Task Demand-dependent Contribution of Frontal-Sensory Cortical Projections to Attentional Behavior in Mice

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Abstract

Top-down attention is a dynamic cognitive process that facilitates the detection of the task-relevant stimuli from our complex sensory environment. A neural mechanism capable of deployment under specific task-demand conditions would be crucial to efficiently control attentional processes and improve goal-directed attention performance in task demand-dependent manner. Previous studies have shown that frontal top-down neurons projecting from anterior cingulate area (ACA) to the visual cortex (VIS; ACA_{VIS}) are required for attentional behavior during the 5-choice serial reaction time task (5CSRTT) in mice. However, it is unknown whether the contribution of this projecting neurons is dependent on the extent of task demand. Here, we examine the differential contribution of ACA_{VIS} projection neurons to the attentional behavior in adult male mice performing two visual attention tasks of varying task demand: the 5CSRTT and 2-choice serial reaction time task (2CSRTT). We found that optogenetic suppression ACA_{VIS} projections immediately before stimulus presentation has no effect during the 2CSRTT in contrast to the impaired performance during the 5CSRTT. Fiber photometry calcium imaging of ACA_{VIS} neuron activity revealed that these neurons, which are recruited after errors during 5CSRTT, are not recruited during 2CSRTT. These results suggest that ACA_{VIS} projections are necessary only when task demand is high and that ACA_{VIS} neuron activity may not provide an error monitoring signal when task demand is low. Collectively, this frontal-sensory ACA_{VIS} projection regulates visual attention behavior during specific high task demand conditions, pointing to a flexible circuit-based mechanism for promoting attentional behavior.

Introduction

Top-down attention is a fundamental cognitive process that facilitates the detection of the most pivotal goal-directed stimuli from our dynamic environment. The frontal cortex, particularly the anterior cingulate cortex area (ACA), has been demonstrated to be a key mediator of top-down control of visual attention across species including rodents (Chudasama et al., 2003; Kim et al., 2016; Koike et al., 2016; Passetti et al., 2002; Pehrson et al., 2013). Among ACA neurons, recent studies in mice have demonstrated that frontal-sensory projections from the ACA to the primary visual cortex (VIS) (ACA_{VIS}) modulate visual discrimination (Moore and Zirnsak, 2017; Zhang et al., 2014) and free-moving attentional behavior (Norman et al., 2021) through modulation of visual cortex processing (Norman et al., 2021). Previous fMRI studies in humans show that the response of the primary visual cortex during attention is dependent on task load (Bahrami et al., 2007; Pinsk et al., 2004; Schwartz et al., 2005). However, it is unknown whether this graded response is driven by top-down input from the frontal cortex. A circuit mechanism capable of deployment under specific task-demand conditions would be crucial to efficiently control attentional processes and improve promote goal-directed attention performance during fluctuating attentional demand. Here, we aimed to determine to what extent frontal-sensory projection neurons are dynamically recruited depending on the task demand.

We hypothesized that ACA_{VIS} projection neurons may only be necessary for visual attentional behavior under conditions of elevated task demand. We tested this hypothesis by subjecting mice to two tasks of varying task demand while selectively manipulating and monitoring ACA_{VIS} neural activity in mice. We used the tasks in which mice were required to sustain and divide their attention in anticipation of a random presentation of a brief stimulus at one location

across select number of response windows; either 5 locations during the 5CSRTT (Carli et al., 1983) (5CSRTT, **Fig.S1A**) or limited to 2 locations during the 2CSRTT (van Gaalen et al., 2009). During these tasks, we employed optogenetics to suppress ACA_{VIS} projection activity, or fiber photometry to monitor ACA_{VIS} neuron activity during these tasks to assess to what extent task demand impacts the contribution of ACA_{VIS} projection neurons to visual attentional behavior.

Materials and Methods

Experimental model: Adult, male C57Bl/6 mice (Charles River Laboratories, MA) were group-housed under a standard 12 hour light/dark cycle in a temperature- and humidity-controlled vivarium. Training was initiated when mice were 9–10 weeks old. Mice were allowed access to water for 2 hours each day and maintained approximately 85-90% of their *ad libitum* weight during behavioral training. Food was available *ad libitum* throughout the experiment. All animal protocols were approved by the Institutional Animal Care and Use Committee at Icahn School of Medicine at Mount Sinai. Data of the 5CSRTT have been analyzed previously (Norman et al., 2021), but only the mice which underwent both 5CSRTT and 2CSRTT with optogenetics (6 out of 8 mice) or fiber photometry recordings (6 out of 8 mice) were re-analyzed in this study.

Viral Strategies and Stereotaxic Procedures: Following procedures previously described in (Norman et al., 2021), mice were anesthetized with 2% isoflurane and head-fixed in a mouse stereotaxic apparatus (Narishige, East Meadow, NY). Bilateral ACA injection sites relative to Bregma area are: AP +0.7mm, ML ±0.2mm, DV -0.7mm; AP +0.2mm, ML ±0.2mm, DV -0.7mm; AP -0.3mm, ML ±0.2mm, DV -0.7mm. Bilateral VIS injection sites relative to lambda are: AP +0.0mm, ML ±3.0mm, DV -0.4mm; AP +0.1mm, ML ±2.85mm, DV -0.4mm; AP +0.1mm, ML

±3.15mm, DV -0.4mm. For circuit-specific *in vivo* imaging of ACA_{VIS} neurons, AAV1-Syn-FLEX-GCaMP6f (Addgene) was injected to the ACA and concurrently, retroAAV2-Cre was injected into VIS, unilaterally. A 1.2 mm length fiber optic ferrule was a 0.48 numerical aperture and a 400 µm core diameter (Doric Lenses, Quebec, Canada) was implanted at the center of ACA injection sites and cemented to the skull using dental cement (C&B Metabond, Parkell, Edgewood, NY; Jet Acrylic, Lang Dental, Wheeling, IL). To optogenetically suppress ACA_{VIS} neurons at terminals in the VIS, AAV2-CamKII-eNphR3.0-eYFP (UNC Viral Vector Core, Chapel Hill, NC) was injected bilaterally into the ACA. For eNpHR3.0 experiments, bilateral LEDs (Amuza, San Diego, CA) of 500 µm diameter that delivered 470nm light were implanted at the VIS. Each infusion (500 nl) was made at 150 nl/min using a microinjector set (Nanoject III) and glass pulled syringe. The syringe was left in place for 1min following the injection to reduce backflow of virus. Behavioral testing occurred at least three weeks after viral injection to allow for maximal viral expression. Mice were excluded if location of fiber optic ferrule implant was inaccurate.

Behavior:

<u>5- and 2-Choice Serial Reaction Time Task (5CSRTT/2CSRTT):</u>

Apparatus: 5CSRTT and 2CSRTT (See also **Fig. S1**) was conducted in eight Bussey–Saksida operant chambers with a touchscreen system (Lafayette Instruments, Lafayette, IL) following procedures previously described in (Norman et al., 2021). Dimensions are as follows: a black plastic trapezoid (walls 20 cm high × 18 cm wide (at screen-magazine) × 24 cm wide (at screen) × 6 cm wide (at magazine)). Stimuli were displayed on a touch-sensitive screen (12.1 inch, screen resolution 600 × 800) divided into five response windows by a black plastic mask (4.0 × 4.0 cm, positioned centrally with windows spaced 1.0 cm apart, 1.5 cm above the floor) fitted in front of the

touchscreen. For the 5CSRTT, all five response windows were available, but during the 2CSRTT, three of the outer response windows were masked and only two adjacent center windows Schedules were designed and data were collected and analyzed using ABET II Touch software (v18.04.17, Lafayette Instrument). The inputs and outputs of the multiple chambers were controlled by WhiskerServer software (v4.7.7, Lafayette Instrument).

Habituation: Following procedures previously described in (Norman et al., 2021), before 5CSRTT training, mice were first acclimated to the operant chamber and milk reward. The food magazine was illuminated and diluted (30%) sweetened condensed milk (Eagle Brand, Borden, Richfield, OH) was dispensed every 40s after mice entered the food magazine. Mice needed to enter the reward tray 20 times during two consecutive 30min sessions before advancing to the next stage. Mice were then trained to touch the illuminated response window. During this phase a white square stimulus was presented randomly at one response window until it was touched. If the mouse touched the stimulus, the milk reward was delivered in conjunction with a tone and magazine light. Touches to nonstimuli had no consequence. After reaching criterion on this phase (20 stimulus touches in 30 min for 2 consecutive days), mice advanced to the 5CSRTT training phase.

5CSRTT Training and Baseline: Following procedures previously described in (Norman et al., 2021), mice were tested 5 days a week, 100 trials a day (or up to 30min). Each trial began with the illumination of the magazine light. After mice exited the food magazine, there was an intertrial interval (ITI) period of 5s before a stimulus was presented at one response window. If a mouse touched the screen during the ITI period, the response was recorded as premature and the mouse was punished with a 5s time-out (house light on). After the time-out period, the magazine light illumination and house light switch off signaled onset of the next trial. After the ITI period, a

stimulus appeared randomly in one of the five response windows for a set stimulus duration (this varied from 32 to 2s, depending on stage of training). A limited-hold period followed by the stimulus duration was 5s, during which the stimulus was absent but the mouse was still able to respond to the location. Responses during stimulus presence and limited holding period could be recorded either as correct (touching the stimulus window) or incorrect (touching any other windows). A correct response was rewarded with a tone, and milk delivery, indicated by the illumination of the magazine light. Failure to respond to any window over the stimulus and limited-hold period was counted as an omission. Incorrect responses and omissions were punished with a 5-s time-out. In addition, repeated screen touches after a correct or incorrect response were counted as perseverative responses. Animals started at stimulus duration of 32s. With a goal to baseline mice at a stimulus duration of 2s, the stimulus duration was sequentially reduced from 32, 16, 8, 4, to 2s. Animals had to reach a criterion (≥50 trials, ≥80% accuracy, ≤20% omissions) over 2 consecutive days to pass from one stage to the next. After reaching baseline criterion with the 2s stimulus duration for 4 out of 5 days, mice began 5CSRTT testing.

5CSRTT Testing: Following procedures previously described in (Norman et al., 2021), during 5CSRTT testing, attention demand was increased by reducing and pseudo-randomly shuffling the stimulus duration to 2.0, 1.5, 1.0, and 0.8s for 4 out of 5 days. Following 5CSRTT testing, mice underwent stereotaxic viral surgery and then were reestablished to baseline criterion before fiber photometry imaging (2.0, 1.5, 1.0, and 0.8s stimulus duration), or optogenetics (2.0 and 1.0s simulus duration) experiments. Between experimental testing days, mice were subjected to 2s stimulus duration training to confirm that the mice maintain stable baseline criterion. Attention and response control were assessed by measuring the following performance: correct percentage ((100 x (correct responses)/(correct responses + incorrect responses + omissions)), percentage

accuracy (100 × correct responses/(correct responses + incorrect responses)), percentage omission (100 × omissions/(omissions + correct responses + incorrect responses)), percentage of premature responses, percentage of perseverative responses, latency to correct response (s), and latency to reward collection (s) after correct choices. A premature response is a response given during the ITI before a stimulus appears. It is a measure of impulsive behavior. A perseverative response is when a response to any screen continues to be given after the correct or incorrect screen has been poked and before retrieval of the reward. It is a measure of compulsive behavior. Correct response latency (CRL) is the difference between the time of stimulus onset and the time of the correct response. It is a measure of attention and processing speed. Reward collection latency (RCL), is the difference between the time of a correct response and the time of reward collection and reflects motivation.

2CSRTT Baseline and Testing: During the 2CSRTT, 3 of the response windows were blocked and only 2 central response windows remained in which the stimulus could appear. Animals had to reach a baseline criterion (≥50 trials, ≥80% accuracy, ≤20% omissions) with the 2s stimulus duration at 1 of 2 locations for 4 out of 5 days to pass before beginning 2CSRTT testing. During 2CSRTT testing, attention demand was increased by reducing and pseudo-randomly shuffling the stimulus duration to 2.0, 1.5, 1.0, and 0.8s for 2 consecutive days.

Optogenetics:

Following procedures previously described in (Norman et al., 2021), TTL signals were sent to a signal generator (Rigol Technologies, Beijing, China) that drove the light at specific time points during the trials. During separate test sessions, LEDs were stimulated during either -5:-2s of ITI, -3:0s of ITI or during the length of the stimulus presentation. One timing of stimulus duration was

used per test day in a counterbalanced manner. During behavioral 5CSRTT and 2CSRTT, continuous 470nm light was delivered bilaterally via LED optic fiber implanted at the VIS pseudo randomly at specific time points throughout a trial during 50% of trials. In our study, we chose to use 470nm LED to suppress eNpHR3.0-expressing ACA neurons, because previous studies found that red-shifted light frequencies resulted in off-target effects in control mice that reduced 5CSRTT performance (White et al., 2018). During optogenetics testing, stimulus duration was pseudo randomly shuffled between 2.0 and 1.0 s.

***In vivo* Fiber Photometry:**

Following procedures previously described in (Norman et al., 2021), experiments were done at least three weeks after viral injection to allow for sufficient viral expression. A mono-fiber optic cannula (MFC 400/430-0.48 1.2 MF2.5 FLT, Doric Lenses) was stereotaxically implanted above the ACA. During recording, a matching fiber optic patch cord (Doric Lenses, MFP_400/430/0.48_1.3) was attached to the implanted fiber optic cannula with cubic zirconia sheathes following procedures previously described in (Norman et al 2021). The fiber optic cable was coupled to two excitation LEDs (Thorlabs, Newton, NJ): 470nm to stimulate GCaMP6f fluorescence in a Ca^{2+}-dependent manner and 405nm which serves as an isosbestic wavelength for GCaMP6f, for ratiometric measurements of GCaMP6f activity, correcting for bleaching and signal artifacts. 470nm excitation was sinusoidally-modulated at 210 Hz and 405nm excitation was modulated at 330 Hz. Light then passed through a GFP (490) or violet (405) excitation filter (Thor labs) and dichroic mirrors before passing through the fiber optic cannula into the brain. GFP excitations emissions passed through the fiber optic cable is passed through the dichroic mirror and emission filters, and through a 0.50 N.A. microscope lens (62-561, Edmund Optics,

Barrington, NJ) and then focused and projected onto a photodetector (Model 2151 Femtowatt Photoreceiver, Newport Corporation, Irvine, CA). A digital fiber photometry processor (RZ5P, Tucker-Davis Technologies, Alachua, FL) was used to demodulate the signal from each LED. Signal was then pre-processed using the Synapse Software Suite (Tucker-Davis Technologies) and collected at a sampling frequency of 1018 Hz. The power at the fiber-optic tip was approximately 10 mW. 405 nm signal was used to detrend 470 nm based on signal bleaching. The ⊿F/F was then calculated as: (470 nm signals – median 470 nm signals) / (median 470 nm signals) based on each individual 30min 5CSRTT or 2CSRTT session. Fiber photometry signal data was divided into bins to assess differences in ACA_{VIS} recruitment depending on overall performance (correct or error) or trial history (previous error or previous correct).

Quantification and statistical analysis

Statistical analyses were performed using Prism (Graphpad, San Diego, CA) and in R v. 3.5.3. Analyses were conducted using general linear mixed models, ANOVA, or t-tests as indicated. Bar graphs and photometry averaged traces are represented as the mean and error bars represent standard error of the mean (s.e.m.)

Results

2CSRTT shows reduced task demand compared to 5CSRTT

To determine whether attentional behavior outcomes depend on the extent of task demand, we compared the performance between mice performing two tasks with varying degrees of task demand: the 5CSRTT and 2CSRTT. Mice were first trained to perform freely moving attentional behavior during the 5CSRTT, an assay of visual attention (**Fig 1.A, S1.A-B**). The task

required mice to sustain and divide their attention across 5 response windows in their lower visual field in anticipation of a white square stimulus appearing briefly at 1 of the 5 locations in automated standardized Bussey-Saksida touch-screen operant chambers (Bari et al., 2008; Carli et al., 1983). Touching the window location where the stimulus presented suggests proper allocation of attention and lead to a reward, while touching the other windows (incorrect) or failing to respond to any response window (omission) were considered to reflect lapses in attentional behavior (Dalley et al., 2004; Koike et al., 2016; Lustig et al., 2013) (**Fig S1.C**). Mice had to reach baseline criteria of 4 out of 5 sessions with ≥50 trials, ≥80% accuracy, ≤20% omission at 2s stimulus duration before 5CSRTT testing in which the stimulus duration length was shuffled between 2.0, 1.5, 1.0, and 0.8 s in a pseudorandomized order. Following 5CSRTT testing, mice began 2CSRTT baselining with 2s stimulus duration in which the number of response windows was reduced to 2. To pass 2CSRTT baselining stage, mice had to reach same baseline criteria as the 5CSRTT (4 out of 5 sessions with ≥50 trials, ≥80% accuracy, ≤20% omission at 2s stimulus duration) before undergoing 2CSRTT testing. We found that task performance of mice was significantly higher during the 2CSRTT compared to the 5CSRTT as indicated by increased correct trials. The improved task performance during the 2CSRTT was driven by improved accuracy with a lesser degree of change in omissions (**Fig. 1B-C**). Furthermore, correct response latency has significantly shorter during the 2CSRTT, suggesting that performance occurred at a faster rate under lower task demand. Reward collection latency(s), premature responses (%), and perseverative responses (%) were similar between the both the 5CSRTT and 2CSRTT, demonstrating that each task engaged similar levels of motivation, impulsivity, and compulsivity, respectively (**Fig. 1F-H**). Collectively, these data support that lower task demand of 2CSRTT compared to 5CSRTT is reflected to better task performance in 2CSRTT.

ACA_{VIS} projections, while required for 5CSRTT, are dispensable for 2CSRTT

While our previous studies have shown that ACA_{VIS} projection neurons are required for attentional behavior during 5CSRTT in mice (Norman et al., 2021; Norman, 2021), it is unknown whether the contribution of this subpopulation of frontal cortex projecting neurons are task demand dependent. To this end, we selectively suppressed ACA_{VIS} axon terminal activity in visual cortex using the inhibitory opsin halorhodopsin eNpHR3.0 at key time points during both the 5CSRTT and 2CSRTT in which the stimulus duration length was shuffled between 2s and 1s in a pseudorandomized order to determine it's effect on task performance (**Fig.2A-B**). Data of the 5CSRTT have been analyzed previously (Norman et al., 2021), but only the mice which underwent both 5CSRTT and 2CSRTT (6 out of 8 mice) were re-analyzed in this study. Viral spread was validated previously (Norman et al., 2021). We found that suppressing ACA_{VIS} projections during the 3s immediately before stimulus presentation (-3:0s) impaired performance during the 5CSRTT, reducing percent correct and increasing percent omission, however, no effect was observed during the 2CSRTT (**Fig.2F-H**). Performance of either task was not affected by adjusting the LED illumination time to earlier in the ITI (-5:-2s) or during stimulus presentation (0:1s) (**Fig.2C-E, I-K**). ACA_{VIS} terminal inhibition during any time point examined had no effect on correct response latency, reward collection latency, premature responses, or perseverative responses during the 2CSRTT (**Fig.S2).** Taken together, ACA_{VIS} projections are causally important for attentional task performance in the period just before stimulus presentation during 5CSRTT with higher task demand, but are dispensable during 2CSRTT with less task demand, suggesting that ACA_{VIS} projections are necessary only when task demand is high.

ACA_{VIS} projection neurons, recruited after errors during 5CSRTT, are not recruited during 2CSRTT

We then examined whether the trial circumstances that regulated ACA_{VIS} projections recruitment depend on task demand during the 5CSRTT and 2CSRTT (**Fig. 3A-B**). To selectively express the calcium indicator, GCaMP6f, into the top-down ACA_{VIS} neurons, we microinjected a Cre-dependent AAV encoding GCaMP6f and a retrograde Cre encoding virus into the ACA and VIS, respectively. The activity of ACA_{VIS} neurons was assessed using fiber photometry as mice performed the 5CSRTT and 2CSRTT. Data of the 5CSRTT have been analyzed previously (Norman et al., 2021), but only the mice which underwent both 5CSRTT and 2CSRTT (6 out of 8 mice) were re-analyzed in this study. Viral spread was validated previously (Norman et al., 2021). During the 2CSRTT, we found that ACA_{VIS} neuron activity within single trials did not differ between correct choices, when attention was properly allocated, and erroroneous trials during both the intertrial interval (ITI) and stimulus presentation (**Fig.3C**). The change in ACA_{VIS} neuron signaling between correct and error trials was similar between the 2CSRTT and 5CSRTT (Norman et al., 2021) (**Fig.3D**). As our recent study showed that ACA_{VIS} neurons are selectively recruited after error trials during 5CSRTT (Norman et al 2021), we next examined ACA_{VIS} neuron activity in relation to the past trial outcomes in 2CSRTT. In contrast to 5CSRTT (Norman et al., 2021), ACA_{VIS} neuron activity during 2CSRTT trials did not differ between trails following errors or after correct trials (**Fig.3E**). When trials during 2CSRTT were classified into four groups based on the combined outcome of the previous and current trials and showed that overall ACA_{VIS} activity also did not correlate with improved performance after an error was made (**Fig. S3**). However, when we compared the change in ACA_{VIS} neuron signaling between correct and error trials between 2CSRTT and 5CSRTT, ACA_{VIS} neuron activity showed a less change during the 2CSRTT than the

5CSRTT (**Fig.3F**). This result suggests that ACA_{VIS} activity may not provide an error monitoring signal when task demand is low. Collectively, the data reveal a task demand-dependent recruitment of ACA_{VIS} projection neurons during the visual attention task following error trials.

Discussion

In this study, we first characterized how attentional behavior outcomes depend on the extent of task demand and showed that the 2CSRTT is less task demanding than the 5CSRTT. We then used circuit-specific optogenetic suppression to show that frontal-sensory ACA_{VIS} projection neurons are only crucial for top-down control of attention behavior in male mice during high task demand conditions. Circuit-selective calcium imaging of ACA_{VIS} projection neurons revealed that the neurons, which are recruited after errors during 5CSRTT, are not recruited during 2CSRTT.

Previous studies in mice revealed a causal role of a specific sub-population ACA neurons projecting to the visual cortex in contributing to attentional behavior (Norman et al., 2021; Norman, 2021), similar to other ACA neurons (Kim et al., 2016; White et al., 2018). However, it remained unclear whether this mechanism was dependent on task demand. Our study provides further insight into defining the role of the specific factor of task demand has in recruiting particular subtypes of neurons. The task demand-dependent recruitment of ACA_{VIS} neurons may support efficient attentional behavior following errors. Challenging task demands amplify behavioral mechanisms of post-error attentional adjustments (Regev and Meiran, 2014) and may differentially recruit neural mechanisms for implementation.

Our work identifies a task demand-dependent mechanism that facilitates the detection of the task-relevant stimuli during the 5CSRTT and 2CSRTT, but these tasks are limited by only including relevant target stimuli(Young et al., 2012; Young and Geyer, 2015). The ability to filter irrelevant sensory stimuli is another key component of attentional behavior. The five-choice continuous performance task (5C-CPT) integrates aspects of the 5CSRTT and with the continuous performance task (CPT), a gold standard of attention task in human patients(Young et al., 2009), that includes nontarget stimuli which require response inhibition. To date, it is unknown whether varying degrees of task demand alter neural recruitment during the 5C-CPT, which includes a response inhibition component, as was observed in the current study using the 5CSRTT. Another limitation of the current study is that all mice performed the 5CSRTT prior to the 2CSRTT. Future studies should randomize the order in which mice were trained on each of the task in order to control for the effect of task order on performance and the contribution of ACA_{VIS} neurons.

Our study provides insight into the neural mechanisms underlying cognitive behavior deficits in psychiatric disease. In low attentional load conditions, attentional performance does not differ between patients with schizophrenia and healthy controls but attentional deficits emerge in those with schizophrenia during high load conditions (Ducato et al., 2008). Our study may ultimately inspire consideration of task demand when designing and interpreting the results of interventions specifically targeting top-down frontal-sensory circuits to improve attention.

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Author Contributions

KJN and HM designed experiments. KJN and HM analyzed and wrote the manuscript with contributions from all co-authors. KJN performed fiber photometry and optogenetics experiments with assistance from JB and HK. JB, CC, and KC performed behavioral training. HM supervised the research.

Figure Legends

Figure 1. 2CSRTT is less task demanding than the 5CSRTT. A) Experimental timeline: Mice were first trained on the 5CSRTT before undergoing 5CSRTT testing. During 5CSRTT testing, with a fixed 5s ITI and pseudorandomized stimulus duration (2.0, 1.5, 1.0, or 0.8s), (8 mice; 2,161 total trials). The same mice were then baselined with 2CSRTT before undergoing 2CSRTT testing (2,354 total trials). **B)** Mice had a significantly increased correct trials during the 2CSRTT

compared to the 5CSRTT(%, two-way repeated measures analysis of variance (RM ANOVA), Main effect: $F_{1,7}$ =15.26, ***P*=0.0059, Interaction: $F_{3,21}$ =3.626, **P*=0.0298, Holm-Sidak multiple comparisons at 2.0, 1.5, 1.0, 0.8 s stimulus duration, *P*=0.7623, 0.1197, *0.0121, ****<0.0001, n=8 mice). **C)** Mice had a significantly increased accuracy during the 2CSRTT compared to the 5CSRTT (%, two-way RM ANOVA, Main effect: $F_{1,7}$ =11.40, **P*=0.0118, Interaction: $F_{3,21}$ =10.64, ****P*=0.0002, Holm-Sidak multiple comparisons at 2.0, 1.5, 1.0, 0.8 s stimulus duration, *P*=0.9781, 0.2574, 0.3198, **0.0017, n=8 mice). **D)** Mice had no difference in omissions during the 2CSRTT compared to the 5CSRTT(%, two-way RM ANOVA, Main effect: $F_{1,7}$ =3.246, *P*=0.1146, Interaction: $F_{3,21}$ =0.2317, *P*=0.8733, n=8 mice). **E)** The 2CSRTT had a significantly reduced correct response latency compared to the 5CSRTT (t_7=7.193, ***P*=0.0002). **F-H)** No difference in **F**) reward collection latency (t_7=0.3012, *P*=0.7720), **G**) premature responses (t_7=1.066, *P*=0.3218), and **H)** perseverative responses (t_7=1.291, *P*=0.2377) was observed between 2CSRTT and 5CSRTT testing. Error bars indicate mean±s.e.m. n.s. = non significant. * indicates *P*<0.05, ** indicates *P*<0.01, *** indicates *P*<0.001, **** indicates *P*<0.0001.

Figure 2. ACA_{VIS} projections, while required for 5CSRTT, are dispensable for 2CSRTT. A) Viral strategy: To express light-gated chloride channel eNpHR3.0 in excitatory ACA neurons, eNpHR3.0 encoding AAV under the CaMK2 promoter was injected in the ACA. An optic fiber was implanted at the VIS to optically suppress ACA_{VIS} projection terminals with temporal precision during the 5CSRTT. **B)** Experimental timeline: Mice were first trained on the 5CSRTT, before viral injection. After allowing three weeks for maximal viral expression, mice underwent 5CSRTT testing. During 5CSRTT testing, with a fixed 5s ITI and pseudorandomized stimulus duration (2.0 or 1.0s), 470nm LED was illuminated during early ITI (-5:-2s), late ITI (-3:0s), or during period of

stimulus presentation (0:1s) during 50% of trials (6 mice; 2,694 total trials). One timing was tested per day. The effect of optogenetic suppression during early ITI, late ITI, or stimulus period on behavioral performance (correct %, omission %, and accuracy %) at each light timing tested. The same mice were then baselined with 2CSRTT before undergoing the same optogenetic manipulation protocol with the 2CSRTT testing (6 mice; 2,844 total trials). **C-E)** Continuous 470nm illumination during ITI -5:-2s had no effect on correct trials (%, general linear mixed model (GLMM), effect of light: $P = 0.1010$, effect of task: $P < 0.0001$, interaction: $P = 0.1412$), omissions (GLMM, effect of light $P = 0.2873$, effect of task: $P < 0.0001$, interaction: $P = 0.2214$), or accuracy (GLMM, effect of treatment (light on vs. off): $P = 0.1796$). **F-H)** Continuous 470nm illumination during ITI -3:0s had an effect on correct trials (GLMM, effect of light: $P = 0.1274$, effect of task: $P < 0.0001$, interaction: $P = 0.0305$) and omissions (GLMM, effect of light $P = 0.0470$, effect of task: $P < 0.0001$, interaction: $P = 0.0008$). Light disrupted correct trials during the 5C task (*$P = 0.0114$), but not during the 2C task ($P = 0.9784$). Light disrupted omissions during the 5C task (***$P = 0.0001$), but not during the 2C task ($P = 0.8267$). Continuous 470nm illumination during ITI -3:0s had no effect on accuracy (GLMM, effect of light: $P = 0.6049$, effect of task: $P < 0.0001$, interaction: $P = 0.6068$). **I-K)** Continuous 470nm illumination during the stimulus period had no effect on correct trials (GLMM, effect of light: $P = 0.4721$, effect of task: $P < 0.0001$, interaction: $P = 0.7455$), omissions (GLMM, effect of light: $P = 0.0242$, effect of task: $P < 0.0001$, interaction: $P = 0.1570$), or accuracy (GLMM, effect of light: $P = 0.2600$, effect of task: $P = 0.0009$, interaction: $P = 0.5844$). Error bars indicate mean ± s.e.m. n.s. = non significant. * indicates $P<0.05$, *** indicates $P<0.001$.

Figure 3. ACA_{VIS} projection neurons, recruited after errors during 5CSRTT, are not recruited during 2CSRTT. A) Intersectional viral strategy: To selectively express GCaMP6f calcium indicator in ACA_{VIS} neurons, Cre-dependent GCaMP6f and retrograde-Cre encoding AAVs were injected bilaterally into the ACA and visual cortex (VIS), respectively. An optic fiber was implanted at the ACA for calcium dependent recordings of ACA_{VIS} neurons using fiber photometry. **B)** Mice (n=6 mice) were first trained on the 5CSRTT, before viral injection. After allowing three weeks for maximal viral expression, fiber photometry recordings of ACA_{VIS} neurons were conducted while mice performed two days of 5CSRTT (6 mice; 753 total trials) testing with a fixed 5s intertrial interval (ITI) and pseudorandomized stimulus duration (2.0, 1.5, 1.0, or 0.8s). After baselining with 2CSRTT, fiber photometry recordings of ACA_{VIS} neurons during 2CSRTT was conducted (6 mice; 668 total trials). **C)** During 2CSRTT testing, there was no difference in averaged ACA_{VIS} activity between correct and error trials (two-way RM ANOVA, $F_{1,5}$=2.424, P=0.1802, n=6 mice) during ITI (-5:0 s) or during the stimulus period (0:2s). **D)** There was no difference between the change in ACA_{VIS} activity (error – correct) between mice performing the 5CSRTT and 2CSRTT (two-way RM ANOVA, Main effect: $F_{1,10}$=0.5005, P=0.4955, Interaction: $F_{1,10}$=1.952, P=0.1926, n=6 mice)**. E)** During 2CSRTT testing, there was no difference in averaged ACA_{VIS} activity between trials following correct and error trials (two-way RM ANOVA, $F_{1,5}$=0.8606, P=0.3962, n=6 mice) during ITI (-5:0 s) or during the stimulus period. **F)** Error bars indicate mean±s.e.m., n.s. = non significant. * indicates P<0.05. **F)** ACA_{VIS} activity was higher (previous error – previous correct) during the 5CSRTT compared to the 2CSRTT (two-way RM ANOVA, Main effect: $F_{1,10}$ = 9.227, *P=0.0125, Interaction: $F_{1,10}$ = 0.02314, P=0.8821, n=6 mice; posthoc analyses,ITI: P=0.0141, stimulus period, P=0.0163). Error bars indicate mean±s.e.m. n.s. = non significant. * indicates P<0.05.

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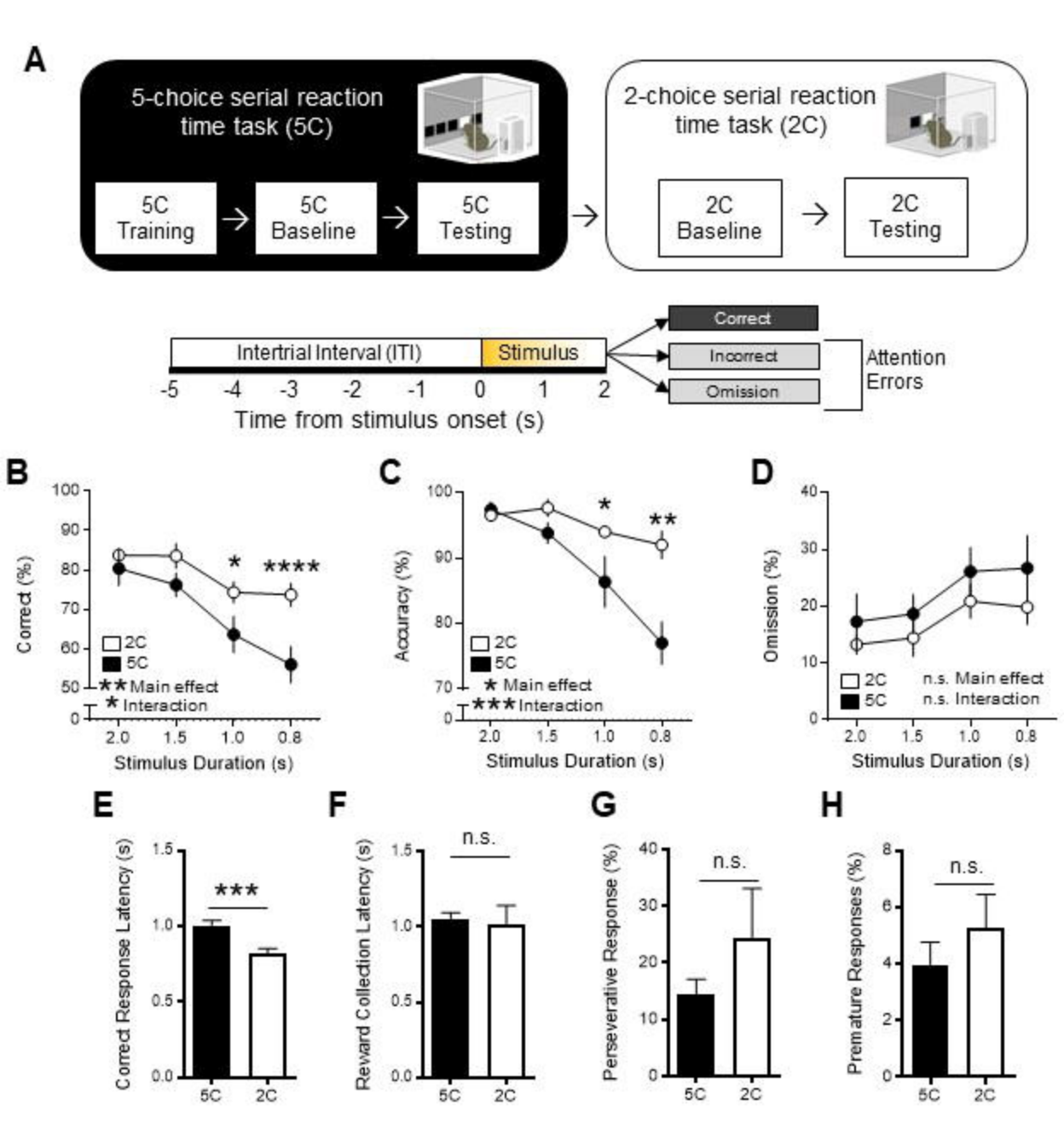
A
5-choice serial reaction time task (5C)
5C Training
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5C Testing
2-choice serial reaction time task (2C)
2C Baseline
2C Testing
Intertrial Interval (ITI)
Stimulus
-5 -4 -3 -2 -1 0 1 2
Time from stimulus onset (s)
Correct
Incorrect
Omission
Attention Errors
B
Correct (%)
2C
5C
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Stimulus Duration (s)
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Accuracy (%)
2C
5C
* Main effect
*** Interaction
Stimulus Duration (s)
D
Omission (%)
2C
5C
n.s. Main effect
n.s. Interaction
Stimulus Duration (s)
E
Correct Response Latency (s)
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5C
2C
F
Reward Collection Latency (s)
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5C
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G
Perseverative Response (%)
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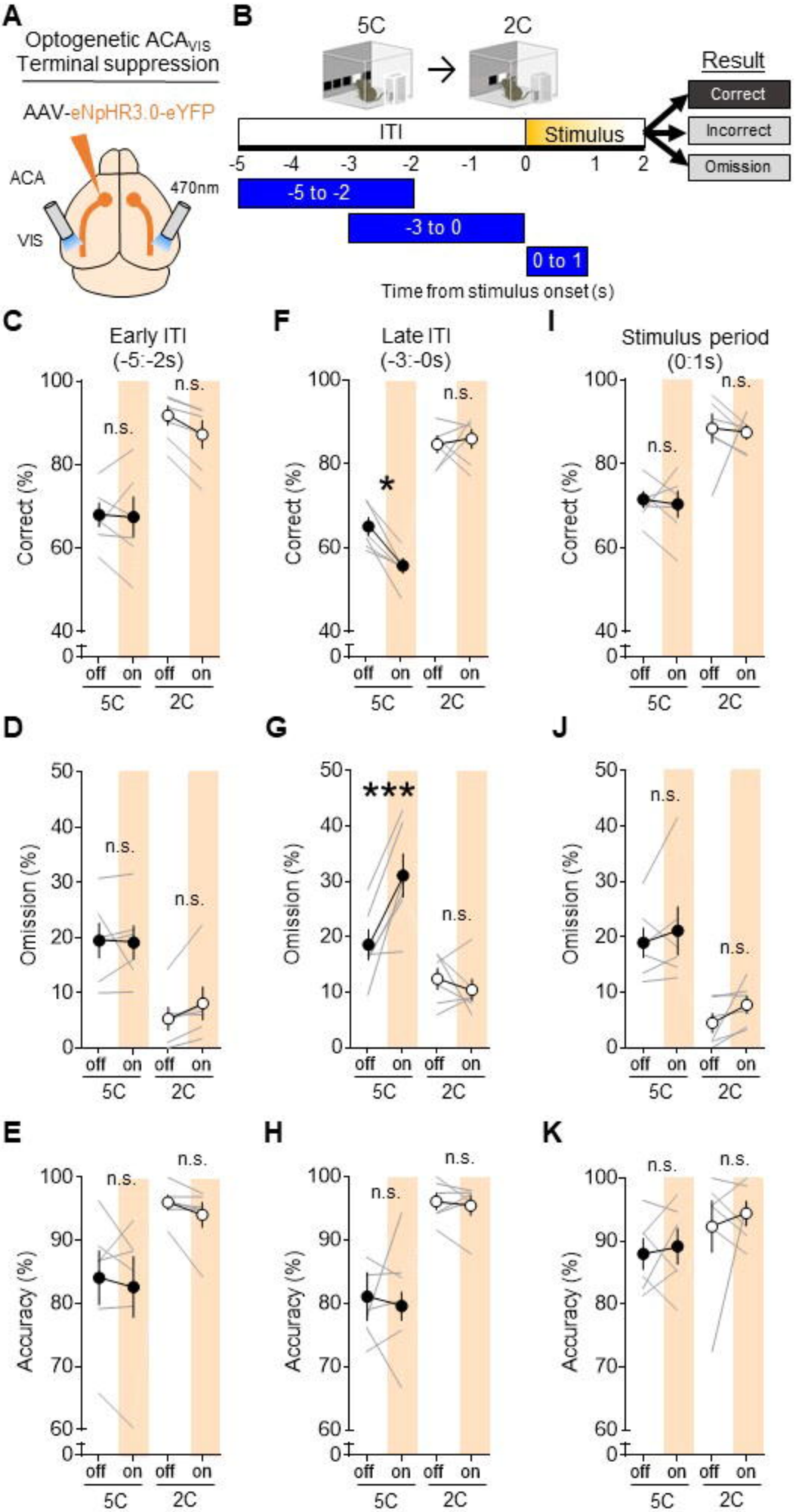
5C
2C
H
Premature Responses (%)
n.s.
5C
2C


A
Optogenetic ACA$_{VIS}$ Terminal suppression
AAV-eNpHR3.0-eYFP
ACA
470nm
VIS
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5C
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Result
Correct
Incorrect
Omission
ITI
Stimulus
-5 -4 -3 -2 -1 0 1 2
-5 to -2
-3 to 0
0 to 1
Time from stimulus onset (s)
C
Early ITI (-5:-2s)
F
Late ITI (-3:-0s)
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Stimulus period (0:1s)
Correct (%)
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Omission (%)
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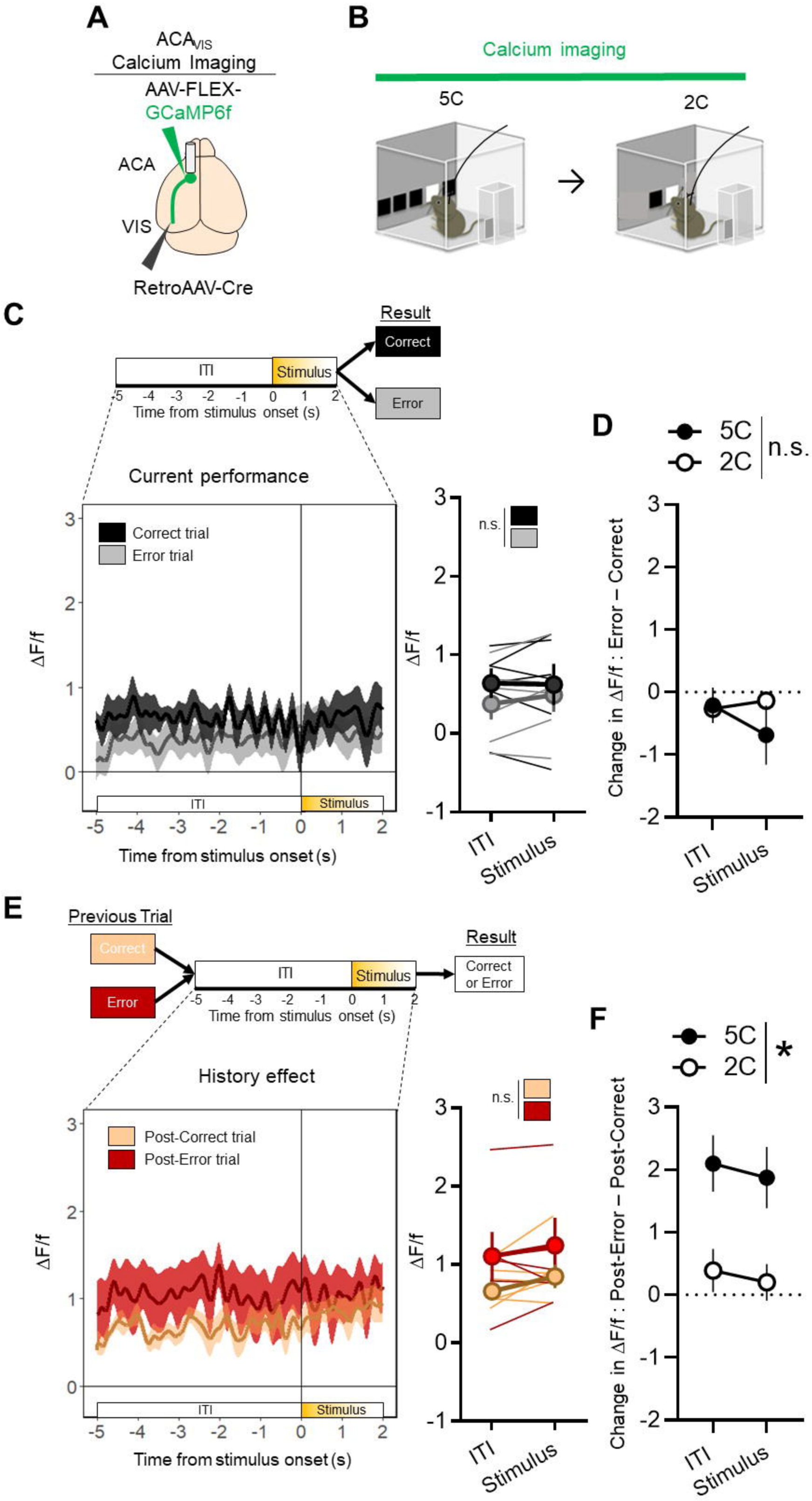
A
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Calcium Imaging
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B
Calcium imaging
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Result
Correct
Error
ITI
Stimulus
Time from stimulus onset (s)
Current performance
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Error trial
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ITI
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n.s.
Change in ΔF/f : Error – Correct
E
Previous Trial
Correct
Error
Result
Correct or Error
History effect
Post-Correct trial
Post-Error trial
F
5C
2C
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Change in ΔF/f : Post-Error – Post-Correct