Anterior cingulate cortex activity regulates effort-based decision making

Abbreviated title: *Anterior cingulate cortex in effortful choice*

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Abstract (150 words)

Prior evidence suggests that the anterior cingulate cortex (ACC) is required for choosing high effort options over less effortful alternatives. Here, rats earned a high-value sucrose reward by lever pressing on a progressive ratio schedule in either the presence (choice) or absence (no-choice) of freely available, low-value lab chow. Disruption of ACC, either via chemogenetic inhibition or excitation, reduced lever pressing for high-value reward in the choice, but not in the no-choice, condition. In vivo calcium imaging revealed cell populations in ACC that selectively responded prior to lever pressing and during sucrose retrieval. These responses were significantly weaker during choice than no-choice sessions, which may have rendered them more susceptible to chemogenetic disruption. Our results suggest that neural responses in ACC encode the relative value of competing outcomes, and that the amount of effort an animal is willing to exert is proportional to the magnitude of this relative value signal.

Keywords: *prefrontal cortex, amygdala, orbitofrontal cortex, DREADDs, miniscopes, single-photon, calcium imaging*

Introduction

Real-world decisions rarely involve choosing between unambiguously favorable vs. unfavorable options. Often, options must be evaluated along multiple dimensions that incorporate an evaluation of the rewards themselves as well as the actions or efforts to procure them (Skvortsova et al., 2014). For example, we typically make decisions between options in comparison, where one outcome may be more costly (i.e. more effortful) yet more preferred than the other.

Outside of the striatum, which has been the major region of study in such effort-based decision making studies (Cousins et al., 1996; Ghods-Sharifi and Floresco, 2010; Nowend et al., 2001; Salamone et al., 2007; Salamone et al., 2003; Salamone et al., 1994; Salamone et al., 1991), the anterior cingulate cortex (ACC) is also involved in the evaluation of both physical and cognitive effort costs (Cowen et al., 2012; Floresco and Ghods-Sharifi, 2007; Hillman and Bilkey, 2010, 2012; Hosking et al., 2014; Schweimer and Hauber, 2005; Walton et al., 2003; Winstanley and Floresco, 2016). Neurons in both rat and primate ACC signal value during economic decision-making (Azab and Hayden, 2017; Hunt and Hayden, 2017; Lapish et al., 2008; Mashhoori et al., 2018). Neural responses in this region also track trial-by-trial outcomes of choices (Akam et al., 2017; Procyk et al., 2000; Seo and Lee, 2007; Shidara and Richmond, 2002), reward history (Bernacchia et al., 2011), reward prediction errors, (Hayden et al., 2009; Hyman et al., 2017; Kennerley et al., 2011), and counterfactual options ('rewards not taken') (Hayden et al., 2009; Mashhoori et al., 2018). Indeed, both anatomical and functional evidence supports the idea that ACC activity supports representations of reward value and action outcomes (Heilbronner and Hayden, 2016; Shenhav et al., 2013).

In rats, effort t-maze and effort discounting tasks typically require the animal to select among different magnitudes of the same reward identity (i.e. more sucrose pellets vs. fewer sucrose pellets). However, a paradigm that involves selecting between qualitatively different reinforcers may also closely model human decisions where we encounter options that are more/less preferred, not more/less of the same reward identity (Cousins and Salamone, 1994; Nowend et al., 2001; Nunes et al., 2013; Randall et al., 2014a; Randall et al., 2015; Randall et al., 2012; Salamone et al., 2007; Salamone et al., 2017; Salamone et al., 1991; Yohn et al., 2016a; Yohn et al., 2016b; Yohn et al., 2016c). The majority of the seminal rodent studies probing ACC in effort-based choice (Floresco and Ghods-Sharifi, 2007; Hauber and Sommer, 2009; Walton et al., 2003; Walton et al., 2002; Winstanley and Floresco, 2016) have used traditional pharmacological, lesion, and electrophysiological approaches, so a fine-grained analysis involving viral-mediated targeting of ACC in effort choice has not yet been reported.

Here, we tested the effects of inhibitory (hM4Di, or G_i) and excitatory (hM3Dq, or G_q) Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) in ACC on the same effortful choice task that we previously probed following lesions (Hart et al., 2017) and pharmacological inactivations (Hart and Izquierdo, 2017). Briefly, our task required rats to choose between working for a preferred reward (sucrose) vs. consuming a concurrently and freely-available, but less preferred reward (standard chow). We assessed the role of ACC on (i) progressive ratio (PR) lever pressing for sucrose pellets (i.e. general motivation), and (ii) PR lever pressing with choice (PRC) of a freely-available alternative (i.e. effortful decision-making: choosing between working for sucrose pellets vs. concurrently available laboratory chow). We also tested the effects of ACC inhibition and excitation on the choice between sucrose pellets vs. chow when these reinforcers were both freely available (i.e. “free choice”). Finally, in a separate

cohort of animals we utilized *in vivo* miniaturized fluorescence microscopy (UCLA "miniscopes") to compare calcium activity in ACC and to evaluate how populations of neurons in this region respond during no-choice (PR) and choice (PRC) sessions.

Results

Histology. Reconstructions of viral spread confirmed that most placements were centered on the targeted region of Cg1 in ACC (**Figure 1**). DREADD expression was driven by a CaMKIIα promoter, which is thought to selectively target projection neurons in cortex (Nathanson et al., 2009; Wang et al., 2013). Viral spread in the G_i and G_q groups was quantified by pixel count using GIMP software (see Methods). There was no significant difference between G_i and G_q groups ($t_{(20)}$=2.03 p=0.056; G_i = 25258 ± 3121 total mean pixels; G_q = 17736 ± 2002 total mean pixels).

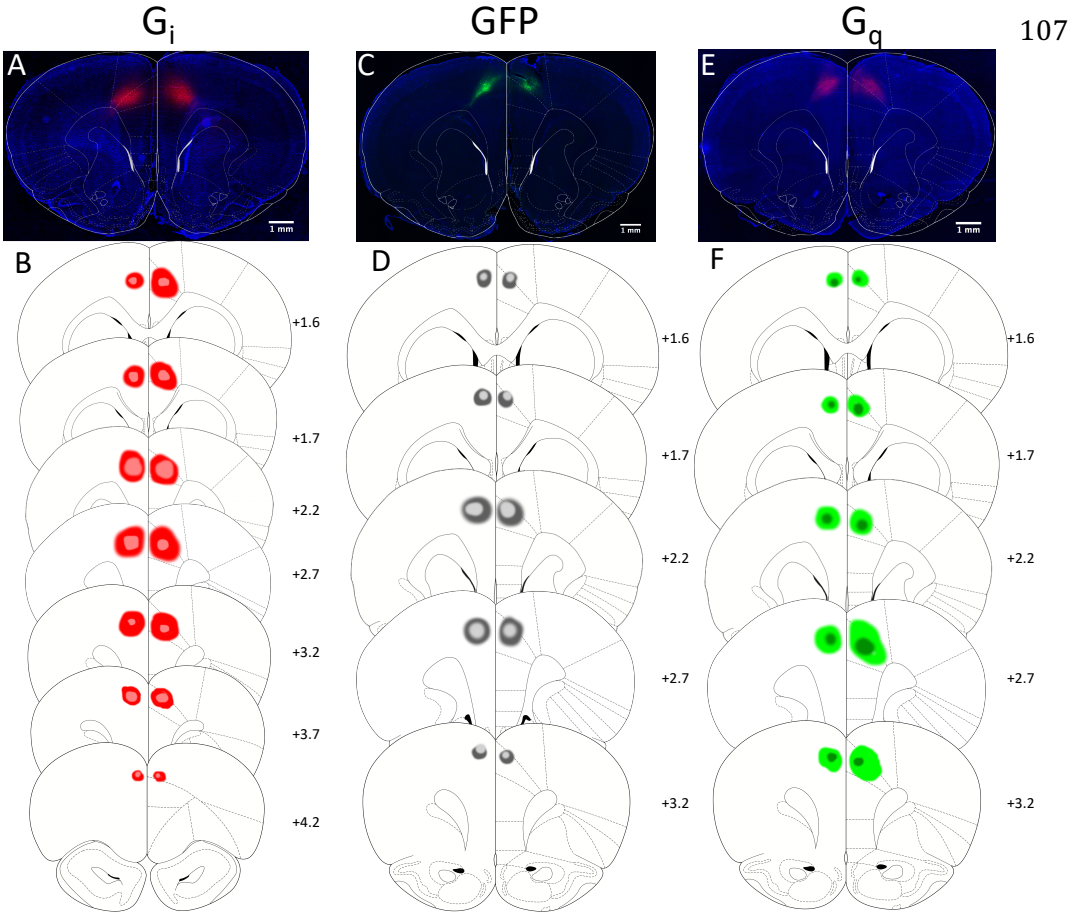
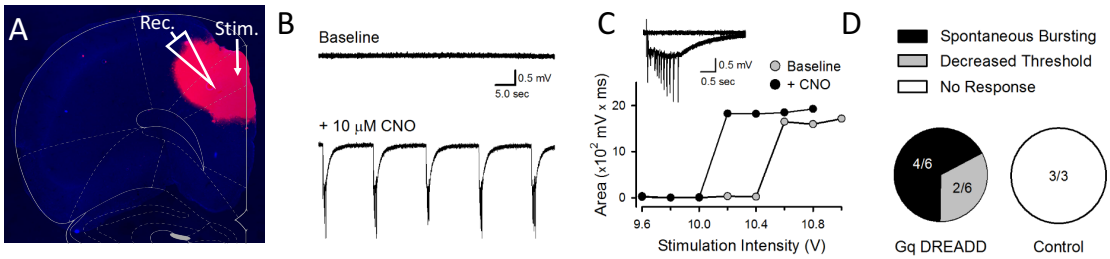


Figure 1. Expression of inhibitory and excitatory DREADDs and null virus eGFP in Anterior Cingulate Cortex. (A) Representative photomicrograph showing hM4Di-mCherry DREADDs under CaMKIIα in ACC, labeled G_i. **(B)** Schematic reconstruction of maximum (red) and minimum (pink) viral spread for all rats. Numerals depict +Anterior-Posterior (AP) level relative to Bregma. Scale bars 1mm. **(C)** Representative photomicrograph showing eGFP (null virus) under CaMKIIα in ACC, labeled GFP. **(D)** Schematic reconstruction of maximum (dark grey) and minimum (light grey) viral spread for all rats. Numerals depict +Anterior-Posterior (AP) level relative to Bregma. Scale bars 1mm. **(E)** Representative photomicrograph showing hM3Dq-mCherry DREADDs expression in ACC, labeled G_q. **(F)** Schematic reconstruction of maximum (light green) and minimum (dark green) viral spread for all rats. Numerals depict +Anterior-Posterior (AP) level relative to Bregma. Scale bars 1mm.

Ex vivo electrophysiological validation of DREADDs. We confirmed the efficacy of our DREADDs in slice recordings. A separate group of rats was prepared with G_q DREADDs in ACC (Cg1) using identical surgical procedures to the main experiments (**Figure 2A**). As described in Methods, a bipolar stimulating electrode was placed near the medial wall in layer I of ACC, and a glass microelectrode filled with ACSF (resistance = 5–10 MΩ) was placed in layer 2/3 of ACC to record field potentials and multiunit responses elicited by layer 1 stimulation. Using similar methods, we have previously reported that application of CNO strongly suppressed evoked field potentials in G_i transfected slices in ACC (Stolyarova et al., 2019). Here, we found that application of CNO induced spontaneous bursting in four out of six G_q transfected slices (spontaneous activity rate was increased from 0 to 0.06 Hz ± 0.01 Hz, inter-burst intervals were 18.3 ± 2.6 seconds (mean ± SEM, range: 12.4 – 31.6 seconds, n=4 slices from 3 rats) (**Figure 2B)**. In two other G_q transfected slices where no spontaneous bursting was observed, CNO application reduced threshold for stimulation induced postsynaptic responses (n=2 slices from 3 rats), with a sharp transition from

133 no response to maximal response as a function of stimulation intensity (**Figure 2C**). CNO had no

134 effect on responses in non-transfected slices (n=3 slices from 3 rats) (**Figure 2D**).



135 **Figure 2. Excitatory hM3Dq DREADDs validation in Anterior Cingulate Cortex.**
136 Electrophysiological effect of bath application of CNO in slice. (**A**) Representative
137 photomicrograph showing hM3Dq-mCherry DREADDs under CaMKIIα in a recording site of a
138 400 μm slice in ACC. **(B)** Spontaneous ACC activity in a G_q DREADD-expressing slice before
139 (top) and after 20 min bath application of 10 μM CNO (bottom). CNO-induced spontaneous
140 bursting was seen in 4 out of 6 G_q DREADD-expressing slices from 3 rats. **(C)** In the two G_q
141 DREADD-expressing slices that failed to show spontaneous bursting there was a decrease in the
142 threshold for stimulation induced postsynaptic responses. Plot shows results from one of these
143 slices before (baseline) and after CNO application. **(D)** Summary of responses to CNO in G_q
144 DREADD-expressing and control slices. CNO did not elicit either spontaneous bursting or
145 decrease threshold for evoked responses in all 3 of the control (non-transfected) slices from 3 rats.

146

147 *Lever press training.* Rats were trained over a series of days to press a lever for food on a

148 progressive ratio (PR) schedule (where the required number of presses to earn each pellet increased

149 throughout the session according to a prescribed formula; see Methods). After reaching criterion,

150 rats received systemic injections of either VEH or CNO (in a counterbalanced repeated measures

151 design) prior to subsequent sessions, which alternated between two different types: 1) progressive

152 ratio choice (PRC) sessions during which a ceramic ramekin containing 18 g of freely available

153 lab chow was introduced to the chamber (modified from Randall et al., 2012), and 2) PR only

154 sessions during which the rat pressed on the progressive ratio schedule with no other food available

155 (as during prior training). During PRC sessions, rats were free to choose between consuming

156 freely-available (but less preferred) chow versus lever pressing for preferred sucrose pellets,

157 whereas during PR sessions, the only reward option was to lever press for sucrose pellets. In
158 general, lever pressing rates were higher during PR sessions than PRC sessions (**Figures 3 and 4**),
159 indicating the free chow draws behavior away from lever pressing (Salamone et al., 2018) and
160 animals emit fewer lever presses in PRC sessions than in PR sessions.

161 *G_i inhibition of ACC.* We found that chemogenetic inhibition of ACC significantly
162 decreased lever pressing during PRC but not PR sessions. A paired-samples t-test revealed CNO
163 significantly reduced the total mean number of lever presses during 30 min PRC sessions
164 ($t_{(10)}$=3.047 p=0.01; VEH = 216.1 ± 52.85 presses; CNO = 173.2 ± 40.27 presses) **(Figure 3A)**.
165 The total amount of chow consumed during 30 min PRC sessions was not affected by CNO
166 treatment ($t_{(10)}$=1.048 p=0.32; VEH = 7.79 ± 0.33 g; CNO = 7.50 ± 0.36 g) **(Figure 3B)**. CNO had
167 no effect on PR responding in the absence of freely available chow ($t_{(10)}$=0.26 p=0.80; VEH =
168 905.1 ± 102.00 presses; CNO = 893.9 ± 128.3 presses) **(Figure 3C)**. A two-way ANOVA (food
169 type- sucrose, chow; injection- VEH, CNO) on the amount of sucrose and chow consumed during
170 free choice testing revealed a significant main effect of food type ($F_{(1,10)}$=12.02 p=0.006; sucrose
171 = 8.59 ± 0.83 g; chow = 4.19 ± 0.34 g), but no significant food type by injection interaction
172 ($F_{(1,10)}$=1.415 p=0.26), or main effect of injection ($F_{(1,10)}$=0.01 p=0.91) was found **(Supplementary**
173 **Figure 1A)**. Hence, food preference was intact and not affected by CNO injection.

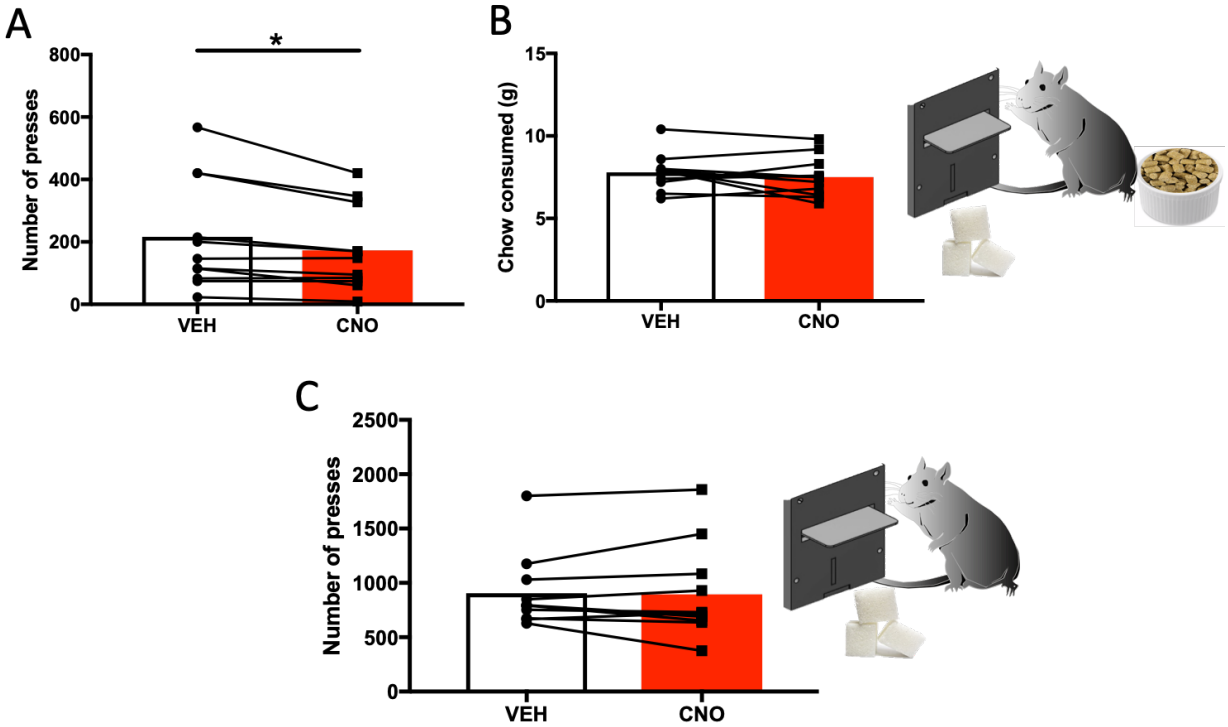


Figure 3. Effects on choice behavior following DREADDs inhibition of Anterior Cingulate Cortex. (A) Mean lever presses during PRC sessions, when rats were presented with both the possibility of lever pressing under a PR schedule for sucrose pellets and freely-available chow. Shown is within-subject, counterbalanced choice behavior under vehicle and CNO. CNO significantly reduced the number of lever presses in this condition. **(B)** Total chow consumed during choice testing was not different between vehicle and CNO conditions. **(C)** Mean lever presses during PR sessions when there was no chow available as an alternative option. Lever pressing was not different between vehicle and CNO conditions. * $p<0.05$.

G_q excitation of ACC. Similar to results for the G_i experiment, we found that chemogenetic excitation of ACC significantly decreased lever pressing during PRC but not PR sessions. Using identical procedures to the inhibition experiment, a separate group of rats was assessed on effortful choice following excitatory G_q DREADDs transfection in ACC. A paired-samples t-test revealed CNO significantly reduced the total mean number of lever presses during 30 min PRC sessions ($t_{(10)}$=2.31 p=0.04; VEH = 210.7 ± 49.44 presses; CNO = 166.6 ± 40.4 presses) **(Figure 4A)**. The total amount of chow consumed during 30 min PRC sessions was not affected by CNO treatment

($t_{(10)}$=0.39 p=0.71; VEH = 7.71 ± 0.36 g; CNO = 7.81 ± 0.42 g) **(Figure 4B)**. CNO had no effect on PR responding in the absence of freely available chow ($t_{(10)}$=1.15 p=0.28; VEH = 1107.00 ± 99.66 presses; CNO = 1209.00 ± 156.3 presses) **(Figure 4C)**. A two-way ANOVA (food type- sucrose, chow; injection- VEH, CNO) on the amount of sucrose and chow consumed during free choice testing revealed a significant main effect of food type ($F_{(1,10)}$=47.63 p<0.001; sucrose = 9.68 ± 0.47 g; chow = 5.10 ± 0.37 g), but no significant food type by injection interaction ($F_{(1,10)}$=2.27 p=0.16) or main effect of injection ($F_{(1,10)}$=0.06 p=0.81), indicating food preference was intact and unaffected by CNO **(Supplementary Figure 1B)**.

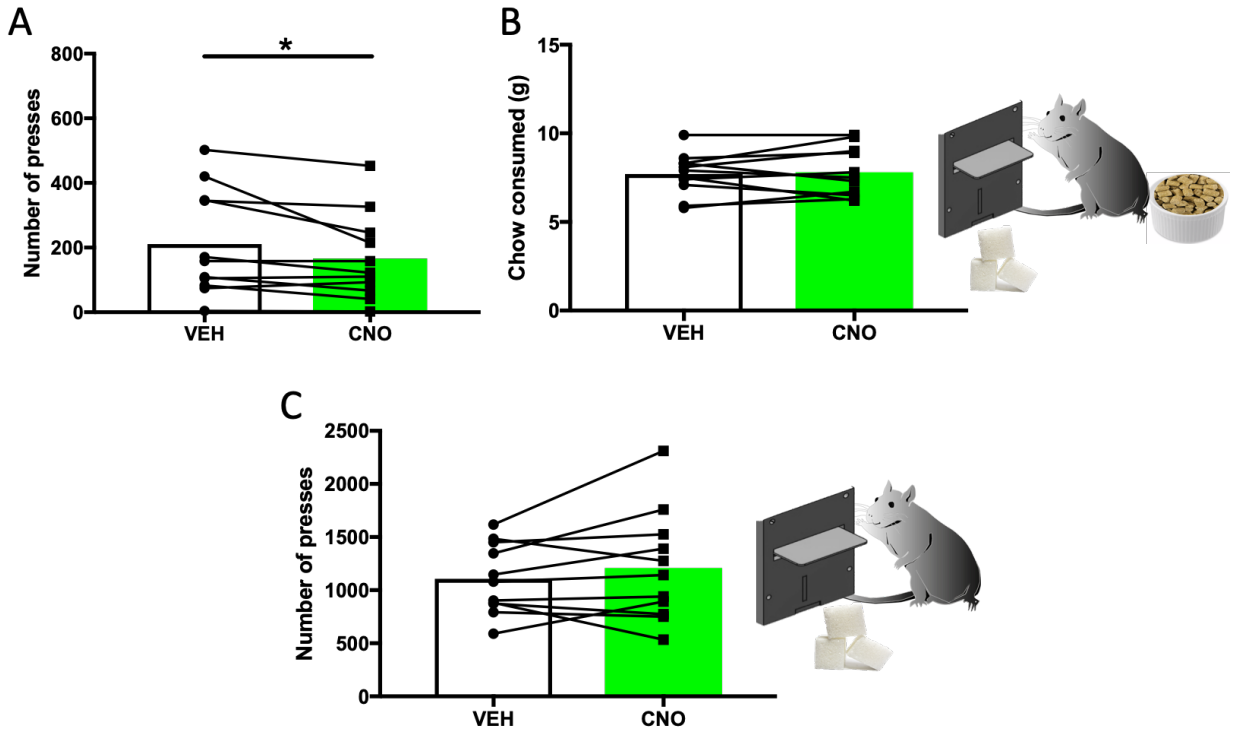


Figure 4. Effects on choice behavior following DREADDs stimulation of Anterior Cingulate Cortex. (A) Mean lever presses during PR sessions, when rats were presented with both the possibility of lever pressing under a PR schedule for sucrose pellets and freely-available chow. Shown is within-subject, counterbalanced choice behavior under vehicle and CNO. CNO significantly reduced the number of lever presses in this condition. **(B)** Total chow consumed during choice testing was not different between vehicle and CNO conditions. **(C)** Mean lever presses during PR sessions when there was no chow available as an alternative option. Lever pressing was not different between vehicle and CNO conditions. * p<0.05.

208 *GFP (null virus) control.* To test whether results for Gi and Gq DREADDs could be
209 explained by virus exposure alone, a control virus carrying only GFP (but no DREADD receptors)
210 was infused into ACC in a separate group of rats. A paired t-test revealed no effect of CNO on the
211 total mean number of lever presses during 30 min PRC sessions ($t_{(9)}$=0.48 p=0.64; VEH = 314.9 ±
212 57.34 presses; CNO = 302.7 ± 59.66 presses) **(Supplementary Figure 2A)**. The total amount of
213 chow consumed during 30 min PRC sessions was not affected by CNO treatment ($t_{(9)}$=0.26 p=0.80;
214 VEH = 6.65 ± 0.99 g; CNO = 6.85 ± 0.34 g) **(Supplementary Figure 2B)**. CNO also had no effect
215 on PR responding in the absence of freely available chow ($t_{(10)}$=0.73 p=0.48; VEH = 1001.00 ±
216 109.4 presses; CNO = 1043.00 ± 120.00 presses) **(Supplementary Figure 2C)**. A two-way
217 ANOVA (food type- sucrose, chow; injection- VEH, CNO) on the amount of sucrose and chow
218 consumed during free choice testing revealed a significant main effect of food type ($F_{(1,9)}$=80.63
219 p<0.001; sucrose = 8.37 ± 0.60 g; chow = 3.19 ± 0.35 g), indicating food preference was intact.
220 No significant food type by injection interaction ($F_{(1,9)}$=1.27 p=0.29), or effect of injection
221 ($F_{(1,9)}$=0.97 p=0.35) was found **(Supplementary Figure 1C)**.

Calcium imaging in rat ACC. A group of 4 rats received infusions of AAV9- CaMKIIα-GCaMP6f and was subsequently implanted with 1.8 mm diameter 0.25 pitch GRIN lenses in Cg1 (**Figure 5**). Rats were then trained on lever pressing and tested during PR and PRC sessions identical to those described above for DREADD experiments. GRIN lens implanted rats were also given "satiety control" (CON) sessions, where rats were pre-fed with chow in the operant chamber, and then were allowed to lever press on a PR schedule in the absence of chow. This was to control for the possibility that neural encoding could be influenced by satiety from chow consumption, regardless of whether chow was freely available during the lever pressing task.

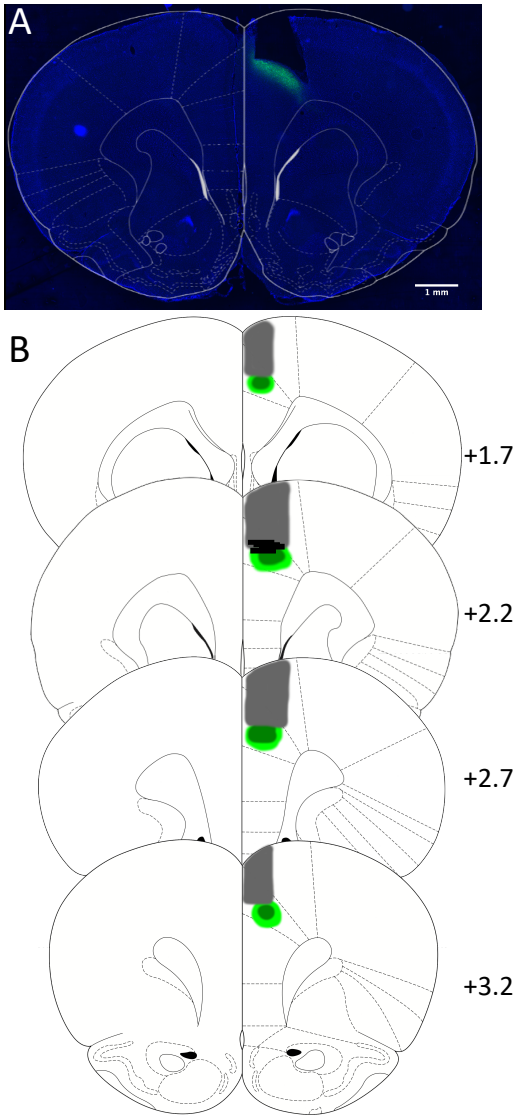


Figure 5. Calcium imaging histology in Anterior Cingulate Cortex. (A) Representative photomicrograph showing GCaMP6f expression and aspiration site for lens placement for ACC imaging. (**B**) Schematic reconstruction of maximum (light green), minimum (dark green) viral spread, and maximum aspiration damage (gray). Black bars represent ACC recording sites. Numerals depict +Anterior-Posterior (AP) level relative to Bregma. Scale bar 1mm.

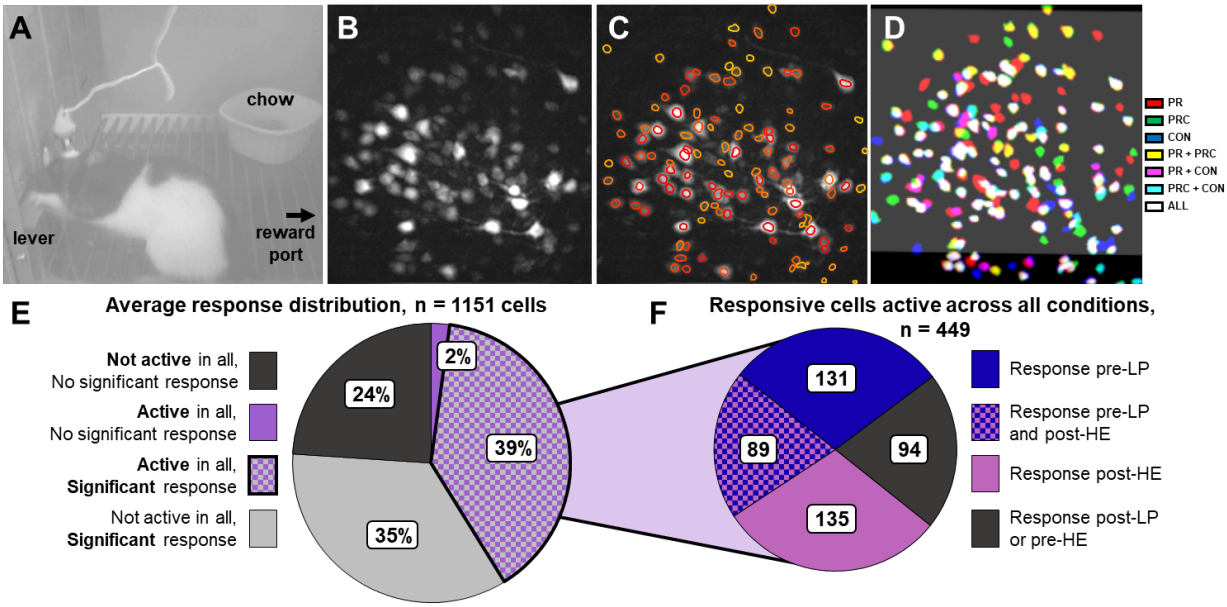


Figure 6. **Anterior Cingulate Cortex calcium imaging. (A)** Example of PRC behavior session with the miniscope on the rat's head; both sucrose and chow options are available, with reward port on the right (just out of view) and chow in the ramekin. **(B)** Maximum projection image of calcium fluorescence from the session in 'A' after motion correction and neural enhancement. **(C)** Same as in 'B' with extracted cell contours overlaid. **(D)** Cell contours matched across three recording sessions (one of each type: PR, PRC, CON); color codes indicate the subset of sessions during which a given cell fired at least one spike. **(E)** Distribution of response properties for all recorded cells (1151 cells from 4 rats). 39% (449) of the total unique 1151 cells were active in at least one of each session type and significantly modulated by behavior. These cells were further analyzed (see Figure 7). **(F)** Further classification of 449 cells that were active in at least one session of each type (PR, PRC, and CON) and had a significant response to either lever press (LP) or reward port head entry (HE) events during at least one session type; We separately analyzed cells which responded before lever-press (pre-LP) and after head-entry (post-HE).

Single cell responses. We recorded a total of 1151 neurons from ACC in 4 animals (136 from rat #1, 567 from rat #2, 254 from rat #3, 194 from rat #4). Each neuron's spike events were extracted by deconvolving its denoised calcium trace (sampling rate = 7.5 Hz; see Methods). A cell's baseline spiking probability was measured during time periods that were at least ±5 s away from any lever press ("LP") or head entry ("HE") into the reward magazine. To analyze neural responses to LP and HE events, perievent time histograms (PETHs) were generated by combining

266 data from all sessions of a given type (PR, PRC, or CON) during which the cell fired at least one
267 spike. Spike probabilities were computed in time bins within ±3 s of the trigger event (LP or HE),
268 and compared against baseline using a binomial probability test (bin width = 133.33 ms, derived
269 from the 7.5 Hz frame rate). Any cell exhibiting at least 3 bins with spike rate exceeding baseline
270 at the $p<.01$ level was classified as "responsive" within the 6 s period surrounding the trigger event.

271 To analyze how responses to HP and LE events varied with session type (PR, PRC, CON),
272 we identified a subset of 449 neurons (39% of all recorded cells) that were: 1) active during at least
273 one session of each type, and 2) significantly responsive to either LP or HE events (or both) during
274 at least one of the three session types (**Figure 6E**). The natural sequence of task behavior caused
275 LP events to often be followed within 3 s by HE events, and likewise, for HE events to often be
276 preceded within 3 s by LP events (**Supplementary Figure 3**). This was because immediately after
277 completing the required number of LPs for a trial, the lever was retracted and the rat went to the
278 magazine where it generated an HE event that closely followed the last LPs of that trial.
279 Consequently, cells that responded after LP events or before HE events could not be
280 unambiguously classified as responsive to one kind of event or the other (LPs versus HEs, because
281 the response was proximal to both kinds of events). By contrast, LP events were rarely preceded
282 within 3 s by HE events, and HE events were rarely followed within 3 s by LP events
283 (**Supplementary Figure 3**). We thus restricted further analysis to cells that consistently responded
284 before but not after LP events ("pre-LP" cells, n=131), or after but not before HE events ("post-
285 HE" cells, n=135), or both ("LP-HE" cells, n=89); cells that responded after LP events or before
286 HE events (n=94) were omitted from further analysis (**Figure 6F**).

287 *Population responses preceding LP events.* For every cell that responded prior to LP events
288 (that is, cells belonging to either the pre-LP or LP-HE cell populations), three LP-triggered PETHs

were generated (one for each session type: PR, PRC, CON; **Figure 7A**). To normalize the response of each cell, the bins of all three PETHs for the cell were divided by the maximum value observed in any bin across all three PETHs. A population average of responses surrounding LP events was computed as the mean of the normalized PETHs (**Figure 7B**). Each cell's summed normalized response during the 3 s preceding LP events was computed for each session type, and it was found that pre-LP responses differed significantly by session type (Friedman's non-parametric ANOVA: p = 8.57e-10; **Figure 7B,C**). Post-hoc comparisons revealed that the pre-LP response was greatest during PR sessions, smallest during PRC sessions, and intermediate during CON sessions, with significant differences between all three session types (Sign rank test: PR > CON, p = 7.94e-04; PR > PRC, p = 3.22e-12; CON > PRC, p = 4.14e-06) (**Figure 7C**).

Population responses following HE events. For every cell that responded after HE events (that is, cells belonging to either the post-HE or LP-HE cell populations), three HE-triggered PETHs were generated (one for each session type: PR, PRC, CON; **Figure 7D**). Population averaged responses surrounding HE events were computed for each of the three session types, using the same methods described above for LP-triggered PETHs (**Figure 7E**). It was found that post-HE responses different significantly by session type (Friedman's non-parametric ANOVA: p = 6.38e-04; **Figure 7E,F**), and post-hoc comparisons revealed that population responses during PR and CON sessions were not significantly different from one another (Sign rank test: PR = CON, p = 0.22), whereas PRC sessions showed a significantly lower post-HE response than either of the other two session types (Sign rank test: PR > PRC, p = 4.23e-05 ; CON > PRC, p = 1.47e-05). This pattern of results suggests that responses of ACC neurons to rewarding outcomes may depend upon the availability of alternative outcomes (see Discussion).

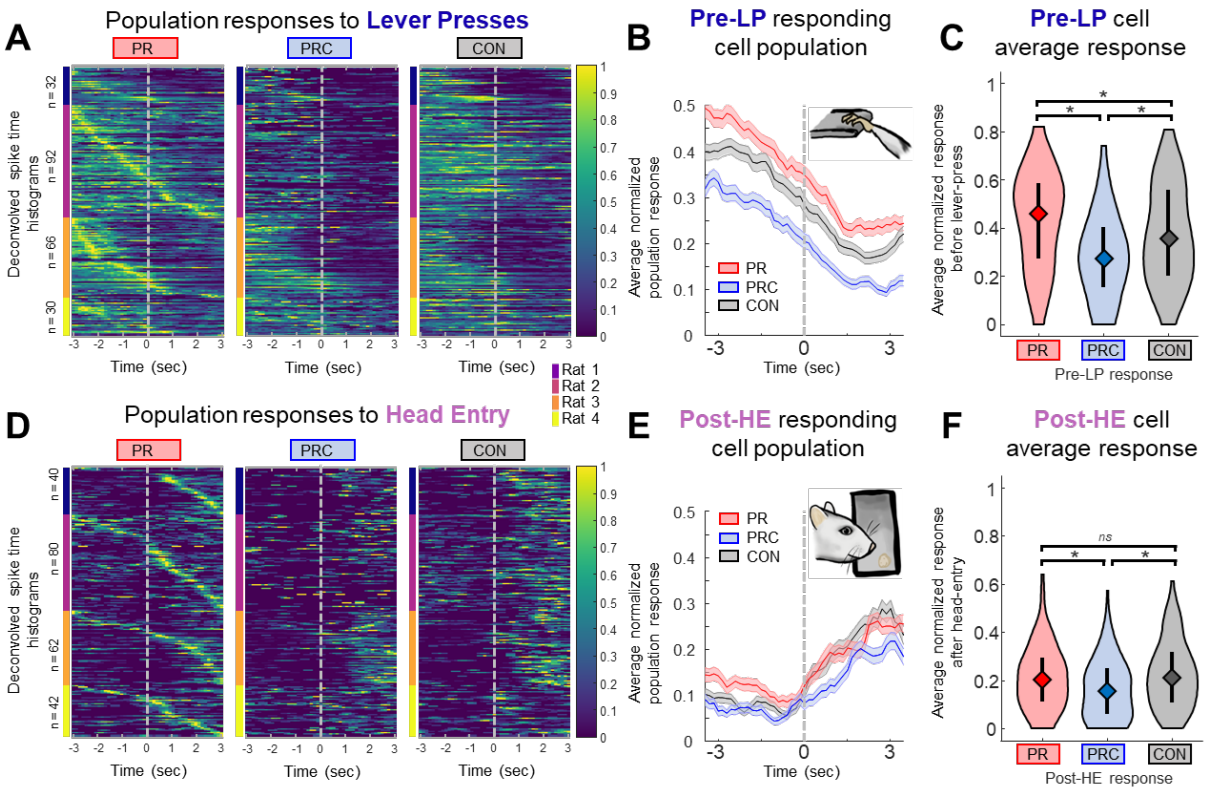


Figure 7. Analysis of Anterior Cingulate Cortex lever press and magazine head entry responsive cells during different session types. (A) Heat plots show normalized PETHs of spike responses for cells classified as "pre-LP" cells. PETHs are sorted first by animal (color code at left) and then by the time bin of their peak activity bin in PR sessions. Cells are matched across session types. **(B)** Mean population response for PR, PRC, and CON sessions; shaded portion denotes 1 standard error. Pre-LP responsiveness was weakest during PRC testing. **(C)** Violin plots of the population activity averaged over the 3 s before lever press. Diamonds mark the median, and lines mark the inner quartiles. Pre-LP responsiveness was weakest during PRC testing. **(D)** Normalized PETHs for cells classified "post-HE" cells. **(E)** Mean population response to HE in PR, PRC, and CON conditions. Post-HE responsiveness was weakest during PRC testing. **(F)** Violin plots for post-HE population activity after HE. Post-HE responsiveness was weakest during PRC testing. *p<.001, *n.s.* not significant.

Discussion

We found that either chemogenetic silencing *or* stimulation of ACC excitatory neurons resulted in decreased PR lever pressing for a qualitatively preferred option, but this effect was only observed when a concurrently available, lower effort alternative was available, and not when lever pressing was the only response option. Chemogenetic manipulations had no effects on the ability to lever

329 press for sucrose or on food preference. CNO administration also had no effects in rats lacking
330 active DREADD (hM4D-G_i or hM3D-G_q) receptors. Slice electrophysiology confirmed robust
331 inhibition (Stolyarova et al., 2019) and excitation in hM4D-G_i and hM3D-G_q transfected slices,
332 respectively. Finally, we found that ACC calcium activity was modulated by the availability of
333 alternative choice options, in a manner suggesting that ACC neurons may encode the relative value
334 of available reward options (see below). Taken together, these findings support a causal role for
335 ACC in the evaluation of effortful behavior, consistent with recent evidence from single-unit
336 recordings suggesting involvement of this region in "biasing decision-making towards behaviors
337 with highest utility" (Porter et al., 2019).

338 **ACC chemogenetic silencing**. The earliest studies probing rat ACC in effort-based choice
339 made use of T-maze tasks where rats selected between the same food option but of different
340 magnitudes (Schweimer and Hauber, 2006; Walton et al., 2003; Walton et al., 2002). The first test
341 of rat ACC in a task where rats chose between qualitatively different options produced negative
342 results (Schweimer and Hauber, 2005); it is possible this was due to pre-training lesions as well as
343 prior behavioral testing, as later work from our lab found that excitotoxic lesions of ACC resulted
344 in decreased PR lever pressing in the context of choice (Hart et al., 2017).

345 Here, hM3Dq and hM4Di receptors were expressed under a CaMKIIα promoter, thus
346 targeting primarily excitatory pyramidal neurons (Nathanson et al., 2009; Wang et al., 2013), in
347 contrast with prior studies using lesions or inactivations that silence all neural activity. It seems
348 likely that ACC exerts its effects on choice behavior via projections to downstream targets, the
349 densest of which are to dorsal striatum and mediodorsal thalamus (Vogt and Paxinos, 2014),
350 though ACC also sends sparser efferents to ventral striatum and amygdala (Gabbott et al., 2005).
351 A recent report demonstrated a role for dorsal striatal dopamine in effort-based choice (Bailey et

352 al., 2018). Interestingly, the role of dorsal striatum in effort-based choice is relatively understudied
353 and could be a ripe avenue for future study given the strong cortical afferentation from ACC.

354 Though reliable, the magnitude of effect observed here with DREADDs was considerably
355 smaller than what we previously observed following lesions (Cohen's d=1.39 lesions vs d=0.28 G_i
356 vs d=0.29 G_q) (Hart et al., 2017), and also smaller than effects we have previously reported from
357 our lab following pharmacological inactivation (Hart and Izquierdo, 2017; 2019) and drug
358 exposure (Hart et al., 2018; Thompson et al., 2017). The smaller effect obtained with DREADDs
359 could be due to several factors. First, unlike NMDA lesions which cause widespread destruction
360 of ACC neurons, DREADD receptors targeted only cells that recognize the CaMKIIα promoter
361 (presumably excitatory projection neurons), and these cells may only have been partially
362 transfected with DREADD receptors. Second, although slice experiments have shown that
363 inhibitory DREADD receptors suppress neural activity in ACC (Stolyarova et al., 2019), and that
364 excitatory DREADDs reliably altered firing patterns of ACC neurons (**Figure 2**), DREADD-
365 induced changes in neural activity may have weaker effects upon behavior than complete
366 pharmacological inactivation of ACC with muscimol, or widespread changes in neural activity
367 following chronic psychostimulant exposure (Hart et al., 2018).

368 **ACC calcium imaging.** We used *in vivo* calcium imaging to investigate how ACC neurons
369 responded during lever pressing and reward retrieval (i.e., reward port head entry) during PR, PRC,
370 and CON sessions (**Figure 7**). A total of 449 neurons from 4 rats were successfully recorded during
371 at least one session of each type and had a significant response to surrounding lever press or head
372 entry. About half of these neurons (220/449) fired significantly above baseline during the 3s time
373 window preceding lever presses in at least one of the three session types (**Figure 7A-C**). These
374 pre-lever responses were significantly lower during CON than PR sessions, and since rats were

375 sated on chow before lever pressing during CON but not PR sessions, it appears that satiety from
376 chow consumption may have attenuated neural responses during the pre-lever period. Consistent
377 with this interpretation, calcium activity during the pre-lever period was also lower during PRC
378 than PR sessions. However, pre-lever activity was even lower during PRC than CON sessions
379 (despite the fact that rats were similarly sated on chow at the start of both PRC and CON sessions),
380 suggesting that satiety on chow plus free chow availability attenuated pre-lever calcium activity
381 even more than satiety alone.

382 About half of the recorded ACC neurons (224/449) fired significantly above baseline
383 during the 3s time window following head entry into the reward magazine, which was the period
384 during which the rat retrieved and consumed the high value sucrose reward (**Figure 7D-F**). These
385 sucrose responses were similar in magnitude during CON and PR sessions, indicating that satiety
386 from prior chow consumption had little effect on ACC responses to sucrose. By contrast, sucrose-
387 related activity was significantly lower during PRC sessions than during PR and CON sessions,
388 indicating that independently of satiety state, free chow availability attenuated sucrose responses
389 of ACC neurons below the levels seen when sucrose was earned in the absence of free chow.

390 These findings suggest that the activity of ACC neurons during sucrose consumption
391 encodes the difference between the value of the sucrose versus the value of other available reward
392 options. If no other options are available (as during PR and CON sessions), then the value of other
393 options is zero, and thus nothing is subtracted from the value of the sucrose reward. But if a lower
394 value reward option (such as lab chow during PRC sessions) is available while the rat is working
395 for sucrose, then the nonzero value of the other option may be subtracted from the value of the
396 sucrose reward, reducing the magnitude of ACC responses during sucrose delivery. If this relative
397 value signal for sucrose in ACC is involved in driving motivated effort to work for sucrose, then

398 it would be expected that the rat should exert less effort for sucrose under conditions where the
399 ACC responses to sucrose are smaller. This is exactly what we observed: sucrose responses of
400 ACC neurons were lower during PRC sessions (where lab chow was available as a competing
401 reward option) than during PR or CON sessons (where sucrose was the only reward option). The
402 reduced motivation to lever press might account in part for the attenuated calcium activity we
403 observed in ACC during the pre-lever period of PRC sessions, compared with PR and CON
404 sessions. However, since pre-lever calcium activity was also lower during CON sessions than PR
405 sessions, satiety may have also attenuated calcium activity prior to lever pressing.

406 In summary, neural activity associated with sucrose pellet collection in ACC may be
407 strongest when sucrose is the only available option, and weakened by the presence of the
408 counterfactual choice. This is consistent with prior evidence that rat ACC encodes "the utility of
409 remote locations" that contain rewards not chosen (Mashhoori et al., 2018), and also with findings
410 that nonhuman primate dorsal ACC is involved in actively signaling the value of leaving a patch
411 in pursuit of another option in foraging tasks (Hayden et al., 2011). Dorsal ACC also signals the
412 value of the rejected (counterfactual) option (Blanchard and Hayden, 2014), which is analogous to
413 the chow option in our task. Unfortunately, because of our task design, it was not easy to accurately
414 identify when rats were consuming chow, and thus it was not possible to analyze neural activity
415 during chow consumption. This would be a fruitful avenue for future work. Additionally, how
416 specific is relative value coding to ACC? The orbitofrontal cortex has long been implicated in the
417 encoding of relative reward value (Tremblay & Schultz, 1999), so it would also be interesting to
418 compare signaling across frontocortical areas in future experiments.

419 **ACC inhibition versus stimulation**. If ACC activity encodes relative value signals that
420 are involved in deriving an animal's motivation to exert effort, then it is natural to predict that

421 disrupting ACC activity at the neural level would alter effort exertion at the behavioral level. We
422 also expected that bidirectional manipulations of neural activity might yield bidirectional effects
423 upon motivated effort. Indeed, impairments in effort induced by dopamine receptor blockade or
424 dopamine depletion can be reversed by several manipulations that enhance dopamine transmission
425 or block adenosine transmission (Farrar et al., 2010; Nunes et al., 2013; Randall et al., 2014a;
426 Yohn et al., 2015a; Yohn et al., 2015b). Further, enhancing dopamine transmission with major
427 psychostimulants (Yohn et al., 2016c), dopamine transporter blockers (Randall et al., 2014b; Yohn
428 et al., 2016a), adenosine A2A receptor antagonists (Randall et al., 2012), or 5-HT2C ligands
429 (Bailey et al., 2018; Bailey et al., 2016) can increase effort output in otherwise untreated rats.

430 We applied a similar rationale for our G_q and G_i DREADD experiments, and tested whether
431 ACC chemogenetic inhibition versus stimulation yielded bidirectional effects upon behavioral
432 responding. This was not the case: lever pressing behavior during PRC sessions was similarly
433 attenuated by both G_q and G_i DREADDs. The contributions of ACC and other frontocortical
434 regions to effort may be complex; for example, blocking inhibitory transmission in orbitofrontal
435 cortex, which presumably increases local excitation, results in decreased PR responding (Munster
436 and Hauber, 2017). Others report a similar effect of local infusion of the GABA-A antagonist
437 bicuculline in prelimbic cortex, finding decreased high-effort choice in a two-lever operant task
438 (Piantadosi et al., 2016). Therefore, in frontal cortex there may be an optimal excitatory/inhibitory
439 ratio for computing relative cost-benefit and, consequently, sending appropriate output to
440 downstream targets. It is possible that the G_q stimulation used here enhanced background noise
441 levels in ACC, decreasing signal-to-noise ratio within the network. It is conceivable this would
442 interfere with normal recruitment of ACC during choice lever pressing, or could even produce
443 local inhibition *in vivo* that we were unable to detect in *ex vivo* slice recordings. The results of

444 ACC stimulation here are consistent with our manipulation introducing noise to otherwise normal
445 neural computations (Mainen and Sejnowski, 1995; Stein et al., 2005), thus impairing behavior in
446 a manner similar to when neural activity is inhibited. Lever pressing rates and neural activity levels
447 in ACC were both lower during PRC sessions than PR or CON sessions. Hence, signal-to-noise
448 ratios in ACC may have been more susceptible to interference by both excitatory and inhibitory
449 DREADDs during PRC sessions, but not during PR or CON sessions.

450 In conclusion, our findings suggest that the role of ACC in effort-based choice may be to
451 discriminate the utility of available choice options by providing a stable population code for the
452 relative value of different reward outcomes. Consequently, a disruption in decision making can
453 occur via changes in signal-to-noise ratio in this region: either by decreases in the signal (i.e. G_i
454 DREADDs), or by increases in background noise (i.e. G_q DREADDs). A better understanding of
455 ACC contributions to effort-based choice may yield insight into the mechanimsms underlying
456 motivational symptoms that occur in a wide range of conditions, particularly depression (Nunes et
457 al., 2013) and addictions (Robinson et al., 2013).

458

459 **Materials and Methods**

460 *Subjects.* Subjects were N=44 adult male Long-Evans rats (n=12 G_i DREADD experiment,
461 n=12 G_q DREADD experiment, n=10 GFP (null virus) control experiment, n=4 calcium imaging
462 experiment, n=6 acute slice recording for validation of DREADDs). Rats were obtained from
463 Charles River Laboratories (Hollister, CA), were PND 60 at the time of arrival to the UCLA
464 vivarium, and were singly-housed for all phases of experiments with the exception of the
465 acclimation period and handling, during which they were pair-housed. All rats were handled for

466 10 minutes in pairs for 5 d after a brief acclimation period (3 d). Rats weighed an average of 309.1
467 g at the beginning of experiments. Three subjects were not included in the final data analyses: one
468 from G_i experiment and one from G_q experiment due to unilateral (not bilateral) viral expression,
469 and one from the GCaMP experiments due to poor stability of imaging over days. Representative
470 photomicrographs and reconstruction of DREADDs expression resulting from histological
471 processing are shown in **Figure 1.**

472 The vivarium was maintained under a 12/12 h reverse light cycle at 22°C, and lab chow
473 and water were available *ad libitum* prior to behavioral testing. Rats were food restricted one day
474 prior to behavioral testing to ensure motivation to work for rewards. Given the sensitivity of the
475 behavioral tests on motivation, special care was taken to maintain consistent food rations
476 throughout the experiment. This was 12 g/d at the beginning of testing, but then decreased to 8 g/d
477 at the beginning of the choice phase (details below). Rats were monitored every other day for their
478 body weight, and were never permitted to drop below their 85% free feeding baseline weight.
479 Training and testing were conducted during the early portion of the dark cycle (~0800 to 1200 H).
480 Experiments were conducted 5-7 d per week, and rats were fed once daily on weekends (12 g)
481 when testing was not conducted. All procedures were reviewed and approved by the Chancellor's
482 Animal Research Committee (ARC) at the University of California, Los Angeles.

483 *Food restriction.* One day before behavioral testing began, rats were singly-housed, the
484 amount of chow given to each rat was reduced to 12 g/d, and rats were given ~10 sucrose pellets
485 (45-mg dustless precision sucrose pellets; Bio-Serv, Frenchtown NJ) in their home cage to
486 acclimate them to the food rewards. Rats were maintained on 12g of food daily and were each fed
487 within 30 min of completing the daily testing. Once rats progressed to the choice task they were

488 given 8 g of chow per d, in addition to the food they consumed during testing. At the time of
489 euthanasia, rats weighed an average of 356.5 g.

490 *Stereotaxic Surgery.* General surgical procedures were the same as those recently published
491 (Hart and Izquierdo, 2017). Rats were anesthetized with isoflurane (5% induction, 2% maintenance
492 in 2L/min O_2). Burr holes were drilled bilaterally on the skull for insertion of 26-gauge guide
493 cannulae (PlasticsOne, Roanoke, VA), after which 33-gauge internal cannulae (PlasticsOne,
494 Roanoke, VA) were inserted. Rats were infused with 0.5 μL of virus at a flow rate of 0.1 μL/minute
495 and injectors were subsequently left in place for 5 additional minutes to allow for diffusion of
496 solution. In the G_i experiment, the virus used was AAV8-CaMKIIα-hM4D(G_i)-mCherry
497 (Addgene, Cambridge, MA). In the G_q experiment, the virus used was AAV8-CaMKIIα-
498 hM3D(G_q)-mCherry (Addgene, Cambridge, MA). In the GFP (null virus) control, the virus used
499 was AAV8-CaMKIIα-eGFP (Addgene, Cambridge, MA). In the imaging experiment, the virus
500 used was AAV9-CaMKIIα-GCaMP6f (Addgene, Cambridge, MA). The coordinates used for the
501 guide cannulae targeting ACC (Cg1) in the G_i, G_q, and GFP control experiments were: AP= +2.0
502 mm, ML= ±0.7 mm, DV= −1.9 mm from Bregma. Four of twelve rats in the G_i experiment
503 received infusions in more anterior Cg1 to compare with other laboratory experiments on decision
504 confidence (Stolyarova et al., 2019), at AP= +3.7 mm, ML= ±0.8 mm, DV= −1.6 mm from
505 Bregma. Since no differences emerged from this differential targeting, we combined the Cg1
506 groups. In the imaging experiment, coordinates were: AP= +2.0 mm, ML= ±0.7 mm, DV= −1.4
507 mm (0.5 μL) from Bregma, and a second 0.5 μL bolus of virus was injected at DV= -0.9 mm.
508 Injectors extended 1 mm beyond the tip of the cannula. Following the 5-minute diffusion time, the
509 cannulae and injectors were removed, incisions were stapled closed, and the rats were placed on a
510 heating pad and kept in recovery until ambulatory before being returned to the vivarium.

511 Three days following viral infusions in the subset of rats receiving AAV9-CaMKIIα-
512 GCaMP6f (for calcium imaging), rats were implanted with 1.8 mm diameter 0.25 pitch GRIN
513 lenses (Edmund optics part 64-519, Barrington, NJ). Following similar surgical procedures, 4
514 anchor screws were secured to the skull, after which a 2.0 mm craniotomy was drilled 0.2 mm
515 lateral to the center of the viral infusion hole. The dura was cleared and approximately 0.5 mm of
516 tissue was aspirated, after which the lens was placed 2.0 mm ventral from the surface of the skull
517 and secured in place with cyanoacrylate glue and bone cement. The lens was protected with Kwik-
518 Sil (World Precision Instruments, Sarasota, FL).

519 Post-operative care for all rats consisted of five daily injections of carprofen (5mg/kg, s.c.)
520 and oral sulfamethoxazole/trimethoprim solution. Two-to-three weeks following lens
521 implantation, a small aluminum baseplate was attached to the animal's head and secured with bone
522 cement. The exposed lens was cleaned with 100% ethanol, and the baseplate was secured in a
523 position where cells in the field of view and vasculature were in focus. A 3D printed cover was
524 secured to the baseplate with an anchor screw at all times when recording was not occurring. Rats
525 were allowed a 5-d free feeding recovery period following viral infusion (G_i, G_q, and GFP
526 experiments) or GRIN lens implantation (imaging experiment) after which they were food
527 restricted and behavioral testing began.

528 *Apparatus*. All behavioral testing was conducted in chambers outfitted with a house light,
529 internal stimulus lights, a food-delivery magazine, and 2 retractable levers positioned to the left
530 and right of the chamber wall, opposite the magazine. All hardware was controlled by a PC running
531 Med-PC IV (Med-Associates, St. Albans, VT).

532 *Miniaturized microscope data collection.* Microscopes were custom built according to
533 plans available at Miniscope.org. Images were acquired with a CMOS imaging sensor (Labmaker,

534 Berlin, Germany) attached to custom data acquisition (DAQ) electronics via a 1.5 mm coaxial
535 cable. Data were transferred to a PC running custom written DAQ software over Super Speed
536 USB. DAQ software was written in C++ and used Open Computer Vision (OpenCV) for image
537 acquisition. 480x752 pixel images were acquired at 30 Hz and written to .avi files. DAQ software
538 simultaneously recorded and time-stamped behavioral data and image data, allowing for offline
539 alignment. All hardware design files and assembly instructions are available at Miniscope.org.
540 Calcium signals were extracted using modified constrained non-negative matrix factorization
541 scripts in Matlab (Mathworks 2016, Natick, MA) (Pnevmatikakis et al., 2016; Zhou et al., 2018).

542 *Lever press training.* Rats were first given fixed-ratio (FR)-1 training where each lever
543 press earned a single sucrose pellet (Bioserv, Frenchtown, NJ). They were kept on this schedule
544 until they earned at least 30 pellets within 30 minutes. Following this, rats where shifted to a
545 progressive ratio (PR) schedule where the required number of presses for each pellet increased
546 according to the formula:

547 $$n_i = 5e^{(i/5)} - 5$$

548 where n_i is equal to the number of presses required on the ith ratio, rounded to the nearest whole
549 number (Richardson and Roberts, 1996), after 5 successive schedule completions. No timeout was
550 imposed. Rats were tested on the PR schedule until they earned at least 30 pellets on any given
551 day (~5 d) within 30 min. Upon meeting the PR performance criterion, a ceramic ramekin
552 containing 18 g of lab chow was introduced (modified from (Randall et al., 2012)) during testing.
553 Rats were free to choose between consuming freely-available but less preferred chow or lever
554 pressing for preferred sucrose pellets. Rats (G_i, G_q, and GFP experiments) were given at least 5
555 choice testing sessions before clozapine-N-oxide (CNO) or vehicle (VEH) injections began
556 (details below).

557 *Drug treatment.* Rats in the G_i, G_q, and GFP control experiments were given either VEH
558 (95% saline, 5% DMSO, 1 mL/kg) or CNO (3.0 mg/kg i.p. in 95% saline, 5% DMSO, 1 mL/kg)
559 (Tocris, Bristol, UK) 45 min prior to test sessions. Three types of test sessions were given: 1) a
560 progressive ratio choice (PRC) session during which rats lever pressed on the PR schedule in the
561 presence of the ceramic ramekin containing lab chow so that they were free to choose between
562 lever pressing for sucrose versus free feeding on chow, 2) progressive ratio (PR) only sessions
563 during which we omitted the ceramic ramekin (so that there was no freely-available lab chow) to
564 assess whether manipulations decreased lever pressing in the absence of choice, and 3) free access
565 to pre-weighed amounts of sucrose pellets and lab chow (18 g) in empty cages (different from their
566 home cages). Following this, any remaining food was collected and weighed to determine rats'
567 food preferences. All sessions were 30 min in duration. In a repeated measures design, VEH or
568 CNO was administered prior to a PRC testing session, a PR only testing session, and a free
569 availability choice testing session, in that order, for each rat. The order of vehicle versus CNO
570 administration was counterbalanced for baseline choice performance. Rats were given at least 48
571 hours between injections, and testing never occurred on consecutive days.

572 *Euthanasia.* Following behavioral testing, rats were sacrificed by sodium pentobarbital
573 overdose (Euthasol, 0.8 mL, 390 mg/mL pentobarbital, 50 mg/mL phenytoin; Virbac, Fort Worth,
574 TX) and perfused transcardially with 0.9% saline followed by 10% buffered formalin acetate.
575 Brains were post-fixed in 10% buffered formalin acetate for 24 hours followed by 30% sucrose
576 cryoprotection for 5 days. 50 μm sections were cover slipped with DAPI mounting medium
577 (Prolong gold, Invitrogen, Carlsbad, CA), and visualized using a BZ-X710 microscope (Keyence,
578 Itasca, IL).

579 *DREADDs quantification.* 50 μm sections taken from each animal were visualized at seven
580 AP coordinates relative to bregma: +4.2 mm, +3.7 mm, +3.2 mm, +2.7 mm, +2.2 mm, +1.7 mm,
581 and +1.6 mm. No fluorescence was observed beyond these coordinates. mCherry fluorescence was
582 drawn by a blind experimenter on a GNU Image Manipulation Program (GIMP) document
583 containing a schematic of each of these seven sections, drawn to scale. Spread was quantified as
584 total pixel count across all seven sections.

585 *Electrophysiological confirmation of DREADDs*. Separate rats were prepared with ACC
586 (Cg1) DREADDs using identical surgical procedures to the main experiments (**Figure 2A**). Slice
587 recordings did not begin until at least four weeks following surgery to allow sufficient hM receptor
588 expression. Slice recording methods were similar to those previously published (Babiec et al.,
589 2017). Six rats were deeply anesthetized with isoflurane and decapitated. The brain was rapidly
590 removed and submerged in ice-cold, oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid
591 (ACSF) containing (in mM) as follows: 124 NaCl, 4 KCl, 25 $NaHCO_3$, 1 NaH_2PO_4, 2 $CaCl_2$, 1.2
592 $MgSO_4$, and 10 glucose (Sigma-Aldrich). 400-μm-thick slices containing the ACC were then cut
593 using a Campden 7000SMZ-2 vibratome. Slices from the site of viral infusion were used for
594 validation. Expression of mCherry was confirmed after recordings were performed, and ACC
595 slices with no transfection were used as control slices. Slices were maintained (at 30°C) in
596 interface-type chambers that were continuously perfused (2–3 ml/min) with ACSF and allowed to
597 recover for at least 2 hours before recordings. Following recovery, slices were perfused in a
598 submerged-slice recording chamber (2–3 ml/min) with ACSF containing 100 μM picrotoxin to
599 block $GABA_A$ receptor-mediated inhibitory synaptic currents. A glass microelectrode filled with
600 ACSF (resistance = 5–10 MΩ) was placed in layer 2/3 ACC to record field excitatory postsynaptic
601 synaptic potentials and postsynaptic responses elicited by layer 1 stimulation delivered using a

602 bipolar, nichrome-wire stimulating electrode placed near the medial wall in ACC. Inhibitory
603 validation in ACC with identical coordinates, reagents, and virus was previously performed by our
604 lab, with the methods and data appearing elsewhere (Stolyarova et al., 2019) and so these
605 experiments were not needlessly repeated. Briefly, we first recorded for 2 minutes without synaptic
606 stimulation to measure spontaneous levels of activity. Presynaptic fiber stimulation (0.2 msec
607 duration pulses delivered at 0.33 Hz) was then delivered and the stimulation intensity was varied
608 in 0.2 V increments to generate an input/output curve and identify the threshold for generation of
609 postsynaptic responses. Stimulation strength was then set to the minimum level required to induce
610 postsynaptic responses in ACC. Once stable responses (measured as the area of responses over a
611 4 second interval) were detected, baseline measures were taken for at least 10 minutes, followed
612 by 20 minutes bath application of 10 μM CNO. In slices where CNO failed to elicit spontaneous
613 activity, we generated a second input/output in the presence of CNO to test for CNO-induced
614 changes in postsynaptic responses evoked by synaptic stimulation. Unless noted otherwise, all
615 chemicals were obtained from Sigma-Aldrich.

616 *Behavioral analyses.* Behavioral data were analyzed using GraphPad Prism v.7 (La Jolla,
617 CA) and SPSS v.25 (Armonk, NY). An alpha level for significance was set to 0.05. Paired samples
618 t-tests (reported as means ± SEM) on data from the choice and PR-only tests were used to test for
619 effects of CNO. Two-way ANOVA was used to test for effects of CNO on food preference in the
620 free choice test.

621 *Calcium image analyses.* Image analyses were performed using custom written MATLAB
622 scripts. First, images were motion corrected using functions based on the Non-Rigid Motion
623 Correction ("NoRMCorre") package (Pnevmatikakis and Giovannucci, 2017), downsampled
624 spatially by a factor of two (240x356 pixels) and temporally by a factor of 4 (to a frame rate of 7.5

625 fps). In order to remove background fluorescence, we performed a neural enhancement processing
626 step as in the min1pipe processing framework (Lu et al., 2018). Briefly, from each frame we
627 constructed a background fluorescence estimate by performing a neuron-sized morphological
628 opening function, and subtracting this background frame from the original frame. This removes
629 large fluorescence artifacts inherent in single photon microscopy, while preserving neural
630 components. This motion corrected, downsampled, and enhanced video was then processed using
631 Constrained Non-negative Matrix Factorization for Endoscopic data, "CNMF-E", (Pnevmatikakis
632 et al., 2016; Zhou et al., 2018). This extracted individual neural segments, denoised their
633 fluorescent signals, demixed cross-talk from nearby neighbors, and deconvolved the calcium
634 transients in order to estimate instances of increased firing activity for each neuron. These
635 estimated spike timings were used to compare neural firing time-locked to specific behavioral
636 instances. Neurons were matched across recording sessions using CellReg (Sheintuch et al., 2017)
637 by matching cells based on their contours and centroid locations.

638 *Imaging behavioral analysis*. For each recording session, we identified every bout of lever
639 pressing (LP) or sucrose magazine head entry (HE). We computed baseline calcium transient
640 probabilities for each cell from time periods not surrounding LPs and HEs ($\pm$5 seconds). Around
641 the beginning of each LP and HE bout we created 6 second ($\pm$3) peri-event time histograms
642 (PETHs) on the inferred spike times for every cell recorded within a session, yielding an average
643 calcium transient rate per bin. We then compared each time bin to the cell's baseline transient
644 probability with a binomial test to evaluate significantly modulated time bins surrounding either
645 LPs or HEs. Cells with at least 3 bins with probability <= .01 were deemed significantly responsive
646 to either behavior. To compare cell responses to LPs or HEs across different session types, we
647 selected cells that were 1) significanty modulated in at least 1 of any session type and 2) was

648 detected in at least 1 session of each type (regardless of significance). PETHs for each cell were
649 averaged across sessions of the same type, and the peak response time was determined from the
650 max time bin of the gaussian smoothed average PETH (3 bin width, 400ms). Cells were split into
651 pre- and post-bout labels based on their summed response magnitude before and after LP or HE
652 (pre: <0, post: >0). Cells with a pre-LP response in either PR, PRC, or CON conditions, and were
653 detected in every condition, were labelled as "pre-LP" cells (**Figure 7A**). Cells with a post-HE
654 response in either PR, PRC, or CON conditions, and were detected in every condition, were
655 labelled as "post-HE" cells (**Figure 7D**). Cells that fell into the post-LP and pre-HE category were
656 not evaluated due to the substantial overlap between behaviors during this time period. The average
657 PETH of the pre-LP and post-HE populations were constructed to yield population response curves
658 (**Figure 7B,E**). Pre-LP cells were evaluated across the three conditions using the average response
659 for each cell during the pre-lever-press time bins (<0 sec) and post HE cells were evaluated using
660 the average response per cell after head-entry (>0 sec) (**Figure 7C,F**).

661 *Satiety Control Condition.* For a subset of calcium imaging sessions, we
662 administered a satiety control condition. For these sessions we capitalized on the fact that rats
663 typically consume chow early in the test session, presented them with the lever and the chow
664 initially, allowed them to consume chow, but then removed the chow once the rat began to lever
665 press. This control condition allowed rats to reach a comparable motivational (more sated) state
666 relative to the PR-only condition, and thus controlled for satiety differences between PR and PRC
667 sessions.

668

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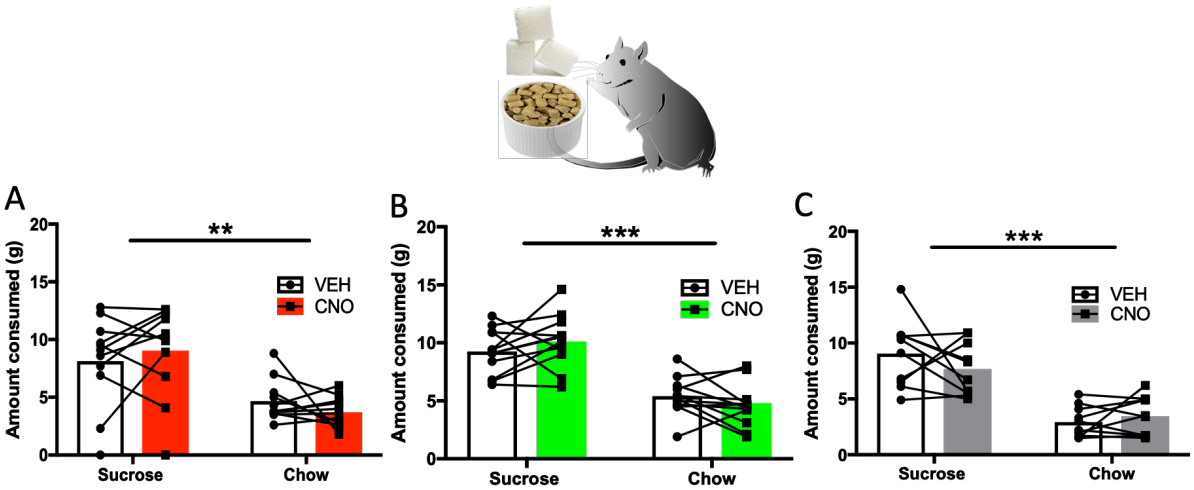
Conflict of Interest: The authors declare no competing financial interests

679 **Supplementary Figures**

680

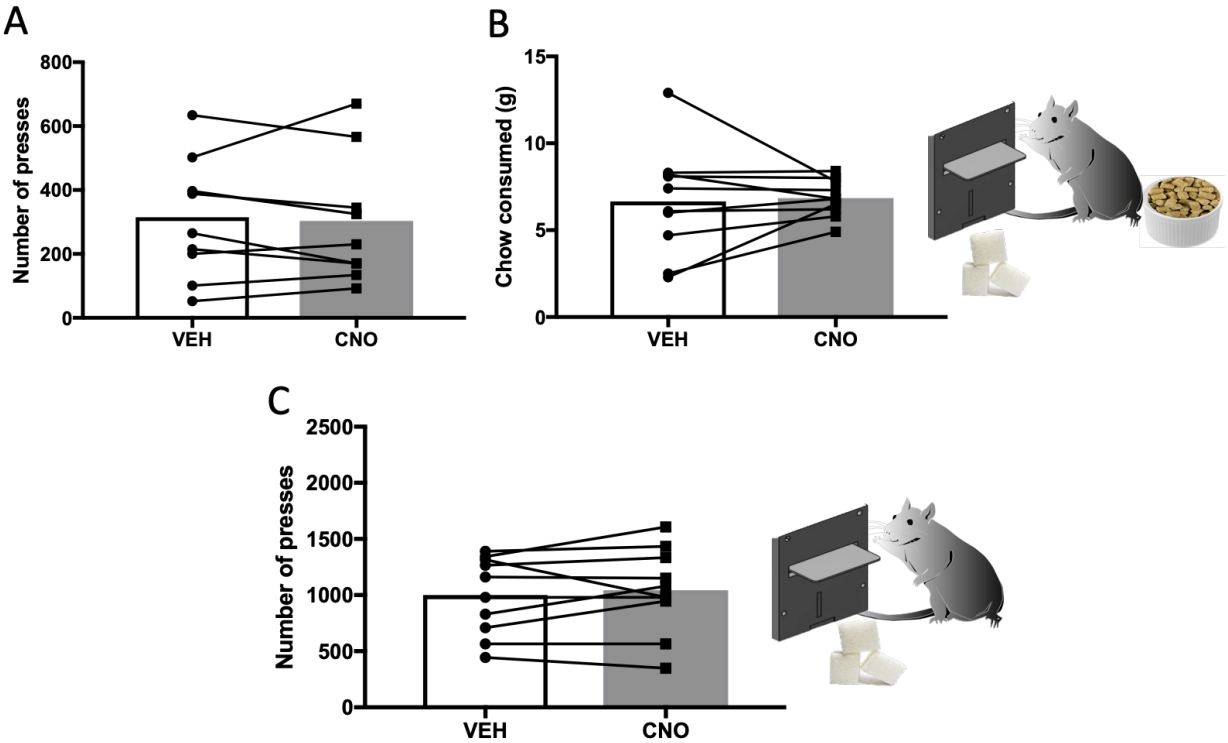
681



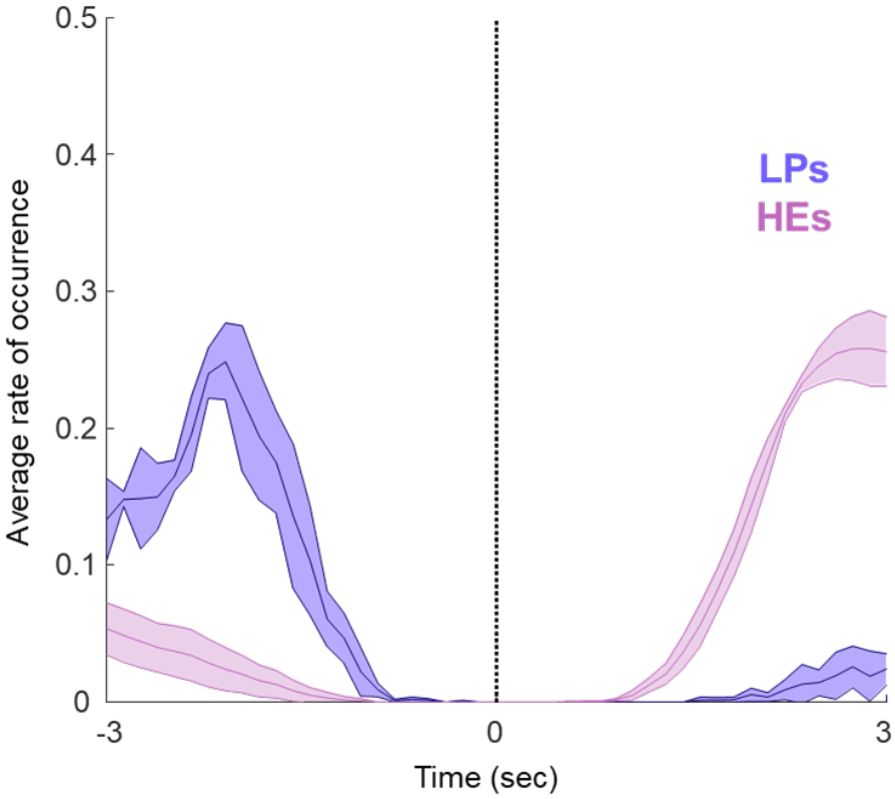
682

683 **Supplementary Figure 1. Free choice behavior in all three treatment groups following CNO**
684 **administration.** Mean consumption of sucrose and chow when rats were presented with both as
685 freely-available options. Shown is within-subject, counterbalanced choice behavior under vehicle
686 and CNO, indicating that food preference was intact: rats preferred the sucrose over chow. **(A)**
687 CNO had no effect on either sucrose or chow consumed in the G_i DREADDs condition. **(B)** CNO
688 had no effect on either sucrose or chow consumed in the G_q DREADDs condition. **(C)** CNO had
689 no effect on either sucrose or chow consumed in the null virus (GFP) condition. **p<0.01,
690 ***p<0.001.

691



Supplementary Figure 2. No effect on effortful choice behavior following CNO administration in GFP (null virus) controls. Shown is within-subject measures under counterbalanced conditions of vehicle and CNO in null virus controls. **(A)** Mean total lever presses during PRC sessions, when rats were presented with both the possibility of lever pressing under a PR schedule for sucrose pellets and freely-available chow. Unlike following active virus, CNO had no significant effect on the number of lever presses in this condition. **(B)** Total chow consumed during choice testing was also not different between vehicle and CNO conditions. **(C)** Mean lever presses during PR testing when there was no chow available as an alternative option. Lever pressing was also not different between vehicle and CNO conditions.



Supplementary Figure 3. Behavior triggered behavior PETH demonstrating behavioral overlap in post-LP and pre-HE periods. Histogram of lever-presses based on head-entries (blue). Histogram of Head-entries based on lever-presses (magenta). The 3 second time window following LPs and preceding HEs show substantial contamination from the other behavior, and were thus excluded from our analysis.

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