Neural activity in the anterior cingulate cortex is required for effort-based decision making

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ABSTRACT

Adaptive decision making requires the evaluation of cost-benefit tradeoffs to guide action selection. Effort-based decision making involves weighing predicted gains against effort costs and is disrupted in several neuropsychiatric disorders. The ACC is postulated to control effort-base choice via its role in encoding the value of overcoming effort costs in rodent effort-based decision making assays. However, temporally precise methods of manipulating neural activity have rarely been applied to effort-based decision making. We developed and validated a mouse version of the barrier T-maze, and used optogenetics to inhibit ACC excitatory neurons at specific times during this task. Bilateral inhibition of ACC rapidly and reversibly impaired preference to exert greater effort for a larger reward when a less rewarded, low effort alternative was available. Equalizing effort for potential choice options led mice to choose the high reward arm of the maze regardless of whether the ACC was inhibited or not. The mechanics of choice behavior were altered during trials where the ACC was inhibited, but there were no effects on overall mobility or tendency to exert effort in an unrelated assay. These findings establish causality between ACC neural activity during a choice trial and effortful action selection during cost-benefit decision making.

SIGNIFICANCE STATEMENT Disturbances in evaluating effort-based costs during decision making occur in depression, schizophrenia, addiction and Parkinson's disease. Precisely resolving the function of prefrontal brain regions in mediating these processes will reveal key loci of dysfunction and potential therapeutic intervention in these disorders.

INTRODUCTION:

The anterior cingulate cortex (ACC) is a prefrontal region that has been associated with a wide variety of behavioral functions related to cognitive control[1,2]. Developing a unifying framework to explain the role of the ACC in cognition has been a longstanding goal of neuroscience research[3]. The ACC has been implicated in numerous aspects of decision making, including computation of reward value and action outcomes, representing reward prediction errors, model-based action selection, exploration of alternative strategies, tracking outcomes across trials, and others[2,4–12]. Although, this suggests a broad range of functions for the ACC in decision making, a role in evaluating the utility of competing choice costs is amongst the most commonly ascribed functions.

One of the earliest and most reliable findings has been from rodent studies implicating the ACC in effort-based decision making tasks. Seminal studies have used the barrier T-maze task, originally developed by Salamone and colleagues to test spatial effort-based decision making[13]. In this task, animals must choose between climbing a vertical barrier to access a larger magnitude reward, or take an unobstructed path to a smaller reward. Rats with lesions encompassing ACC, but not neighboring prefrontal regions, exhibit deficits in this task, tending to post-operatively alter their preference to choose higher effort for larger rewards[14–16]. Although this result was not replicated in at least one study that attempted to use mice instead of rats[17]. The role of the ACC in effort-related choice tasks that involve operant lever pressing have been more mixed, but also suggest a role for this region[18–21]. These studies clearly implicate the ACC in rodent effort-based decision tasks, but have primarily used lesions or pharmacology to establish causal linkages. These approaches generally affect larger regions, produce observations lacking temporal precision and cannot address cell-type specific contributions in ACC.

The temporal relationship between the activity of ACC neurons and effort-based decision making has been explored using electrophysiological approaches. Recordings of population and single neuron activity from rodent ACC find representations of choice options with the highest utility based on integrating effort cost and reward value[22–25]. These studies find clear encoding of effort-related value, but also find high degrees of heterogeneity in neuron responding suggesting that ACC subpopulations may perform unique functions. Other studies have begun to explore the encoding of effort-related behaviors at the ensemble level using miniaturized head-mounted microscopes, or multielectrode recordings[20,26]. Similarly, local field potential recordings between

ACC and reward related regions implicate oscillatory activity in spatial effort-based decision making[27–29]. Taken together, these studies further implicate neural activity in the ACC in the computation of effort-related value during behavioral assays utilizing rats. These data indicate that the ACC can represent the value of competing response options when effort costs are operative on a trial-by-trial basis and at specific points during spatial effort-based decision making. However, few studies thus far have utilized temporally precise optogenetic manipulation during effort-based decision making, and none to our knowledge have done so in the barrier T-maze task.

In this study, we attempt to resolve this deficit by using an optogenetic approach to rapidly and reversibly inhibit ACC excitatory neurons during effort-based decision making in the barrier T-maze. We used mice in this study to clarify conflicting results as to the role of ACC in effort-related behavior in mice vs. rats, and also to open the possibility of using the full arsenal of transgenic tools available only in this species. We developed a protocol for training and testing mice in this behavior. Mice readily learned to overcome a barrier for a larger reward, and we validated that the behavior of mice in this task was sensitive to adjustments in choice utility based on reward magnitude or effort cost. Optogenetic inhibition of bilateral ACC rapidly and reversibly disrupted preference for high effort, high reward choices on a trial-by-trial basis. When effort cost was equalized mice were able to adapt to choose the more rewarded arm, suggesting that recall or reward preference were not affected. Mice were slower to make choices when the ACC was inhibited, but there was no effect on movement in an open field or general tendency to exert effort in response to acute, tail suspension stress.

MATERIALS AND METHODS:

All experiments were conducted in accordance with procedures established by the administrative panels on laboratory animal care at the University of California, Los Angeles.

Animals: All experiments use C57BL/6J wildtype mice of either sex (12-18 weeks of age), bred in house or ordered from Jackson Laboratories.

Viral vectors and targeting/expression: Adult mice were stereotaxically injected at 7-10 weeks of age with 600 nl of $1.5x10^{13}$ vg/ml AAV1-CKIIa-stGtACR2-FusionRed (AddGene) or $7x10^{12}$ vg/ml AAV1-CKIIa-mCherry control at bilateral ACC (antero-posterior (AP) +1.4, medio-lateral (ML) ±0.35, dorso-ventral (DV) -1.3mm relative to bregma). Bilateral 200 µm diameter, 0.37NA fiber optic cannulae (Newdoon) were implanted over ACC at +1.4 AP, ±0.35 ML, -1.1 DV. Implants were affixed onto the skull using Metabond dental cement (Parkell). Targeting of viral constructs/cannulae were confirmed via post-hoc histology without immunostaining.

T-maze Behavior Apparatus: Our T-Maze was constructed in-house, using white corrugated plastic (Coroplast). Removable, transparent food cups were inserted into slots in each arm. Barriers were constructed from diamond pattern wire mesh, and were 5 or 10 cm in vertical height with ~45 degrees sloping ramp on the backside, placed at a standard position in the maze. Removable guillotine style doors were inserted to create a start box, and also to block off one arm of the maze during forced trials or after mice made a choice on a given trial. Experiments were conducted inside specialized chambers with an open front, LED lighting and ports to pass cabling in and out. The maze was fixed in place within the chamber and a webcam was mounted overhead in a fixed location (ELP 2.8-12mm Varifocal 1.3 megapixels, 30 fps). Mazes were hand-operated by an experimenter standing in front of the chamber.

T-maze Behavioral Procedures: Mice were housed under standard 12-h light/dark cycles, and all experiments were performed during the light portion of the cycle. Mice were group housed and allowed to fully recover post-operatively (~1 week). Mice were habituated to human handling and food intake was restricted until mice were 80-85% of the *ad libitum* feeding weight. Throughout the experiment, mice were weighed and fed daily rations. Reward pellets were peanut butter chips (Reese's), precisely cut into small cubes (~0.01g; tolerance 0.08-0.12g). A random subset of pellets were weighed to ensure consistent sizes. After mice reached target weight, they are allowed to explore the maze with 20 food pellets scattered throughout. Once mice ate all of the food pellets in <5 minutes, behavior was further shaped so that mice ran trials and could easily locate single food pellets in each reward cup. Thereafter, one maze arm was designated as high reward (HR, 3 pellets) and the other as low reward (LR, 1 pellet). The HR arm was counterbalanced (left vs. right) across mice, with mice trained against any inherent initial side biases. On each training day, mice were forced to

sample each arm and then ran 15 choice trials. Once mice learned to choose the HR arm (>70%, ~2-7days), barrier training commenced. Mice were trained on a 5cm, then 10cm barrier in the HR arm. If mice refused to climb the barrier, additional forced trials were introduced to ensure adequate sampling of HR arm (up to 4/day). Training was complete when mice achieved >70% high effort/HR choices on 2 consecutive days (~5-10 days). For some experiments, the reward differential was manipulated and/or a second 10cm barrier was introduced in the LR arm to equalize effort cost between choices. Mice ran behavior trials 5 days per week, with food deprivation maintained during the weekend. After each trial was completed, mice were placed in a home cage during the inter-trial interval (ITI), while the maze was reset (timed to ~60 seconds). Trials were run consecutively for each individual mouse and the maze was cleaned thoroughly with 70% ethanol in between mice.

Tail suspension test (TST): TST mice were suspended by taping their tail to a bar ~12 inches above the tabletop, within 4 inch wide stalls of a custom apparatus constructed for this purpose. A 1.5 ml microcentrifuge tube was cut and placed over the tail base to prevent the mouse from climbing its tail during testing. Per standard protocols, and to ensure a stable base level of struggling, the first 2 minutes of the TST was not analyzed. The TST for optogenetics experiments was 14 minutes in duration and only one mouse was run at a time. Optogenetic stimulation was delivered in 4x, 3 minute blocks (OFF-ON-OFF-ON) with laser stimulation parameters as per below. The apparatus was thoroughly cleaned between uses. TST videos were analyzed manually on a per minute basis by an experimenter that was blinded to the conditions being tested and using an established method[30].

Open field (OF): OF testing was conducted in a standard sized arena (40x40cm), with a height of 30cm on each side. Video was collected from above and subsequent analysis was done using Bioviewer software to automatically track mice in the maze. Open field testing was 8 minutes in duration and mouse was placed by hand into the lower right corner of the apparatus. Optogenetic stimulation was delivered in 4x, 2 minute blocks (OFF-ON-OFF-ON) with laser stimulation parameters as per below. Mean velocity in the apparatus, during total light OFF vs. ON epochs was analyzed as a measure of overall activity level. The OF apparatus was cleaned thoroughly between uses.

Optogenetic Protocols: For optogenetic inhibition during behavior, light stimulation was delivered via a fiberoptic cable fed through a rotary joint commutator to a bifurcated optogenetic patch cable (Doric Lenses), and attached to a 150 mW, 473 nm fiber coupled laser (Shanghai Lasercentury). For optogenetic inhibition the laser was triggered with commercial software and external pulse generator (Prizmatix), or triggered manually. Light power was measured at the beginning of each behavior session using a light power meter (Thorlabs), measured as 5mW total light power (2.5 mW/side). Light OFF and ON trials were interspersed pseudorandomly within each set of behavior trials and initiated during defined regions of the T-maze. For some experiments the laser was turned on and off manually based on the location of the mouse in the maze or during specific times during the intertrial-interval (ITI) or in the start box. Mice were maintained in the start box of the maze for 6 seconds before opening the door to initiate a trial. For all optogenetic experiments, mice first ran two forced trials to either arm, followed by 16 choice trials each day (8 light OFF and 8 light ON). We delivered blue laser light to optogenetically inhibit the bilateral ACC during specific times during the ITI, while the mouse was in the start box of the maze, or during spatiotemporal period extending from start gate through the choice point of the T-maze. Light was delivered during discrete periods designated for optogenetic testing. Mice were run up to 3 consecutive days with a given maze arrangement. There was no apparent change in responsiveness to inhibition over the course of the experiments. T-maze experiments ended with 1-3 days in which a second barrier was inserted to equalize effort between conditions. Because control mice typically chose the HR barrier arm on most trials, we did not run them on the 2 barrier condition. The OF and TST were performed as described above to examine effects of optogenetic inhibition on overall effort and mobility.

Behavior Analyses: In all cases, the experimenter manually recorded the choice of mice in the T-maze, and these data were cross-checked against recorded videos to verify accuracy, and correct timing of laser stimulation. These choice data are reflected in plots of %HR choice throughout. In our initial validation dataset, we manually annotated videos to determine the length of each choice trial from the time point just after the start until the point at which the tip of the mouse's nose reached the reward cup in either arm. For subsequent optogenetic experiments, we employed a more detailed, automated analysis of choice trials. This analysis was not done for the validation data due to difficulties with automated tracking of mice in the original version of the

maze. For automated analyses we first used DeepLabCut (DLC)[31] to train a neural network to track multiple points on the mouse during choice trials. We used a point centered on the implant as our main tracking point for subsequent analysis of time in zones for mice in distinct parts of the maze. Maze zones were defined in a consistent manner based on standardized markings on the maze itself. The barrier, reward cups and start gate were placed in identical locations for each trial based on specialized slots or marks on the outside of the maze. We used BehaviorDEPOT[32] software and custom MATLAB code to define the choice made on each trial and to divide the maze into zones for further analysis. We calculated time in each zone and parsed these based on choice (HR vs. LR) and laser status (light OFF vs. ON).

Statistics: Unless otherwise specified, nonparametric tests or ANOVA was used to assess significance. Statistics were calculated using custom MATLAB code or Graphpad Prism. All statistical parameters, including test statistics, correction for multiple comparisons, and sampling of repeated measurements are stated in the main figure legends. Error bars represent ±SEM. The code and data used to generate and support the findings of this study are available from the corresponding author upon reasonable request.

RESULTS:

Behavioral Validation

Most studies exploring the role of the ACC in spatial, effort-based choice tasks have done so in rats. In order to investigate the role of the ACC in mouse models, we designed and validated a barrier T-maze training and testing procedure for use in mice (see methods). We built a custom, scaled down version of a standard rat T-maze, with manually removable guillotine style doors, wire mesh barriers of different sizes and transparent food cups to hold reward pellets (**Fig. 1A and B**). Mice were food deprived, habituated to human handling and behavior was shaped such that mice sought food in the reward wells. Animals were trained using 3 peanut butter pellet rewards in a high reward (HR) arm and a one pellet in the low reward (LR) arm (**Fig. 1A**). Mice were trained to a threshold of >70% HR choices, first without a barrier and then sequentially with 5 and 10 cm wire mesh barriers before initiating experimental testing procedures (**Fig. 1C**). Mice initially ran 1 forced trial to either arm, followed by 16 choice trials on each day of training and testing.

In order to validate that the behavior of mice in our T-maze assay was sensitive to manipulations of reward differential between HR vs. LR arms or effort cost (barrier), we performed an initial validation experiment in wildtype mice (**Fig. 2**). Mice were trained in the maze as described, then we systematically changed the number of HR reward pellets and/or the amount of effort associated with HR vs. LR arms of the maze and recorded the resulting pattern of choices made by mice in the maze (**Fig. 2A and B**). For consistency, we have used 'HR' to indicate the original high effort, high reward arm of the maze throughout including when the reward amount is lowered to be equivalent to the LR arm (**Fig. 2B**). Mice initially chose the high effort, high reward arm ~50% of the time, gradually approaching ~80% HR choices during the final stage of training (**Fig. 2B**). Reducing the reward differential (from 3:1 to 2:1 to 1:1) resulted in mice making fewer HR choices, particularly after changing to a 1:1 reward pellet ratio (no differential) such that there was no additional utility associated with climbing the barrier (**Fig. 2B**). HR choices increased slightly after reinstating a 2:1 reward differential, though HR choices remained lower than during the 2:1 phase that preceded this change (**Fig. 2B**). These data indicate that mice respond as predicted to adjust their willingness to exert effort in proportion to the degree to which effort was associated with more rewarding outcomes (**Fig. 2B**). The lower proportion of HR choices after reinstating a reward differential may be related to the influence of recent history of reward in the maze. Finally, in order to determine whether mice are sensitive to changes in the amount of effort, independent of reward differential, we inserted a second 10 cm barrier in the LR arm of the maze. Once both choices incurred an equivalent effort cost, mice rapidly adjusted their choice strategy to choose the more highly rewarded arm on nearly every trial (**Fig. 2B**). Thus mice in our assay actively organize their behavior to optimize the utility of choices based on reward magnitude relative to effort costs. We were also able to observe changes in mean choice strategy occurring within a day following changes in task contingencies (**Fig. 2C**). This suggests that there is learning that occurs both within and between sessions when the maze effort and reward contingencies are adjusted.

The time that it takes animals to perform decision making tasks is a relevant variable and may reflect the relative degree to which different decision making systems are engaged during action selection[33,34]. We hypothesized that if mice are deliberatively making choices in our maze in a goal-directed manner, this would be reflected in the time it takes to complete decision making trials. Thus we manually calculated trial length

using overhead videos collected during decision making behavior (**Fig. 2D**). At the start of 10 cm barrier training mice were significantly slower to complete high effort, HR trials compared to LR trials (**Fig. 2E**). However, at the completion of the training period, there was no difference in the time required to complete trials regardless of whether mice made HR vs. LR choices (**Fig. 2E**). Importantly, this suggests that at the conclusion of training there was no additional time cost associated with making a higher effort choice, underscoring the value of this assay for disambiguating effort from time-related costs which is a known issue with operant effort-based decision making tasks that require lever pressing[35]. Subsequent changes in reward differential had minimal impact on trial length, though a slight difference between HR and LR trial lengths did emerge, which may reflect an increase in cognitive effort associated with high effort choices when associated reward rates are dynamic or uncertain (**Fig. 2F and G**). Finally, when a second barrier was added to make effort equivalent for both options LR trial length increased significantly relative to HR choices in the 1 barrier condition (**Fig. 2G**). Overall, these data demonstrate that mice pursue goal-directed choice strategies in our assay in a manner that is highly sensitive to relative reward magnitude and effort cost. Thus, this approach is a valid strategy for studying the neural basis of effort-based, cost-benefit decision making in mouse models.

Optogenetic inhibition of the ACC disrupts effort-based decision making

In order to investigate the spatiotemporal requirement for ACC activity during effort-based decision making we used a viral, optogenetic approach. We stereotaxically targeted virus expressing an inhibitory opsin (stGtACR2) or a control fluorophore (mCherry) to excitatory neurons in ACC and delivered blue laser light via bilateral fiberoptic cannulae (**Fig. 3A-C**). This approach consistently targeted a relatively restricted region of dorsal, anterior ACC (Cg1; **Fig. 3E**). We hypothesized that neural activity in this region would be required during the process of making effort-related choices on a trial-by-trial basis. To test this hypothesis, we trained mice as previously described and tested the effects of optogenetic inhibition during discrete phases of our training and testing protocol (**Fig. 3D**). Following T-maze behavior, we tested the effects of our manipulation on overall mobility in an open field (OF) and in a more generalized test of effortful behavior using the tail suspension test (TST) (**Fig. 3D**). During T-maze testing, we directly compared light OFF vs. light ON trials that were interleaved in a pseudorandomly organized pattern during each testing day (**Fig. 3F**).

In order to test the acute necessity of ACC activity for effort-related decision making, we first decided to investigate the requirement for neural activity during discrete times during the trial cycle in our behavior. Mice were placed into the start box of the maze for 6 seconds before lifting the start gate to initiated a trial, with an ~60 second intertrial interval (ITI) (**Fig. 4A**). Light was delivered to inhibit the ACC during discrete phases of this cycle and we compared the %HR choices for light OFF vs light ON trials. Light delivered during the trial itself was spatially restricted to cover the approach/stem and choice point regions of the T-maze such that mice would need to commit to a choice while the activity of ACC was actively suppressed (**Fig. 4B**). We initially inhibited the ACC from the end of the ITI through the choice point of the maze and observed a robust reduction in high effort, HR choices for light ON vs. OFF trials (**Fig. 4C**). We observed a similar reduction in HR choices when light was delivered during the ITI and in the start box, but not during the trial itself (**Fig. 4D**). However, there was no effect of inhibiting the ACC on %HR choices when light ON was limited to the home cage ITI period, but turned off when mice were placed into the maze start box (**Fig. 4E**). Likewise, there was a highly significant reduction in high effort, HR choices when the ACC was inhibited only while mice were performing the choice part of the trial itself (between lifting the start gate and committing to a choice) (**Fig. 4F**). There was no effect of light ON vs. OFF for mCherry control mice in any of the 4 conditions (**Fig. 4C-F**). These data demonstrate that the activity of excitatory neurons in the ACC is required on a trial-by-trial basis for mice to choose to exert greater effort for a larger reward, when a less effortful, lower reward alternative is available. Moreover, these results highlight a requirement for ACC activity during the period of time between when mice enter the start box and prepare to initiate a trial, through commitment to a particular choice. The requirement for activity in the start box was somewhat surprising. Although we cannot rule out that this reflects some delay in re-establishing meaningful, task-related ACC representations after an extended period of inhibition, this finding suggests that ACC activity may be important during a choice-planning phase that precedes physical action selection at the choice point.

To better interpret the effects of manipulations on decision making in the barrier T-maze it is common to test the effects of adding a barrier to the LR arm. This equalizes the effort requirement associated with the two options and can help to disambiguate whether effort is the critical variable driving the effects of a manipulation.

To better understand our results, we added a second 10 cm barrier and compared light OFF vs. ON during the choice phase of trials (**Fig. 4G**). While mice initially persisted in choosing the LR arm when the ACC was inhibited, they gradually adjusted their behavior to choose the HR arm on both light OFF and ON trials (**Fig. 4H**). This result suggests that some degree of learning must occur in order to overcome the effects of ACC inhibition on effort-based choices. However, this result also suggests that ACC activity ultimately does not disrupt preference for larger rewards, ability to surmount a barrier, or capacity to recall the position of preferred outcomes.

In order to better understand the effects of inhibiting ACC on T-maze decision making we conducted a separate experiment in which inhibition delivered in 3 day blocks during trials themselves (**Fig. 5A and B**). This experimental design is more similar to how barrier T-maze lesion experiments have typically been conducted, allowing better comparison between pharmacology or lesion studies and the temporally precise optogenetic manipulations used in our study. In this experiment, we initially inhibited the ACC during three consecutive days after mice reached criteria in the no barrier training condition (**Fig. 5C**). There was minimal impact of inhibiting the ACC during choice trials that occurred before a barrier was introduced, although mice were slightly more likely to choose the LR arm when the light was ON (**Fig. 5C**). Similar to our other data, there was a large and highly significant disruption in high effort choices after barrier training when ACC activity was suppressed, and this effect was stable over at least several days (**Fig. 5D**). When a second barrier was added to the maze, we again saw that inhibiting ACC continued to bias mice towards LR choices, but this effect was limited to the first day of the 2 barrier block (**Fig. 5E**). There was no effect of light alone in a similar 3 day block in mCherry expressing control mice indicating observed results are not due to effects of laser by itself (**Fig. 5F**). Taken together, these data indicate that ACC activity is minimally required for reward-based decision making in the absence of an associated effort cost. However, once there is an effort-cost associated with decision making, mice are stably impaired in selecting to exert greater effort for a larger reward.

Detailed analysis of effects of ACC inhibition on effort-based decision making during choice trials

Analysis of trial times as outlined in experiments validating our mouse barrier T-maze decision making assay indicates that detailed timing of choice trials is sensitive to manipulations of reward differential and effort costs (**Fig. 2**). We therefore predicted that a more detailed analysis of decision making behavior during specific times during effort-based choice trials might reveal a more nuanced understanding of the effects of silencing the ACC on effort-based decision making. An advantage of employing spatial effort-based decision making tasks like the barrier T-maze, rather than operant chambers, is that task events in which specific aspects of cost-benefit decision making are segregated in time and space[35]. For example, in our assay, action selection based on evaluation of costs and benefits is spatiotemporally segregated from the exertion of effort (climbing barrier). The most commonly employed alternative assays involve lever pressing in an operant chamber in which mice must decide whether it is worth it to continue pressing a lever for a preferred reward over pursuing a less desired alternative. We hypothesized that the effects of inhibiting ACC activity on T-maze decision making would impact the time it takes mice to make choices in our maze and that these effects might be distinct for different zones within the maze. For optogenetics experiments we therefore optimized our approach for detailed tracking and analysis of mice performing choice trials. For these experiments we utilized white barriers and transparent food cups to enhance contrast between subject and background to facilitate segmentation of animals during behavior. We then collected videos from trials in which optogenetic inhibition of ACC was conducted only during the trial itself and used DeepLabCut[31] to train a neural network for precise keypoint tracking of mice (**Fig. 6A**). Our lab has recently collaborated to develop BehaviorDEPOT, an open-source, flexible software package that allows for customizable data analysis approaches based on keypoint tracking during rodent behavioral assays[32]. We developed customized BehaviorDEPOT modules for use with DLC-based tracking of mice in T-maze effort-based decision making assays[32]. We defined spatial zones within the maze where distinct task events occur, including approach, choice and high-effort/low-effort zones (**Fig. 6C**). The choice zone was defined as the point at which mice can visually identify the presence of the barrier in the HR arm (**Fig. 6C and D**). Although HR and LR choice trials are similar in duration (**Fig. 2D-G**), mice often pause briefly before climbing the barrier. Thus we defined a narrow choice zone and used a point on the head as the tracking point such that choice time was not affected by such micropauses and could be compared between HR and LR choice trials (**Fig. 6C and D**).

BehaviorDEPOT uses automated algorithms to process keypoint tracking data to segregate choice trials based on position as the mouse traverses zones sequentially during a choice. The specific sequence further defines whether the individual trials correspond to HR or LR choices, and we have validated performance of this process by comparison with manual annotations (**Fig. 6A**). We then assign the identify of trials as light OFF vs. light ON and automatically collect trial statistics segregated by zone and trial identity (light OFF/ON, HR/LR) (**Fig. 6A**). We processed and analyzed videos this way for testing days in which ACC was inhibited during choice trials only (corresponding with 'approach' and 'choice' zones) (**Fig. 6B and C**). We analyzed the time mice spent in the approach and choice zones of the maze segregated by choice type for one and two barrier conditions (**Fig. 6E-L**). When both HR and LR choices were grouped together, there was a significant increase in approach zone time for light ON vs. OFF trials, but not for choice zone (**Fig. 6E**). When we examined HR choices by themselves, time in approach and choice zones was significantly greater for the light ON condition, but no significant effects for LR choices (**Fig. 6 F and G**). Counter to our original prediction, these data indicate that inhibiting ACC during decision making has the largest and most consistent effect on the time it takes mice to traverse the approach zone. This might suggest that mice are typically evaluating choice options as they approach the maze choice point, and that inhibition of ACC impairs this process. The fact that inhibiting ACC significantly increases time in both zones for HR, but not LR choices suggests that overcoming the effects of ACC inhibition on preventing HR choices may incur the greatest cognitive effort. In order to examine the relative effect of inhibiting ACC in each zone for each choice type, we plotted the ratio of light ON to light OFF time. There were no significant effects, but there was a trend level effect for all choices when comparing approach to choice zones ($p=0.10$; **Fig. 6H**). The effects of ACC inhibition on time in approach and choice zones were similar for the two barrier conditions, with increased time in both zones during light ON trials for all choices, and HR choices, but not LR choices (**Fig. 6I-K**). The relative ratio of light ON to light OFF times for approach and choice zones were not different for any of the choice types in the 2 barrier condition (**Fig. 6L**). These data support the conclusion that inhibiting the ACC during decision making affects both the tendency of mice to choose high effort, HR maze arm as well as the actual trajectory of mice as they make a given type of choice in the maze.

Optogenetic inhibition of ACC does not affect overall mobility or effortful responses in the tail suspension test

Our results in the barrier T-maze assay indicate that ACC activity is required for mice to choose to exert greater effort for a larger reward, when a less effortful alternative is available (**Fig. 3-5**). Moreover they indicate that inhibiting the ACC disrupts the trajectory of mice in the maze above and beyond changing the types of choices that animals make (**Fig. 6**). We therefore wondered if inhibiting the activity of the ACC would have more generalized effects on willingness or ability of mice to exert effort during behavior that is not explicitly reward-based or goal-directed. To investigate this we examined the effects of inhibiting ACC activity in mice in the tail suspension test (TST). The TST is a widely employed test for depression-related behavior in which mice are exposed to an inescapable acute stressor (tail suspension). Mice confronted with tail suspension stress either engage in bouts of effortful escape related behavior or adopt an energy conserving immobile posture. While immobility has generally been taken as a measure of 'behavioral despair' – a purportedly depression-like response – a more conservative interpretation is that both immobility and struggling can be considered potentially adaptive responses. In this case, we are not making any claims about relevance to depression and only using the assay as a measure of tendency to exert effort. Inhibiting neural activity in ACC during the TST does not reduce struggling behavior (**Fig. 7A and B**). This suggests that inhibiting the ACC does not cause generalized reductions in effort, and that the effects observed during decision making reflect an effect weighing the value of effort in the context of alternative actions.

We also found that when the ACC is inhibited mice are slower when approaching the maze choice point, and in particular are slower when making HR choices with the light ON (**Fig. 6**). We therefore wondered whether this reflects generalized deficits in mobility, or is specific to situations that involve explicit decision making. To test this, we observed the effects of inhibiting the ACC when mice are exploring an open field (OF). We find that there is no difference in the average velocity of mice during light OFF vs. ON epochs for either stGtACR2 expressing mice or mCherry controls (**Fig. 7C**). This suggests that mice are slower during effort-related choice trials because of the specific demands associated with decision making. This further supports the idea that mice are slower to make choices in our assay due to impediments in cognition associated with suppression of activity in the ACC.

DISCUSSION:

Here we report on the development and validation of a mouse version of the barrier T-maze assay, and demonstrate that inhibition of ACC during choice trials rapidly and reversibly disrupts effort-based decision making.

Validation of Mouse Barrier T-maze Assay

Mice quickly adapt their choice strategy consequent to changes in the reward differential under constant effort costs. Similarly, when the effort associated with each choice is made equal by adding a second barrier into the LR arm, mice quickly adjust to choose the more rewarded arm on nearly every trial. Updates in choice strategy after each change are apparent on the first day following contingency switches, but typically take 2-3 days to stabilize. Likewise, observing changes in trial length as a proxy for the mechanics of decision making behavior reveals adjustments during the training phase, and after contingency switches. Mice are initially slow to complete high effort choices after the barrier is added, but by the end of training the time it takes to complete both high and low effort trials is not significantly different. This is important because it suggests that time and effort costs are not conflated in subsequent experiments, and that differences in choice time observed with manipulations are not due to a change in the relative number of high vs. low effort choices. Finally, we see that trial length is longer after a change in contingencies, which may reflect altered effort-related cognition under dynamic or uncertain constraints. These results indicate that mice make choices in this assay that are influenced by effort-reward tradeoffs that are similar to those seen in rat experiments.

Optogenetic Inhibition of the ACC Disrupts Effort-Based Decision Making

Previous studies of effort-based decision making utilized lesions or pharmacologic manipulation of target brain regions to evaluate their requirement in this task. These studies have been the foundation of claims for the involvement of ACC in effort-based decision making, but address causality with low temporal precision. One recent, well designed study utilized chemogenetics to activate or inhibit ACC during an operant effort-based choice task[20]. We elected to use optogenetic inhibition (stGtACR2) in our study to manipulate neural activity briefly during parts of a trial. This approach is useful because it can address the requirement for activity during specific parts of a choice trial which may correspond to distinct steps in making an effort-based choice. The

use of a spatial task is likewise useful because evaluation of effort-reward tradeoffs and action selection occur prior to exertion of effort. Similarly, because ACC remains active during the outcome of choices, our approach presumably leaves ACC activity associated with an outcome intact. As predicted, we saw that inhibiting the ACC from the start of a trial through action selection disrupts preference for high effort, high reward choices seen when ACC is active during trials within the same session. There were no apparent effects on subsequent choices after a trial in which ACC was inhibited, and equalizing effort by adding a second barrier restored preference for the higher magnitude reward. This indicates that mice retain the capacity to climb barriers and relative preference for larger reward. Notably, mice do not immediately return to selecting the high reward arm, but adjust choice strategy gradually within a session, or after the first day during a multi-day design. Similar findings are reported in some of the original lesion studies. This suggests that mice require some learning to update their model, and are perhaps using a relatively more automated (rather than model-based) approach during decision making.

Effects of the timing of optogenetic inhibition during effort based choice trials

The largest effect of inhibition in our task occurred when the ACC was silenced during the trial itself, suggesting that ACC is important for evaluating and/or selecting high effort actions. Surprisingly though, inhibition that included the time during which mice are in the start box, but not during the trial itself, is sufficient to impair effortful choices. This may suggest that placement into the start box itself triggers a particular action plan, and that inhibition of ACC during this time disrupts the representation of effortful choice strategies. Alternatively, it is possible that after-effects of inhibition (e.g., post-inhibitory rebound), disturbs task-relevant activity patterns necessary for evaluating effort-reward contingencies during the trial. Hart et al, recently found that both decreasing and increasing activity with DREADDs could disrupt effort-based decision making, suggesting that it is the organization of ACC activity that is critical and that more activity may functionally act as a lesion if it is not appropriately organized[20]. Of note, we sometimes observed that during light ON trials stGTCAR2 mice would turn and wait at the barrier or even reverse their initial selection of the effortful arm. Similar behavior was reported in one of the lesion studies of this task during the first few post-surgery trials. This suggests that, in addition to its role in planning and executing choices, ACC may also be important for maintaining effort after action selection.

Further studies are warranted to disambiguate these potential functions and to understand whether they are mediated by different ACC circuits. Similar to Hart et al, our approach utilized a promoter with some specificity for excitatory neurons (CaMKII), so our results may be driven by effects on excitatory rather than inhibitory ACC neurons. However, we have not validated the degree to which expression is specific to excitatory neurons in ACC. The ACC is composed of many different cell-types with broad connectivity to cortical and subcortical targets. A distributed network characterized by prefrontal, ventral striatal and other subcortical structures has been implicated in mediating effort-based decision making[14,35–37]. In particular, basolateral amygdala, ventral striatum, anterior insula, and habenula have all been implicated in effort-related tasks. Further, mesolimbic dopamine pathways seem to exert particular influence over effort-based tasks. Future studies should investigate the specific bidirectional circuits mediating interactions between these regions during effort-based decision making tasks. In particular, exploration of cell-type specific circuits using temporally precise methods like optogenetics should prove fruitful approaches.

Effects of optogenetic inhibition on the mechanics of effort-based choices

Our T-maze validation data indicates that the timing of choice trials is influenced by changes in effort-reward contingencies. The behavior of mice at maze choice points can reflect the underlying neural processes that drive decision making, including whether mice are using deliberative or more automated strategies[33]. Recently, open-source algorithms have been developed to neural networks for precisely tracking mice during behavior experiments[31]. We used DeepLabCut[31] to track mice during decision making in the barrier T-maze, and wrote custom code for detecting trials and calculating behavioral metrics in specific zones of our maze using BehaviorDEPOT[32]. We find that when the ACC is inhibited during single barrier choice trials, mice take longer to approach the maze choice point, but take a similar amount of time to traverse the actual choice point. This suggests that cognition related to evaluating potential choice options may take place as mice run up the stem of the T-maze, and that absent critical task-related representations in the ACC during decision making, mice may be less certain of the most advantageous response. This interpretation fits well with the proposed role of ACC neurons in representing the utility of the potential choices as has been demonstrated from electrophysiological recordings from ACC single neurons during similar spatial, effort-based choice tasks[23–25]. These representations may also be necessary for model-based action selection processes required during

such tasks[5]. Alternatively, it is possible that the increased time during approach is related to a disturbance in more procedural action and reflects engagement of deliberative mechanisms that cannot select the more advantageous high effort option because of a requirement for the ACC in this process as well[33,34]. While the ACC was recently implicated in model-based action selection, some evidence suggests it may also be involved in more procedural actions[34]. It is important to note as well that our optic fiber placements in these experiments, while located primarily in ACC are near the border with prelimbic regions which are also important for aspects of evaluative decision making[34]. Notably, when looking only at high effort, high reward choices, the difference between trials where the ACC was silenced and those where it is not becomes significant for both approach and choice zones. Thus these effects cannot be attributed to differences in the proportion of barrier vs. non-barrier trials. Another important function of the ACC is in resolving conflicts between competing neural representations. In this case, one could consider the choice of high and low effort choices to be in conflict (avoidance of effort cost in conflict with approach for a larger reward). In this model, the ACC may be necessary to resolve such conflicts and the increased time it takes mice to choose the higher effort option involves the greater cognitive load associated with overcoming this type of conflict, without the assistance of the ACC. Future work that explores the contribution of the ACC and its inputs and outputs should focus on understanding function in behavioral assays that can better distinguish these processes in effort-based decision making.

Effects of optogenetic inhibition on mobility and effort in other contexts

Inhibiting the ACC impacts the decision about whether to exert greater effort and significantly slows mice during the process of making decisions in this behavior. Thus, we wondered whether these effects were specific to the context of pursuing rewards or might to some degree result from a generalized impairment in mobility or capacity to exert effort in other situations that do not involve an overt choice. The prefrontal cortex, and specific neuron subpopulations within the prefrontal cortex can modulate effortful, depression-related behavior[38–41]. However, these effects have primarily been explored using methods that exogenously drive increased activity in neurons of interest. We show that optogenetic inhibition of excitatory neurons in the ACC does not significantly alter effortful responses to tail suspension stress. Although the direct relevance of this finding to depression is questionable, it does demonstrate that acutely inhibiting the ACC does not universally

impair the ability of mice to exert effort. This suggests that effects we observe on effortful choices in our assay are to some degree specific to a context in which mice must actively choose whether to exert effort to achieve an objective when a less effortful alternative is available. Similarly, we find that inhibiting the ACC does not affect the overall mobility of mice exploring an open field. Thus, the consistent increase in the time it takes mice to make choices in our assay, when the ACC is silenced cannot be solely explained by an impairment in the movement per se. These findings further clarify the nature of the effects we report in effort-based decision making. Future studies addressing the effects of these same manipulations in other types of decision making assays that involve effort other types of response costs would be useful.

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Competing Interests The authors declare no competing interests

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FIGURES:

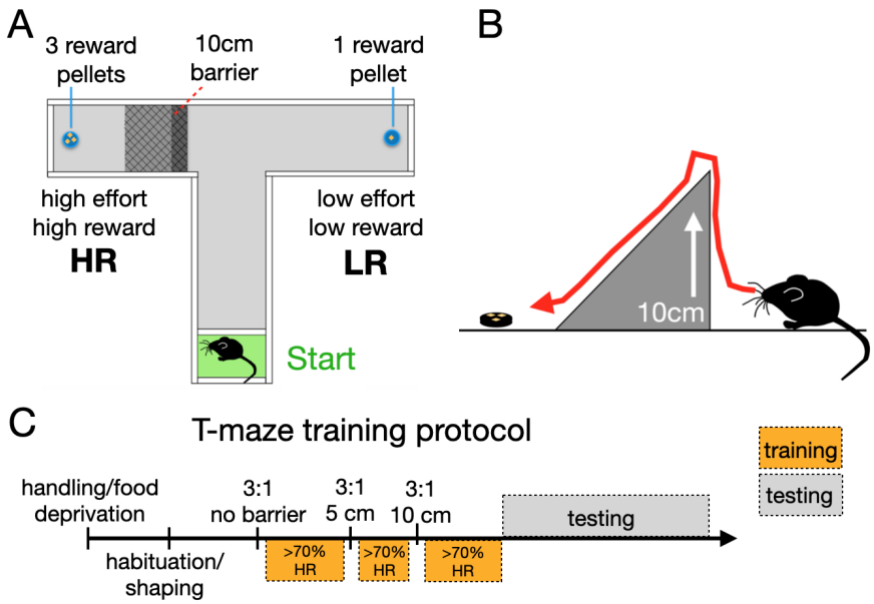

Figure 1 Mouse barrier T-maze schematic and training protocol

A) Barrier T-maze design with 10cm wire mesh barrier, and 3:1 reward differential for high effort, high reward (HR) vs. low effort, low reward (LR) arms. **B**) Side view of barrier showing 10 cm vertical side with 45 degree slope down to reward cup. **C**) Overview of general approach for training and testing mice in the assay - Following habituation/shaping, mice are trained to >70% HR choices with no barrier, 5cm barrier and 10cm barriers preceding testing phase.

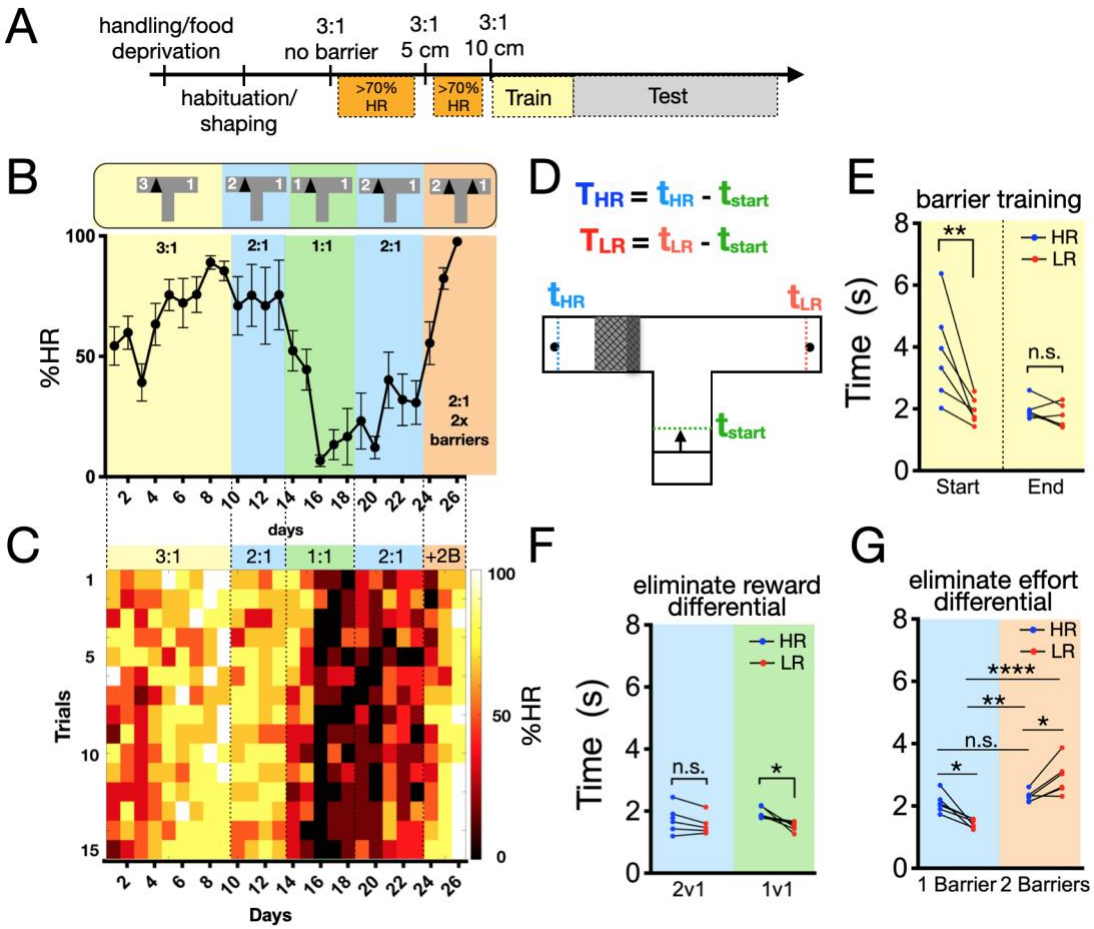

Figure 2 Validation of T-maze training and testing protocol in mice

A) For validation experiment, resutls are shown for the 3:1 +10cm barrier training portion of the task, followed by manipulations of reward differential or barrier arrangement (Test). **B**) Outcome of training and testing phase shown as %HR choices. Reward in high effort arm is systematically changed, and a second barrier is added to equalize effort in the final phase. **C**) Same data as B, but shown with mean %HR on a per trial basis to reveal within session changes. To examine behavior in greater detail, we calculated the time it takes mice to complete HR and LR choices (from start of a trial through reaching reward cup). **D**) Schematic representation of how trial lengths were calculated for **E-G. E**) Quantification of trial length for start vs. end of barrier training, [$F_{Interaction}(1,20)=5.84$, $P=0.025$, $F_{Time}(1,20)=9.21$, $P=0.007$, $F_{Choice}(1,20)=8.58$, $P=0.008$], 2-way ANOVA with Sidak posthoc test. **F**) Quantification of trial length before vs. after eliminating reward differential (2v1-to-1v1), [$F_{Interaction}(1,19)=1.20$, $P=0.288$, $F_{Reward}(1,19)=0.363$, $P=0.55$, $F_{Choice}(1,19)=5.98$, $P=0.024$], 2-way ANOVA with Sidak posthoc test. **G**) Quantification of trial length before vs. after eliminating effort differential (1Barrier-to-2Barriers), [$F_{Interaction}(1,20)=20.97$, $P=0.0002$, $F_{Barrier}(1,20)=37.14$, $P<0.0001$, $F_{Choice}(1,20)=0.037$, $P=0.85$], 2-way ANOVA with Sidak posthoc test. N=6 mice, n.s. = not significant, *p<0.05, **p<0.01, ***p<.001, ****p<0.0001.

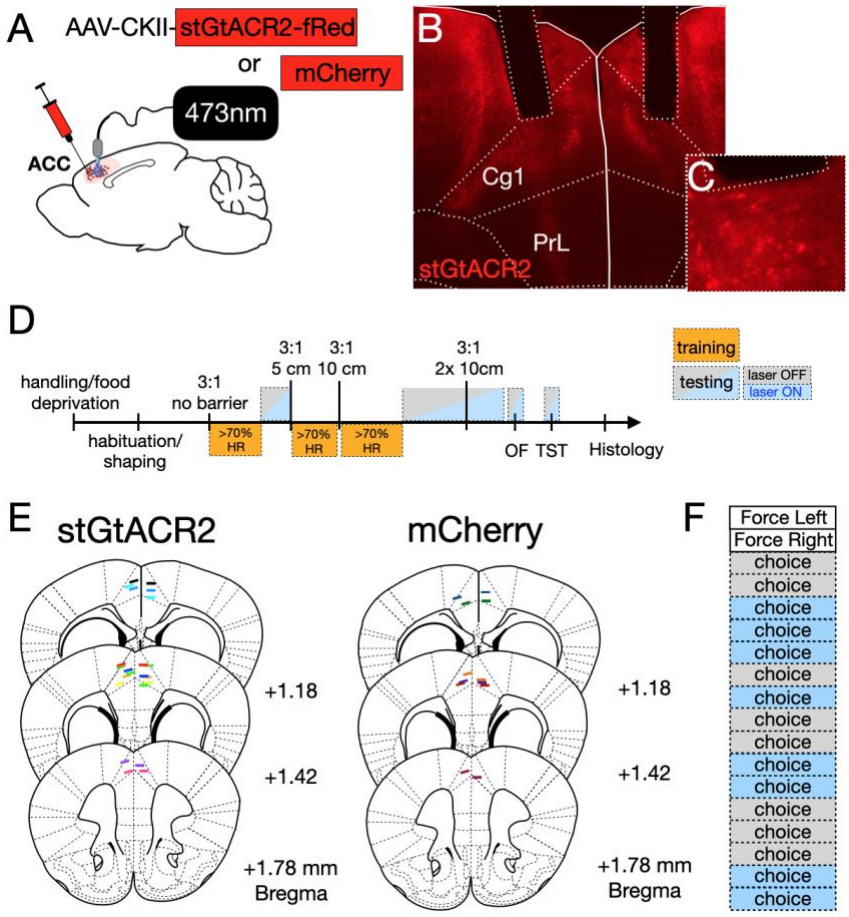

Figure 3 Experimental design and optic cannula for ACC silencing experiments

A) Bilateral ACC was injected with virus expressing an inhibitory opsin (stGtCAR2) or control (mCherry), and implanted with fiberoptic cannulae. **B**) Histology showing representative virus + cannula targeting (one cannula was implanted at a 15 degree angle). **C**) High magnification image showing membrane expression of stGtCAR2 under optic fiber. **D**) Overview of training and testing protocol for optogenetic inhibition; optogenetic testing followed no barrier and/or barrier training phases, and during an open field (OF) and tail suspension test (TST). **E**) Targeting of optic cannulae. **F**) Example of a typical test day with 2 forced and 16 choice trials with light delivered in a pseudorandom pattern.

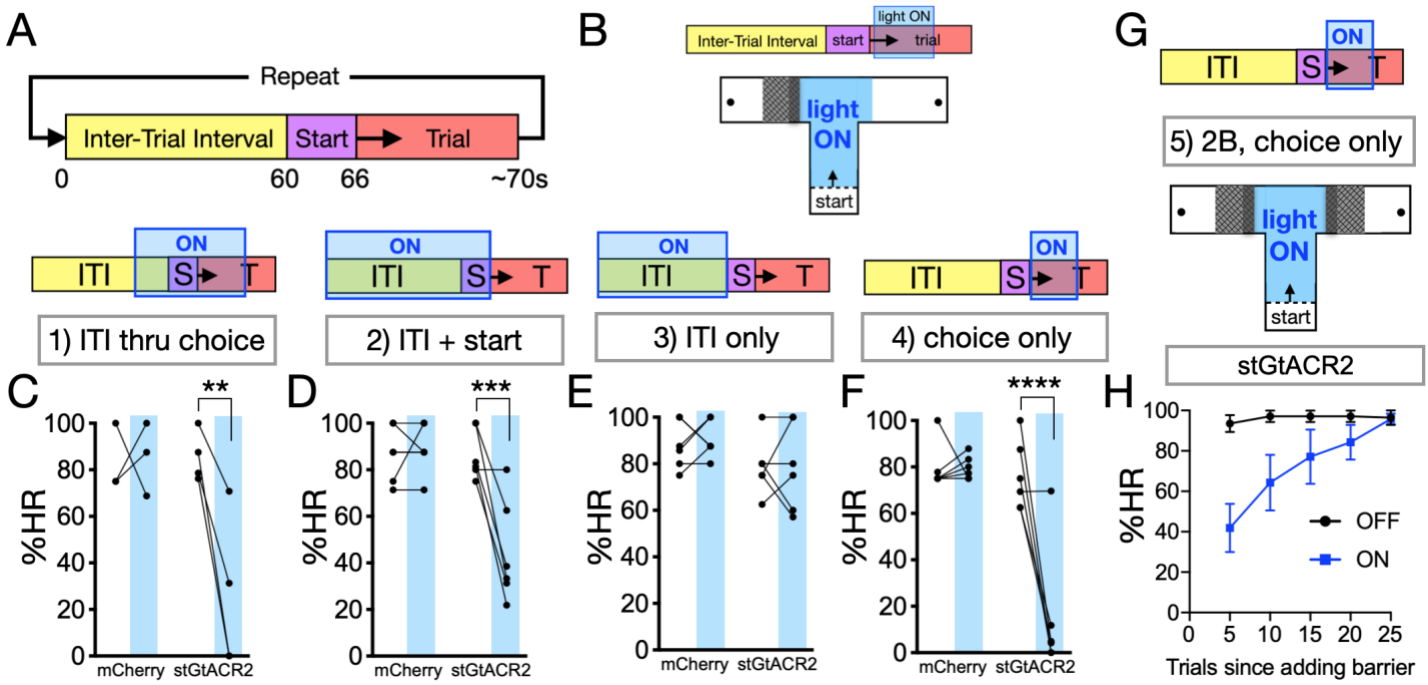

Figure 4 Optogenetic inhibition of bilateral ACC reversibly disrupts high effort choices

A) Schematic representing trial cycle on which light OFF/ON epochs are overlaid. **B**) Example of light ON overlaid for choice only, light ON during trial itself extends spatially from start through choice point. **C**) Quantification of %HR choices for light OFF vs. ON, end of ITI through choice point, mCherry and stGtACR2; (blue shading is light ON) [$F_{Interaction}(1,10)=7.13$, $P=0.024$, $F_{Laser}(1,10)=6.20$, $P=0.032$, $F_{Virus}(1,10)=6.14$, $P=0.033$], 2-way ANOVA with Sidak posthoc test. **D**) Quantification %HR for light OFF vs. ON, ITI + start only [$F_{Interaction}(1,20)=13.6$, $P=0.002$, $F_{Laser}(1,20)=11.2$, $P=0.003$, $F_{Virus}(1,20)=14.0$, $P=0.001$], 2-way ANOVA with Sidak posthoc test. **E**) Quantification %HR for light OFF vs. ON, ITI only [$F_{Interaction}(1,20)=0.054$, $P=0.82$, $F_{Laser}(1,20)=0.048$, $P=0.83$, $F_{Virus}(1,20)=3.98$, $P=0.06$], 2-way ANOVA with Sidak posthoc test. **F**) Quantification %HR for light OFF vs. ON, choice only [$F_{Interaction}(1,20)=20.8$, $P=0.0002$, $F_{Laser}(1,20)=25.8$, $P=0.0001$, $F_{Virus}(1,20)=20.6$, $P=0.0002$], 2-way ANOVA with Sidak posthoc test. **G**) 2 Barrier control continuation for stGtACR2 choice only condition. **H**) %HR for 2 Barrier control, consecutive trials quantified in blocks of 5 for light OFF vs. ON. For (**C**), mCherry (n=3), stGtACR2 (n=4). For (**D-H**), mCherry (n=5), stGtACR2 (n=6); *p<0.05, **p<0.01, ***p<.001, ****p<0.0001.

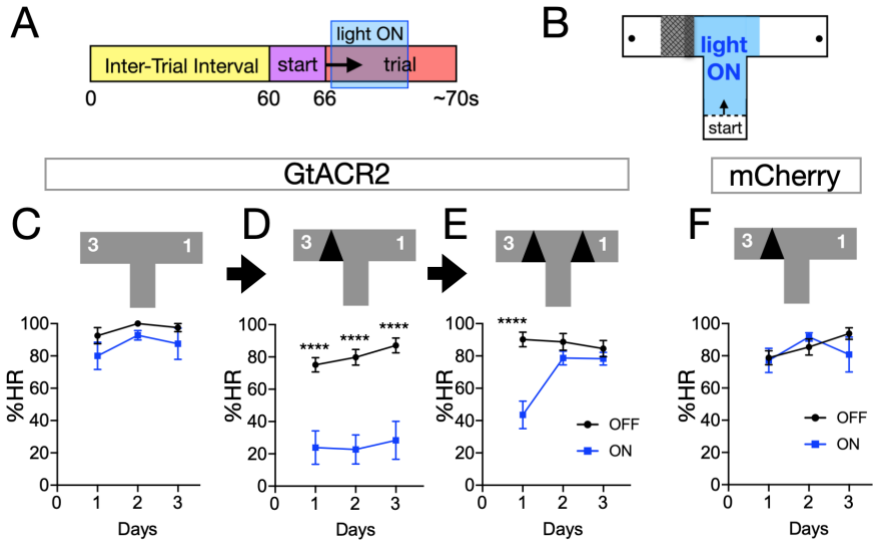

Figure 5 Optogenetic inhibition of bilateral ACC reversibly disrupts high effort choices across days

A-B) Schematic for light ON vs. OFF during choice trials delivered across sequential 3 day blocks. **C**) Quantification of %HR choices for light OFF vs. ON, 3:1 differential no barrier condition [$F_{Interaction}(2,24)=0.10$, $P=0.904$, $F_{Days}(2,24)=1.53$, $P=0.24$, $F_{Laser}(1,24)=4.30$, $P=0.049$], 2-way ANOVA with Sidak posthoc test (n=5). **D**) Quantification %HR for light OFF vs. ON, 3:1 one barrier condition [$F_{Interaction}(2,42)=0.11$, $P=0.89$, $F_{Days}(2,42)=0.76$, $P=0.47$, $F_{Laser}(1,42)=91.99$, $P<0.0001$], 2-way ANOVA with Sidak posthoc test (n=8). **E**) Quantification %HR for light OFF vs. ON, 3:1 two barriers condition [$F_{Interaction}(3,38)=9.40$, $P<0.0001$, $F_{Days}(3,38)=8.96$, $P=0.0001$, $F_{Laser}(1,38)=11.34$, $P=0.002$], 2-way ANOVA with Sidak posthoc test (n=7). **F**) Quantification %HR for light OFF vs. ON, mCherry control condition 3:1 one barrier condition [$F_{Interaction}(2,26)=1.20$, $P=0.32$, $F_{Days}(2,26)=2.10$, $P=0.14$, $F_{Laser}(1,26)=0.33$, $P=0.57$], 2-way ANOVA with Sidak posthoc test (n=6). *p<0.05, **p<0.01, ***p<.001, ****p<0.0001.

Figure 6 Effect of optogenetic inhibition of ACC on time in T-maze zones

A) Schematic representation of T-maze video processing: keypoint tracking with DeepLabCut (DLC) and analysis of tracking data using custom BehaviorDEPOT code. **B**) Behavior videos with inhibition during trial/choice only used for tracking analysis. **C**) Schematic representation of T-Maze zones assigned for subsequent analysis. **D**) DLC tracking for a mouse in the choice zone of the maze (zone analysis uses head mounted implant as tracking point (green dot)). **E**) Quantification of time in approach and choice zones for light OFF vs. ON trials, one barrier condition - all choices [$F_{Interaction}(1,28)=4.41$, $P=0.045$, $F_{Zone}(1,28)=2.47$, $P=0.13$, $F_{Laser}(1,28)=14.35$, $P=0.0007$], 2-way ANOVA with Sidak posthoc test. **F**) Quantification for HR choices only [$F_{Interaction}(1,16)=1.16$, $P=0.30$, $F_{Zone}(1,16)=0.011$, $P=0.92$, $F_{Laser}(1,16)=20.9$, $P=0.0003$], 2-way ANOVA with Sidak posthoc test. **G**) Quantification for LR choices only [$F_{Interaction}(1,20)=0.47$, $P=0.50$, $F_{Zone}(1,20)=3.58$, $P=0.07$, $F_{Laser}(1,20)=6.05$, $P=0.02$], 2-way ANOVA with Sidak posthoc test. **H**) Quantification of relative time in each zone for light ON vs. OFF stratified by choice type [$F_{Interaction}(2,32)=0.40$, $P=0.67$, $F_{Choice}(2,32)=0.43$, $P=0.66$, $F_{Zone}(1,32)=4.87$, $P=0.035$], 2-way ANOVA with Sidak posthoc test. **I**) Quantification of time in approach and choice zones for light OFF vs. ON trials, two barrier condition - all choices [$F_{Interaction}(1,24)=0.013$, $P=0.91$, $F_{Zone}(1,24)=2.39$, $P=0.14$, $F_{Laser}(1,24)=15.03$, $P=0.0007$], 2-way ANOVA with Sidak posthoc test. **J**) Quantification for HR choices only [$F_{Interaction}(1,24)=0.0006$, $P=0.98$, $F_{Zone}(1,24)=1.84$, $P=0.19$, $F_{Laser}(1,24)=15.22$, $P=0.0007$], 2-way ANOVA with Sidak posthoc test. **K**) Quantification for LR choices only [$F_{Interaction}(1,16)=0.1.78$, $P=0.20$, $F_{Zone}(1,16)=1.52$, $P=0.24$, $F_{Laser}(1,16)=0.12$, $P=0.74$], 2-way ANOVA with Sidak posthoc test. **L**) Quantification of relative time in each zone for light ON vs. OFF stratified by choice type [$F_{Interaction}(2,32)=0.90$, $P=0.42$, $F_{Choice}(2,32)=3.33$, $P=0.049$, $F_{Zone}(1,32)=1.66$, $P=0.21$], 2-way ANOVA with Sidak posthoc test. *p<0.05, **p<0.01, ***p<.001, ****p<0.0001.

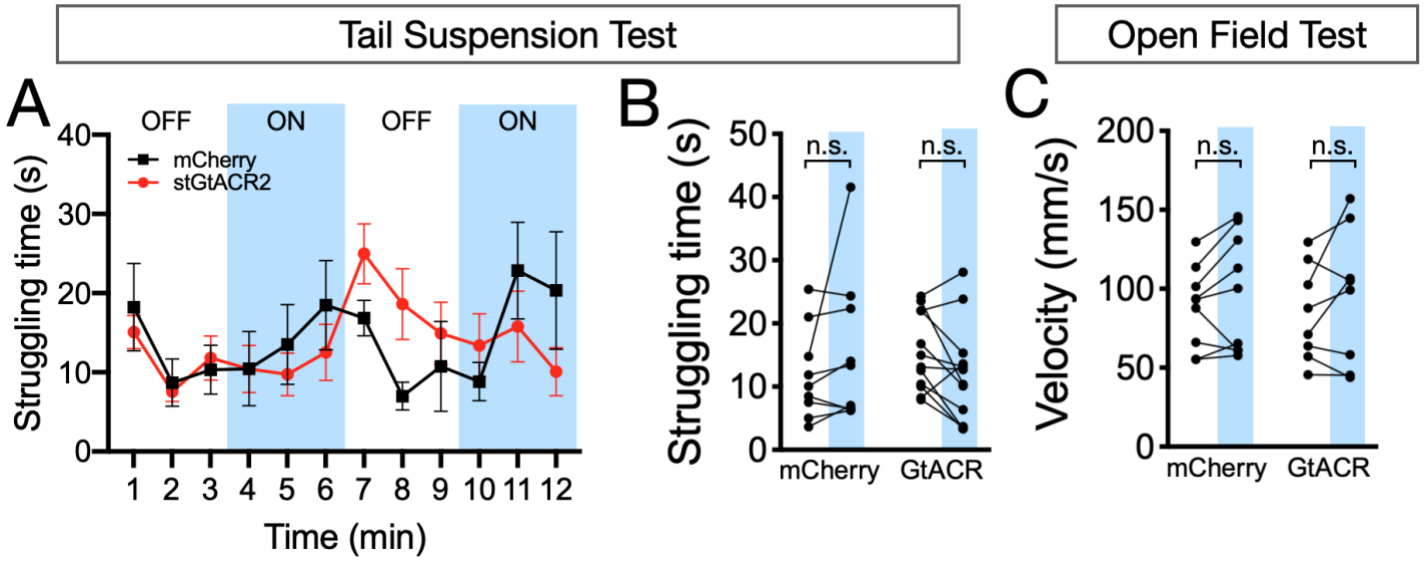

Figure 7 Optogenetic inhibition of ACC has no effect on tail suspension test or open field tests.

A) Quantification of mean struggling time calculated for each minute of the tail suspension test with optogenetic inhibition of ACC (blue shading = light ON). **B**) Summary data for light OFF vs. ON for mCherry (n=9) and stGtACR2 (n=12) conditions [$F_{Interaction}(1,38)=1.96$, $P=0.17$, $F_{Virus}(1,38)=0.0021$, $P=0.96$, $F_{Laser}(1,38)=0.0028$, $P=0.96$], 2-way ANOVA with Sidak posthoc test. **C**) Quantification of mean velocity for open field test with optogenetic inhibition of the ACC, data for light OFF vs. ON for mCherry (n=9) and stGtACR2 (n=8) conditions [$F_{Interaction}(1,30)=0.0033$, $P=0.95$, $F_{Virus}(1,30)=0.076$, $P=0.78$, $F_{Laser}(1,30)=0.68$, $P=0.42$], 2-way ANOVA with Sidak posthoc test. n.s. = not significant.