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Disentangling the role of NAc D1 and D2 cells in hedonic eating

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Abstract

Consumption of highly palatable foods is thought to be influenced by the central reward circuits that control both the hedonic component ('liking') of food and the motivation ('wanting') to eat it. The nucleus accumbens (NAc), a key brain center implicated in these processes, contains two main populations: D1 and D2 dopamine receptor-expressing neurons. The precise contribution of these cells to these reward-associated behaviors is still unresolved and establishing a causal relationship between activation of each neuronal subtype and its effect on motivated behaviors has proven challenging. To understand the forces underlying eating behavior that may lead to overconsumption, we assessed 'liking' and 'wanting' in separate sets of experiments. First, in a free palatable food consumption paradigm, we analyzed licking microstructure to demonstrate that both NAc D1 and D2 cell activity are more strongly correlated with 'liking' than 'wanting'. Second, we evaluated motivation to retrieve a reward ('wanting') in an operant task. Both NAc D1 and D2 cell activity encoded motivation during the reward-approach phase, after the cue and before consumption. Lastly, we show that opto-excitation of NAc D1 cells, but not D2 cells, intensifies incentive motivation to overconsume the optostimulation-paired palatable food in a two-bottle choice task. This demonstrates an important role for NAc D1 cells in preference formation and promoting consumption of palatable foods. Our results suggest a fundamental difference: NAc D1 cells promote reinforced behaviors under an operant paradigm or a Pavlovian association task. In contrast, NAc D2 cells are essential for initiating retrieval of palatable food but, once consumed, reduced activity of these cells allows prolonged eating episodes.

Keywords

Motivation, dopamine, D1, D2, nucleus accumbens, reward, fiber photometry, optogenetics, food preference

Introduction

Overweight and obesity, and eating disorders generally, have increased in recent decades (Hoek, 2016; Tavolacci et al., 2021). Thus, it is crucial to better understand the factors that contribute to the development of different eating disorders. In the modern obesogenic environment, palatability and availability of foods play a major role in the development of disorders of overeating (Corsica and Hood, 2011; King, 2013). Food consumption is not only motivated by the homeostatic need to monitor, restore and maintain energy balance. Homeostatic signals can also be overridden, causing us to engage in non-homeostatic eating that is driven by the sensory and/or hedonic properties of palatable foods with high fat and/or sugar contents. This 'hedonic' eating, activates brain regions involved in reward, resulting in increased motivation to consume food, and increased energy consumption (Frank et al., 2019; Leigh and Morris, 2018; Volkow et al., 2011) despite a state of satiety (Zheng and Berthoud, 2008).

The 'incentive salience theory' of reward has provided a valuable framework for more than a decade for investigating the role of food hedonics in the context of appetitive behavior in humans and animals (Berridge and Robinson, 1998, 2003). This theory postulates that reward is not a unitary process but comprises of an affective pleasure component referred to as 'liking' and a non-affective motivational component referred to as 'wanting'. 'Liking' is the hedonic component that reflects the immediate experience or anticipation of eating a pleasurable food (Mela, 2006). 'Wanting' is the incentive motivation (reward-seeking) that can lead to increased appetite, food cravings and also to overconsumption of food (Berridge and Robinson, 1998; Berthoud and Morrison, 2008; Finlayson et al., 2007). 'Incentive salience theory' has been studied in the framework of the regulation of food intake, specifically as a model for interpreting studies linking the function of the reward system with food intake or obesity. Looking at these psychological traits in eating behaviors (Berridge, 1996, 2009; Finlayson and Dalton, 2012), overeating in some individuals might reflect the abnormal functioning of either the 'wanting' or 'liking' mechanisms, and there are indications from subjects with eating disorders that 'liking' and 'wanting' of obesogenic foods can also be dissociated (Benton, 2010).

It is widely known that dopaminergic neurotransmission in the nucleus accumbens (NAc) plays a key role in behaviors related to natural rewards and drugs of abuse (Hyman and Malenka, 2001; Kelley and Berridge, 2002; Koob and Le Moal, 2001; Robinson and Berridge, 2003; Soares-Cunha et al., 2016b). In the NAc, two important classes of cells are medium spiny neurons expressing D1 dopamine receptors (D1 cells) and those expressing D2 dopamine receptors (D2 cells) (Surmeier et al., 1996). The precise contribution of D1 and D2 cells in reward-associated behaviors is still unresolved, and the establishment of a causal relationship between activation of each neuronal subtype and its effect on motivated behaviors has proven challenging. D1 cell activation is canonically considered to be related to positive rewarding events leading to persistent reinforcement, while activation of D2 cells is thought to facilitate aversion (Kupchik et al., 2015; Lobo et al., 2010; Trifilieff et al., 2013). However, the current literature is controversial regarding the functionality of these cells and their role in motivation-related behaviors (Natsubori et al., 2017; Soares-Cunha et al., 2016a; Soares-Cunha et al., 2020; Yang et al., 2018).

Therefore, increased understanding of which cells in the dopaminergic system underly brain substrates of 'liking' and 'wanting' may open an avenue to comprehending the impact of food rewards on eating behaviors linked to obesity and other eating disorders. In addition, it may provide insight into brain circuits and mechanisms underlying both hedonic and motivational components of reward, including mechanisms that are common to both natural and artificial rewards.

This paper aims to further understand the differential contribution of NAc D1 and D2 cells to different components of palatable food consumption, namely 'liking' (hedonic/pleasurable) and 'wanting' (incentive motivational value/desire/craving). We assess 'liking' and 'wanting' from several different angles using multiple behavioral paradigms, while employing neuronal recording or optogenetic tools and analytical techniques, enabling us to better understand the forces underlying eating behavior that may lead to overconsumption.

Results

NAc D1 cells exhibit transiently elevated activity, and D2 cells exhibit suppressed activity, during periods of free consumption of a palatable food reward

To probe the neural mechanisms underpinning reward consumption, we used *in vivo* fiber photometry to record GCaMP6s calcium indicator fluorescence selectively expressed in D1 cells (in D1 receptor-cre mice) or D2 cells (in adenosine A2a receptor gene (Adora2a)-cre mice) (Gerfen et al., 2013) (**Figure 1a**).

To determine how D1 and D2 cells represented solutions with different palatability, mice were first given free access to water from a bottle (**Figure S1a**). Once they reliably consumed this, they were allowed to freely consume a highly palatable food (milkshake, **Figure 1b,c**) over the course of five daily sessions, in the absence of nutrient deficit.

We found that before licking, both D1 and D2 cells transiently increased their activity, which peaked just after licking onset. In both D1 and D2 cells, the peak was more pronounced in response to milkshake than water, possibly reflecting hedonic processes (**Figure 1b-d**). However, after consumption onset, the activity patterns of the two populations differed; D1 cells returned to baseline, while D2 cell activity was suppressed below the baseline as the licking bout continued (**Figure 1d**). Both cell types continued to respond to milkshake over subsequent sessions, but the D1 peak reduced over sessions, and the D2 suppression was exaggerated (**Figure 1e**). These results demonstrate that D1 and D2 cells do not act as a single homogeneous population during consumption.

Milkshake consumption within a session (total number of licks) was significantly higher than water, and this was consistent across sessions (**Figure S1c,d**). This indicates more positive hedonic reactions to milkshake than water (**Figure S1d**). However, the amount consumed provides an incomplete readout of ingestive behavior (Davis and Smith, 1992). Therefore, to further probe the driving forces behind these changes in overall consumption, we investigated different aspects of free consumption of water vs. a palatable food (milkshake). In particular, if it would be possible to differentiate between 'wanting' and 'liking' parameters (Berridge, 2009). We therefore analyzed licking microstructure (Davis, 1989), since it provides several measures to evaluate pleasure ('liking') and motivation ('wanting') during liquid-food consumption in rodents (Davis and Smith, 1992; Dwyer et al., 2011; Galistu and D'Aquila, 2013; Johnson, 2018a; Robinson and McCool, 2015). Specifically, increases in the duration of single licks, licking bout duration and bout size (lick intensity determined by number of licks in a bout), and decreases in inter-lick interval (ILI), have been suggested to represent increased hedonic impact and palatability, while increases in bout number and decreases in inter-bout interval represent increased incentive salience (Davis, 1973; Davis and Smith, 1992; Dwyer, 2012; Higgs and Cooper, 1998; Robinson and McCool, 2015; Smith, 2001)(**Figure S1b**).

We found that changes in 'liking' parameters paralleled greater 'liking' for milkshake over water; lick duration, bout duration and bout size increased significantly, while inter-lick interval (ILI) decreased significantly, between the water and first milkshake sessions (**Figure 1f and S1e**). Therefore, as expected, animals displayed more positive hedonic reactions ('liking') to the milkshake than to water.

122 Lick duration also kept increasing over milkshake sessions, suggesting an increased hedonic
123 component of milkshake consumption in later sessions (**Figure 1f, left**).

124 Bout number increased, and inter-bout interval (IBI) decreased significantly between water and the
125 first milkshake session, suggesting that mice were more motivated ('wanted') to consume milkshake
126 (**Figure 1g**). IBI also kept decreasing across milkshake sessions, suggesting 'wanting' for milkshake
127 increased over sessions (**Figure 1g, right**).

128 We then performed multiple regression analysis to investigate whether *in vivo* calcium activity of NAc
129 D1 and D2 cells during consumption of milkshake across all 5 sessions could predict the level of 'liking'
130 or 'wanting' of the palatable food consumed. This analysis can determine key photometry signal
131 parameters, among the multiple parameters that exist, to predict specific aspects of palatable food
132 consumption. In D1 cells, the minimum and maximum signal values were significant predictors of bout
133 duration ('liking'), explaining around 30% of the variability in bout duration (as shown by the R^2 change
134 of models 1 and 2, **Figure S2a, top right**), with negative and positive betas, respectively. The R^2 change
135 values showed that the higher the initial increase (and to a smaller extent, the lower the subsequent
136 decrease), the longer the bout (**Figure S2a**). In D2 cells, we found that the area under the curve and
137 the minimum signal during the 3 sec following lick onset were significant predictors of bout duration,
138 explaining when combined around 40% of the variability in bout duration (**Figure S2b, top right**). More
139 specifically, the more the D2 cell signal decreases, the longer the bouts are and the higher the 'liking'
140 is (negative beta coefficients) (**Figure S2b**). Interestingly, in a similar analysis of inter-bout interval, IBI
141 ('wanting' – **Figure S3**), the significant predictors in the photometry signals only explained a very small
142 amount of IBI variability and were thus poor predictors overall.

143 Together, these findings show that *ad libitum* consumption of milkshake induces both 'liking' and
144 'wanting' to eat. These two main components underlying palatable food consumption are more
145 positively associated with consuming milkshake than water. However, we detected a dissociation
146 between 'liking' and 'wanting', with the signals of both D1 and D2 cells being more strongly correlated
147 with 'liking' than 'wanting'. Specifically, the initial increase in NAc D1 cell activity at licking onset and
148 the sustained decrease in NAc D2 cell activity during eating are good predictors of 'liking' parameters.
149

150 ***Increasing NAc D2 cell activity increases consumption by increasing 'wanting' despite a decrease in***
151 ***'liking'***

152 We next employed optogenetics to investigate how the roles of NAc D1 and D2 cells differ during *ad*
153 *libitum* palatable food consumption. Based on previous findings (Kravitz et al., 2012; Lobo et al., 2010),
154 we initially hypothesized that activation of NAc D1 cells would lead to increased consumption, whereas
155 activation of NAc D2 cells – counteracting the natural suppression in activity described earlier – would
156 decrease 'liking'. However, it would not necessarily affect overall intake, depending on its effect on the
157 'wanting' aspect of consumption. To test our prediction, we injected the optogenetic Cre-dependent
158 excitatory actuator ChrimsonR or a control GFP virus, into the NAc of D1- or D2(A2a)-cre mice, and
159 implanted optical fibers bilaterally in the NAc (**Figure 2a**). Consistent with our photometry data and
160 our initial prediction, optogenetic activation of D2 cells contingent to eating decreased 'liking'
161 (increased ILI, **Figure 2c**). Unexpectedly, it increased 'wanting' (increased bout number, **Figure 2d**)
162 leading to increased overall consumption (increased number of licks, **Figure 2b**). This suggests that the
163 'wanting' aspect triggered by optical activation overrode 'liking' in driving overall consummatory
164 behavior. Conversely, contrary to our hypothesis, D1 cell activation did not impact overall consumption
165 via effects on either 'liking' or 'wanting' parameters (**Figure 2b-d**). This would mean that sustained
166 activity of D1 cells beyond the naturally occurring transient increase described in **Figure 1d-e** does not
167 promote further 'liking', nor does it induce 'wanting'.

168 Next, we sought to test whether silencing NAc D1 and D2 cells using the inhibitory opsin eNpHR3.0
169 could also influence consumption of a palatable food. We hypothesized that D1 cell silencing would
170 induce a decrease in consumption, while D2 cell silencing would prolong consumption. However,
171 silencing D1 cells did not lead to a significant decrease in overall consumption (**Figure 2b**), but induced
172 a significant increase in 'liking' (decreased ILI, **Figure 2c**) with no significant effect on 'wanting' (**Figure**
173 **2d**). The influence of inhibitory post-ingestive mechanisms such as satiation, which increase as
174 ingestion proceeds, could have been a confounding factor to D1 cell inhibition, because they may
175 already have reduced lick number and bout number to their floor (D'Aquila, 2010; Sclafani and Ackroff,
176 2004).

177 Inhibition of D2 cells did not lead to any significant difference in lick or bout number compared to
178 controls (**Figure 2c,d)**, suggesting that a further decrease of the natural suppression in activity
179 observed during consumption (**Figure 1d,e**) may not be possible and therefore may not yield any
180 stronger consummatory effect.

181 Taken together, these results show that during *ad libitum* consumption, only D2 cell activation leads
182 to an overall increase in eating, and this is mediated by an increase in 'wanting', despite a concomitant
183 decrease in 'liking'. However, these findings are limited because an *ad libitum* paradigm only examines
184 baseline motivation, and is not designed to evaluate motivation to work for a reward. Therefore, we
185 next probed the role of D1 and D2 cells in a different motivational task designed to interrogate
186 'wanting'.
187
188 ***NAc D1 and D2 cell activity increases with anticipation during a motivational task to retrieve***
189 ***palatable food, which is followed by a D2 cell activity decrease upon consumption onset***

190 To probe 'wanting' further, we paired reward with a cue, which allowed us to investigate the
191 attribution of 'incentive salience' to the cue. We exposed mice to a Pavlovian 'motivation to retrieve a
192 reward' task in which they received up to 15 milkshake rewards announced by a sound cue, which they
193 had to retrieve from a food magazine. This did not require the mice to develop learning strategies. This
194 allowed us to monitor three parameters: the number of entries to the food magazine, the time to
195 retrieve and consume the milkshake after cue onset (latency), and the length of food-magazine entries.
196 (**Figure 3a,b**).

197 Photometry recordings showed sustained increased activity in D2 cells during the approach phase
198 (after milkshake delivery cue and before consumption; **Figure 3c, right**). D1 cells, however, only
199 transiently increased their activity following milkshake delivery cue (**Figure 3c, left**). An important
200 distinction in the behavior of D1 cell activity must be made: in contrast to the *ad libitum* paradigm
201 where D1 cell signal increased prior to licking onset, here, in a motivational task, the increase in signal
202 was specifically aligned to the reward delivery cue. During food magazine visits, i.e., during milkshake
203 consumption, the neural dynamics observed were similar to the first *ad libitum* study, with a sustained
204 decrease in the activity of D2 cells and only a small initial increase in D1 cells (**Figure 3d**).

205 As in the first experiment, we were interested to see if the natural D1 and D2 cell activity recorded
206 with photometry holds any predictive information about components of consummatory behavior of a
207 palatable food. Correlation analysis of 'wanting' behavioral parameters and D2 cell activity around food
208 magazine visit after a milkshake delivery cue revealed that latency can be well predicted by the average
209 D2 cell activity before the food magazine visit, and by the standard deviation (Std) of the pre-food
210 magazine visit – **Figure S4a**). The higher the signal before the food magazine visit, the smaller the
211 latency. This correlation analysis also showed that the number of food magazine visits can be well
212 predicted by the median signal before the food magazine visit (**Figure S4b**). The higher the signal pre-
213 food magazine visit, the more visits occurred. The average length of a food magazine visit was also

214 found to be well predicted by the minimum of D2 cell activity *during* consumption, and by the standard
215 deviation of the signal before the visit (**Figure S4c**); the lower the minimum, the longer the food
216 magazine visit.

217 Therefore, latency and number of visits correlated with D2 cell signals *before* visits occurred, while the
218 length of a visit was determined by the signals *during* the visit. These data also suggest that suppression
219 of D2 cell activity *during* feeding favors consumption, which was investigated further in the next
220 experiment.

221 ***Inhibition of D2 or D1 cells reduces motivation to retrieve a reward, while activation of only D1 cells***
222 ***enhances motivation***

223 Because both D1 and D2 cells were modulated during the motivation task (**Figure 3c,d**), we aimed to
224 causally test the effect of cell-type-specific activation and inhibition on motivated behavior ('wanting').
225 We optogenetically manipulated NAc D1 and D2 cells at either reward (milkshake) delivery cue or at
226 food magazine visit (first visit following a delivery cue) (**Figure 3a,b**). D2 cell silencing at either
227 milkshake delivery cue, or at food magazine visit significantly decreased number of food magazine
228 entries (**Figure 3e,f**), and inhibiting D2 cells after milkshake delivery cue significantly decreased the
229 average length of the subsequent food magazine visit (**Figure 3i**). These results are in line with our
230 photometry findings (**Figure 3c, right**) suggesting a role for D2 cell activity in the anticipation of a
231 reward ('wanting'). Optogenetic activation of D1 cells at either milkshake delivery cue or at food
232 magazine visit significantly increased the number of food magazine entries (**Figure 3e,f**). Moreover,
233 silencing D1 cells during the reward delivery cue increased the average latency (**Figure 3g**). This means
234 that inhibiting D1 cells during the approach phase delayed the approach. Thus, the behavioral results
235 obtained with D1 cell activation were expected based on the photometry findings (**Figure 3c,d, left**):
236 increase in D1 cell activity may reflect a higher incentive motivation to approach the palatable food,
237 causing the mice to be more focused on obtaining the reward and being more reactive to the cue.

238 Together, our data indicate that both D1 and D2 cell activity during the approach phase of a palatable
239 food (preceding consumption) encode incentive motivation or 'wanting'.
240
241 ***Modulation of NAc D1 and not D2 cells influence hedonic preference learning (hedonic shifting)***

242 We showed in our two previous experimental paradigms that consumption of a palatable food
243 (milkshake) elicits, as expected, 'liking' responses. We investigated whether pairing milkshake,
244 constituting a conditioned stimulus (CS), with optogenetic stimulation (unconditioned stimulus, US) of
245 either D1 or D2 cells could shift natural preference for one flavor to another. We hypothesized that
246 optogenetic stimulation of either population would establish a learned preference for the least
247 innately preferred milkshake flavor by increasing the hedonic value ('liking') of the CS (milkshake).

248 To assess innate preference, mice were first presented with two bottles, each containing a different
249 flavor of milkshake, but with identical nutritional composition ('pre-test'). The least preferred flavor
250 was then coupled to optogenetic stimulation of either D1 or D2 cells during a daily conditioning session
251 over several days, while the preferred flavor was presented unpaired with optogenetic stimulation in
252 a separate daily session (**Figure 4a**). Following conditioning, we performed a 'choice-test' to determine
253 if the paired flavor was now able to trigger incentive motivation ('wanting') and become equally, or
254 possibly more, attractive than the previously preferred, unpaired flavor. During conditioning, increased
255 consumption from the paired milkshake flavor over the unpaired one would indicate a hedonic change
256 ('liking'). During the choice test, with respect to the 'liking'/'wanting' hypothesis, disruption of a
257 reward-oriented behavior by optogenetic manipulation of the NAc could, in principle, be due to a
258 disruption of either 'liking' or 'wanting' or, presumably, a combination of both.

259 During optogenetic activation of D2 cells, consumption of the paired and unpaired flavors across the
260 conditioning phase did not differ (**Figure 4c**). Note that during this conditioning phase, each flavor was
261 presented in separate sessions, hence the absence of difference between the amounts consumed,
262 unlike the difference observed in the 'pre-test' (**Figure S5a, right**). However, optogenetic stimulation
263 of D1 cells led to a gradual increase in the consumption of the paired flavor, so that the mice consumed
264 higher amounts of milkshake in the late paired conditioning sessions compared to GFP controls (**Figure**
265 **4b**). During the choice-test, an increase in consumption of the paired flavor compared to the pre-test
266 levels was noted while a decrease was observed in the unpaired flavor, leading to significantly different
267 'choice-test – pre-test' deltas between the two flavors (**Figure 4d, left**). There was no significant change
268 in preference in control GFP mice (**Figure 4d, right**), suggesting repeated exposure to the flavors did
269 not alter preference.

270 In addition, we used lick microstructure analysis to assess potential changes in 'liking' and 'wanting'
271 produced by flavor preference conditioning. During the conditioning sessions, stimulation of D1, but
272 not D2 cells increased 'liking' (increased lick duration and decreased inter-lick duration) over time
273 (**Figure 5a,b, left inserts**). This indicates an increased palatability of the flavor paired with D1 cell
274 optostimulation, which is of relevance because conditioned increases in palatability have been shown
275 to be important for driving overeating (Johnson, 2018b). Moreover, inter-bout interval decreased over
276 conditioning sessions (**Figure 5d, left insert**), indicating an increase in 'wanting', while motivation to
277 consume the unpaired flavor decreased over sessions, as indicated by decreasing bout numbers and
278 initially increasing IBI (**Figure 5c,d, middle inserts**). During the choice-test, when both flavors were
279 once again presented simultaneously (with no optogenetic stimulation on either of the flavors at this
280 stage), licking microstructure indicated an increased 'liking' of the paired flavor (higher lick duration,
281 and lower ILI, **Figure 5a,b, right inserts**) – effects not observable in the unpaired flavor. 'Wanting' was
282 also increased for the paired flavor (increased bout number, decreased IBI, **Figure 5c,d, right inserts**)
283 and decreased for the unpaired flavor (**Figure 5d, right insert**).

284 These results show an overall preference for the milkshake flavor coupled with D1 cell stimulation,
285 thus demonstrating an important role for D1 cells in promoting the consumption of preferred palatable
286 foods.

287

288 **Discussion**

289 Our study reveals distinct patterns of NAc D1 and D2 cell activity during different behavioral paradigms
290 intended to assess different psychological processes of hedonic behaviors: hedonia ('liking' a food) and
291 motivation ('wanting' the food). We found that NAc D1 and D2 cells do not act as a single homogeneous
292 population in regulating these processes. In a free palatable food consumption paradigm a dual role
293 of NAc D2 cells is linked to both 'liking' and 'wanting', where increased D2 cell activity signals 'wanting'
294 and decreased activity enhances 'liking', with the magnitude of natural inhibition following palatable
295 food consumption being a good predictor for 'liking'. In contrast, D1 cells promoted 'liking' during free
296 consumption. In the operant task, we showed that NAc D1 cell activity after cue and right after eating
297 onset drives the motivation to retrieve palatable food, while reduced activity of D2 cells after food
298 magazine visits drives the motivation to remain in the magazine. Lastly, we show that NAc D1 cell
299 stimulation induced hedonic shifting in a Pavlovian task and overconsumption of palatable food. This
300 effect was specific to NAc D1 cells, as stimulation of NAc D2 cells during opto-paired food conditioning
301 had no effect across conditioning days, or during the choice test (See results summary, **Figure 6**).

302

303 **The role of NAc D1 and D2 cells during periods of free consumption of palatable food**

304 The NAc is part of a neural circuit governing consumption of palatable food (Kelley et al., 2005) and
305 neuronal subpopulations in the NAc shell encode rewarding stimuli and palatability (Roitman et al.,
306 2005; Villavicencio et al., 2018). Pharmacological manipulations in rodents and in hyper-dopaminergic
307 knockout mice showed that 'wanting' and 'liking' sucrose could be separated with an increase in
308 'wanting' but a constant decrease in 'liking' (Berridge and Valenstein, 1991; Pecina et al., 2003; Smith
309 and Berridge, 2005; Wyvell and Berridge, 2000) and that 'wanting' was increased with prolonged
310 sucrose consumption (Avena et al., 2005). Here, using photometry and optogenetics, we showed a
311 differential role between NAc D1 and D2 cells during free consumption of a palatable food. Activity of
312 NAc D2 cells was linked to both 'liking' and 'wanting' but with opposing actions; high D2 cell activity
313 signaled 'wanting', and low D2 cell activity enhanced 'liking'. Importantly, the increased activity in D2
314 cells was similar across milkshake sessions, whereas inhibition of this signaling following the onset of
315 milkshake consumption was greater after repeated milkshake sessions (**Figure 1e**). A multiple
316 regression analysis indicated that this magnitude of inhibition (area under the curve) after
317 consumption was a good predictor for 'liking' (bout duration, **Figure S2b**). Previous findings showed
318 that during consumption of sucrose, a decrease in firing rate in a subset of NAc neurons immediately
319 before and during a lick bout is required to initiate and maintain consummatory behavior (Taha and
320 Fields, 2005; Taha and Fields, 2006). The present study supports the idea that sustained D2 cell
321 depression maintains the actions resulting in palatable food consumption and hedonic effects
322 (Carlezon and Thomas, 2009; Krause et al., 2010; Roitman et al., 2005; Taha and Fields, 2005; Taha and
323 Fields, 2006). We showed that D1 cells on the other hand promote 'liking' right after consumption
324 onset. D1 receptors have been shown to play a role in incentive learning (Beninger, 1983), and so may
325 be important during the initial phase of instrumental learning, when reward is novel and unpredictable
326 (Schultz, 1998) and in the primary response to drugs of abuse (Self, 2014). This may explain why D1
327 cell activity was highest during the first milkshake session (**Figure 1e**). Supporting this interpretation,
328 in a multiple regression analysis, the maximum D1 cell signal following lick onset was a significant
329 predictor of bout duration; the larger the initial increase, the longer the licking bout (**Figure S2a**).
330

331 **Contribution of NAc D1 and D2 cells to reward-associated cues**

332 D1 and D2 cells displayed distinct temporal activity during food magazine visits in the motivational
333 task; D1 cell activity increased after onset of the food delivery cue, whereas D2 cell activity was
334 increased before, but suppressed after, food magazine entry (**Figure 3c,d**). These results are in line
335 with previous findings from drug-addiction literature showing that NAc D1 cells increase their activity
336 before entering a drug-paired chamber, while D2 cells decrease their activity after (Calipari et al.,
337 2016). Thus it may be that in our task, D1 cell activity drives the motivation to enter the food magazine,
338 while reduced activity of D2 cells after food magazine visit onset would drive the motivation to remain
339 in the magazine. This might suggest that D1, but not D2 cell signaling drives reward- and
340 reinforcement-related behaviors. In particular, we show a causal role of temporally specific cue-
341 elicited D1 cell signaling, which can further demonstrate the role of this cell type in motivation to
342 retrieve rewards. Activation of D1 cells when animals entered the food magazine or at cue-onset
343 increased the number of food magazine visits. This may imply that the D1 cell signals guide animals to
344 seek reward, whereas suppressing D1 cell activity at either food magazine or cue onset doesn't lead to
345 any changes in the number of visits compared to the control group. However, inhibition of D1 cells at
346 cue onset delayed approach. D1 cell activity may thus drive higher incentive motivation to reach the
347 palatable food (reward) by increasing focus on the cue, and amplifying 'wanting' for a particular
348 incentive target (the high-caloric palatable food). This could contribute to intense urges to indulge in
349 those foods, leading to overeating. Attenuating or eliminating D1 cell signals, may decrease the
350 associative learning of environmental stimuli (reward-associated cue) with reward. Our experiments
351 were conducted once the animals learnt the association between cue and reward and the neuronal

352 response that was measured was to the reward-predictive cue (Schultz, 1997; Wise and Rompre,
353 1989). Pharmacological studies investigating the role of NAc D1 and D2 receptors in response to drugs
354 of abuse have also shown that both D1 and D2 receptors have a role in associative learning (Graham
355 et al., 2007). Dopamine responses to cues predicting drug availability can lead to drug seeking behavior
356 and over time these responses to drug-associated cues are dampened (Graham et al., 2007; Self, 2014).
357 Our findings that D1 cells in the context of associative learning are in line with previous publications
358 demonstrating that D1-like receptors are essential in reward related learning including instrumental
359 learning (Ranaldi and Beninger, 1995; Sutton and Beninger, 1999) as well as driving motivation to
360 action (Fibiger, 1993). More specifically, systemic administration as well as local infusion into the NAc
361 of dopamine D1-like receptor antagonists have been shown to attenuate food-reinforced lever
362 pressing and to dampen the rewarding effects of palatable food (Aberman et al., 1998; Beninger et al.,
363 1987; Cousins et al., 1994; Koch et al., 2000; McDougall et al., 1991). Furthermore, optogenetic
364 activation of NAc D1 cells enhanced drug-induced conditioned place preference (Koo et al., 2014; Lobo
365 et al., 2010). Pharmacological activation of NAc D1 receptors produced similar findings, showing that
366 D1 cell activity drives incentive motivation to consume food and drug of abuse (Natsubori et al., 2017;
367 Schmidt et al., 2006; Wakabayashi et al., 2004).

368 On the other hand, D2 cell silencing at the time of milkshake delivery cue and at the time of food
369 magazine visit significantly decreased the number of food magazine entries. Also, silencing natural D2
370 cell activity after milkshake delivery cue also significantly decreased the average length of a
371 subsequent food magazine visit. These observations suggest a role for D2 cell activity in the
372 anticipation of a reward ('wanting'). These effects on motivation, although inconsistent with some
373 previous findings which suggested that D2 cells are associated with negative rather than positive
374 valence events (Hoek, 2016; Kravitz et al., 2012; Lobo et al., 2010), were in line with other more recent
375 findings which have shown that D2 cell activation increased motivation to obtain a reward in a
376 progressive ratio task (Soares-Cunha et al., 2018; Soares-Cunha et al., 2016b).
377

378 **NAc D1 cell stimulation drives hedonic shifting and overconsumption of palatable food**

379 In the hedonic-shifting choice test after the final optogenetic stimulation pairing, D1- but not D2-cell
380 activity significantly increased preference for the milkshake flavor that was previously least preferred.
381 This indicates that D1 cell optostimulation induced a hedonic preference via an association between
382 the flavor and the rewarding effects of stimulation (**Figure 4d**). This was not observed in control mice.
383 In addition, during the conditioning sessions of D1 cell optostimulation, an increase in time spent
384 consuming the opto-paired flavor was observed across sessions (**Figure 4b**). This may reflect a hedonic
385 experience which alters associative hedonic/'liking' processes by selectively enhancing D1 cell activity.
386 This facilitates the association between flavor and opto-stimulation and promotes hedonic shifting that
387 in turn leads to increased consumption of that flavor as observed in the choice test. This effect was
388 specific for NAc D1 cells; stimulation of NAc D2 cells during conditioning had no effect either across
389 conditioning days, or on the choice test.

390 In this experiment we were able to induce a learned shift in preference for a particular flavor when D1
391 cell optogenetic stimulation was coupled with a less preferred flavor. This is based on a Pavlovian
392 association formed between NAc D1 cell activation (US) and the least preferred milkshake flavor (CS),
393 which changed the taste preference to that flavor. This suggests that hedonic 'liking' of a flavor
394 stimulus can be determined not only by flavor itself, but also by the relevant 'brain' state at the time
395 of formation of Pavlovian associations, for example by a change in perceived palatability. In a similar
396 manner, in the context of drug addiction, it was shown that NAc D1 cell activity preceding entry to the
397 drug-paired compartment in a conditioned place preference test is needed for drug-associative
398 learning to take place (Calipari et al., 2016; Lobo et al., 2010).

399 That our findings are not always in line with previous literature may reflect the discordance between
400 previous studies themselves: the precise NAc subregion targeted plays a role in the results obtained
401 (de Jong et al., 2019; Yang et al., 2018), the pattern (frequency, length) of the optogenetic modulation
402 pattern also has an impact on the direction of the effects (Soares-Cunha et al., 2020), and the
403 experimental paradigm also plays a crucial role depending on the cues and responses it entails (Nicola
404 et al., 2004).
405
406 **Clinical implications for the role of NAc D1 and D2 cell activity in 'liking' versus 'wanting' in**
407 **overconsumption and eating disorders**

408 Our findings may carry potential implications for human eating disorders. 'Hedonic eating' does not
409 necessarily involve pleasure, but can also be driven by a motivation to eat which can be dissociated
410 from the drive caused by a nutrient deficit (Lowe and Butryn, 2007; Lutter and Nestler, 2009). It is
411 already well known that in humans, high-calorie foods, especially sweet and fatty ones, promote
412 overconsumption. The incentive theory of 'liking'/'wanting' was significant in the study of food intake
413 regulation, modelling associations between the function of the reward system and feeding or obesity.
414 Studies tried to show that different neural mechanisms are responsible for 'wanting' a certain food
415 than 'liking' it. For more than a decade, human studies of obesity have used 'liking' and 'wanting' to
416 try to better understand 'wanting' food can differ in some individuals, and override 'liking' to cause
417 excessive cue-trigged 'wanting' (craving) that will drive overeating and lead to obesity (Berthoud et al.,
418 2011; Devoto et al., 2018; Gearhardt et al., 2014; Schulte et al., 2017; Stice and Yokum, 2016). Incentive
419 sensitization theory, originally postulated for drug addiction (Robinson and Berridge, 1993), was then
420 applied to eating disorders to try to explain how some individuals may experience 'wanting' to eat
421 palatable foods (Berridge, 1996, 2009). Neuroimaging studies suggest that in humans diagnosed with
422 obesity or binge-eating disorders, overeating is triggered by visual cues associated with palatable food
423 (Frankort et al., 2011; Rothemund et al., 2007; Stoeckel et al., 2008). For example, increased brain
424 activity in response to palatable foods was positively correlated with self-reported craving rates or
425 'wanting' to eat compared to healthy controls (Polk et al., 2017; Simon et al., 2016; Wolz et al., 2017).
426 This evidence is very similar to findings in individuals suffering from drug addiction (Cameron et al.,
427 2017; Carter et al., 2016; Devoto et al., 2018; Fletcher and Kenny, 2018; Volkow et al., 2017; Westwater
428 et al., 2016).

429 To our knowledge, our study is the first to approach aspects of NAc dependent reward consumption
430 using such a multi-faceted approach. We incorporated observation, inhibition and excitation of NAc
431 D1 and D2 cells, multiple behavioral paradigms investigating different aspects of 'liking' and 'wanting',
432 including analysis of lick microstructure, and multivariate analysis. In summary, we show that NAc D1
433 cell activity plays a role in reinforced behaviors induced by palatable foods in an operant paradigm or
434 a Pavlovian association test. However, in a palatable food free-access paradigm D1 cells encode only
435 the hedonic property of the palatable food. NAc D2 cells on the other hand, have a role in the drive to
436 retrieve food, showing elevated activity prior to eating onset in a motivational task. Once eating starts,
437 suppressed D2 cell activity allows for prolonged eating episodes. This would support the idea that
438 evolutionary processes led to these two cell populations that can independently and mutually
439 influence hedonic eating. D1 cells are prone to lead to a maladaptive cycle of increased 'liking' and
440 'wanting', thus driving overeating, while D2 cells cannot reinforce both processes and are faced with a
441 tradeoff between increasing 'wanting' by being more active or allowing 'liking' by remaining silent.
442 These findings, which temporally distinguish between the different psychological processes of food
443 reward and reinforcement, support the incentive salience hypothesis (Berridge, 1996; Berridge and
444 Robinson, 1998). They may assist the design of future studies and improve diagnostic criteria of
445 addiction and eating disorders.

446 ## Materials and Methods

447 ### Animals and housing conditions

448 All animal procedures were carried out in accordance with the Animal Welfare Ordinance (TSchV
449 455.1) of the Swiss Federal Food Safety and Veterinary Office and were approved by the Zurich
450 Cantonal Veterinary Office. Mice were kept on a reversed 12-h/12-h light/dark cycle and provided with
451 standard chow pellets *ad libitum*. In order to avoid food deprivation for behavioral testing, mice were
452 provided with 2% citric acid in water *ad libitum* in their home cage (Urai et al., 2021). All experiments
453 were performed during the dark phase. Both adult male and female mice were used for the
454 experiments (see Table 1) and mice were at least 7 weeks old before surgeries were performed. As no
455 differences were found in the results between sexes, males and females were pooled.

456 ### Viruses and surgeries

457 For specific targeting of D1 cells and D2 cells, D1-cre (B6.FVB(Cg)-Tg(Drd1-cre)EY262Gsat/Mmucd) and
458 Adora2a-cre (Adora2aTg(Adora2a-cre)KG139Gsat, originally obtained from the Jackson Laboratory)
459 mouse lines were used, respectively. For photometry recordings, 6 mice from each line were injected
460 bilaterally with a pAAV9-CAG-Flex.GCaMP6s.WPRE.SV40 virus (Addgene, titre ≥ 1×10^{13} vg/mL) in the
461 medial NAc shell (D1-cre and D2(A2a)-cre mice; bregma +1.4mm, midline ± 1.35mm, depth 3.9 mm, at
462 an angle of 10°) and were then implanted bilaterally with optic fibers (200 µm diameter, NA 0.37, Doric
463 Lenses), using the same coordinates (except that the depth of the fiber tip was reduced to 3.8mm).

464 For optogenetic modulation, mice (see Table 1) were injected with pAAV9-EF1a-DIO-ChrimsonR-
465 mRuby2-KV2.1-WPRE-8V40 (Addgene, titer: $1x10^{13}$ vg/mL) or ssAAV-9/2-hEF1a-dlox-
466 eNpHR3.0_iRFP(rev)-dlox-WPRE-hGHp(A) (ETHZ/UZH Viral Vector Facility, titre: 7.2 x 10^{12} vg/mL) or
467 pAAV-flex-GFP (Addgene, titer: 2.5 x 10^{13} vg/mL) for activation, inhibition and control respectively.
468 Viruses and fiber implants were positioned at the same coordinates as the photometry cohort. Virus
469 expression was assessed using standard histology methods.

470 For stereotaxic surgeries (Kopf Instruments), a small craniotomy of 0.5 mm in diameter was performed
471 above the injection sites using a microdrill. Injections were performed using a Nanoject III injector and
472 glass capillaries (Süd-Laborbedarf Gaunting); 120nL of virus were injected on each side at a rate of
473 1nL/sec before optic fibers were slowly inserted and fixed to the skull using Super-Bond (Sun Medical
474 Co. Ltd). Mice were allowed 6 weeks to recover before starting experiments.
475
476 *Table 1 : Mice implanted in each group and line.*

	Adora2a-cre	**D1-cre**
Photometry – GCaMP6s	6 (4m + 2f)	6 (3m + 3f)
Optogenetics – ChrimsonR	5 (3m + 2f)	6 (3m + 3f)
Optogenetics – eNpHR3.0	6 (3m + 3f)	6 (3m + 3f)
Optogenetics – GFP	6 (3m + 3f)	6 (3m + 3f)

477

478 ### Photometry recordings

479 Photometry recordings were performed using a camera-based bundle-imaging fiber photometry
480 system (Doric Lenses) using interleaved stimulation illumination produced by two LEDs (2 excitation
481 wavelengths: 405 nm and 465 nm / 2 cycles, Resolution 1200 x 1200, Effective Sampling Rate 20 Hz).
482 Fluorescence produced by 405-nm excitation provided a real-time control for motion artifacts.
483 Recordings were performed using the Doric Neuroscience Studio software.

484 **Optogenetics**

485 Optogenetic activation of D1-ChrimsonR and D2-ChrimsonR cells was performed using a red laser
486 (635nm, Laserglow Technologies). Laser output frequency was 4-ms pulses at 20Hz, driven by an
487 Arduino board. Optogenetic inhibition of D1-eNpHR3.0 and D2-eNpHR3.0 cells was achieved with a
488 yellow laser (589 nm, Laserglow Technologies). The laser delivered a constant light when inhibition
489 was triggered and ramped down at the end of an inhibition bout to avoid neuronal activity rebound.
490 D1-GFP and D2-GFP control mice were split evenly between opto-inhibitory and opto-excitatory
491 stimulation patterns.

492 The lasers were connected to the bilateral fiber implants using a 1x2 fiber-optic rotary joint (Doric
493 Lenses) and yielded an output power of ~8mW at the end of each fiber tip. Laser pulse onset
494 parameters are described in each relevant experiment section.

495 **'Motivation to retrieve a reward' task**

496 *Pre-surgery screening:* Prior to surgeries, both photometry and optogenetic cohorts mice were
497 screened in this simple reward delivery task. Mice were habituated to the experimental chambers
498 (Bussey-Saksida Mouse Touch Screen Chambers installed in sound-proof chambers, Lafayette
499 Instruments) for 20 min per day for two days. The task schedule was designed and controlled by ABET
500 II software (Lafayette Instrument). On three consecutive days, mice could receive up to 15 milkshake
501 deliveries (Energy milk, strawberry flavor, Emmi Schweiz AG; 20 µL/delivery; to which mice had been
502 previously habituated in the home cage) during a maximum of 30 min. The session ended when
503 whichever of the 15 deliveries or 30 min came first. The inter-trial interval varied pseudo-randomly,
504 30 ± 10 sec, counted from the moment a mouse exited the food magazine. Only those able to perform
505 the task in 10 min or less were implanted. The house light (white light) was on during the sessions. The
506 tray light was turned on at each milkshake delivery and turned off when the mouse visited the food
507 magazine. Milkshake delivery was associated with a sound cue.

508 *Photometry cohort:* The same paradigm used for pre-surgery screening was run again on 3 consecutive
509 days while photometry signals were being recorded (see *Photometry Recordings* section for details).

510 *Optogenetics cohort:* Here, the task structure was similar to the pre-surgery screening task, except the
511 mouse performed the task over two days only. The first day was identical to the pre-screening task
512 and served as a baseline. On the second day, the task was identical but 10s of optogenetic stimulation
513 either started from milkshake delivery cue (D1-eNpHR3.0, D2-eNpHR3.0 and GFP controls) or started
514 at food magazine visit onset (D1-ChrismonR, D2-ChrismonR, and GFP controls). After a few-weeks
515 wash-out period this 2-day experiment was repeated, only this time mice which had received
516 optogenetic stimulation during milkshake delivery now received it during the food magazine visit and
517 *vice versa*. In these tasks the activation and inhibition patterns were the same as those described in
518 the *Optogenetics* section, repeated continuously over the 10 sec each stimulation lasted for. Instead
519 of lasers, light was delivered using a PlexBright optogenetic stimulation system (Plexon Inc.) consisting
520 of an Optogenetic Controller (Plexon Inc.) and orange LEDs for activation (compact LED, 620 nm, Plexon
521 Inc.) or lime LEDs for inhibition (compact LED, 550 nm, Plexon Inc.). The output at the end of each
522 patchcord (200/230 µm, 0.5NA, Plexon Inc.) was ~5mW. The patterns were designed in and controlled
523 by Radiant software (Plexon Inc.) which was itself triggered by ABET II software during the task.
524

525 ***Ad libitum* consumption experiment**

526 Mice were first habituated to spring-based tip bottles (EBECO) in the home cage using water.
527 Habituation to the arenas (20x20cm^2 arenas, Habitest modular mouse test cage, Coulbourn
528 Instruments) and patchcords (200 µm, low-autofluorescence, Doric Lenses) was performed on 2

529 consecutive days before the experiment started. The test cages were placed in closed, sound-
530 attenuating chambers (Coulbourn instruments) and illuminated using infra-red light only. Licks were
531 detected using a capacitive sensor (Capacitive Touch Breakout, Sparkfun) and recorded at 500Hz
532 throughout the sessions using a National Instruments board (USB-X Series DAQ, NI-6343) and the
533 LabView software (National Instruments). Weights consumed were recorded at the end of each
534 session. Mice were not food deprived for these experiments.

535 *Photometry cohort:* On experimental days, mice were placed in the arenas for 20-min long sessions
536 (one session/day for each mouse) with free access to one 'spring' bottle containing water (days 1-3),
537 strawberry milkshake (days 4-8). Concomitant photometry signals were recorded as described in the
538 *Photometry Recordings* section above.

539 *Optogenetics cohort:* Over a 20-min session with access to milkshake, laser stimulation started
540 (according to the patterns described in the *Optogenetics* section) every time a mouse started licking
541 and continued for the duration of licking.

542 **Hedonic shifting experiment**

543 For this experiment only ChrimsonR mice (and their respective GFP controls) were used. Mice were
544 habituated in the home-cage to two novel flavors of milkshake which were matched for their calorie
545 and macro-nutrient contents: a chocolate-flavored and a vanilla-flavored milkshake ('Milk Choco
546 Mountain' Protein, 'Vanilla Drive', respectively, Chiefs AG Schweiz).

547 *Pre-test phase:* this phase served to test the natural preference of mice for one flavor over the other.
548 Testing was performed over two consecutive days with 20-min sessions in the same Coulbourn arenas
549 as described in the *Ad libitum consumption Experiment* section. Two 'spring' bottles (EBECO), each with
550 a different flavor of milkshake were freely available. The positions of the bottles in the cage were
551 counterbalanced across mice. Licking and amounts consumed were recorded as described in the *Ad*
552 *libitum consumption experiment* section. The time spent licking at each bottle on the second day of
553 the pre-test phase was used to determine which was each mouse's preferred flavour.

554 *Conditioning phase:* Two 10-min conditioning sessions were run, one in the morning, and one in the
555 afternoon for 7 consecutive days. In each of these sessions only one flavor was available at a time, and
556 the order of the sessions was swapped each day (see **Figure 4a**). Licking at the mouse's initially-less-
557 preferred flavor triggered optogenetic activation for as long as the mouse kept licking, using the
558 patterns and equipment described in the *Optogenetics* and *Ad libitum consumption experiment*
559 sections respectively.

560 *Choice-test phase:* 24 hours after conditioning, mice were assessed for their preference again. During
561 the choice-test, both flavors were present simultaneously for 20-min with no optogenetic stimulation
562 on either flavor.

563 Throughout the conditioning and choice-test phases, but not for the initial preference pre-test phase,
564 mice were mildly food-deprived.
565
566 **Data Analysis**

567 ***Licking microstructure analysis***

568 Based on previous literature (Johnson, 2018a; Robinson and McCool, 2015), licking behavior was
569 analyzed by looking at the following parameters:

570 - Single lick durations, defined as an uninterrupted high signal.

571 - Inter-lick intervals (ILI), defined as an uninterrupted low in the signal of up to 250ms. An upper
572 boundary was set in accordance to Johnson (2018a), to distinguish them from inter-bout intervals.

573 - Number of licking bouts, a bout was defined as a licking episode of at least 500ms, allowing for
574 interruptions within a bout of up to 250ms.

575 - Licking bout duration, length of a given bout in seconds.

576 - Licking bout size, number of individual licks within a given bout.

577 - Inter-bout intervals (IBI), defined as interruptions in licking of more than 250ms allowing for licking
578 occurrences of up to 250ms, i.e. a short lick isn't considered a bout and thus does not interrupt a given
579 IBI.

580 For the regression analysis (see below), licking bouts had a slightly more inclusive definition, starting
581 with at least 100 ms of licking and allowing for pauses of up to 1 sec.

582 ***Multiple regression analysis***

583 Correlations between photometry signals and 'liking'/'wanting' parameters were performed using the
584 SPSS (IBM Corp. Version 28) linear regression tool. A subset of 8 parameters depicting the photometry
585 signal around a licking bout onset was selected: maximum, minimum and area under the curve (AUC)
586 in the 5 secs following lick onset; mean and median signal over the 3 sec before lick onset; mean and
587 median signal over the 3 sec following lick onset and standard deviation of the non-z-scored signal in
588 the 5 sec preceding lick onset. Because aiming to fit too many variables in a regression model can lead
589 to spurious results, we used all parameters simultaneously in a first step only, to assess correlations
590 between predictors and see which predictors had the highest and most significant correlations with
591 the outcome variable. If predictors were highly correlated between themselves (Pearson's r >8.5,
592 VIF>10, tolerance<0.1, checking expression of each predictor in eigen vector base) then only the
593 predictor with the most significant correlation with the outcome variable was kept. Then, in a second
594 step, another linear regression was performed keeping only the non-collinear predictors. A final block-
595 wise (hierarchical) regression was done keeping only the predictors that showed significant
596 correlations with the outcome variable and/or model coefficients significantly different from 0. This
597 final block-wise approach – which started with the predictor yielding the highest significant
598 standardized model parameter (β) and continued in decreasing order of βs – allowed us to evaluate
599 from which predictor adding one more predictor to the model did not yield better predictive power
600 (looking at the p value associated to the F-ratio change). Predictors whose addition to the model gave
601 a significant F-ratio change (i.e., improved the model) are reported in **Figures S2-4**. Case-wise
602 diagnostics allowed us to evaluate the adequacy of our dataset, namely whether 95% of data points
603 had standardized residuals within ±2, and around 1% of the sample at most had standardized residuals
604 over 2.5. Assumptions of the model (absence of heteroscedasticity, linearity) were evaluated by
605 plotting standardized residuals against standardized predicted values. Normal distribution was
606 evaluated via the observed vs. predicted cumulative probability plots. The Durbin-Watson index was
607 used to evaluate whether the model's residuals were independent (index should be as close to 2, and
608 at least >1 and <3). As our aim here was to evaluate which aspects of the photometry signal (i.e.,
609 neuronal activity) best predicted 'liking' and/or 'wanting', but not to apply the model to future
610 datasets, we did not use bootstrapping. Nonetheless, to assess the validity of our model for a general
611 population, cross-validation was performed using the 'adjusted R^2 value' – a value as close as possible
612 to R^2 indicated that the model generalizes well to the population beyond the sample data.

613 ***'Motivation to retrieve a reward' task analysis***

When analyzing photometry signals around MS delivery, trials where the mouse entered the food magazine within 5 sec after MS delivery cue were not included so that signals did not include MS consumption, but only MS anticipation (**Figure 3c**). For analyses of signals around food magazine visits, trials for which the mouse exited the food magazine just after entering it were discarded (**Figure 3d**). When calculating the average food magazine visit length, visits of less than 2 seconds were excluded to avoid skewing the mean to short values of very brief – and mostly non-consummatory – visits (**Figure 3i,j**).

Statistical analysis

Data was analyzed using Matlab R2019b (MathWorks). All statistical tests and descriptive statistics were performed using the SPSS software (IBM Corp. Version 28). Sex was taken as a factor in the analysis but did not reveal significant differences in all comparisons. Therefore, since sex was not the primary independent variable of interest in this study, and given the lack of statistical differences, we pooled data from both sexes for all further analyses. Tests and their results are presented in the figure legends. Unless otherwise stated, data are presented as mean ± SEM, and a p-value ≤ 0.05 was considered to indicate significance. In the motivation task (**Figure 3e-j**), outlier trials (defined as points whose absolute z-score is >3.29) were excluded from the statistical analysis.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Main Figures and Figure Legends

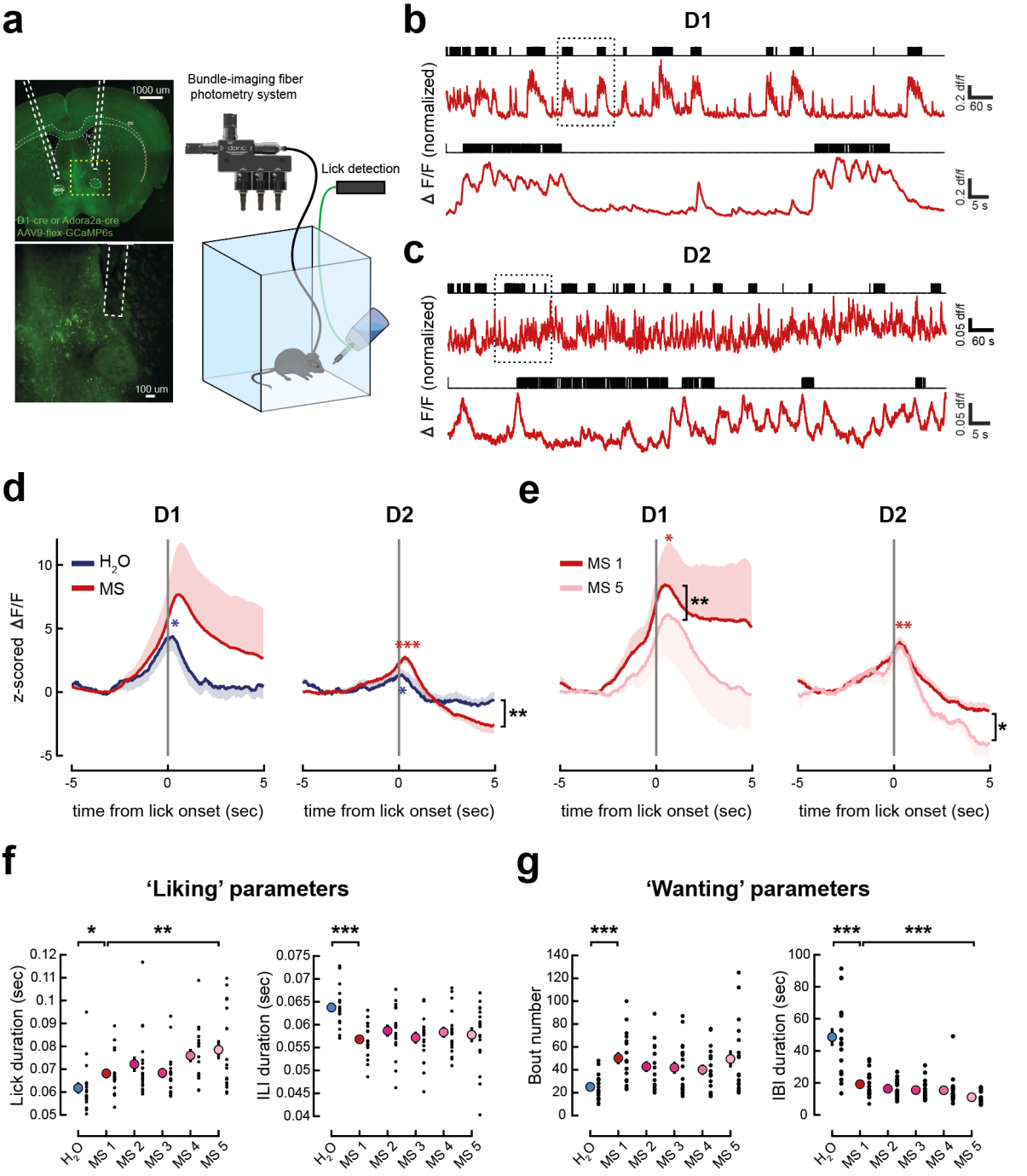


Figure 1. NAc D1 cells exhibit transiently elevated activity, and D2 cells exhibit suppressed activity, during periods of free consumption of palatable food.

(a) Schematic of experimental set-up for photometry recordings and lick detection during an *ad libitum* consumption paradigm. Left: Illustration of fiber placement. Dashed yellow box indicates location of zoom below.

(b) Top: Example photometry recording from a 20-min session with access to milkshake (MS) from a D1-GCaMP6s mouse. Licks indicated by black raster plot above. Bottom: Zoom of photometry signal showing only 2 min indicated by black dotted square above; licks shown above as a black raster plot.

(c) As in (b) but for a D2-GCaMP6s mouse.

(d) Photometry signals around water (H_2O, blue) or MS (red) lick onset, averaged over all licking bouts in D1-GCaMP6s mice (left, N=6) and D2-GCaMP6s mice (right, N=5). MS signals are an average of 5 sessions. Mean

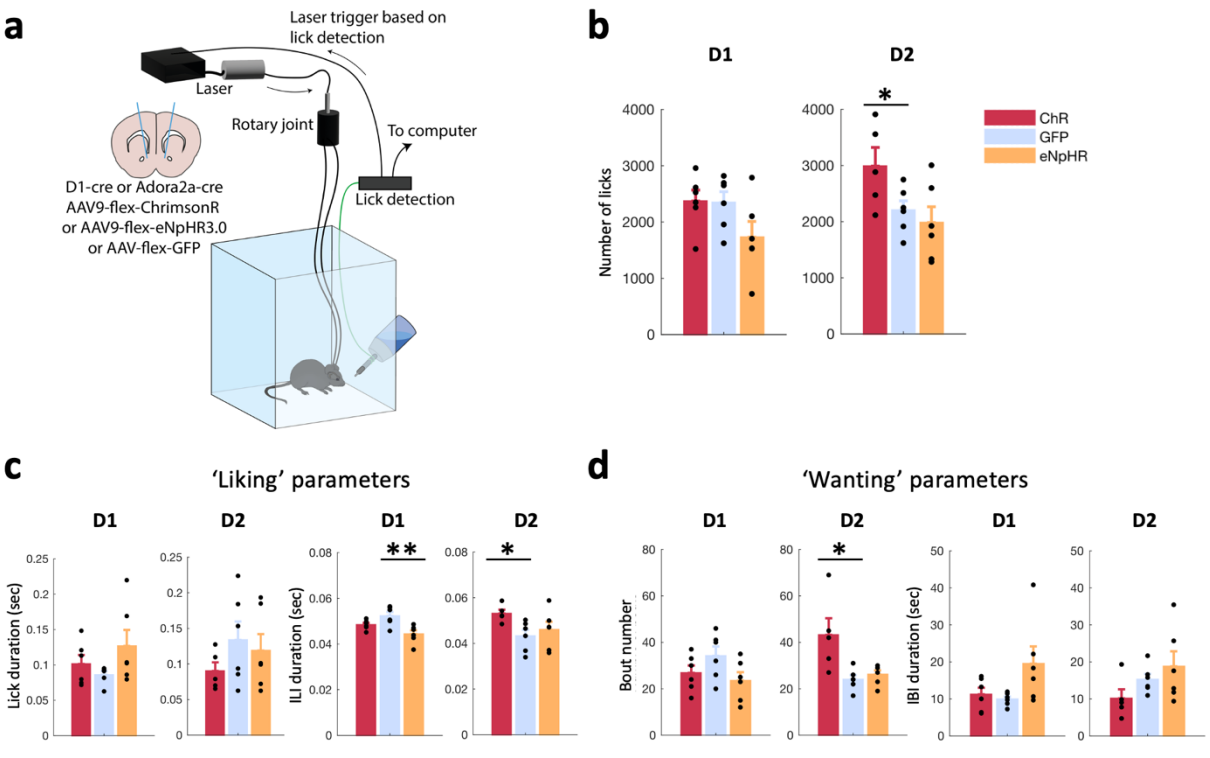
across bouts and animals is shown, shaded area represents the SEM. D1-GCaMP6s mice, one-sample t-tests: max post lick onset water ($t(5)=3.972$, $p=0.011$), delta max-min ($t(5)=8.457$, $p<0.001$). D2-GCaMP6s mice, one-sample t-tests: max at lick onset water ($t(4)=3.437$, $p=0.026$), max at lick onset MS ($t(4)=7.848$, $p=0.001$), min post lick onset MS ($t(4)=-3.526$, $p=0.024$). Paired-samples t-test: min post lick onset MS vs. water ($t(4)=5.407$, $p=0.006$).

(e) As in (d) but comparing the first and last MS sessions. D1-GCaMP6s mice, one-sample t-tests: max post lick onset MS 1 ($t(5)=2.687$, $p=0.043$), MS 5 ($t(5)=1.856$, N.S.). Paired-samples t-test: max MS 1 vs. MS 5 ($t(5)=6.257$, $p=0.002$). D2-GCaMP6s mice, one-sample t-tests: max post lick onset MS 1 ($t(4)=7.628$, $p=0.002$), MS 5 ($t(3)=2.858$, N.S.), delta max-min MS 5 ($t(5)=4.274$, $p=0.008$). Paired-samples t-test: min post lick onset MS 1 vs. MS 5 ($t(3)=3.088$, $p=0.05$).

(f) Lick duration (left) and inter-lick interval (ILI, right) during a session with access to water (blue dot) and 5 sessions with access to MS (red and pink dots). D1- and D2-GCaMP6s cohorts pooled. Mean ± SEM values are shown in color, with individual values from each mouse overlayed in black. One-way RM-ANOVA: lick duration water vs. MS 1 ($F(1,17)=4.799$, $p=0.043$), MS 1 to MS 5 ($F(4,68)=3.607$, $p=0.010$), MS 1 vs. MS 5 ($F(1,17)=9.961$, $p=0.006$); ILI: water vs. MS 1 ($F(1,17)=38.238$, $p<0.001$), MS 1 to MS 5 ($F(4,68)=0.924$, N.S.), N=18.

(g) As in (f) for lick bout number (left) and inter-bout interval (IBI, right). One-way RM-ANOVA: bout number water vs. MS 1 ($F(1,17)=45.661$, $p<0.001$), MS 1 to MS 5 ($F(4,68)=2.268$, N.S.); IBI water vs. MS 1 ($F(1,17)=44.170$, $p<0.001$), MS 1 to MS 5 ($F(4,68)=5.318$, $p=0.001$), MS 1 vs. MS 5 ($F(1,17)=35.626$, $p<0.001$), N=18.

MS: Milkshake. p-values reported on the figures as follows: *$p\leq0.05$, **$p<0.01$, ***$p<0.001$



669

670 **Figure 2. NAc D2 cell natural activity increases consumption by increasing 'wanting' despite a**
671 **decrease in 'liking'.**

672 (a) Schematic of experimental set-up for closed-loop optogenetic control based on recorded licks in an *ad libitum*
673 consumption paradigm. Left: fiber placement and viruses used.

674 (b) Total number of licks recorded in a 20-min *ad libitum* session with access to milkshake (MS) in D1-cre and
675 D2(A2a)-cre mice, expressing either ChrimsonR (ChR, red), eNpHR3.0 (eNpHR, orange) or the GFP (blue) reporter.
676 D1: One-way ANOVA (opsin: $F(2,15)=2.564$, N.S.). D2: One-way ANOVA (opsin: $F(2,14)=3.837$, $p=0.047$),
677 independent-samples t-test: ChR vs GFP ($t(9)=2.231$, $p=0.05$).

678 (c) 'Liking' parameters consisting of lick duration (left) and inter-lick interval (ILI, right) across one session with
679 access to MS in D1-cre and D2(A2a)-cre mice. Mean ± SEM values are shown in color, with individual values from
680 each mouse overlayed in black. D1: ILI, one-way ANOVA (opsin: $F(2,15)=7.516$, $p=0.005$), post-hoc independent
681 t-test with Bonferroni correction eNpHR vs. GFP ($p=0.004$). D2: ILI, one-way ANOVA (opsin: $F(2,14)=3.049$,
682 $p=0.080$), independent-samples t-test ChR vs GFP ($t(9)=2.963$, $p=0.016$). Lick duration: one-way ANOVA (opsin:
683 D2: $F(2,14)=0.950$, N.S., D1: $F(2,15)=1.813$, N.S.).

684 (d) As in (c) for 'wanting' parameters, consisting of number of licking bouts (left) and inter-bout interval (IBI,
685 right). D1: one-way ANOVAs (opsin), Bout number ($F(2,15)=2.235$, N.S.), IBI: ($F(2,15)=3.180$, N.S.). D2: Bout
686 number, one-way ANOVA (opsin: $F(2,14)=6.642$, $p=0.009$), post-hoc independent-samples t-test ChR vs GFP
687 ($p=0.014$). IBI, one-way ANOVA (opsin: $F(2,14)=2.076$, N.S.).

688 p-values reported on the figures as follows: *$p \leq 0.05$, **$p<0.01$.

689

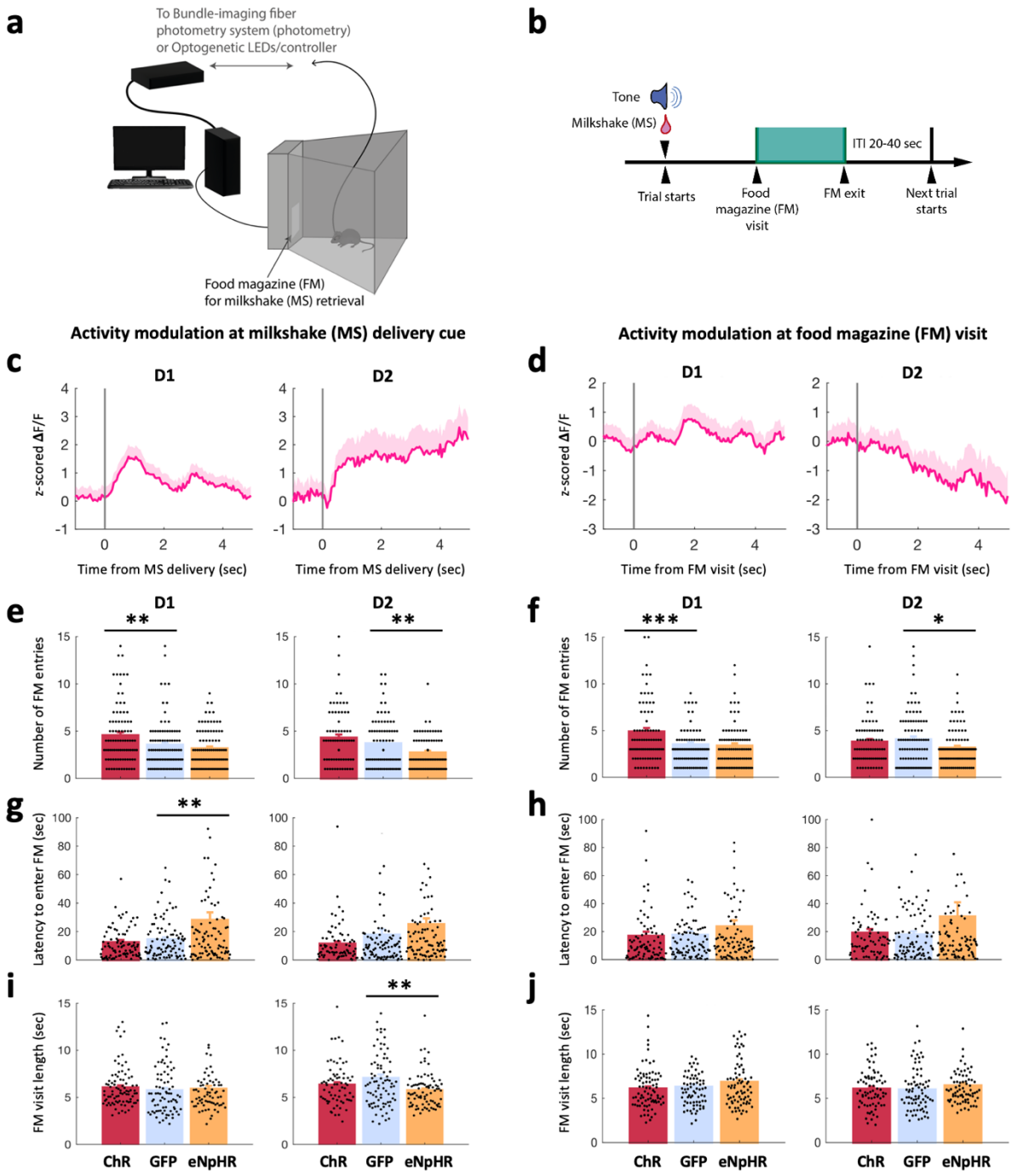


Figure 3. NAc D1 and D2 cell activities increase with anticipation during a motivational task & inhibition of D2 or D1 cells reduces motivation to retrieve a reward.

(a) Schematic of the chamber coupled with photometry recordings or with optogenetic control triggered by the recorded behavior (see Methods).

(b) Schematic of a milkshake delivery trial structure.

(c) Photometry signals around milkshake (MS) delivery time (associated with a sound cue) in D1-GCaMP6s (left) and D2-GCaMP6s (right) mice. Values are an average across mice and over all trials over 2 sessions run on two different days. The line represents mean across trials and animals; shaded areas represent the SEM.

(d) As in (c) but showing photometry signals around food magazine (FM) visits.

(e) Number of food magazine entries in D1-cre and D2(A2a)-cre mice expressing ChrimsonR (ChR), eNphR3.0 (eNpHR) or GFP when optogenetic stimulation occurs during 10sec following MS delivery cue. Each trial is counted as an individual data point. D1, one-way ANOVA (opsin: $F(2,265)=8.630$, $p<0.001$), post-hoc with Bonferroni correction: ChR vs. GFP ($p=0.003$). D2, one-way ANOVA (opsin: $F(2,250)=9.828$, $p<0.001$), post-hoc with Bonferroni correction eNpHR vs. GFP ($p=0.006$).

(f) As in (e) but when optogenetic stimulation occurs during 10sec following the first food magazine entry of each trial. D1, one-way ANOVA (opsin: $F(2,264)=9.597$, $p<0.001$), post-hoc with Bonferroni correction ChR vs. GFP ($p=0.001$). D2, one-way ANOVA (opsin: $F(2,252)=3.403$, $p<0.035$), post-hoc with Bonferroni correction eNpHR vs. GFP ($p=0.032$).

(g) As in (e) but for the latency to enter the FM following MS delivery cue (optogenetic stimulation at MS delivery cue). D1, one-way ANOVA (opsin: $F(2,264)=9.820$, $p<0.001$), post-hoc with Bonferroni correction eNpHR vs. GFP ($p=0.003$). D2, one-way ANOVA (opsin ($F(2,246)=5.158$, $p=0.006$), post-hoc tests N.S.

(h) As in (g) but when optogenetic stimulation occurs during 10sec following the first food magazine visit of each trial. D1: one-way ANOVA (opsin: N.S.). D2: one-way ANOVA (opsin: N.S.).

(i) As in (e) but for the average length of a food magazine visit (optogenetic stimulation at MS delivery cue). D1, N.S. D2, one-way ANOVA (opsin: $F(2,209)=5.408$, $p=0.005$), post-hoc with Bonferroni correction eNpHR vs. GFP ($p=0.004$).

(j) As in (i) but when optogenetic stimulation occurs during 10sec following the first FM visit of each trial. D1: one-way ANOVA (opsin: N.S.). D2: one-way ANOVA (opsin: N.S.).

FM: Food magazine, MS: Milkshake. p-values reported on the figures as follows: *$p\leq0.05$, **$p<0.01$, ***$p<0.001$.

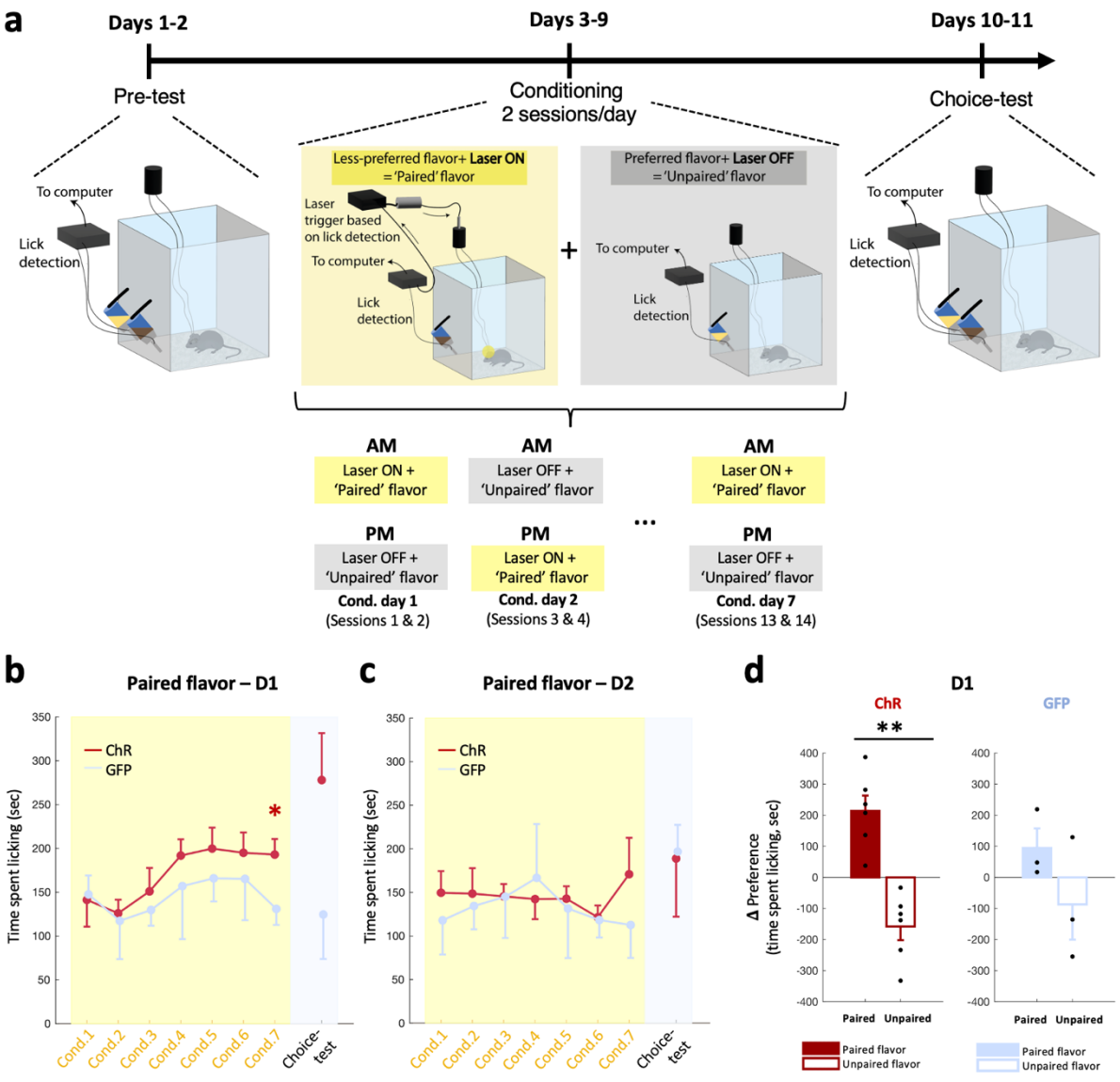


Figure 4. Modulation of NAc D1 and not D2 cells influence hedonic preference learning (hedonic shifting).

(a) Schematic of the experimental design for lick detection and closed-loop optogenetic activation on the paired-flavor (laser ON during conditioning (Cond.) phase only, yellow-shaded cartoon).

(b) Time spent licking on the paired-flavor spout during conditioning sessions and last choice-test session in D1-cre mice expressing ChrimsonR (ChR) and GFP. One-sided independent-samples t-test: ChR vs. GFP (Cond.7: $t(7)=2.160$, $p=0.034$; Choice-test: $t(7)=1.804$, $p=0.057$).

(c) As in (b) but in D2(A2a)-cre mice. Mixed-design ANOVA: N.S.

(d) Change in preference expressed as the difference (**Δ**) choice-test – pre-test in time spent licking on a given spout (paired & unpaired) in D1-ChR (left) and D1-GFP (right) mice. ChR, paired-samples t-test paired vs. unpaired flavors: $t(5)=4.754$, $p=0.003$ – one-sided. GFP: $t(2)=1.074$, $p=0.198$ – one-sided.

ChR: ChrimsonR, Cond.: Conditioning. p-values reported on the figures as follows: *$p \leq 0.05$, **$p<0.01$.

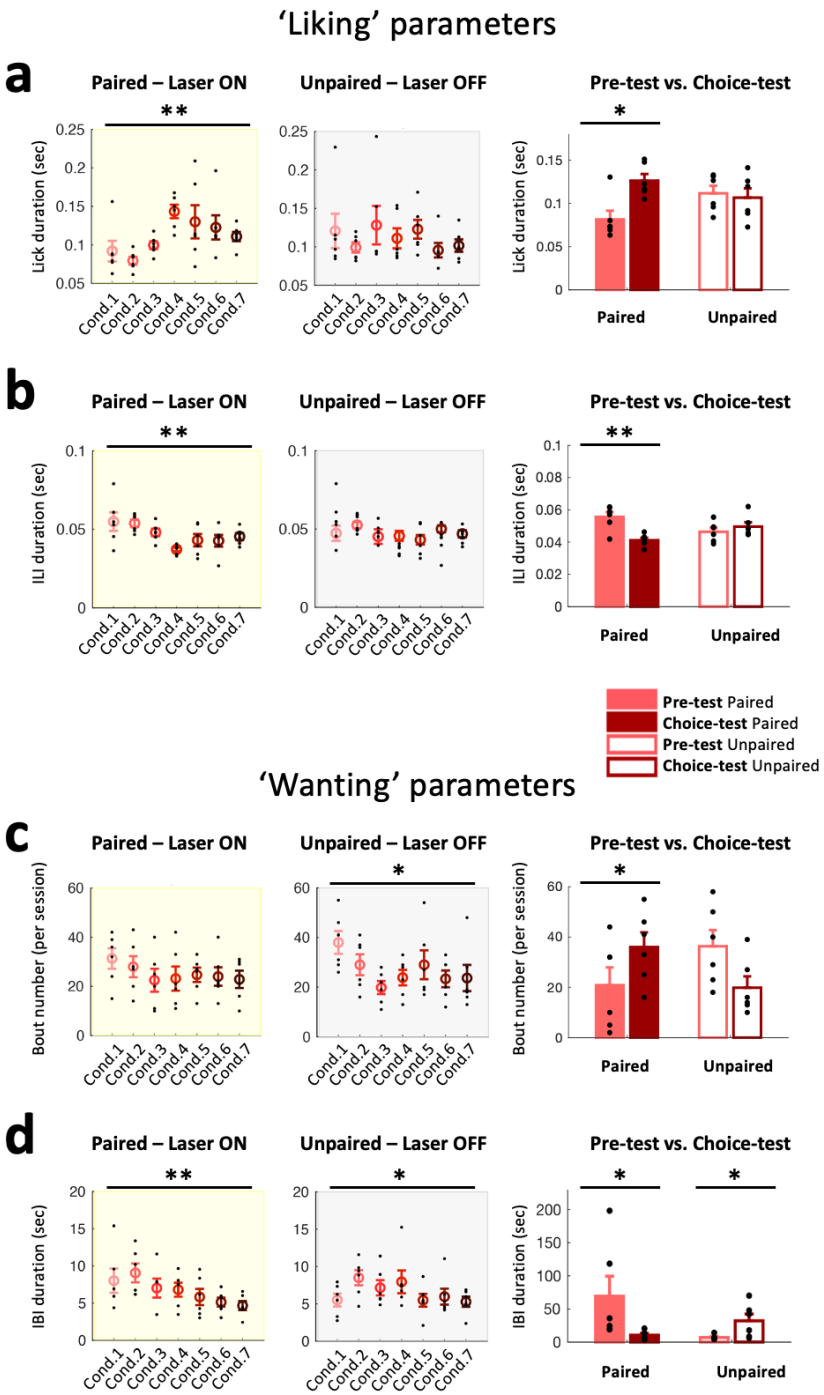


Figure 5. Activation of NAc D1 cells induces a preference shift *via* an increase in 'liking' and 'wanting' for the optoactivation-paired flavor.

(a) Single-lick duration across conditioning (Cond.) sessions (left & middle inserts) and pre-test & choice-test sessions (right insert) in the paired and unpaired flavors in D1-ChR mice. Values are plotted (in color) as mean ± SEM, with individual values from each mouse overlayed in black. Conditioning, paired flavor: RM-ANOVA (day: $F(6,30)=3.386$, $p=0.006$ – one-sided), one-sided paired-sample t-test Cond.1 vs. Cond.3: $t(5)=-2.675$, $p=0.022$; unpaired flavor (day: $F(6,30)=0.825$, N.S.). Choice-test vs. pre-test, paired flavor: one-sided paired-sample t-test, $t(5)=-3.188$, $p=0.012$; unpaired flavor, $t(5)=0.310$, N.S.

(b) As in (a) but for inter-lick interval (ILI) durations. Conditioning, paired flavor: RM-ANOVA (day: $F(6,30)=3.870$, $p=0.003$ – one-sided), Cond.1 vs. Cond.4 (one-sided paired-sample t-test, $t(5)=3.212$, $p=0.012$); unpaired flavor (day: $F(6,30)=1.334$, N.S.). Choice-test vs. pre-test, paired flavor: one-sided paired-sample t-test, $t(5)=4.422$, $p=0.003$; unpaired flavor: $t(5)=-0.834$, N.S.

(c) As in (a) but for the number of licking bouts. Conditioning, paired flavor: RM-ANOVA (day: $F(6,30)=0.872$, N.S.); unpaired flavor (day: $F(6,30)=2.588$, $p=0.019$ – one-sided), Cond.1 vs. Cond.7 (one-sided paired-samples t-test, $t(5)=2.330$, $p=0.034$). Choice-test vs. pre-test, paired flavor: one-sided paired-samples t-test, $t(5)=-1.986$, $p=0.05$; unpaired flavor, $t(5)=1.800$, $p=0.066$.

(d) As in (a) but for the inter-bout interval (IBI) durations. Conditioning, paired flavor: RM-ANOVA (day: $F(6,30)=3.176$, $p=0.008$ – one-sided), Cond.1 vs. Cond.7 (one-sided paired-samples t-test, $t(5)=2.074$, $p=0.046$); unpaired flavor (day: $F(6,30)=2.280$, $p=0.031$ – one-sided), Cond.1 vs. Cond.7 (one-sided paired-samples t-test, $t(5)=0.276$, $p=0.397$), Cond.1 vs. Cond.2 ($t(5)=-7.664$, $p<0.001$). Choice-test vs. pre-test, paired flavor: one-sided paired-samples t-test, $t(5)=1.961$, $p=0.05$; unpaired flavor: $t(5)=-2.471$, $p=0.028$.

ChR: ChrimsonR, Cond.: Conditioning, IBI: inter-bout interval, ILI: inter-lick interval. p-values reported on the figures as follows: *$p\leq0.05$, **$p<0.01$.

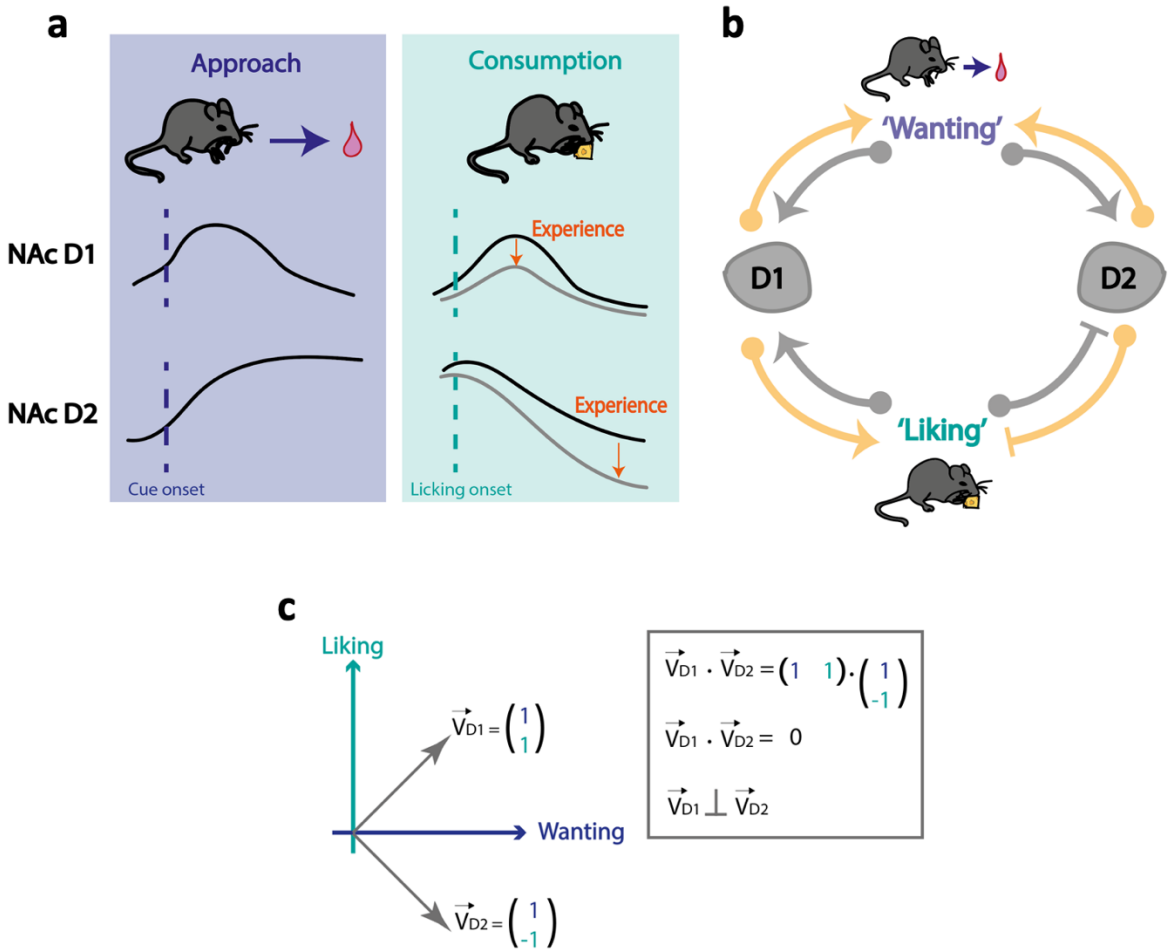


Figure 6. NAc D1 cells positively induce 'wanting' and 'liking' while D2 cells positively encode 'wanting' but negatively induce 'liking'.

Summary of the findings from the different experimental paradigms to test the distinctive patterns of NAc D1 and D2 cell activity relative to distinct psychological processes of hedonic behaviors: 'liking' (hedonia) and 'wanting' (motivation). (a) Summary of D1 and D2 cell activity in the NAc around food approach and following food consumption onset, and the effect of repeated exposure to the reward. NAc D1 cells show transient activation following a reward-predicting cue (dotted line) and following consumption onset (dotted line). The increase following feeding onset fades with daily repeated exposure to the food reward (orange arrow). NAc D2 cells show a sustained increase in activity following a food-predicting cue and their activity drops following feeding onset; this drop becomes lower during the consumption bout and with repeated daily exposure to the food reward (orange arrow). (b) Hypothetical model of the mutual relationship between behaviors (approaching and consuming rewards) and NAc D1 and D2 cell activity. NAc D1 cell activation induces both 'liking' and 'wanting' processes (yellow arrows), and reversely, D1 cells are naturally activated during behaviors associated with 'wanting' and/or 'liking' (grey arrows). NAc D2 cell activation, on the other hand, leads to increased 'wanting' and decreased 'liking' (yellow arrows). Reversely, behaviors associated with 'wanting' (e.g., following a food-predicting cue and during food approach) lead to an increase in D2 cell activity while behaviors associated with 'liking' (e.g., during food consumption) lead to a sustained decrease in D2 cell activity in the NAc. (c) If considering 'liking' and 'wanting' as a 2D space underlying hedonic eating, our data show that D1 and D2 cells are orthogonal in that space and can themselves form a base of this 2D space. This would support the idea that evolutionary processes led to these two cell populations that can independently and mutually influence hedonic eating. D1 cells are prone to lead to a maladaptive cycle of increased 'liking' and 'wanting', thus driving overeating, while

D2 cells cannot reinforce both processes and are faced with a tradeoff between increasing ‘wanting’ by being more active or allowing ‘liking’ by remaining silent.

Supplementary figures

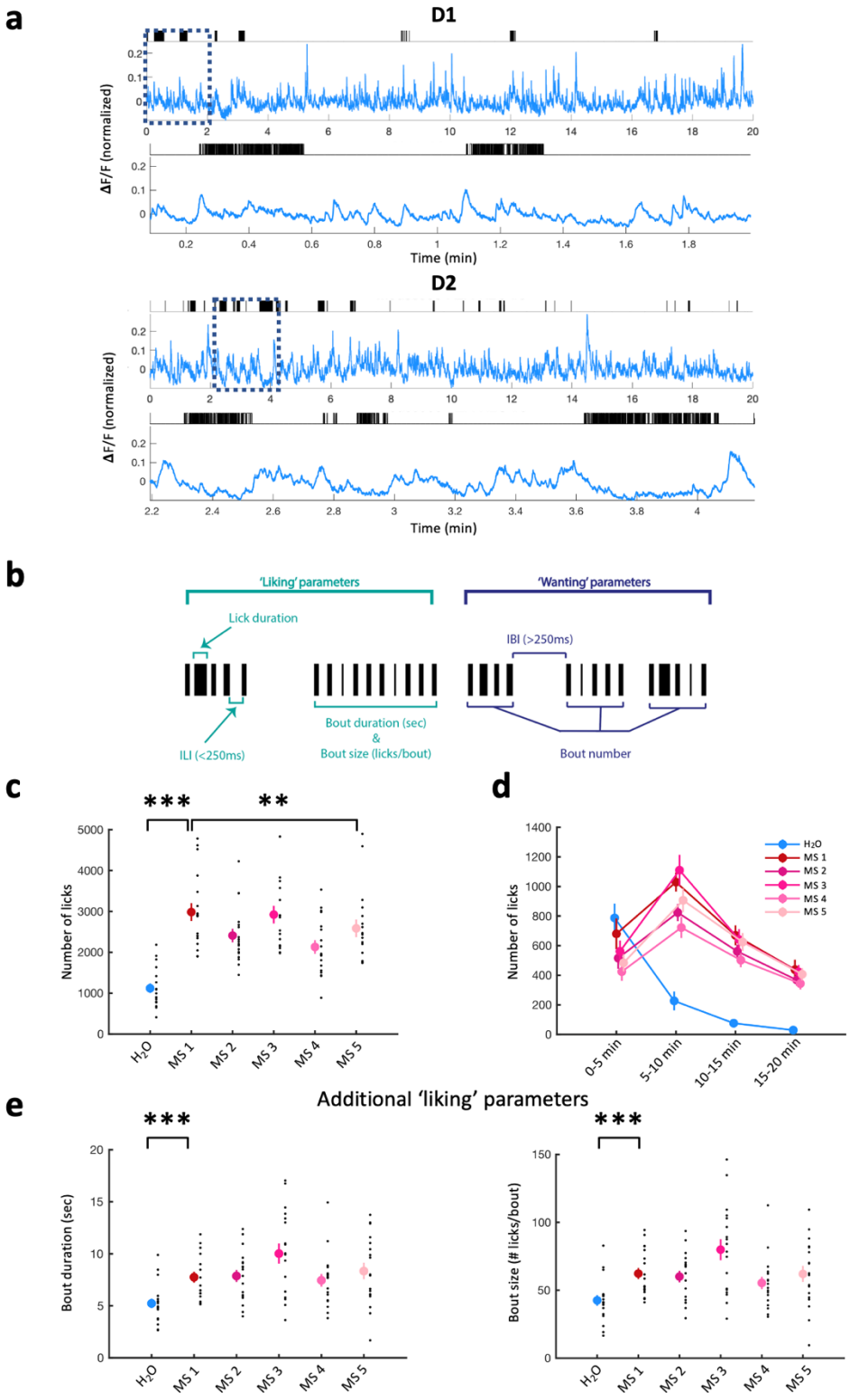


Figure S1. An increase in palatability is reflected in hedonic behavior ('liking') parameters.

(a) Top: Example photometry recording from a 20-min session with access to water from a D1-GCaMP6s mouse with licks indicated as a black raster plot above. Below: Zoomed-in photometry signal from the same session and animal showing only 2 min; licks shown above as a black raster plot. Photometry signals are (465nm signal – fitted 405 signal)/fitted 405nm signal. Bottom: As in top graph, but for a D2-GCaMP6s mouse.

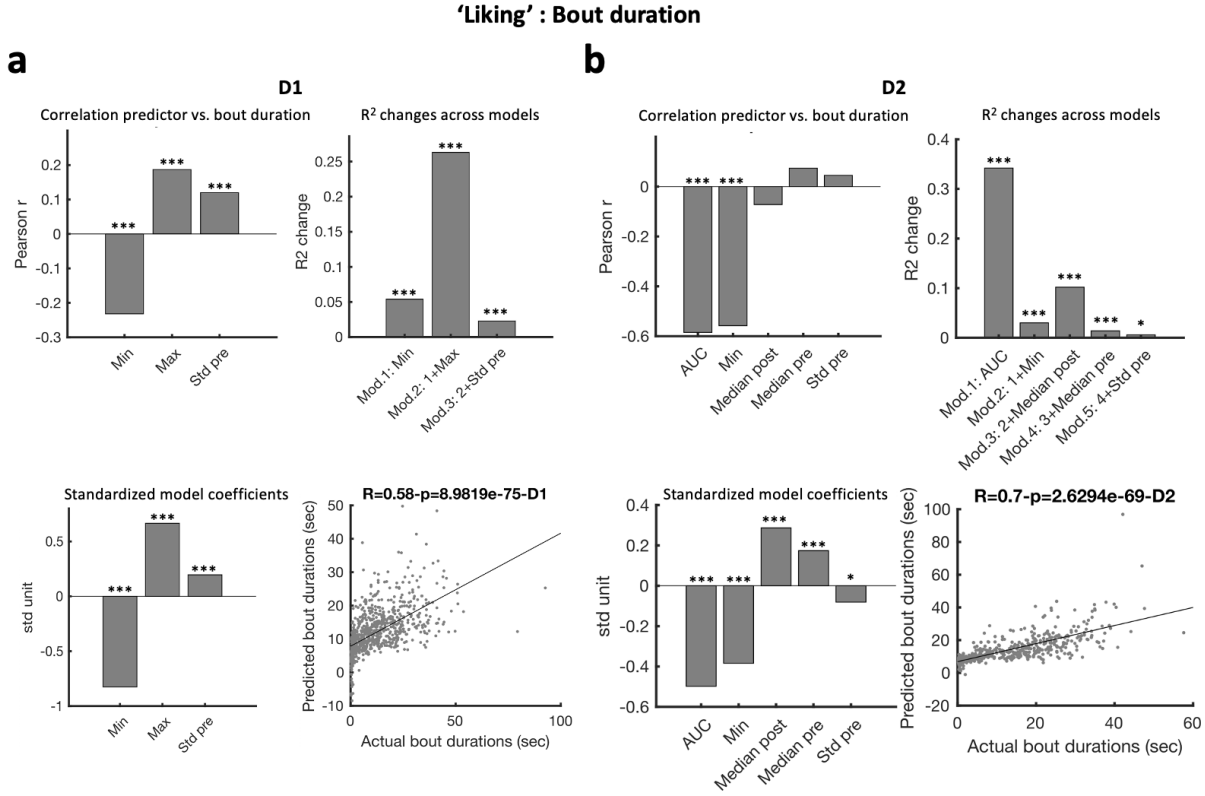
(b) Description of lick microstructure parameters associated with 'liking' and 'wanting'.

(c) Lick number during daily 20-min sessions with access to water (H_2O, first session) and milkshake (MS - 5 sessions). Values are plotted (in color) as mean ± SEM, with individual values from each mouse overlayed in black. D1-GCaMP6s and D2-GCaMP6s mice are pooled, N=18. One-way repeated measures ANOVA: water vs. MS 1 ($F(1,17)=147.327$, $p<0.001$), MS 1 to MS.5 ($F(4,68)=19.895$, $p<0.001$), MS 1 vs. MS 5 ($F(1,17)=11.006$, $p=0.004$).

(d) Lick number over 5-min blocks across daily 20-min sessions with access to water (first session) and MS (5 sessions). Values are plotted (in color) as mean ± SEM, N=18.

(e) Additional parameters calculated from lick recordings traditionally correlated with 'liking': bout duration (left) and bout size (i.e., number of licks per bout, right) across one session with access to water and 5 sessions with access to MS. D1-GCaMP6s and D2-GCaMP6s mice are pooled. Values are plotted (in color) as mean ± SEM, with individual values from each mouse overlayed in black, N=18. One-way repeated measures ANOVA, Bout duration: water vs. MS 1 ($F(1,17)=25.529$, $p<0.001$), MS 1 to MS 5 ($F(4,68)=4.245$, $p=0.004$), MS 1 vs. MS 5 ($F(1,17)=0.675$, $p=0.423$); Bout size: water vs. MS 1 ($F(1,17)=26.825$, $p<0.001$), MS 1 to MS 5 ($F(4,68)=5.282$, $p=0.001$), MS 1 vs. MS 5 ($F(1,17)=0.003$, $p=0.956$)

MS: Milkshake, IBI: inter-bout interval, ILI: Inter-lick interval. p-values reported on the figures as follows: **$p\leq0.01$, ***$p<0.001$.



	B	Std. B	β
(Constant)	3.383 (b_0)	0.594	
Min	-0.96 (-b_1)	0.05	-0.826***
Max	0.45 (b_2)	0.03	0.666***
Std pre	0.003 (b_3)	0.001	0.196***

R^2=0.340, Adjusted R^2=0.337. ***p<0.001

Bout duration = b_0 - b_1****Min*** + b_2****Max*** + b_3****Std pre***

	B	Std. B	β
(Constant)	7.573 (b_0)	0.784	
AUC	-0.003 (-b_1)	0	-0.498***
Min	-0.785 (-b_2)	0.109	-0.384***
Median post	0.76 (b_3)	0.128	0.287***
Median pre	0.578 (b_4)	0.146	0.174***
Std pre	-0.006 (-b_5)	0.003	-0.081*

R^2=0.494, Adjusted R^2=0.489. *p<0.05, ***p<0.001

Bout duration = b_0 - b_1****AUC*** - b_2****Min*** + b_3****Median post*** + b_4****Median pre*** - b_5****Std pre***

Figure S2. Photometry signal features of NAc D1 and D2 cells around palatable food consumption are good predictors of 'liking' of the food consumed.

(a) Multiple regression analysis results using variables extracted from the photometry signals (Max: Maximum value in the 5 sec following lick onset, Min: Minimum value in the 5 sec following lick onset, AUC: area under the curve over the 5 sec following lick onset, Mean pre: mean signal over the 3 sec before lick onset, Mean post: mean signal over the 3 sec following lick onset, Median pre: median signal over the 3 sec before lick onset, Median post: median signal over the 3 sec following lick onset, Std: standard deviation of the non-z-scored signal in the 5 sec preceding lick onset – see Methods) of D1-GCaMP6s mice (N=6) around licking onset to predict licking bout duration. Top left: Pearson's r values of single correlations between predictors and bout duration. Top right: R^2 change evaluating whether a given regression model (Mod.) significantly improves the prediction of bout duration compared to the previous model; the p-value associated with the R^2 change is reported above each bar. The R^2 change indicates whether the amount of variance in the outcome (bout duration) can be explained by the added predictor. For model 1 (Minimum (Min) as the only predictor) the R^2 change shows that using the Min to predict bout duration significantly improves predictions compared to using the grand mean of bout duration. For model 2 (Minimum and Maximum (Max) included as predictors) the R^2 change shows that including the Max of the photometry signal around licking onset in addition to the Min significantly improves the model's performance in predicting bout duration, with similar reasoning for subsequent models. Middle left: Standardized coefficients of the final model; standardized coefficients allow to compare the relative contribution of each predictor, regardless of the units used to measure them (the higher the absolute value of a standardized coefficient, the higher the contribution to the model). The p-values associated with these contributions (i.e., whether they are

834 significant, regardless of their magnitude) is reported above each bar. Middle right: Plot of the actual vs.
835 predicted bout duration with the R and p values reported on top. Bottom: model coefficients (B), standard
836 deviation for the coefficients (Std. B) and standardized model coefficient (β) of each predictor in the final model,
837 of which the equation is reported below the table.

838 (b) As in (a) but for D2-GCaMP6s mice (N=5).
839
840 AUC: area under the curve, Max: maximum, Min: minimum, pre: before licking onset, post: following licking
841 onset, Std: standard deviation. p-values reported on the figures as follows: *p≤0.05, ***p<0.001.

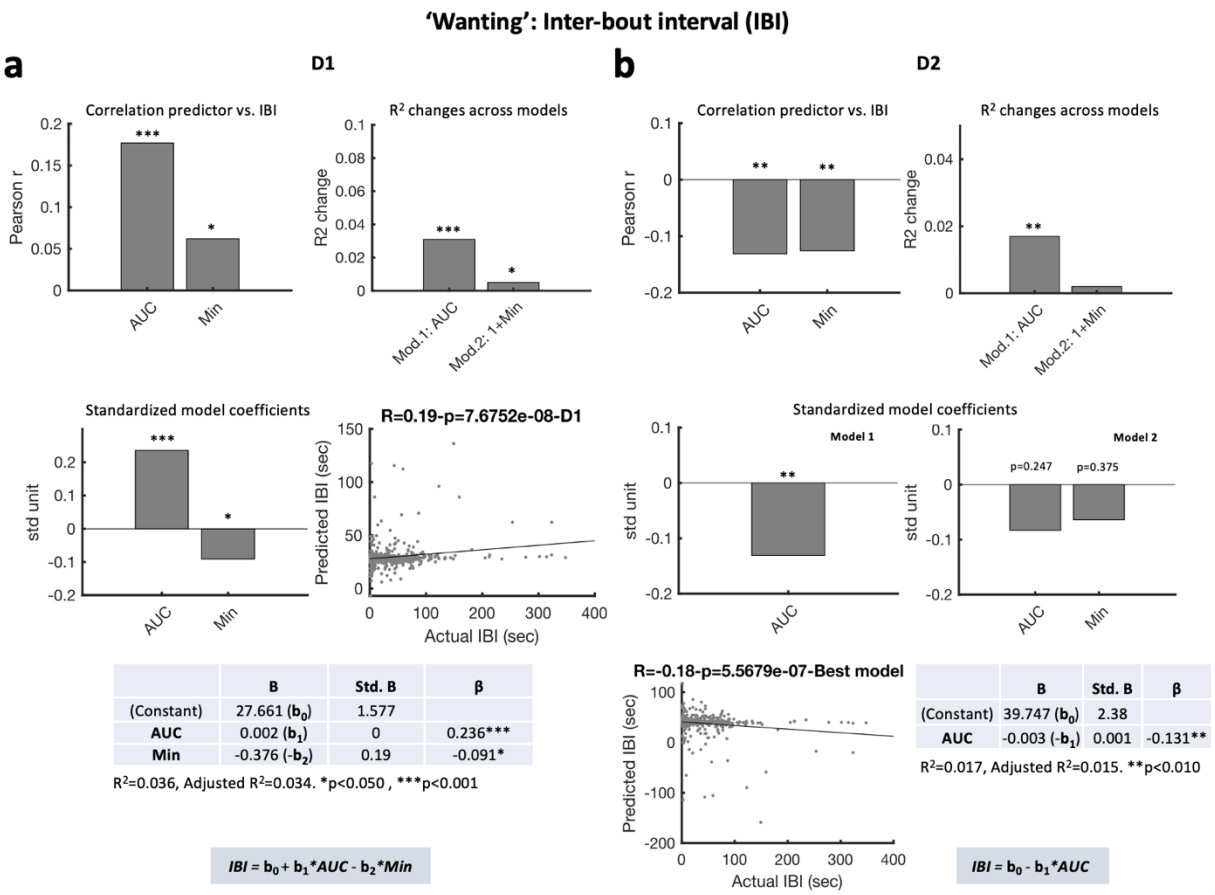
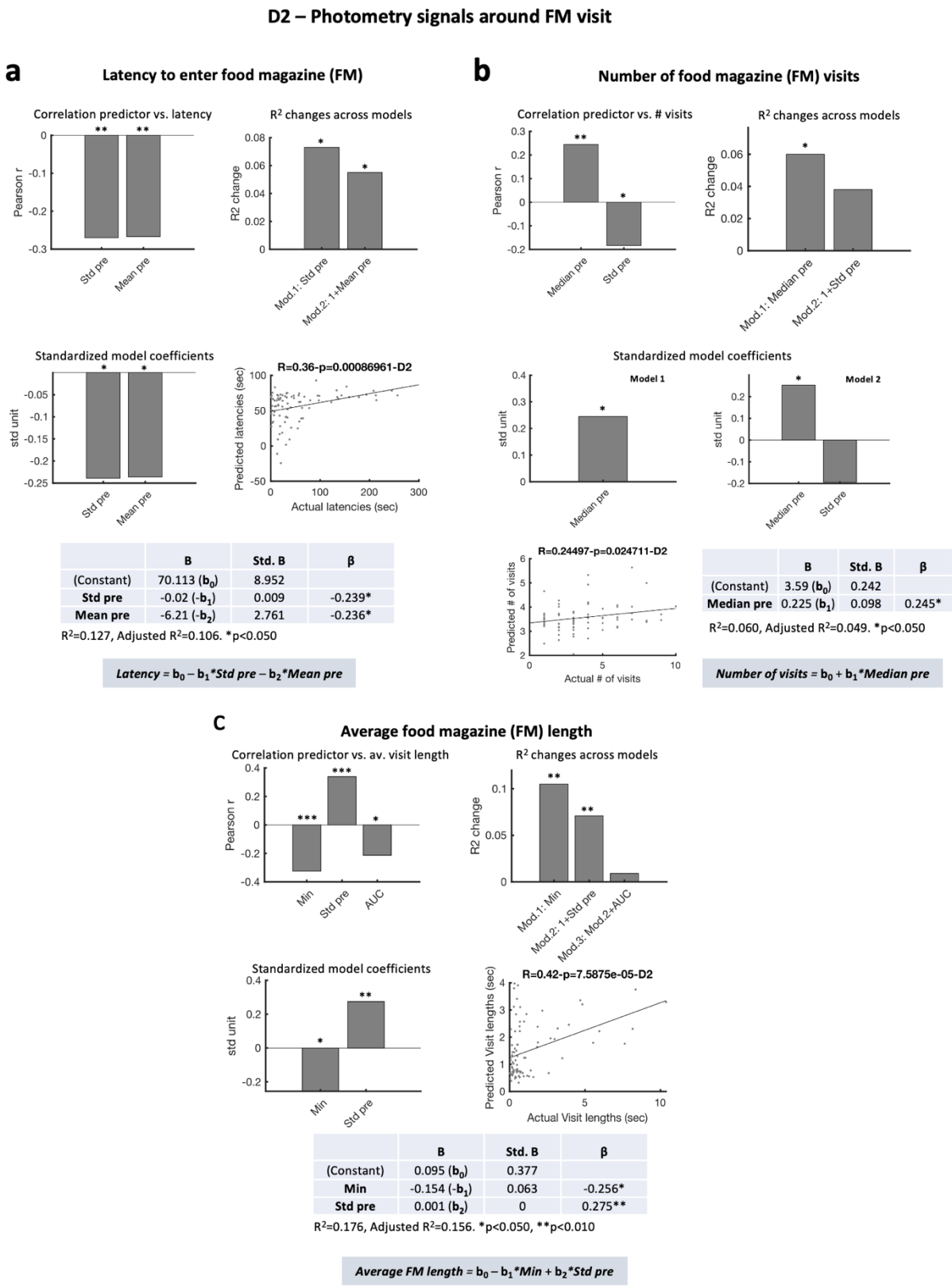


Figure S3. Photometry signal features of NAc D1 and D2 cells around palatable food consumption are not good predictors of 'wanting' of the food consumed.

(a) As in Figure S2a but for prediction of inter-bout intervals (IBI) in D1-GCaMP6s mice (N=6).

(b) As in (a) but for D2-GCaMP6s mice (N=5). Note that here although both the AUC and Min predictors were – individually – significantly correlated with IBI (top left), only the model considering the AUC yielded a significant improvement in predicting IBI duration compared to using the grand mean of IBI duration (i.e., no better predicting power of model 2 compared to model 1) (top right and middle right).

AUC: area under the curve, IBI: inter-bout interval, Min: minimum, Std: standard deviation. p-values reported on the figures as follows: *p≤0.05, **p≤0.01, ***p<0.001.



	B	Std. B	β
(Constant)	70.113 (b_0)	8.952	
Std pre	-0.02 ($-b_1$)	0.009	-0.239*
Mean pre	-6.21 ($-b_2$)	2.761	-0.236*

R^2=0.127, Adjusted R^2=0.106. *p<0.050

Latency = $b_0 - b_1$***Std pre*** $- b_2$***Mean pre***

	B	Std. B	β
(Constant)	3.59 (b_0)	0.242	
Median pre	0.225 (b_1)	0.098	0.245*

R^2=0.060, Adjusted R^2=0.049. *p<0.050

Number of visits = $b_0 + b_1$***Median pre***

	B	Std. B	β
(Constant)	0.095 (b_0)	0.377	
Min	-0.154 ($-b_1$)	0.063	-0.256*
Std pre	0.001 (b_2)	0	0.275**

R^2=0.176, Adjusted R^2=0.156. *p<0.050, **p<0.010

Average FM length = $b_0 - b_1$***Min*** $+ b_2$***Std pre***

Figure S4. The decrease in D2 cell activity during consumption favors longer licking bouts.

(a) Multiple regression analysis results using variables extracted from the photometry signals of D1-GCaMP6s mice (N=6) around food magazine (FM) visit onsets (Max: Maximum value in the 5 sec following FM visit onset, Min: Minimum value in the 5 sec following FM visit onset, AUC: area under the curve over the 5 sec following FM

visit onset, Mean pre: mean signal over the 3 sec before FM visit onset, Mean post: mean signal over the 3 sec following FM visit onset, Median pre: median signal over the 3 sec before FM visit onset, Median post: median signal over the 3 sec following FM visit onset, Std: standard deviation of the non-z-scored signal over the 5 sec preceding FM visit onset – see Methods) to predict the latency to enter the FM following a milkshake delivery (see Methods). Top left: Pearson's r values of single correlations between predictors and FM visit latency. Top right: R^2 change evaluating whether a given regression model (Mod.) significantly improves the prediction of FM visit latency compared to the previous model; the p-value associated with the R^2 change is reported above each bar. The R^2 change indicates whether the amount of variance in the outcome (latency to enter the FM) can be explained by the added predictor. For model 1 (Standard deviation of the signal in the 5 sec before FM visit (Std pre) as the only predictor) the R^2 change shows that using the Std pre to predict latency significantly improves predictions compared to using the grand mean of latencies. For model 2 (Std pre and Mean pre included as predictors) the R^2 change shows that including the mean photometry signal over the 3 sec before FM visit in addition to the Std pre significantly improves the model's performance in predicting latency to enter the FM. Middle left: Standardized coefficients of the final model; standardized coefficients allow to compare the relative contribution of each predictor, regardless of the units used to measure them (the higher the absolute value of a standardized coefficient, the higher the contribution to the model). The p-values associated with these contributions (i.e., whether they are significant, regardless of their magnitude) is reported above each bar. Middle right: Plot of the actual vs. predicted FM visit latency with the R and p values reported on top. Bottom: model coefficients (B), standard deviation of the coefficients (Std. B) and standardized model coefficient (β) of each predictor in the final model, of which the equation is reported below the table.

(b) As in (a) but for prediction of number of food magazine visits. Note that here although both the Median pre and Std pre predictors were – individually – significantly correlated with the number of FM visits (top left), only the model considering the Median pre yielded a significant improvement in predicting number of FM visits compared to using the grand mean of the number of visits (i.e., no better predicting power of model 2 compared to model 1) (top right and middle right).

(c) As in (a) but for the average food magazine visit length.

AUC: area under the curve, FM: food magazine, Min: minimum, pre: before FM visit onset, Std: standard deviation. p-values reported on the figures as follows: *$p \leq 0.05$, **$p \leq 0.01$, ***$p < 0.001$.

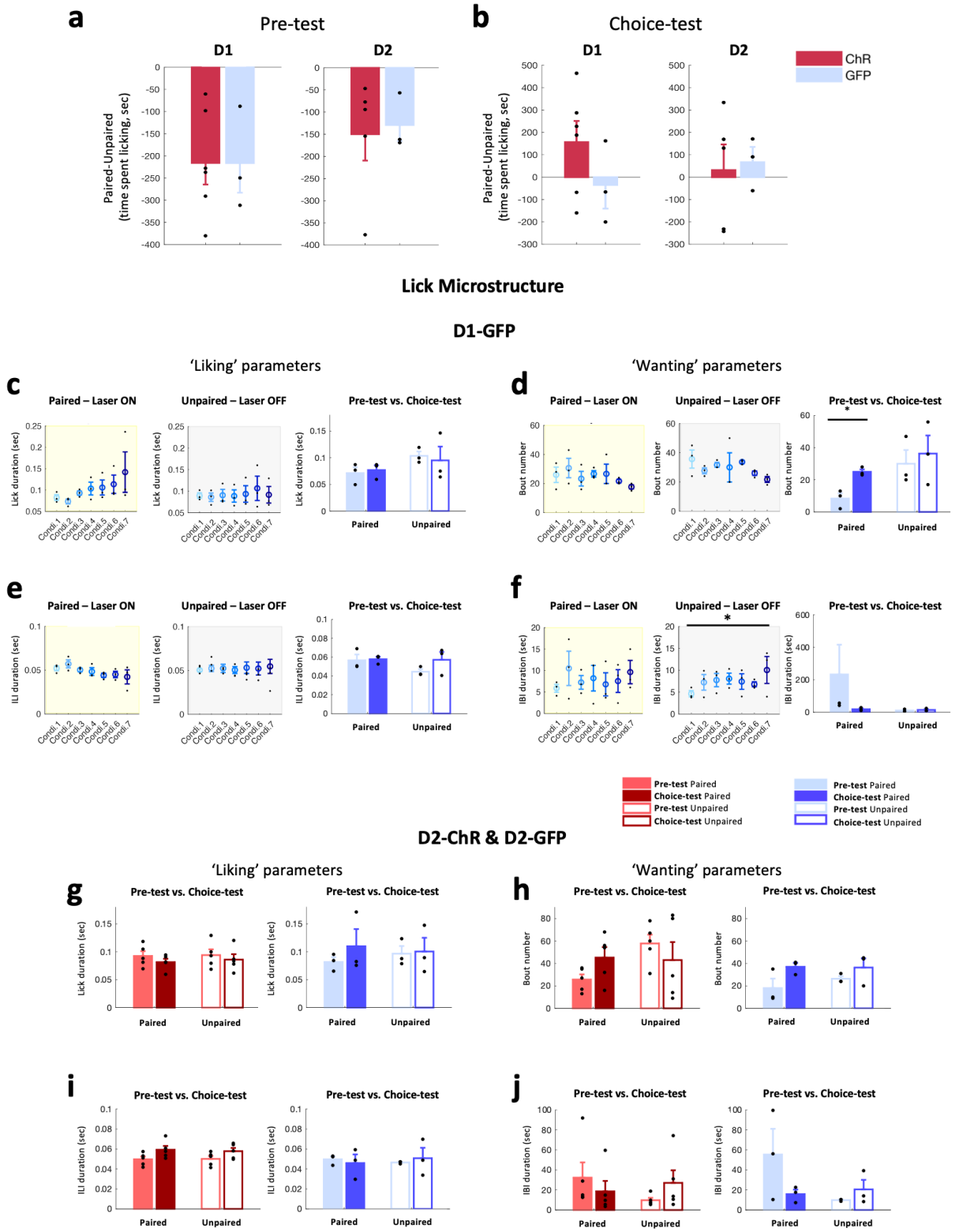


Figure S5. Activation of D2 cells does not lead to a shift in flavor preference for the optoactivation-paired flavor.

(a) Difference in the time spent licking the paired and unpaired flavors during the pre-test session of the hedonic shifting experiment in D1-cre (left) and D2(A2a)-cre mice (right) expressing ChrimsonR (ChR) or GFP. One-way ANOVA (opsin), D1: $F(1,7)<0.001$, $p=0.994$, D2: $F(1,6)=0.062$, $p=0.812$.

(b) As in (a) but for the time spent licking each flavor on the choice-test session. One-way ANOVA (opsin), D1: $F(1,7)=1.526$, $p=0.257$, D2: $F(1,6)=0.048$, $p=0.833$.

(c) Single-lick duration calculated from lick recordings indicating 'liking' across the conditioning (Cond.) sessions (left & middle inserts) and pre-test & choice-test sessions (right insert) in the paired and unpaired flavors in D1-GFP mice. Values are plotted (in color) as mean ± SEM, with individual values from each mouse overlayed in black. RM-ANOVA – one-sided: Conditioning, paired flavor (day: $F(6,12)=1.705$, N.S.), unpaired flavor (day: $F(6,12)=0.405$, N.S.); Choice-test vs. pre-test, paired flavor (test day: $F(1,2)=3.865$, N.S.), unpaired flavor (test day: $F(1,2)=0.224$, N.S.).

(d) As in (c) but for the number of licking bouts. RM-ANOVA – one-sided: Conditioning, paired flavor (day: $F(6,12)=0.815$, N.S.), unpaired flavor (day: $F(6,12)=0.974$, N.S.); Choice-test vs. pre-test, paired flavor (test day: $F(1,2)=12.563$, $p=0.036$), unpaired flavor (test day: $F(1,2)=0.113$, N.S.).

(e) As in (c) but for inter-lick interval (ILI) durations. RM-ANOVA – one-sided: Conditioning, paired flavor (day: $F(6,12)=1.528$, N.S.), unpaired flavor (day: $F(6,12)=1.128$, N.S.); Choice-test vs. pre-test, paired flavor (test day: $F(1,2)=0.044$, N.S.), unpaired flavor (test day: $F(1,2)=3.277$, N.S.).

(f) As in (c) but for the inter-bout interval (IBI) durations. RM-ANOVA – one-sided: Conditioning, paired flavor (day: $F(6,12)=0.689$, N.S.), unpaired flavor (day: $F(6,12)=13.824$, $p=0.033$); Choice-test vs. pre-test, paired flavor (test day: $F(1,2)=1.307$, N.S.), unpaired flavor (test day: $F(1,2)=0.183$, N.S.).

(g) Single-lick duration during the pre-test & choice-test sessions in the paired and unpaired flavors in D2-ChR (left) and D2-GFP (right) mice. Values are plotted (in color) as mean ± SEM, with individual values from each mouse overlayed in black. RM-ANOVA: Choice-test vs. pre-test, D2-ChR: paired flavor (test day: $F(1,4)=1.307$, N.S.), unpaired flavor (test day: $F(1,4)=0.599$, N.S.); D2-GFP: paired flavor (test day: $F(1,2)=0.517$, N.S.), unpaired flavor (test day: $F(1,2)=0.011$, N.S.).

(h) As in (g) but for the number of licking bouts. RM-ANOVA: Choice-test vs. pre-test, D2-ChR: paired flavor (test day: $F(1,4)=2.538$, N.S.), unpaired flavor (test day: $F(1,4)=0.423$, N.S.); D2-GFP: paired flavor (test day: $F(1,2)=7.367$, N.S.), unpaired flavor (test day: $F(1,2)=0.906$, N.S.).

(i) As in (g) but for inter-lick interval (ILI) durations. RM-ANOVA: Choice-test vs. pre-test, D2-ChR: paired flavor (test day: $F(1,4)=6.971$, N.S.), unpaired flavor (test day: $F(1,4)=1.887$, N.S.); D2-GFP: paired flavor (test day: $F(1,2)=0.141$, N.S.), unpaired flavor (test day: $F(1,2)=0.153$, N.S.).

(j) As in (h) but for the inter-bout interval (IBI) durations. RM-ANOVA: Choice-test vs. pre-test, D2-ChR: paired flavor (test day: $F(1,4)=0.394$, N.S.), unpaired flavor (test day: $F(1,4)=2.593$, N.S.); D2-GFP: paired flavor (test day: $F(1,2)=2.962$, N.S.), unpaired flavor (test day: $F(1,2)=1.342$, N.S.).

ChR: ChrimsonR, Cond.: conditioning, IBI: inter-bout interval, ILI: inter-lick interval. p-values reported on the figures as follows: *$p \leq 0.05$.

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