A special role for Anterior Cingulate Cortex, but not Orbitofrontal Cortex or Basolateral Amygdala, in choices involving information

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This work was supported by R01 DA047870 (Izquierdo), BCS-1844144 (Blaisdell) and UC's Chancellor Postdoctoral Fellowship (González). The authors have no competing interests to declare that are relevant to the content of this article.

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Abstract

Humans and non-humans make decisions under uncertainty. Choosing an option that provides information can improve decision making. However, subjects often choose information that cannot be used. In a procedure that promotes such paradoxical choices, animals choose between two alternatives: The richer option is followed by a cue that is reinforced 50% of the time (No-info) and the leaner option is followed by one of two cues, one always followed by food, and the other always followed by no food (Info). Since decisions involve comparing the subjective value of options after integrating all their features perhaps including information value, preference for information may rely on corticoamygdalar circuits. To test this, male and female Long-Evans rats were prepared with bilateral inhibitory DREADDs in the anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), basolateral amygdala (BLA), or null virus infusions as a control manipulation. Using a counterbalanced design, we inhibited these regions after stable preference was acquired and during learning of new Info and No-info cues. We found that inhibition of ACC, but not OFC or BLA, selectively destabilized choice preference in female rats without affecting latency to choose or response rate to cues. Fit of a logistic regression revealed that previous choice was a strong predictor of preference in control animals, but not in female rats following ACC inhibition. Additionally, BLA inhibition tended to decrease the learning of new cues that signaled the Info option. The results reveal a sex-dependent and special role for ACC in maintaining choice preference in decisions involving information.

Keywords: chemogenetics, frontal cortex, DREADDs, information, choice, paradoxical, curiosity, explore-exploit, information-seeking, value-based decision making

The environment is full of unpredictable events, and information that reduces uncertainty about such events allows an organism to better predict and prepare for the future. However, several psychiatric conditions are characterized by a strong preference for information, or an Intolerance of Uncertainty, including Autism Spectrum Disorder, Substance Use Disorders, Attention-Deficit Hyperactivity Disorder, Generalized Anxiety Disorder, and Obsessive-Compulsive Disorder (Boulter et al., 2014; Dugas et al., 2004; Jenkinson et al., 2020; Mandali et al., 2021; Tolin et al., 2003). Intolerance of uncertainty has been linked to 'pathological doubt' during which the preference for information is dramatically increased (Tolin et al., 2003).

Obtaining information can be crucial for survival. However, if information cannot be used to modify action, a bias toward information can be considered paradoxical or even suboptimal because organisms should not invest resources to obtain information that does not affect the outcome of a choice. Imagine a situation in which an organism is choosing between two sources of delayed reward; if rewards are signaled before each choice, it will be worth to invest in that information to choose the best option. In contrast, if the outcomes are signaled *after* the choice, information is useless, since the organism cannot change its choice. The latter has been broadly studied at the behavioral level. In the so-called paradoxical or suboptimal choice task (Stagner & Zentall, 2010), animals are presented with two alternatives, one providing a lower rate of reinforcement with different stimuli indicating presence (S+) or absence (S-) of delayed food (i.e. Info), and another one (S3) providing a higher rate of reinforcement but with non-differential stimuli signaling food (i.e. No-Info) (See **Figure 1A**).

Animals often prefer the leaner but informative option despite the difference in reinforcement rates between alternatives (Fortes et al., 2016; Macias et al., 2021; Stagner & Zentall, 2010) .

The neural substrates of reinforcement uncertainty have been broadly researched, with evidence pointing to a distributed network that involves prefrontal cortex (PFC) (Rushworth & Behrens, 2008), striatum, hippocampus, basolateral amygdala (BLA) and mediodorsal thalamus (Soltani & Izquierdo, 2019; Winstanley & Floresco, 2016). Nevertheless, to our knowledge there is no investigation of the brain using this behaviorally well-documented paradoxical choice procedure, and a paucity of rodent studies on the value of non-instrumental information and its neural substrates, compared to primate (Iigaya et al., 2019; Iigaya et al., 2016). When human subjects are assessed on choices between two cued alternatives- an informative vs. a non-informative one (but with no difference in overall reinforcement rate), subjects reliably prefer advanced information and BOLD signal in ventromedial PFC tracks the value of the anticipation of reward (Iigaya et al., 2019; Iigaya et al., 2016). Neural correlates of uncertainty have been found in different subregions of the PFC in several species, among them, orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) (Rushworth & Behrens, 2008). Electrophysiological recording studies in rats and monkeys demonstrate that activity in OFC is associated with stimulus (cue) value and expected uncertainty, or probabilistic risk (Jenni et al., 2023; Jo & Jung, 2016; Namboodiri et al., 2019; Riceberg & Shapiro, 2017; Stolyarova & Izquierdo, 2017). Similarly, studies have shown that ACC neurons signal value, uncertainty of predictions about rewards or punishments (Jezzini et al., 2021; Monosov, 2017), and track trial-by-trial outcomes of choice (Procyk et al., 2000; Shidara & Richmond, 2002). On the other hand, inactivation of BLA decreases choice of uncertain options in probabilistic-discounting tasks (Ghods-Sharifi et

al., 2009; St Onge et al., 2012; Stopper & Floresco, 2011) suggesting the contribution of BLA may be in biasing choice towards larger rewards, especially when the delivery of these rewards is uncertain. Furthermore, selectively disrupting PFC-to-BLA connections increases choice of the larger yet increasingly uncertain reward, implying that communication between these two regions serves to modify choice biases (St Onge et al., 2012). Finally, lesions to either OFC or BLA (or their connection), results in slower learning about which option has the better payout, suggesting that these areas form a functional circuit for the adaptation of reward-maximizing strategies (Zeeb & Winstanley, 2011, 2013).

In this experiment, we examined the specific contributions of ACC, OFC, and BLA to this seemingly 'suboptimal' choice phenomenon via chemogenetic manipulation. On the additional evidence that these regions participate in decision confidence under uncertainty (Lak et al., 2014; Stolyarova, Rakhshan, Hart, O'Dell, et al., 2019), we hypothesize that they may play vital, yet dissociable roles in decisions involving information value. To study this, we inactivated these regions during stable preference and during the learning of new informative and non-informative cues. We found that inhibition of ACC, but not OFC or BLA, destabilized choices involving information in females and reduced their ability to use previous choices to guide current decisions.

Materials and Method

Animals.

Sixty-six Long Evans rats (*Rattus Norvegicus*), 36 females and 30 males, acquired from Envigo served as subjects. Subjects were between aged post-natal days (PND) 90 and 140 at the

start of the experiment. Subjects were pair-housed before and single-housed after surgeries in transparent plastic tubs with wood shaving bedding in a vivarium maintained on a reverse 12-hr light cycle. Experiments were conducted during the dark portion of the cycle at a minimum of five days per week. A progressive food restriction schedule was imposed prior to the beginning of the experiment to maintain rats at 85% of their initial free-feeding weights. Water was always available in their home-cages. The procedures used in this experiment were conducted under approval and following the guidelines established by the Chancellor's Animal Research Committee at UCLA.

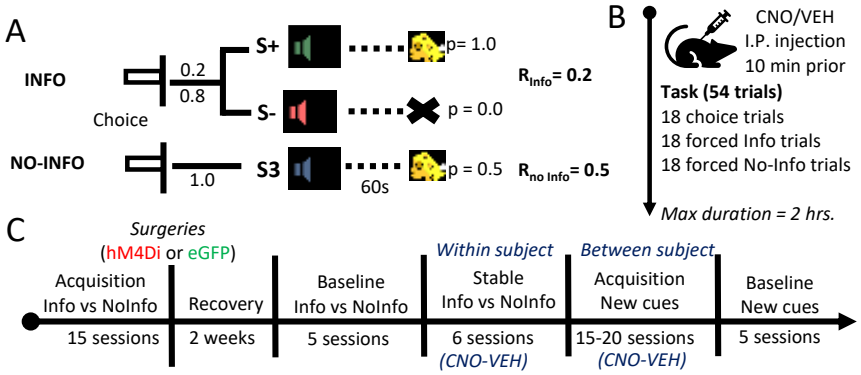

Figure 1. Task structure and experimental timeline. **(A)** Rats choose between two levers (left or right). After pressing once, both levers retract and an auditory cue commences. If the rat chooses the 'Info' alternative, on 20% of the trials, tone S+ plays for 60s always ending with the delivery of one sugar pellet; the other 80% of the trials sound S- plays for 60s always ending without food. If the rat chooses the 'No-Info' option, a third sound S3 plays for 60s ending with the delivery of one sugar pellet in 50% of the trials. (**B**) CNO or VEH was administered 10 min before starting the task. Each session consisted of 54 trials in which rats received 18 choice trials (both levers available to choose), 18 forced Info trials (only the lever associated with the Informative alternative was available) and 18 forced No-Info trials (only the No-Info lever was available). Animals were required to complete the session in 2 hrs. (**C**) Rats were trained on the task over 15 sessions before they underwent bilateral viral infusion of inhibitory hM4Di DREADDs or null virus enhanced Green Fluorescent Protein (eGFP) in ACC, BLA, or OFC. After two weeks of recovery, baseline performance was reestablished for 5 sessions before administrating CNO and VEH (order counterbalanced) for three consecutive sessions with a wash-out day between drugs. Then, the three originally trained auditory cues were replaced with three new auditory cues, while maintaining the rest of the structure as before. Rats received CNO or VEH for 15 to 20 sessions before receiving 5 final sessions of the task without drug.

Viral Constructs.

To express DREADDs on putative projection neurons in ACC, OFC, or BLA, an adeno-associated virus AAV8 driving the hM4Di-mCherry sequence under the CaMKIIa promoter was used (AAV8-CaMKIIa-hM4D(Gi)-mCherry, packaged by Addgene, viral prep #50477-AAV8). A virus without the hM4Di DREADD gene but containing the fluorescent tag enhanced Green Fluorescent Protein, eGFP (AAV8-CaMKIIa-EGFP, packaged by Addgene, viral prep #50469-AAV8) was infused into ACC, OFC, or BLA as a null virus control. This allowed us to control for non-specific effects of surgical procedures (i.e., craniotomy, anesthesia), exposure to AAV8, and non-specific effects of drug or injections.

Behavioral apparatus.

This experiment was conducted using operant testing chambers, measuring 30 x 25 x 20 cm (L x W x H). Each chamber was housed in separate sound- and light- attenuating environmental isolation chests (ENV-008, Med Associates, Georgia, VT). The front and back walls and ceiling of the chambers were constructed of clear Plexiglas, the side walls were made of aluminum, and the floors were constructed of stainless-steel rods measuring 0.5 cm in diameter, spaced 1.5 cm center-to-center.

Each chamber was equipped with a pellet-dispenser (ENV-203-45, Med Associates) with a cup type pellet receptacle (ENV-200R1M, Med Associates). When activated, one sucrose pellet was delivered into the cup. The opening of the cup was equipped with an infrared beam and photodetector to record entries into the food niche. A 3.5-cm wide operant lever was positioned one cm to the left of the food niche on the metal wall.

A speaker (ENV-224DM) on the ceiling of the chamber delivered a siren (cycling between 1500 and 1900 Hz at a 0.5 s rate), a 1000-Hz tone and a white noise, all were 8 dB above background to serve as the initial S+, S- and S3; and ~~a~~ another siren (cycling between 4000 and 3500 Hz at a 0.5 s rate), a 3000-Hz tone and a click train (4/s) 8 dB above background served as a second set of S+, S- and S3 cues, counterbalanced across subjects. A diffuse incandescent light (ENV-227M, Med Associates) was located on the bottom panel of the right-side chamber wall, 6 cm from the ceiling.

Surgical procedures.

After completing the *Training*, rats were anesthetized with isoflurane for bilateral infusion of ACC, OFC or BLA inhibitory (Gi) DREADDS (AAV8-CaMKIIα-hM4D(Gi)-mCherry, Addgene, Cambridge, MA, viral prep #50477-AAV8) or eGFP (AAV8-CaMKIIa-EGFP, Addgene, Cambridge, MA, viral prep #50469-AAV8). Craniotomies were created and a 26-gauge guide cannula (PlasticsOne, Roanoke, VA) were lowered into ACC (AP: +3.7, ML: ±0.8, DV: -2.4 from skull surface), BLA (AP: -2.5, ML: ±5.0, DV: -8.6 and -8.3 from skull surface) or OFC (AP: +3.7 and +4.0, ML: ±2.5, DV: -4.6 and -4.4 from skull surface); after which a 33-gauge internal cannula (PlasticsOne, Roanoke, VA) was inserted. Animals were infused with two bilateral sites of injections in BLA (first volume at lower coordinate = 0.2 µL, then 0.1 µL; total volume = 0.3 µL), two bilateral sites in OFC (more posterior and ventral coordinate = 0.2 µL, then 0.15 µL; total volume = 0.35 µL) and one bilateral site in ACC (total volume of 0.3 µL) of virus. All injections were infused at a flow rate of 0.1 µL/min. After infusion, the cannula was left in place for ten additional minutes to allow for diffusion.

Drug treatment.

Before testing, rats were given intraperitoneal (i.p.) injections of vehicle (VEH: 95% saline + 5% DMSO) or clozapine-N-oxide, CNO (3 mg/kg CNO in 95% saline + 5% DMSO) 10 min prior to beginning the behavioral task. This was a shorter time than some other work (Hart et al., 2020; Ye et al., 2023) to account for the longer duration of behavioral testing sessions as in a previous experiment (Stolyarova, Rakhshan, Hart, O'Dell, et al., 2019). CNO and VEH were administered during *Stable Info vs No Info* condition in a within-subject design (**Figure 1B**), where animals received 3 sessions of CNO (or VEH) followed by a washout day with no injection or training, and then 3 sessions of VEH (or CNO) followed by another washout day, such that the order of drug administration was counterbalanced across animals. Following this, CNO or VEH was administered during *Acquisition of new cues* in a between-subject design (drug condition counterbalanced) for 15-20 sessions.

Behavioral procedure.

Pretraining. Before training, 10 sucrose pellets were given in the rat home cage to avoid neophobia to the food. On the first day, rats were trained to eat pellets from the pellet tray by delivering one pellet every 20 ± 15 s in the chamber (actual intertrial interval (ITI) values = 5, 10, 15, 20, 25, 30, and 35 s) for a total of 40 pellets. On days 2 and 3, rats were trained in an autoshaping procedure to lever press the left and right lever (lever presented in alternate order). Reinforcements were delivered following each lever press (i.e., a continuous reinforcement schedule) for a maximum of 40 pellets within a session, or after 30 min elapsed.

Training of the Paradoxical choice task. The training stage of the task was comprised of two types of trials: choice and forced trials (**Figure 1C**). In choice trials, rats were required to make a choice between two levers available simultaneously. A choice trial started with the

simultaneous insertion of both levers and the houselight turning on. A choice was made by pressing a lever one time. After the choice was made, if the animal chose the Info alternative, both levers retracted and the houselight turned off. If the lever associated with the Info option was pressed, 20% of the time an auditory cue (S+) was presented for 60 s always ending with food; the other 80% of the time another auditory cue (S-) was on for 60 s never ending with food. The total percentage of reinforced trials was 20%. If the rat chose the No-Info alternative, a third auditory cue (S3) was presented for 60 s and ended with food on half of the trials. The percentage of reinforcement on this alternative was 50%. On forced trials, only one lever was presented at a time, following the same contingencies described above. All trials were separated by a 10-s intertrial-interval (ITI) during which all stimuli were off. A single session constituted 54 trials, 18 choice trials, and 36 forced trials. The maximum duration of a session was set to 120 min. The assignment of sounds to S+, S- or S3 and the lever side for each option was counterbalanced across animals but remained the same for individual animals throughout training. This condition lasted 15 sessions.

Stable preference. Approximately two weeks after surgery, rats received five sessions of training as described above as a reminder of the task, used as a measure of baseline preference (Baseline Info vs No Info, **Figure 1B**). After this, rats received three sessions of the task with an i.p. injection of CNO and three sessions with an injection of VEH (order counterbalanced across animals). After the three-session round of injections, animals underwent a washout day in which they were not tested on the task. This condition lasted 6 sessions (8 days).

New cues preference. Three new tones were introduced to replace the auditory cues used in previous conditions as S+, S- and S3. This was used to evaluate the effect of inhibition of

BLA, OFC, or ACC in learning about new informative cues. Aside from the auditory cues, all else remained identical. Animals received a minimum of 15 sessions of training and stability criteria (no more than 20% change over the last three sessions) with a maximum of 20 sessions (acquisition of new cues, **Figure 1B**). Finally, animals received 5 sessions of the same procedure without drug to establish baseline performance (baseline new cues, **Figure 1B**)

Histology.

At the conclusion of the experiment, rats were euthanized by an overdose of sodium pentobarbital (Euthasol, 0.8 mL, i.p.; Virbac, Fort Worth, TX) and transcardially perfused with phosphate buffered saline (PBS) followed by 10% buffered formalin acetate. Brains were extracted and post-fixed in this solution for 24 hours followed by 30% sucrose cryoprotection. Tissue was sectioned in 40-µM thick slices and cover slipped with DAPI mounting medium (Prolong gold, Invitrogen, Carlsbad, CA) visualized using a BZ-X710 microscope (Keyence, Itasca, IL), and analyzed with BZ-X Viewer software (**Figure 2A**).

We have previously validated efficacy of our CNO-activated DREADDs ex-vivo in slice (Aguirre et al., 2023; Hart et al., 2020) and behaviorally (Aguirre et al., 2023; Stolyarova, Rakhshan, Hart, O'Dell, et al., 2019) in OFC, ACC, and BLA. A subset of 40 µm coronal sections containing ACC were also stained for c-Fos, following an adapted Abcam protocol for dry mounted slides (Schneider Gasser et al., 2006). In this protocol, mounted tissue was marked with a hydrophobic pen, any medium was added with a pippette and removed using a vacuuum. The tissue was incubated for 22-24 h at 4 °C in a solution of primary antibody (1:2000 rabbit polyclonal to c-Fos, Abcam, Cambridge, MA) with 5% normal goat serum (Abcam, Cambridge, MA), and 0.1% TritonX-100 (Sigma, St. Louis, MO) in PBS. After the incubation, the

tissue was washed with PBS 3 times during a 5-min period, then the brain slides incubated in a secondary antibody for 90 min protected from light at room temperature (0.1% TritonX-100, 5% normal goat serum, PBS solution with 1:500 goat anti-rabbit Alexa 488; Abcam, Cambridge, MA). Slides were washed again as described above. Then, tissue was incubated during 3 min with quenching reagent (1:1 ratio) to reduce background. Slides were washed with PBS and then cover-slipped with fluoroshield DAPI mounting medium (Abcam, Cambridge, MA).

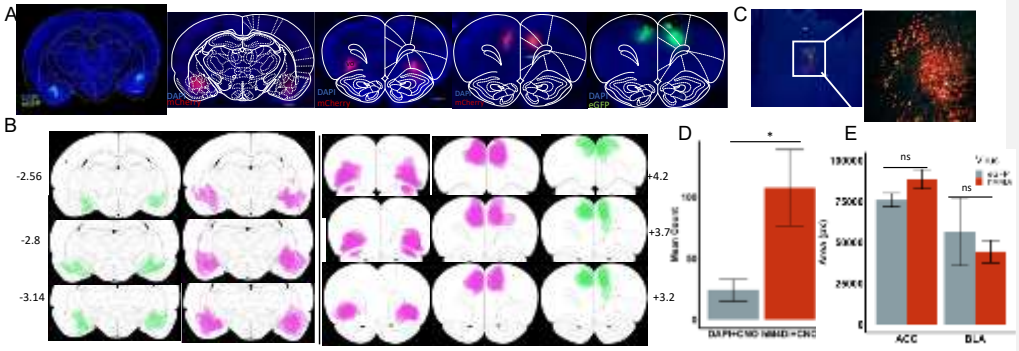

Figure 2. Inhibitory DREADDs in ACC, BLA and OFC; Validation of ACC inhibition via c-fos immunohistochemistry. **(A)** Representative placement of inhibitory hM4Di DREADDs and eGFP null virus at Anterior-Posterior (AP) level +3.7 for ACC and OFC, and -2.8 for BLA relative to Bregma. Note, there were no eGFP for OFC. **(B)** Reconstructions of placement of inhibitory hM4Di DREADDs (pink) and eGFP null virus (*green*) at AP level +4.2, +3.7 and +3.2 for ACC, OFC, and AP -2.56, -2.8 and -3.14 for BLA relative to Bregma. **(C)** Representative image showing DAPI (blue), hM4Di-mCherry (red), c-fos immunoreactivity (green) and their overlap after injections of CNO in ACC at 10x and 20x. **(D)** Mean cell count of 3 images per condition, hM4Di+CNO, DAPI+CNO for ACC. **(E)** Mean spread of viral expression across brain regions, pixel quantification was done using ImageJ software. ns= nonsignificant, *p<0.05, Mann-Whitney U test.

c-Fos inmunoreactivity quantification images were visualized with a 20× objective with a 724 µm by 543 µm field of view using a confocal microscope (Model LSM 900, Zeiss, Germany). For each region, three images were taken from two or three separate coronal sections from both hemispheres at the same approximate AP coordinate (ACC +3.7 mm). To verify DREADD-mediated inhibition of neurons in ACC after CNO administration we compared the number of c-

fos–positive cells in the hM4Di-expressing regions to the number of c-fos–positive cells in neighboring (non-hM4Di-expressing, DAPI) areas. Cell counts were conducted using ImageJ software (**Figure 2C**). We found greater overlap number of c-fos positive cells in hM4Di+ than DAPI+ cell areas (Mann-Whitney U test: W= 0, p = 0.04) (**Figure 2D**). DREADDs and eGFP expression was determined by matching histological sections to a standard rat brain atlas (Paxinos and Watson, 2007) and quantifying fluorescence using ImageJ (Rueden et al., 2017) where two independent raters measured the area of fluorescent pixels for each animal per hemisphere (**Figure 2E)**.

An independent sample t-test was performed comparing viral spread for ACC hM4Di ($Mean_{ACC\ hM4Di}$= 88542.87) against ACC eGFP ($Mean_{ACC}$ eGFP= 75795.5), the same analysis was conducted to compare BLA hM4Di ($Mean_{BLA\ hM4Di}$= 44399.54) and BLA eGFP ($Mean_{BLA\ eGFP}$= 56519.33). No comparison with eGFP was conducted for OFC hM4Di ($Mean_{OFC\ hM4Di}$= 100132), though there were no significant differences between ACC and OFC hM4Di expression (p=0.26) and we show below that control groups were no different from each other and could be collapsed. We found no significant differences in spread between DREADDs hM4Di and control eGFP for ACC (t(18.52)= -1.77, p = 0.093, -95% CI [27850, 2355]) or for BLA t(2.46)= 0.56, p = 0.624, 95% CI [-66729, 90969]).

Data Analysis.

All analyses were performed via custom-written code in MATLAB (MathWorks, Inc., Natick, MA). There were 3 main conditions in our analyses: 1) preference 2) latency to choose, and 3) response rate during the 60-s cue duration.

Learning and performance data were analyzed with a series of mixed-effects General Linear Models (GLMs) (*fitglme* function; Statistics and Machine Learning Toolbox) first in omnibus analyses that included all factors (drug, virus and sex), with all brain regions (ACC, BLA and OFC). Analyses were further pursued pending significant predictors and interactions in the full model. All post-hoc tests were corrected for the number of comparisons (Bonferroni).

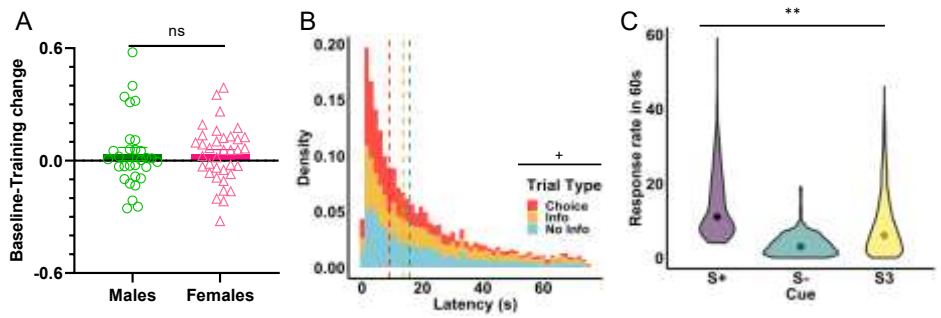

Figure 3. Preference was stable, and response latency and response rate (RR) exhibited the typical phenotype. (A) Mean change in preference for the info alternative between the last three sessions of training and baseline after surgery, with individual rats represented as scatter plots. Note that change in preference scale (y axis) ranges between -1 to 1. **(B)** Density of response latencies on the last three sessions of training are shown for each trial type. Dashed lines indicate the median latency. A marginally significant difference was found between trial types, where responses in choice trials were faster than in Forced (Info and No Info) trials. **(C)** Violin plots of the distribution of total RR during the 60s cue presentations are presented for each cue during the last three sessions of training. Dots indicate median RR for each cue. A significant effect of cue was found. **p<0.01, +p=0.06, ns = nonsignificant.

We also fit a logistic regression, a type of GLM for binary classification, to each rat's data that belonged to either an experimental group vs. control condition. We aimed to predict the probability of choice using 3 features: 1) Latency to choose, 2) Previous choice, and 3) Previous reward. We used only free-choice data for the outcome variable, and the previous choice and reward as either from free-choice or forced choice data. The full model corresponds to *y ~ [1 + PrevChoice + PrevReward + PrevLatency]*, with a binomial distribution.

Statistical significance for all analysis was noted when p-values were less than 0.05, and p-values between 0.05 and 0.06 were noted as trending toward significance.

Results

Control group. Our control group was created combining the animals that were infused with the eGFP virus, but also animals that were originally assigned to the eGFP group but wherein the histological analysis revealed either no-expression (n=9) or unilateral expression (n=4) compared with bilateral expression of eGFP (n=9). To assess if these groups could be collapsed in further analyses, we performed a GLM for each phase of the experiment. Using the mean of the last 3 sessions of training, a GLM was conducted for mean preference using virus, sex, and control group (eGFP, unilateral, or no-expression) as between-subject factors, and individual rat as random factor (full model: $y \sim [1 + sex * control\ group + (1 \mid rat)]$. We found no significant effect of control group (p= 0.54), sex (p = .335), or control group * sex interaction (p= .955). Similarly, a GLM was performed on *preference change* during the stable preference phase of the experiment, adding drug as a within-subject factor (full model: $y \sim [1 + drug * sex * control\ group + (1 + drug \mid rat)]$. We found no significant predictors or interactions. Based on these results, the animals in the control groups were collapsed and treated as a single group for subsequent analysis, and added to the 'virus' factor as a fourth group (ACC hM4Di, BLA hM4Di, OFC hM4Di, and control).

Training: Rats developed a consistent preference, responded quickly during choice trials and responded more to the informative cue (S+).

Choice. Training data for preference for the info option were first analyzed across all sessions, to evaluate learning of preference on the task. A GLM was conducted for preference for the info alternative using sex as between-subject factor, session as within-subjects factor, and individual rat as random factor using the following formula: *y ~ [1 + sex*session + (1+session| rat)*]. We found a main effect of session (GLM: $\beta_{session}$= -0.008, t(868)=-2.09, *p* =0.03) and sex (GLM: β_{sex}= -0.12, t(868)=-2.34, *p* =0.02) but no significant interaction (GLM: $\beta_{session*sex}$= 0.006, t(868)=1.13, *p* =0.26) (see **Supplement 1A**).

To evaluate that the observed preference remained consistent within each animal, we compared preference in no-drug conditions prior to any drug experience. To do so, the last 3 sessions of preference data for training were averaged, and compared to the preference in the last three sessions of the baseline after surgery. A GLM comparing the averaged preference during training and baseline phase was performed, in which phase (training and baseline) was a within-subject factor, and sex was a between-subject factor (full model: *y ~ [1 + phase * sex (1 + phase| rat)*]. No significant effects (p_{sex}= 0.93, p_{phase}= 0.26) or interactions ($p_{sex*phase}$= 0.73) were found, suggesting that preference remained stable across conditions (See **Figure 3A**).

Latencies. A GLM was conducted on median latencies in the last three sessions of training using trial type (forced info, forced no-info, and choice) as within-subject factors, sex as a between-subject factor, and individual rat as a random factor using the following formula: *y ~ [1 + trial type * sex + (1 + trial type| rat)*]. The results yielded a trend for an effect of trial type (GLM: $\beta_{trial\ type}$= 6.16, t(179)=1.85, *p* =0.06) wherein rats tended to be faster during choice trials (Median$_{Choice}$= 10.6 s, SEM±1.69) than either of the forced trials (Median$_{Forced\ Info}$=21.8

s,SEM±3.97, and $Median_{Forced\ No\text{-}Info}$= 18.1 s, SEM±3.81) (See **Figure 3B**). This result replicates what previous literature has shown in this task in pigeons and starlings (González et al., 2023).

Response Rate. The median response rate (RR) during the 60 s cue was analyzed for the last 3 sessions of training. A GLM was conducted for median RR using sex as between-subject factor, and individual rat and cue (S+, S- and S3) as random factors using the following formula: *y ~ [1 + sex + (1|rat:cue)*]. Cue was introduced as a random factor to account for the fact that rats were presented with different frequencies of cues given the programmed contingencies (e.g., S+ present only 20% of all Info trials) but this difference also depended on the rat's choice. For instance, an animal that chooses the No-Info alternative exclusively during choice trials would have more presentations of cue S3 than any other cue. We found a main effect of cue (GLM: β_{cue}= -2.11, t(185)=-2.89, p = 0.004), indicating that RR was greater for the cue predicting food ($Median_{S+}$= 11 presses/minute, SEM±0.34) than to the no-info cue ($Median_{S3}$= 6 presses/minute ,SEM±0.14), but the lowest RR was to the cue predicting absence of food ($Median_{S-}$= 3 presses/minute , SEM±0.06). This also is in line with previous work in which the lowest RR was reported for S-, and typically a greater RR for S+ than S3 (Gonzalez & Blaisdell, 2021; Hinnenkamp et al., 2017).

Info Preference: Inhibition of ACC destabilized preference in female but not male rats.

Choice. Stable preference was analyzed by averaging the 3 sessions of baseline, and then comparing this choice preference with the 3 sessions of CNO and 3 sessions of VEH for each subject. We compared changes in stable preference by calculating the absolute difference between preference in baseline-to-CNO and baseline-to-VEH conditions (See **Figure 4A**). A GLM

was conducted for *absolute preference change* using drug as a within-subject factor, virus and sex as between-subject factors, and individual rat as random factor using the following formula: *y ~ [1 + virus * drug * sex + (1 + drug| rat)*]. We found a significant interaction of drug*virus (GLM: $\beta_{drug*virus}$= 0.065, t(124)=4.88, $p = 3.24^{e-06}$) and sex*drug*virus (GLM: $\beta_{sex*drug*virus}$ = -0.065, t(124)=-3.15, p = 0.002). Thus, we were justified to conduct follow-up analyses with individual group comparisons as follows: *y ~ [1 + virus * drug * sex + (1 + drug| rat)*]. For the comparison of ACC hM4Di vs Control, we found a significant interaction of drug*virus (GLM: $\beta_{drug*virus}$= 0.074, t(68)=5.58, $p = 4.61^{e-07}$) and drug*virus*sex interaction (GLM: $\beta_{sex*virus*drug}$ = -0.077, t(68)= -3.70, p = 0.0004). Comparisons of OFC hM4Di vs. Control and BLA hM4Di vs. Control were not significant. Post-hoc analysis using Bonferroni correction and sex as a covariate for ACC hM4Di group resulted in a significant effect of drug (t(29)= 3.66, p =0.002). In contrast, there was no significant effect of drug in the control group (t(41)= 1.59, p = 0.239). Overall, these results found that ACC inhibition causes a desestabilization on preference for the female rats.

Latencies. Latency to choose was analyzed by calculating the median for CNO and VEH sessions (See **Figure 4B**). A GLM was conducted for median latency using drug as within-subject factor, virus and sex as between-subject factors, and individual rat as a random factor using the following formula: *y ~ [1 + trial type * virus * drug * sex + (1 + trial type + drug| rat)*]. We found a main effect of trial type (GLM: $\beta_{trial\ type}$= 5.61, t(380)=2.19, p =0.028) indicating that rats were faster in choosing during choice trials ($Median_{Choice}$= 8.4 s, SEM±0.4) than forced trials ($Median_{Forced\ Info}$=14.8 s,SEM±0.66, and $Median_{Forced\ No\text{-}Info}$= 15.1 s, SEM±0.62). Note that we did not find any difference by sex or virus, suggesting that the change in preference observed in the female rats following ACC inhibition did not affect decision's speed.

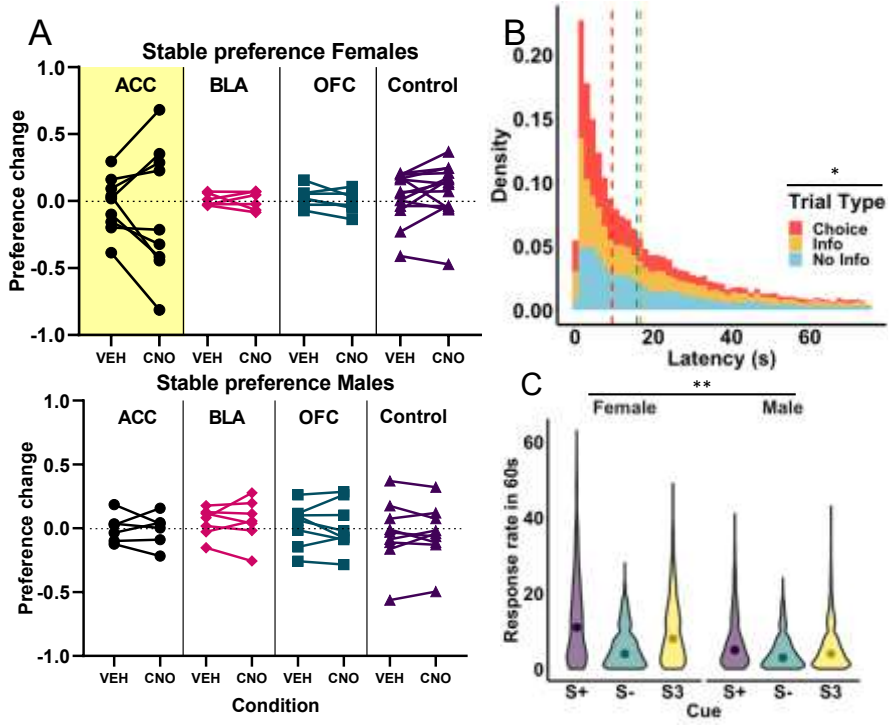

Figure 4. Inhibition of ACC destabilized info preference in female but not male rats, while latencies and response rate (RR) were unaltered by inhibition. **(A)** Mean change in preference for the info alternative between the last three sessions of baseline after surgery minus the preference during VEH and CNO administration for females (top) and males (bottom). Note that a positive change indicates a shift in preference towards the non-info option, whereas a negative change indicates a preference shift toward the info option. Values around 0 indicate no change in preference. **(B)** Density of latencies to choose across drug conditions is shown for each trial type. Dashed lines indicate the median latency. A significant difference between trial types was found, where choice trials were faster than Forced (Info and No Info) trials. **(C)** Violin plots of the distribution of total RR during the 60s cue presentations are presented for each cue for all drug conditions for females (left) and males (right). Dots indicate median response rate for each cue. Females responded more than males and a significant effect of cue was found. **p<0.01, *p=0.06.

360

361 *Response rate*. RR during the 60s cue duration was also analyzed by calculating the

362 median during CNO and VEH sessions (See **Figure 4C**). A GLM was conducted for median RR

363 using drug as within-subject factor, virus, and sex as between-subject factors, and individual rat

and cue (S+, S- and S3) as random factors using the following formula for the full model: *y ~ [1 + cue * virus * drug * sex + (1 + drug| rat:cue)*]. As in training, cue was defined as a random factor given that there were different probabilities of experiencing each cue based on an individual rat's choices. We found a main effect of sex (GLM: β_{sex}= -7.87, t(379)=-2.17, *p* = 0.03), with females showing higher response rates ($Median_{Female}$= 7 presses/min , SEM±0.06) than males ($Median_{Male}$= 4 presses/min , SEM±0.46).

Learning of new cues: Inhibition of BLA tended to decrease learning of new informative cues.

Choice. New cues preference for the info alternative was analyzed across all sessions, to evaluate learning about the informative and non-informative cues for rats administered CNO or VEH. Similar to above, a GLM was conducted for preference using session as within-subject factor, virus, sex, brain and drug as between-subject factors, and individual rat as random factors using the full model formula: *y ~ [1 + session* virus * drug * sex + (1 + session| rat)*]. We found a main effect of drug*sex (GLM: $\beta_{drug*sex}$= -0.54, t(978)= -3.00, *p* = 0.003), sex*virus (GLM: $\beta_{sex*virus}$= -0.17, t(978)= -2.28, *p* = 0.02), and drug*sex*virus (GLM: $\beta_{drug*sex*virus}$= 0.39, t(978)=-3.96, *p* = 7.89^{e-05}). Given this result, we excluded session as a factor, and sex was added as a covariate according to the formula: *y ~ [1 + sex + virus * drug + (1| rat)*]. We found a main effect of virus (GLM: β_{virus}= -0.077, t(989)=-2.03, *p* = 0.04), and drug*virus interaction (GLM: $\beta_{drug* virus}$= 0.13, t(989)=-2.77, *p* = 0.005).

The interaction above justified a GLM analysis on preference separated by brain region vs. the control group using the formula: *y ~ [1 + virus * drug + (1| rat)*]. Separate GLMs each for OFC hM4Di and for ACC hM4Di compared to the control group resulted in no significant predictors or interactions. Only a GLM for BLA hM4Di vs control resulted in a main effect of

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virus (GLM: β_{virus}= -0.13, t(533)=-2.75, p = 0.006) and marginal interaction of virus*drug (GLM: $\beta_{drug*virus}$= 0.14, t(533)=1.87, p = 0.06). However, post-hocs tests using Bonferroni correction did

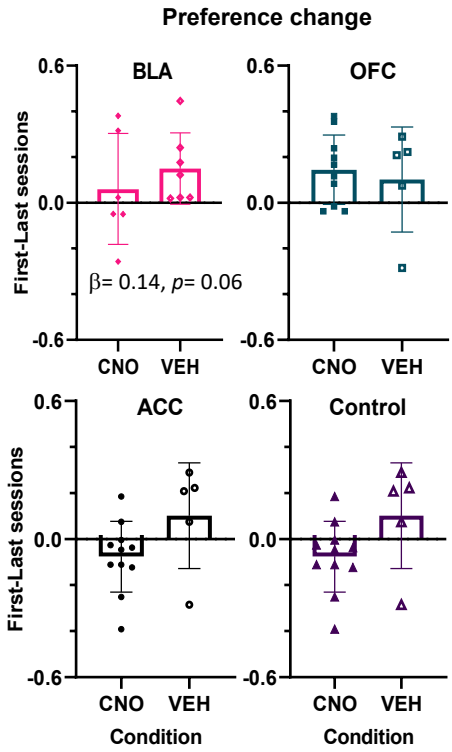

Figure 5. Effect of inhibition on info preference change during acquisition of new cues. Average change in preference for the info alternative, between the first and last three sessions of new cues acquisition for BLA, OFC, ACC and control animals during VEH or CNO administration. Note that a positive change indicates a shift in preference towards the non-info option, whereas a negative change indicates a preference shift toward the info option. Values around 0 indicate no change in preference. There was a trend for a decrease in learning the new info option following BLA inhibition. p=0.06 (drug x virus mixed-effects GLM).

not result in a significant effect of drug in either BLA hM4Di (p = .127) or control (p = .528) groups (**Figure 5**).

Latencies. We performed GLM analysis on median latencies separated by brain region and compared against the control group: *y ~ [1 + session*sex * virus*drug*trial type + (1+trial type*session| rat)*]. Though we highlight several 3- and 4-way interactions in **Table 1A-C**, there was no significant interaction of drug * virus.

RR. We performed GLM analysis on median RR separated by brain region and compared against the control group: *y ~ [1 + session*sex* virus*drug*trial type + (1+trial type*session| rat)*]. No significant effects were found for BLA vs control. RR for ACC vs control found a significant effect of session*drug (*p* = 0.48) and session*cue*drug (p = 0.035). RR for OFC vs control found only an effect of session*cue*drug (*p* = 0.041). However, there was no significant interaction of drug * virus in any group (**Supplement 1B**).

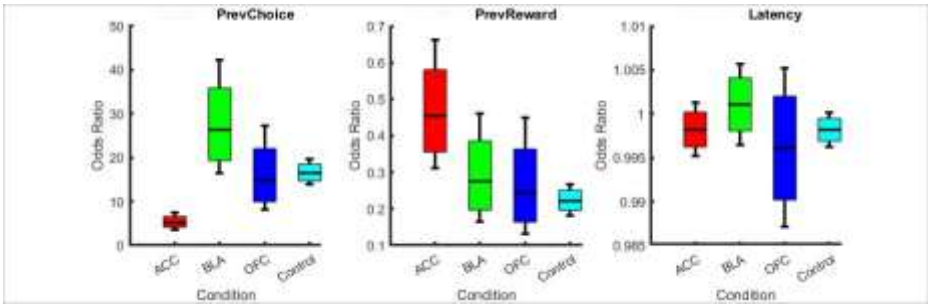

Figure 6. ***Female rats do not use previous choices to guide choices involving information during ACC inhibition.*** Each panel represents a feature of the model (Previous Choice, Previous Reward, and Latency) for female animals following CNO administration in four different groups: ACC hM4Di, BLA hM4Di, OFC hM4Di and Control. The y-axis is the odds ratio of the predictive feature. Box, error bars, and line represent 80% confidence interval, 95% confidence interval, and mean odds ratio, respectively. Mean model R^2 for control group = 0.4613, ACC group = 0.4189, OFC group = 0.4613, BLA group = 0.5181.

Previous choices are not predictive of current information choices in ACC-inhibited females

To further understand the nature of the effect of ACC inhibition on choice behavior, we fit a logistic regression, a type of GLM for binary classification, to each rat's data that belonged to either the test group (female ACC hM4Di-CNO) or control (female control) condition. We aimed to predict the probability of choice using 3 features: 1) Latency to choose, 2) Previous choice, and 3) Previous reward. We used only free-choice data for the outcome variable, and the previous choice and reward as either from free-choice or forced choice data. The full model corresponds to [*y ~ 1+ PrevChoice + PrevReward + PrevLatency]*, with a binomial distribution.

We computed the odds ratio and 95%, 80% CI for each condition's regression fit: If an odds ratio was greater than 1, it represented an increase in the odds of the outcome happening given a one-unit increase in the predictor. If the odds ratio was less than 1, it represented a decrease in the odds of the outcome happening given a one-unit increase in the predictor. And finally, an odds ratio of exactly 1 signified that the value of the predictor had no effect on the probability of the outcome.

The model resulted in a significant effect of Previous Choice ($t(651)= 11.19$, $p = 4.38^{e-29}$) and Previous Reward ($t(651)= -3.07$, $p = 0.002$), but no effect of Latency ($t(651)= -0.84$, $p = 0.40$). We found that in the control group, previous choice was a strong predictor for the next choice (n= 95.45%). Similarly, most BLA hM4Di animals (n = 91%) showed that previous choice was a good predictor of future choices. Interestingly, a similar result to ACC inhibition occurs with the OFC hM4Di group in which previous choices do not predict future behavior well. Finally, both control and BLA hM4Di groups used previous reward as a predictor (86.36% of rats showed a significant effect). In contrast, only 20% of ACC-inhibited animals showed good

prediction of choice from previous reward, suggesting that inhibition of ACC changes choice patterns uniquely (**Figure 6**).

These findings reveal dissociable patterns of choice prediction across different brain areas but also suggest that previous choices have the most predictive impact on subsequent behavior for all conditions. Conversely, previous rewards reduce the odds of the event of interest across all conditions and Latency instead has no predictive impact (Odds Ratio close to 1).

Discussion

Despite the well-characterized roles for OFC and ACC in decision-making (Bromberg-Martin & Monosov, 2020; Izquierdo, 2017; Sosa et al., 2021), few studies find a clear dissociation between the role of both regions in rodents. For example, both OFC and ACC are important in confidence report (Stolyarova et al. 2019; Lak et al. 2014) and both are also involved in stimulus-based reversal learning (Ye et al. 2023). In the present study, we found a clear dissociation between ACC and OFC that may shed light on their individual contributions in decision-making about non-instrumental information, and point to a hierarchy of functions within rodent frontal cortex. Specifically, we found that ACC inhibition rendered animals' decisions about information more stochastic, also corroborated by the logistic regression model which showed that previous choice was not a good predictor of future choices when ACC was offline. The pattern of results on latencies and response rate also indicates the effect on decision-making is not due to performance decrements (i.e., we found intact latencies and response rates). Interestingly, the logistic regression revealed a similar finding of impairment in

the use of previous choices following OFC inhibition, but this did not translate into a significant change in behavior. Thus, it could be that "monitoring" is a general feature of rodent frontal cortex, with ACC exerting a more prominent role than OFC. Indeed, ACC has been linked more to the representation of reward opportunities across the environment at different timescales while also keeping track of animals' previous actions (Kolling et al., 2016; Spitmaan et al., 2020; Wittmann et al., 2016), suggestive of a more metacognitive or performance monitoring role (Kane et al., 2022; Stolyarova, Rakhshan, Hart, O'Dell, et al., 2019; Takeuchi et al., 2022; van Veen et al., 2004). Instead, BLA inhibition did not alter stable performance but it resulted in a tendency to affect learning during the acquisition of new informative cues, supporting a role of BLA in biasing choices towards larger rewards in uncertain environments (Ghods-Sharifi et al., 2009; St Onge et al., 2012).

Our results presented here also replicate the vast literature investigating the behavior behind the preference for the informative, yet "suboptimal" alternative. As other researchers have previously reported, the preference for the informative option is more variable in rats than pigeons or starlings when the informative option results in less food (Stagner & Zentall, 2010; Vasconcelos et al., 2015), indicating perhaps different sensitivities to information and/or reinforcement history between species. In terms of latencies to make a choice, we replicate what has been observed in several studies: animals respond faster on "true" choice trials than forced choice trials. This counterintuitive result has been discussed by ecologists to explain how animals did not evolve to make simultaneous but rather sequential choices in nature, such that latencies during forced choice trials can be used to predict preference (Kacelnik et al., 2010). Finally, response rate during the cue presentation followed the typical pattern reported

previously: animals respond more in the presence of the always and partially reinforced cue, and do not respond to the non-reinforced cue (Gonzalez & Blaisdell, 2021; Hinnenkamp et al., 2017). It is important to highlight that animals do not have to respond during the duration of the cue; however, they typically do, suggesting a Pavlovian association established between the cue and the outcome. This association we believe is central to the preference, in which a stable preference emerges when an association between the reinforced cue (S+) and the outcome and the non-reinforced cue (S-) and the outcome is established (González et al., 2023).

A similar variation of the task presented in this study was used to assess preference for non-instrumental information in humans and monkeys~~' subjects~~. In those studies, subjects are given a choice between two alternatives, one that provides informative cues that indicate the outcome of the trial, and another providing non-informative cues that do not indicate the outcome of the trial. Similar to our task, the information provided by the cues does not influence or change the outcome. However, humans and monkeys were also informed about the quantity (money for humans and juice for monkeys) of the outcome in a given trial. The results showed that macaque monkeys and humans prefer information, and they were willing to sacrifice water/money to obtain immediate information about the outcomes. One group found that OFC neurons encode variables that are relevant in learning and decision-making but do not integrate these variables into a single value (Blanchard et al., 2015). This result is consistent with our findings in which OFC inactivation resulted in an altered pattern of using previous choice information revealed by the logistic regression, suggesting problems in retrieving the memory of previous choice, but this did not translate into a real change in preference. ACC may have a higher-level role in sustaining information-seeking (Blanchard et

al., 2015). For example, activity ramps up in this region in anticipation of information becoming available to resolve uncertainty before reward delivery (White et al., 2019). Our results support the monitoring role of ACC in decisions about information, where inhibition altered the integration of value, destabilizing preference.

We found that ACC inhibition desestabilized preference, but only in female animals. We did not find changes in latency to choose or response rate, indicating that the observed changes were not due to motor impairment or due to changes in the association between cues and outcomes. The change in preference with ACC inhibition is therefore unlikely due to problems in accessing overall value of each alternative. Previous studies have determined that ACC does not support simple effort or the hedonic value of a given alternative (i.e., one option). Instead, it computes the value *across* options, that is, it tracks what is the overall "best" option (Hart et al., 2020; Hart et al., 2017). Here, we found that change in preference following ACC inhibition was not in any particular direction. If animals with ACC offline had issues in accessing the overall value of the best option, in this case the no-info alternative, we would have observed an increase in preference for the info alternative. In contrast, we found that preference became more stochastic. The change in preference may also indicate a reduced ability to link previous actions (in this case, the previous lever press) to the current trial. However, this is also unlikely because the motor response to lever press did not change (latencies were unaffected), nor did the assigned value of the cues (response rate to cues also did not change). We propose instead that ACC inhibition results in an impairment in accessing the value of information. Previous research using this paradigm indicates that the contrast between both informative cues (i.e., the difference in information between the S+ and the S-) is essential for the development of a

preference for information. Previous research has already suggested that ACC is important for information-seeking behavior; however, previous studies reported this following electrophysiological recording and neuroimaging in monkeys and humans, respectively (Blanchard et al., 2015; Bromberg-Martin & Hikosaka, 2009, 2011; Bromberg-Martin & Monosov, 2020; Wallis, 2012). Our study provides the first causal evidence of the importance of ACC in decision-making involving non-instrumental information.

Sex differences in the involvement of ACC in value-based decision making have been reported before. In a recent study, Cox et al. (2023) found that ACC inhibition disrupted the relationship between value of each alternative and motivation to engage in the task in female mice. However, they did not find changes in preference as we found in our study. This group also reported that the ACC-to-dorsomedial striatal neuron pathway represents negative outcomes more strongly in female than males rats, suggesting different sensitivity to negative feedback (Cox et al., 2023). Similarly, in tasks involving decision-making under risk, it has been determined that female rats are more risk-averse (Orsini et al., 2016), indicating that female and male rats use different strategies to make decisions (Orsini et al., 2021). In our task, similar to Cox et al. (2023), we did not find differences in preference between female and male rats before inhibition. However, the destabilization of preference might indicate that females rely more strongly on ACC to keep track of reward statistics. Indeed, our model of trial-by-trial choice indicated that when ACC was inhibited, previous choice was not a good predictor of preference, whereas it remained so in other conditions.

Similar to the results in rodents, groups studying humans subjects have uncovered sex differences in strategy in decision-making (Chen et al., 2021; Chowdhury et al., 2019), usually in tasks involving risk (van den Bos et al., 2013). These sex differences are important to understand because, on the one hand, there is a higher prevalence of depression or anxiety-related disorders in women (Cyranowski et al., 2000); and on the other hand, there is increasing evidence that neuropsychiatric conditions such as ADHD, bipolar disorder, and autism show different onset, symptom severity, and prognosis dependent on sex (Grissom & Reyes, 2019; Hwang et al., 2020). This suggests differential involvement of circuits involved in decision-making by sex, and our study contributes to the effort in finding the neural mechanisms behind these potential differences.

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Table 1A. Median Latency to choose for BLA vs Control in New Cues acquisition

New Cues acquisition, γ = median latency to choose for BLA vs Control							
Formula: γ ~ [1 + Sex*Trial type*Session*Virus*Drug + (1 + Trial Type*Session | Rat)]							
Coefficients	***β***	**SE**	**tStat**	**DF**	***p***	**CI_L**	**CI_U**
Intercept	-87.38	38.91	-2.25	2098	0.025	-163.680	-11.081
Session	**9.01**	**3.50**	**2.58**	**2098**	**0.010**	**2.151**	**15.867**
Trial type	**76.63**	**22.68**	**3.38**	**2098**	**0.001**	**32.146**	**121.110**
Drug	75.00	40.49	1.85	2098	0.064	-4.413	154.410
Sex	105.17	61.50	1.71	2098	0.087	-15.427	225.770
Virus	47.33	34.01	1.39	2098	0.164	-19.357	114.020
Session*Trial type	**-6.23**	**1.79**	**-3.48**	**2098**	**0.001**	**-9.746**	**-2.719**
Session*Drug	**-5.63**	**2.41**	**-2.34**	**2098**	**0.020**	**-10.354**	**-0.907**
Trial type*Drug	**-53.63**	**24.14**	**-2.22**	**2098**	**0.026**	**-100.960**	**-6.294**
Session*Sex	-8.48	5.52	-1.54	2098	0.124	-19.301	2.333
Trial type*Sex	**-83.86**	**35.90**	**-2.34**	**2098**	**0.020**	**-154.250**	**-13.459**
Drug*Sex	-67.09	66.74	-1.01	2098	0.315	-197.980	63.801
Session*Virus	-4.27	3.07	-1.39	2098	0.164	-10.283	1.746
Trial type*Virus	-37.37	19.86	-1.88	2098	0.060	-76.328	1.583
Drug*Virus	-30.11	36.44	-0.83	2098	0.409	-101.570	41.351
Sex*Virus	-39.07	47.99	-0.81	2098	0.416	-133.180	55.048
Session*Trial type*Drug	**3.91**	**1.30**	**3.00**	**2098**	**0.003**	**1.353**	**6.468**
Session*Trial type*Sex	**6.97**	**2.83**	**2.46**	**2098**	**0.014**	**1.423**	**12.519**
Session*Drug*Sex	4.84	3.92	1.24	2098	0.217	-2.845	12.527
Trial type*Drug*Sex	66.58	39.98	1.67	2098	0.096	-11.816	144.970
Session*Trial type*Virus	**3.21**	**1.57**	**2.04**	**2098**	**0.041**	**0.127**	**6.302**
Session*Drug*Virus	2.48	2.14	1.16	2098	0.247	-1.719	6.686
Trial type*Drug*Virus	28.24	21.76	1.30	2098	0.195	-14.437	70.920
Session*Sex*Virus	4.15	4.33	0.96	2098	0.338	-4.340	12.643
Trial type*Sex*Virus	43.57	28.02	1.56	2098	0.120	-11.371	98.509
Drug*Sex*Virus	12.20	53.38	0.23	2098	0.819	-92.482	116.870
Session*Trial type*Drug*Sex	**-4.20**	**2.14**	**-1.96**	**2098**	**0.050**	**-8.406**	**-0.003**
Session*Trial type*Drug*Virus	-2.05	1.16	-1.76	2098	0.078	-4.339	0.229
Session*Trial type*Sex*Virus	-3.65	2.22	-1.64	2098	0.101	-8.004	0.710
Session*Drug*Sex*Virus	-1.68	3.10	-0.54	2098	0.589	-7.765	4.411
Trial type*Drug*Sex*Virus	-35.32	31.94	-1.11	2098	0.269	-97.958	27.328
Session*Trial type*Drug*Sex*Virus	2.29	1.70	1.35	2098	0.178	-1.046	5.626

Table 1B. Median Latency to choose for ACC vs Control in New Cues acquisition

New Cues acquisition, γ = median latency to choose for ACC vs Control							
Formula: γ ~ [1 + Sex*Trial type*Session*Virus*Drug + (1 + Trial Type*Session | Rat)]							
Coefficients	***β***	**SE**	**tStat**	**DF**	***p***	**CI_L**	**CI_U**
Intercept	-87.35	37.06	-2.36	2254	0.019	-160.03	-14.67
Session	**9.02**	**3.28**	**2.75**	**2254**	**0.006**	**2.59**	**15.44**
Trial type	**76.58**	**21.75**	**3.52**	**2254**	**0.000**	**33.93**	**119.23**
Drug	74.88	39.01	1.92	2254	0.055	-1.63	151.38
Sex	103.80	59.10	1.76	2254	0.079	-12.09	219.69
Virus	30.09	22.53	1.34	2254	0.182	-14.09	74.26
Session*Trial type	**-6.23**	**1.69**	**-3.69**	**2254**	**0.000**	**-9.54**	**-2.92**
Session*Drug	**-5.63**	**2.28**	**-2.46**	**2254**	**0.014**	**-10.11**	**-1.15**
Trial type*Drug	**-53.54**	**23.26**	**-2.30**	**2254**	**0.021**	**-99.15**	**-7.93**
Session*Sex	-8.43	5.18	-1.63	2254	0.104	-18.59	1.73
Trial type*Sex	**-85.65**	**34.84**	**-2.46**	**2254**	**0.014**	**-153.98**	**-17.32**
Drug*Sex	-62.43	64.61	-0.97	2254	0.334	-189.13	64.28
Session*Virus	-2.88	1.94	-1.48	2254	0.139	-6.69	0.93
Trial type*Virus	-24.33	12.92	-1.88	2254	0.060	-49.66	1.00
Drug*Virus	-25.60	22.65	-1.13	2254	0.259	-70.01	18.82
Sex*Virus	-26.26	30.43	-0.86	2254	0.388	-85.94	33.42
Session*Trial type*Drug	**3.91**	**1.24**	**3.15**	**2254**	**0.002**	**1.47**	**6.34**
Session*Trial type*Sex	**7.04**	**2.69**	**2.62**	**2254**	**0.009**	**1.77**	**12.32**
Session*Drug*Sex	4.65	3.73	1.25	2254	0.212	-2.66	11.97
Trial type*Drug*Sex	68.59	38.74	1.77	2254	0.077	-7.38	144.55
Session*Trial type*Virus	**2.07**	**1.00**	**2.07**	**2254**	**0.039**	**0.11**	**4.03**
Session*Drug*Virus	1.86	1.35	1.38	2254	0.169	-0.79	4.50
Trial type*Drug*Virus	18.89	13.30	1.42	2254	0.156	-7.20	44.97
Session*Sex*Virus	2.52	2.70	0.93	2254	0.351	-2.78	7.81
Trial type*Sex*Virus	26.34	17.31	1.52	2254	0.128	-7.60	60.29
Drug*Sex*Virus	13.15	32.93	0.40	2254	0.690	-51.42	77.72
Session*Trial type*Drug*Sex	**-4.29**	**2.05**	**-2.09**	**2254**	**0.037**	**-8.31**	**-0.27**
Session*Trial type*Drug*Virus	-1.33	0.73	-1.83	2254	0.068	-2.76	0.10
Session*Trial type*Sex*Virus	-2.19	1.38	-1.58	2254	0.113	-4.89	0.52
Session*Drug*Sex*Virus	-1.27	1.93	-0.66	2254	0.512	-5.06	2.52
Trial type*Drug*Sex*Virus	-19.13	19.38	-0.99	2254	0.324	-57.13	18.88
Session*Trial type*Drug*Sex*Virus	1.28	1.05	1.22	2254	0.222	-0.78	3.34

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740 **Table 1C. Median Latency to choose for OFC vs Control in New Cues acquisition**

New Cues acquisition, γ= median latency to choose for OFC vs Control							
Formula: γ ~ [1 + Sex*Trial type*Session*Virus*Drug + (1 + Trial Type*Session | Rat)]							
Coefficients	***β***	**SE**	**tStat**	**DF**	***p***	**CI_L**	**CI_U**
Intercept	-87.97	38.67	-2.27	2191	0.023	-163.80	-12.14
Session	**9.04**	**3.51**	**2.58**	**2191**	**0.010**	**2.16**	**15.91**
Trial type	**76.99**	**22.54**	**3.42**	**2191**	**0.001**	**32.80**	**121.19**
Drug	75.99	40.65	1.87	2191	0.062	-3.72	155.70
Sex	101.06	61.57	1.64	2191	0.101	-19.69	221.80
Virus	79.18	74.28	1.07	2191	0.287	-66.49	224.85
Session*Trial type	**-6.25**	**1.79**	**-3.49**	**2191**	**0.000**	**-9.76**	**-2.74**
Session*Drug	**-5.68**	**2.42**	**-2.34**	**2191**	**0.019**	**-10.42**	**-0.93**
Trial type*Drug	**-54.23**	**24.33**	**-2.23**	**2191**	**0.026**	**-101.94**	**-6.52**
Session*Sex	-8.30	5.54	-1.50	2191	0.134	-19.17	2.57
Trial type*Sex	**-81.78**	**36.03**	**-2.27**	**2191**	**0.023**	**-152.44**	**-11.11**
Drug*Sex	-57.16	67.19	-0.85	2191	0.395	-188.93	74.61
Session*Virus	-8.48	6.39	-1.33	2191	0.185	-21.02	4.07
Trial type*Virus	-63.45	43.64	-1.45	2191	0.146	-149.02	22.13
Drug*Virus	-38.31	75.65	-0.51	2191	0.613	-186.67	110.05
Sex*Virus	-64.81	103.36	-0.63	2191	0.531	-267.50	137.88
Session*Trial type*Drug	**3.94**	**1.31**	**3.00**	**2191**	**0.003**	**1.36**	**6.51**
Session*Trial type*Sex	**6.87**	**2.84**	**2.42**	**2191**	**0.016**	**1.30**	**12.45**
Session*Drug*Sex	4.39	3.95	1.11	2191	0.266	-3.35	12.13
Trial type*Drug*Sex	60.90	40.45	1.51	2191	0.132	-18.43	140.23
Session*Trial type*Virus	**6.46**	**3.29**	**1.97**	**2191**	**0.049**	**0.02**	**12.90**
Session*Drug*Virus	3.99	4.48	0.89	2191	0.373	-4.79	12.77
Trial type*Drug*Virus	40.93	45.60	0.90	2191	0.370	-48.50	130.35
Session*Sex*Virus	7.42	8.95	0.83	2191	0.407	-10.14	24.97
Trial type*Sex*Virus	85.71	60.74	1.41	2191	0.158	-33.39	204.82
Drug*Sex*Virus	15.76	108.19	0.15	2191	0.884	-196.41	227.93
Session*Trial type*Drug*Sex	**-3.95**	**2.16**	**-1.82**	**2191**	**0.068**	**-8.19**	**0.30**
Session*Trial type*Drug*Virus	-3.55	2.45	-1.45	2191	0.147	-8.35	1.25
Session*Trial type*Sex*Virus	-7.51	4.61	-1.63	2191	0.104	-16.56	1.54
Session*Drug*Sex*Virus	-2.15	6.36	-0.34	2191	0.735	-14.62	10.32
Trial type*Drug*Sex*Virus	-58.67	65.25	-0.90	2191	0.369	-186.63	69.30
Session*Trial type*Drug*Sex*Virus	3.99	3.49	1.14	2191	0.254	-2.86	10.83

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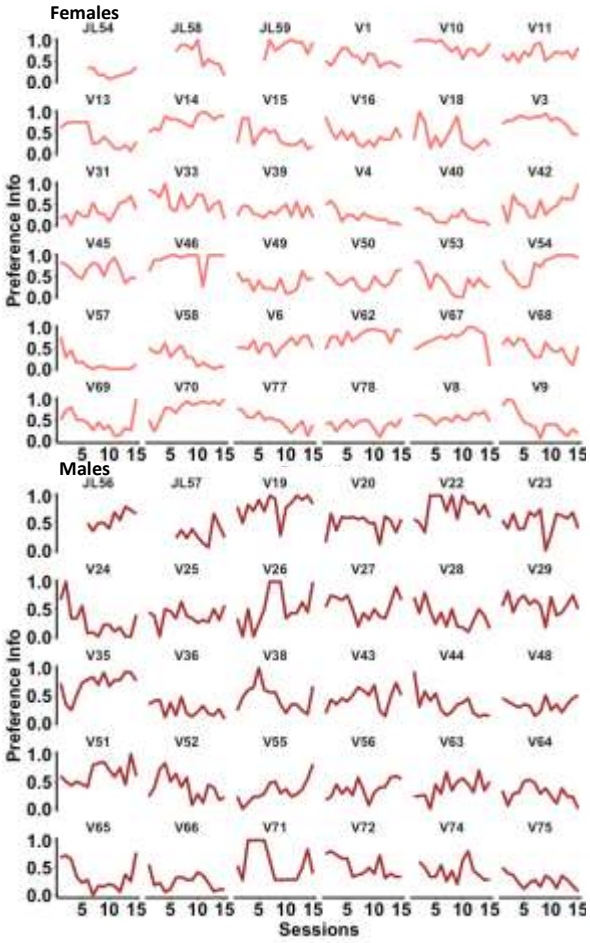
Appendix



Supplement 1A. Acquisition curves during initial training by rat. Rats received 15 sessions of training prior to the stereotaxic surgery to facilitate learning and testing. Color indicates the sex of the animal.

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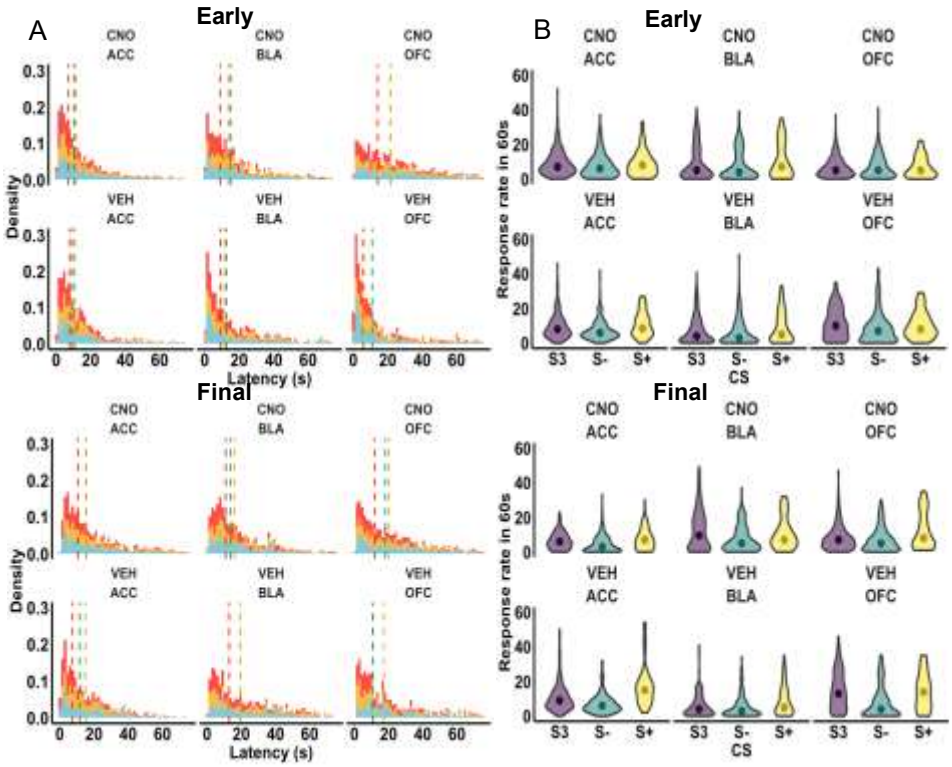

Supplement 1B. Changes in latency occurred across all brain regions over time; No significant changes were observed in RR. **(*A*)** Density of latencies to choose for experimental groups shown for each trial type. Dashed lines indicate the median latency**. (B)** Violin plots of the distribution of total RR during the 60s cue presentations are presented for each cue for all drug conditions for the experimental groups. Dots indicate median RR for each cue.