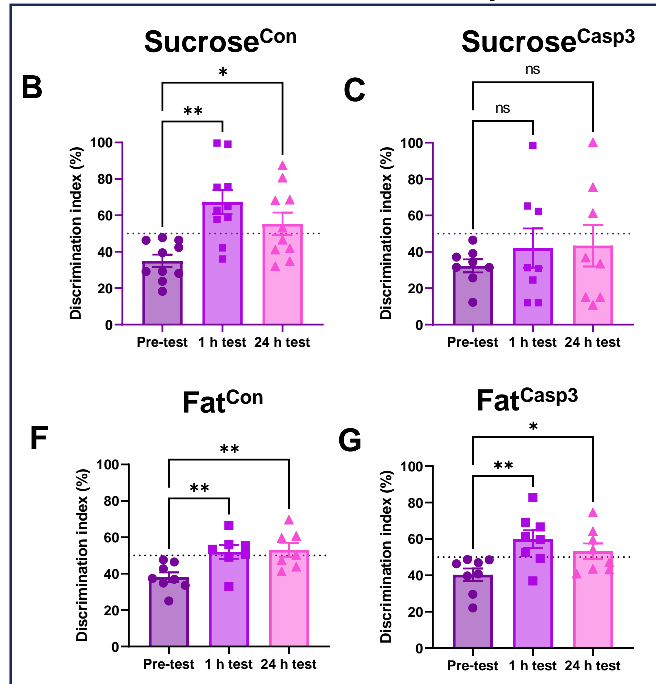

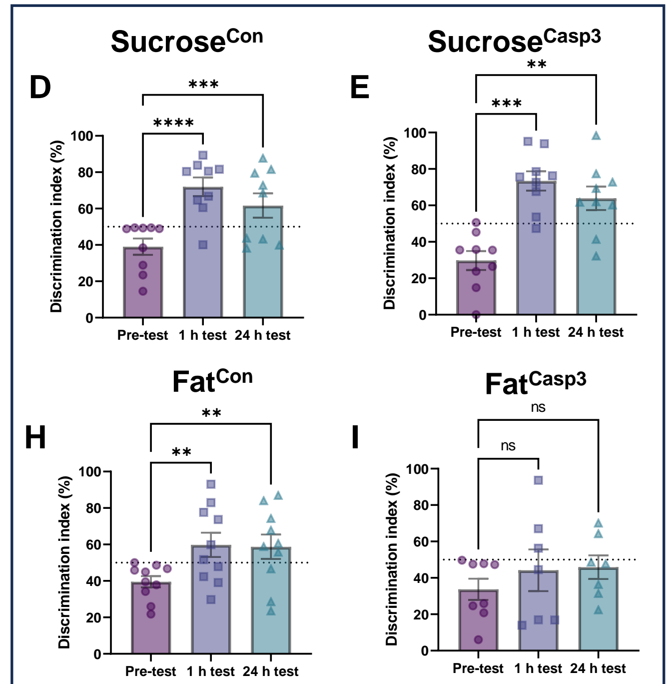

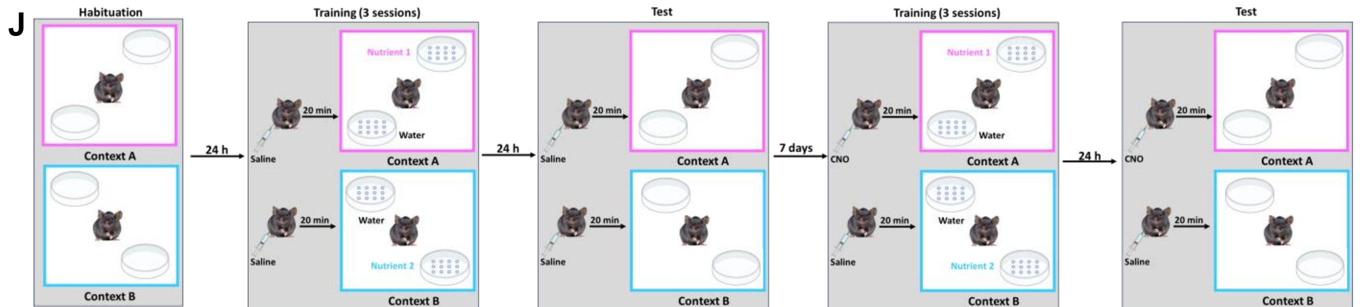

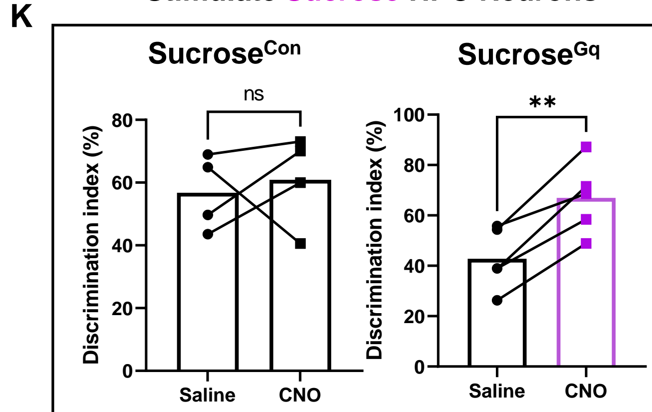

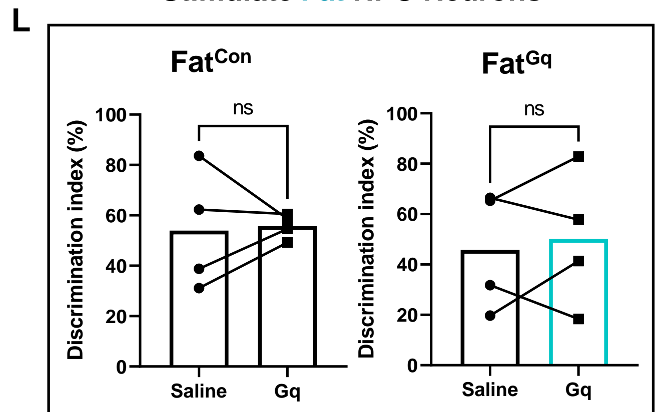

Figure 4

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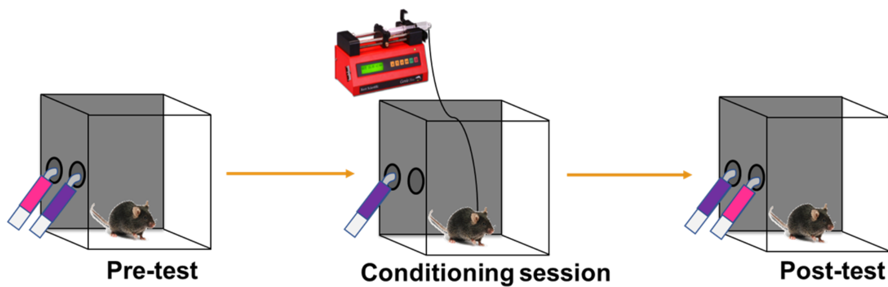

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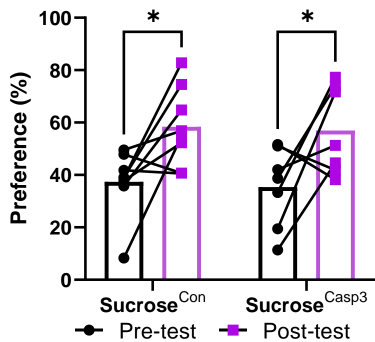

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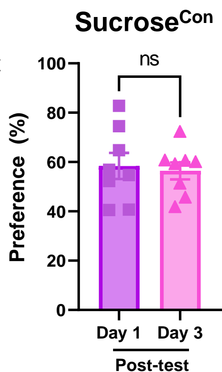

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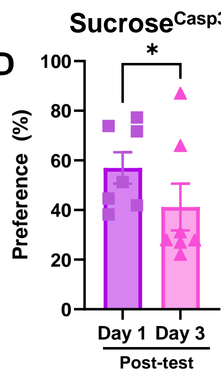

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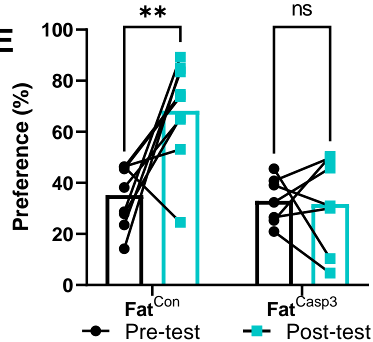
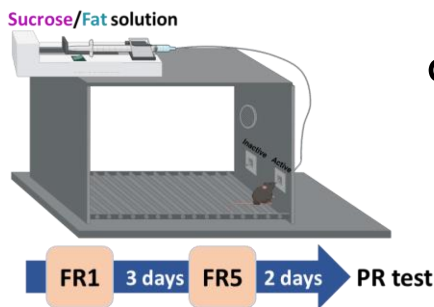


Delete nutrient-responsive HPC Neurons

F



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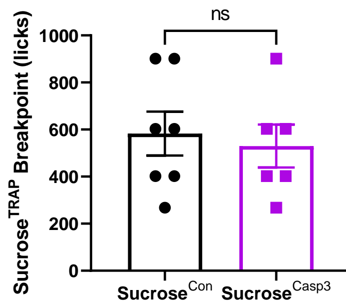

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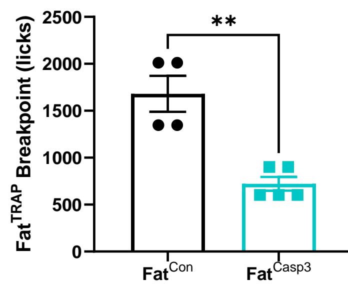
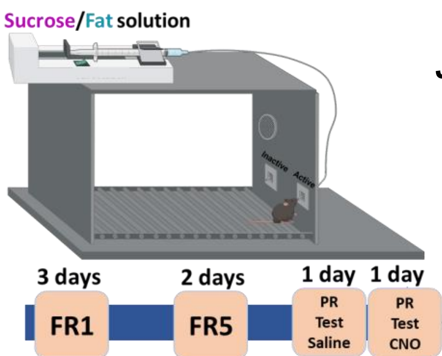


Stimulate nutrient-responsive HPC Neurons

I



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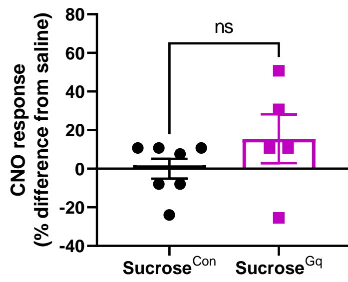

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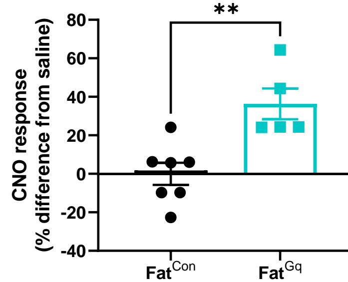

Figure 5

Delete Sucrose HPC Neurons

Delete Fat HPC Neurons