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

Kevin J. Norman[1-5], Julia Bateh[1-5], Priscilla Maccario[1-5], Christina Cho[1-5], Keaven Caro[1-5], Hiroyuki Koike[1-5], Hirofumi Morishita[1-5]*

[1]Department of Psychiatry, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029 USA, [2]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029 USA, [3]Department of Ophthalmology, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029 USA, [4]Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029 USA, [5]Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029 USA,

* Corresponding Author and Lead Contact: Hirofumi Morishita, M.D., Ph.D.

One Gustave L. Levy Place Box 1230, New York, NY 10029 USA,

Tel.: +1 212 824 8975, Fax: +1 646 537 9584

E-mail address: hirofumi.morishita@mssm.edu

Abstract (244/250 words)

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 promote goal-directed attention performance in task-demand dependent manner. Previous studies have shown that frontal top-down neurons projecting from the 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.

60 **Significance Statement** (119/120)

61 Task demand-dependent recruitment of neural circuits would be essential for efficient attentional
62 control. Anterior cingulate area projections to the visual cortex (ACA_{VIS}) are required for attentional
63 behavior in mice, but it is uncertain whether this is task-demand dependent. We first established
64 that visual attentional performance is dependent on task demand, before using an inhibitory
65 optogenetic approach to demonstrate that ACA_{VIS} projections play a causal role in the anticipatory
66 attention only during high task demand conditions. Also, circuit-selective fiber photometry
67 recordings showed that ACA_{VIS} projections are recruited following errors during high, but not low,
68 task demand conditions. This finding advances our understanding of flexible circuit-based
69 mechanisms for promoting attentional behavior that may underlie task-demand dependent behavior
70 deficits in psychiatric disorders.

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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., 2021a) through modulation of visual cortex processing (Norman et al., 2021b). 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 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)

106 (5CSRTT, **Fig.1-1**) or limited to 2 locations during the 2CSRTT (van Gaalen et al., 2009). During
107 these tasks, we employed optogenetics to suppress ACA_{VIS} projection activity, or fiber photometry
108 to monitor ACA_{VIS} neuron activity during these tasks to assess to what extent task demand impacts
109 the contribution of ACA_{VIS} projection neurons to visual attentional behavior.
110

111 **Materials and Methods**

112 **Experimental model:** Adult, male C57Bl/6 mice (Charles River Laboratories, MA) were group-
113 housed under a standard 12 hr light/dark cycle in a temperature- and humidity-controlled vivarium.
114 Training was initiated when mice were 9–10 weeks old. Mice were allowed access to water for 2
115 hours each day and maintained approximately 85-90% of their *ad libitum* weight during behavioral
116 training. Food was available *ad libitum* throughout the experiment. All animal protocols were
117 approved by the Institutional Animal Care and Use Committee at Icahn School of Medicine at Mount
118 Sinai. Data of the 5CSRTT have been analyzed previously(Norman et al., 2021b), but only the mice
119 which underwent both 5CSRTT and 2CSRTT with optogenetics (6 out of 8 mice) or fiber photometry
120 recordings (6 out of 8 mice) were re-analyzed in this study.
121

122 **Viral Strategies and Stereotaxic Procedures:** Following procedures previously described in
123 (Norman et al., 2021b), mice were anesthetized with 2% isoflurane and head-fixed in a mouse
124 stereotaxic apparatus (Narishige, East Meadow, NY). Bilateral ACA injection sites relative to
125 Bregma area are: AP +0.7mm, ML ±0.2mm, DV -0.7mm; AP +0.2mm, ML ±0.2mm, DV -0.7mm;
126 AP -0.3mm, ML ±0.2mm, DV -0.7mm. Bilateral VIS injection sites relative to lambda are: AP
127 +0.0mm, ML ±3.0mm, DV -0.4mm; AP +0.1mm, ML ±2.85mm, DV -0.4mm; AP +0.1mm, ML
128 ±3.15mm, DV -0.4mm. For circuit-specific *in vivo* imaging of ACA_{VIS} neurons, AAV1-Syn-FLEX-

129 GCaMP6f (Addgene) was injected to the ACA and concurrently, retroAAV2-Cre was injected into
130 VIS, unilaterally. A 1.2 mm length fiber optic ferrule was a 0.48 numerical aperture and a 400μm
131 core diameter (Doric Lenses, Quebec, Canada) was implanted at the center of ACA injection sites
132 and cemented to the skull using dental cement (C&B Metabond, Parkell, Edgewood, NY; Jet Acrylic,
133 Lang Dental, Wheeling, IL). To optogenetically suppress ACA_{VIS} neurons at terminals in the VIS,
134 AAV2-CamKII-eNpHR3.0-eYFP (UNC Viral Vector Core, Chapel Hill, NC) was injected bilaterally
135 into the ACA. For eNpHR3.0 experiments, bilateral LEDs (Amuza, San Diego, CA) of 500μm
136 diameter that delivered 470nm light were implanted at the VIS. Each infusion (500 nl) was made at
137 150 nl/min using a microinjector set (Nanoject III) and glass pulled syringe. The syringe was left in
138 place for 1min following the injection to reduce backflow of virus. Behavioral testing occurred at
139 least three weeks after viral injection to allow for maximal viral expression. The location of the
140 cannula was mapped onto blank coronal slice templates taken from Paxinos and Franklin mouse
141 brain atlas as previously described(Norman et al., 2021b). Mice were excluded if cannula location
142 implant was inaccurate.
143
144 **Behavior:**
145 <u>5- and 2-Choice Serial Reaction Time Task (5CSRTT/2CSRTT):</u>
146 *Apparatus:* 5CSRTT and 2CSRTT (See also **Fig. 1-1**) was conducted in eight Bussey–Saksida
147 operant chambers with a touchscreen system (Lafayette Instruments, Lafayette, IL) following
148 procedures previously described in (Norman et al., 2021b). Dimensions are as follows: a black
149 plastic trapezoid (walls 20 cm high × 18 cm wide (at screen-magazine) × 24 cm wide (at screen) ×
150 6 cm wide (at magazine)). Stimuli were displayed on a touch-sensitive screen (12.1 inch, screen
151 resolution 600 × 800) divided into five response windows by a black plastic mask (4.0 × 4.0 cm,

152 positioned centrally with windows spaced 1.0 cm apart, 1.5 cm above the floor) fitted in front of the
153 touchscreen. For the 5CSRTT, all five response windows were available, but during the 2CSRTT,
154 three of the outer response windows were masked and only two adjacent center windows.
155 Schedules were designed and data was collected and analyzed using ABET II Touch software
156 (v18.04.17, Lafayette Instrument). The inputs and outputs of the multiple chambers were controlled
157 by WhiskerServer software (v4.7.7, Lafayette Instrument).

158 *Habituation:* Following procedures previously described in (Norman et al., 2021b), before
159 5CSRTT training, mice were first acclimated to the operant chamber and milk reward. The food
160 magazine was illuminated and diluted (30%) sweetened condensed milk (Eagle Brand, Borden,
161 Richfield, OH) was dispensed every 40s after mice entered the food magazine. Mice needed to
162 enter the reward tray 20 times during two consecutive 30min sessions before advancing to the next
163 stage. Mice were then trained to touch the illuminated response window. During this phase, a white
164 square stimulus was presented randomly at one response window until it was touched. If the mouse
165 touched the stimulus, the milk reward was delivered in conjunction with a tone and magazine light.
166 Touches to nonstimuli had no consequence. After reaching criterion on this phase (20 stimulus
167 touches in 30 min for 2 consecutive days), mice advanced to the 5CSRTT training phase.

168 *5CSRTT Training and Baseline:* Following procedures previously described in (Norman et al.,
169 2021b), mice were tested 5 days a week, 100 trials a day (or up to 30min). Each trial began with
170 the illumination of the magazine light. After mice exited the food magazine, there was an intertrial
171 interval (ITI) period of 5s before a stimulus was presented at one response window. If a mouse
172 touched the screen during the ITI period, the response was recorded as premature and the mouse
173 was punished with a 5s time-out (house light on). After the time-out period, the magazine light
174 illumination and house light switch off signaled onset of the next trial. After the ITI period, a stimulus

175 appeared randomly in one of the five response windows for a set stimulus duration (this varied from
176 32 to 2s, depending on stage of training). A limited-hold period followed by the stimulus duration
177 was 5s, during which the stimulus was absent but the mouse was still able to respond to the location.
178 Responses during stimulus presence and limited holding period could be recorded either as correct
179 (touching the stimulus window) or incorrect (touching any other windows). A correct response was
180 rewarded with a tone, and milk delivery, indicated by the illumination of the magazine light. Failure
181 to respond to any window over the stimulus and limited-hold period was counted as an omission.
182 Incorrect responses and omissions were punished with a 5-s time-out. In addition, repeated screen
183 touches after a correct or incorrect response were counted as perseverative responses. Animals
184 started at stimulus duration of 32s. With a goal to baseline mice at a stimulus duration of 2s, the
185 stimulus duration was sequentially reduced from 32, 16, 8, 4, to 2s. Animals had to reach a criterion
186 (≥50 trials, ≥80% accuracy, ≤20% omissions) over 2 consecutive days to pass from one stage to
187 the next. After reaching baseline criterion with the 2s stimulus duration for 4 out of 5 days, mice
188 began 5CSRTT testing.

189 *5CSRTT Testing*: Following procedures previously described in (Norman et al., 2021b), during
190 5CSRTT testing, attention demand was increased by reducing and pseudo-randomly shuffling the
191 stimulus duration to 2.0, 1.5, 1.0, and 0.8s for 4 out of 5 days. Following 5CSRTT testing, mice
192 underwent stereotaxic viral surgery and then were reestablished to baseline criterion before fiber
193 photometry imaging (2.0, 1.5, 1.0, and 0.8s stimulus duration), or optogenetics (2.0 and 1.0s
194 stimulus duration) experiments. Between experimental testing days, mice were subjected to 2s
195 stimulus duration training to confirm that the mice maintain stable baseline criterion. Attention and
196 response control were assessed by measuring the following performance: correct percentage ((100
197 x (correct responses)/(correct responses + incorrect responses + omissions)), percentage accuracy

198 (100 × correct responses/(correct responses + incorrect responses)), percentage omission (100 ×
199 omissions/(omissions + correct responses + incorrect responses)), percentage of premature
200 responses, percentage of perseverative responses, latency to correct response (s), and latency to
201 reward collection (s) after correct choices. A premature response is a response given during the ITI
202 before a stimulus appears. It is a measure of impulsive behavior. A perseverative response is when
203 a response to any screen continues to be given after the correct or incorrect screen has been poked
204 and before retrieval of the reward. It is a measure of compulsive behavior. Correct response latency
205 (CRL) is the difference between the time of stimulus onset and the time of the correct response. It
206 is a measure of attention and processing speed. Reward collection latency (RCL) is the difference
207 between the time of a correct response and the time of reward collection and reflects motivation.

208 *2CSRTT Baseline and Testing:* Immediately following 5CSRTT testing, the mice underwent
209 baselining for the 2CSRTT. During the 2CSRTT, 3 of the response windows were blocked and only
210 2 central response windows remained in which the stimulus could appear. Animals had to reach a
211 baseline criterion (≥50 trials, ≥80% accuracy, ≤20% omissions) with the 2s stimulus duration at 1 of
212 2 locations for 4 out of 5 days to pass criteria before beginning 2CSRTT testing. During 2CSRTT
213 testing, attention demand was increased by reducing and pseudo-randomly shuffling the stimulus
214 duration to 2.0, 1.5, 1.0, and 0.8s for 2 consecutive days. A separate cohort of naïve mice underwent
215 2CSRTT training and testing, without prior 5CSRTT experience, using the same criteria as the
216 5CSRTT but with only 2 central response windows.

217

218 **Optogenetics:**

219 Following procedures previously described in (Norman et al., 2021b), transistor-transistor logic
220 signals were sent to a signal generator (Rigol Technologies, Beijing, China) that drove the light at

221 specific time points during the trials. During separate test sessions, LEDs were stimulated during
222 either -5:-2s of ITI, -3:0s of ITI or during the length of the stimulus presentation. One timing of
223 stimulus duration was used per test day in a counterbalanced manner. During behavioral 5CSRTT
224 and 2CSRTT, continuous 470nm light was delivered bilaterally via LED optic fiber implanted at the
225 VIS pseudo randomly at specific time points throughout a trial during 50% of trials. In our study, we
226 chose to use 470nm LED to suppress eNpHR3.0-expressing ACA neurons, because previous
227 studies found that red-shifted light frequencies resulted in off-target effects in control mice that
228 reduced 5CSRTT performance (White et al., 2018). During optogenetics testing, stimulus duration
229 was pseudo randomly shuffled between 2.0 and 1.0 s.
230
231 ***In vivo* Fiber Photometry:**
232 Following procedures previously described in (Norman et al., 2021b), experiments were done at
233 least three weeks after viral injection to allow for sufficient viral expression. A mono-fiber optic
234 cannula (MFC 400/430-0.48 1.2 MF2.5 FLT, Doric Lenses) was stereotaxically implanted above the
235 ACA. During recording, a matching fiber optic patch cord (Doric Lenses, MFP_400/430/0.48_1.3)
236 was attached to the implanted fiber optic cannula with cubic zirconia sheathes following procedures
237 previously described in (Norman et al., 2021b). The fiber optic cable was coupled to two excitation
238 LEDs (Thorlabs, Newton, NJ): 470nm to stimulate GCaMP6f fluorescence in a Ca^{2+}-dependent
239 manner and 405nm which serves as an isosbestic wavelength for GCaMP6f, for ratiometric
240 measurements of GCaMP6f activity, correcting for bleaching and signal artifacts. 470nm excitation
241 was sinusoidally-modulated at 210 Hz and 405nm excitation was modulated at 330 Hz. Light then
242 passed through a GFP (490) or violet (405) excitation filter (Thor labs) and dichroic mirrors before
243 passing through the fiber optic cannula into the brain. GFP excitations emissions passed through

244 the fiber optic cable is passed through the dichroic mirror and emission filters, and through a 0.50
245 N.A. microscope lens (62-561, Edmund Optics, Barrington, NJ) and then focused and projected
246 onto a photodetector (Model 2151 Femtowatt Photoreceiver, Newport Corporation, Irvine, CA). A
247 digital fiber photometry processor (RZ5P, Tucker-Davis Technologies, Alachua, FL) was used to
248 demodulate the signal from each LED. Signal was then pre-processed using the Synapse Software
249 Suite (Tucker-Davis Technologies) and collected at a sampling frequency of 1018 Hz. The power
250 at the fiber-optic tip was approximately 10 mW. 405 nm signal was used to detrend 470 nm based
251 on signal bleaching. The ⊿F/F was then calculated as: (470 nm signals – median 470 nm signals) /
252 (median 470 nm signals) based on each individual 30min 5CSRTT or 2CSRTT session. Fiber
253 photometry signal data was divided into bins to assess differences in ACA_{VIS} recruitment depending
254 on overall performance (correct or error) or trial history (previous error or previous correct).
255

256 **QUANTIFICATION AND STATISTICAL ANALYSIS**

257 Statistical analyses were performed using Prism (Graphpad, San Diego, CA) and in R v. 3.5.3.
258 Analyses were conducted using general linear mixed models, ANOVA, or t-tests as indicated. Bar
259 graphs and photometry averaged traces are represented as the mean and error bars represent
260 standard error of the mean (s.e.m.)
261

262 **Results**

263 **2CSRTT shows reduced task demand compared to 5CSRTT**

264 To determine whether attentional behavior outcomes depend on the extent of task demand,
265 we compared the performance between mice performing two tasks with varying degrees of task
266 demand: the 5CSRTT and 2CSRTT. Mice were first trained to perform freely moving attentional

267 behavior during the 5CSRTT, an assay of visual attention (**Fig 1.A, 1-1.A**). The task required mice
268 to sustain and divide their attention across 5 response windows in their lower visual field in
269 anticipation of a white square stimulus appearing briefly at 1 of the 5 locations in automated
270 standardized Bussey-Saksida touch-screen operant chambers (Bari et al., 2008; Carli et al., 1983).
271 Touching the window location where the stimulus presented suggests proper allocation of attention
272 and lead to a reward, while touching the other windows (incorrect) or failing to respond to any
273 response window (omission) were considered to reflect lapses in attentional behavior (Dalley et al.,
274 2004; Koike et al., 2016; Lustig et al., 2013) (**Fig 1-1.C**). Mice had to reach baseline criteria of 4 out
275 of 5 sessions with ≥50 trials, ≥80% accuracy, ≤20% omission at 2s stimulus duration before 5CSRTT
276 testing in which the stimulus duration length was shuffled between 2.0, 1.5, 1.0, and 0.8 s in a
277 pseudorandomized order. Following 5CSRTT testing, mice began 2CSRTT baselining with 2s
278 stimulus duration in which the number of response windows was reduced to 2. To pass 2CSRTT
279 baselining stage, mice had to reach same baseline criteria as the 5CSRTT (4 out of 5 sessions with
280 ≥50 trials, ≥80% accuracy, ≤20% omission at 2s stimulus duration) before undergoing 2CSRTT
281 testing. We found that task performance of mice was significantly higher during the 2CSRTT
282 compared to the 5CSRTT as indicated by increased correct trials. The improved task performance
283 during the 2CSRTT was driven by improved accuracy with a lesser degree of change in omissions
284 (**Fig. 1B-C**). Furthermore, correct response latency was slower during the 5CSRTT, while reward
285 collection latency(s), premature responses (%), and perseverative responses (%) were similar
286 between both the 5CSRTT and 2CSRTT, demonstrating that each task engaged similar levels of
287 motivation, impulsivity, and compulsivity, respectively (**Fig. 1F-H**). Of note, there was no difference
288 in 2CSRTT performance between mice with or without prior 5CSRTT testing, suggesting that the
289 order of training does not influence attentional behavior (**Fig.1-2**). Collectively, these data support

290 that lower task demand of 2CSRTT compared to 5CSRTT is reflected to better task performance in
291 2CSRTT.

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

293 While our previous studies have shown that ACA_{VIS} projection neurons are required for
294 attentional behavior during 5CSRTT in mice (Norman et al., 2021a; Norman et al., 2021b), it is
295 unknown whether the contribution of this subpopulation of frontal cortex projecting neurons are task
296 demand dependent. To this end, we selectively suppressed ACA_{VIS} axon terminal activity in visual
297 cortex using the inhibitory opsin halorhodopsin eNpHR3.0 at key time points during both the
298 5CSRTT and 2CSRTT in which the stimulus duration length was shuffled between 2s and 1s in a
299 pseudorandomized order to determine its effect on task performance (**Fig.2A-B**). Data of the
300 5CSRTT have been analyzed previously (Norman et al., 2021b), but only the mice which underwent
301 both 5CSRTT and 2CSRTT (6 out of 8 mice) were re-analyzed in this study. Viral spread was
302 validated previously (Norman et al., 2021b). We found that suppressing ACA_{VIS} projections during
303 the 3s immediately before stimulus presentation (-3:0s) impaired performance during the 5CSRTT,
304 reducing correct trials and accuracy, however, no effect was observed during the 2CSRTT (**Fig.2F-**
305 **H**). Performance of either task was not affected by adjusting the LED illumination time to earlier in
306 the ITI (-5:-2s) or during stimulus presentation (0:1s) (**Fig.2C-E, I-K**). ACA_{VIS} terminal inhibition
307 during any time point examined had no effect on correct response latency, reward collection latency,
308 premature responses, or perseverative responses during the 2CSRTT (**Fig.2-1).** Taken together,
309 ACA_{VIS} projections are causally important for attentional task performance in the period just before
310 stimulus presentation during 5CSRTT with higher task demand, but are dispensable during 2CSRTT
311 with less task demand, suggesting that ACA_{VIS} projections are necessary only when task demand
312 is high.

313

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

316 We then examined whether the trial circumstances that regulated ACA_{VIS} projections
317 recruitment depend on task demand during the 5CSRTT and 2CSRTT (**Fig. 3A-B**). To selectively
318 express the calcium indicator, GCaMP6f, into the top-down ACA_{VIS} neurons, we microinjected a
319 Cre-dependent AAV encoding GCaMP6f and a retrograde Cre encoding virus into the ACA and VIS,
320 respectively. The activity of ACA_{VIS} neurons was assessed using fiber photometry as mice
321 performed the 5CSRTT and 2CSRTT. Data of the 5CSRTT have been analyzed previously (Norman
322 et al., 2021b), but only the mice which underwent both 5CSRTT and 2CSRTT (6 out of 8 mice) were
323 re-analyzed in this study. Viral spread was validated previously (Norman et al., 2021b). During the
324 2CSRTT, we found that ACA_{VIS} neuron activity within single trials did not differ between correct
325 choices, when attention was properly allocated, and erroneous trials during both the intertrial interval
326 (ITI) and stimulus presentation (**Fig.3C**). The change in ACA_{VIS} neuron signaling between correct
327 and error trials was similar between the 2CSRTT and 5CSRTT (Norman et al., 2021b) (**Fig.3D**). As
328 our recent study showed that ACA_{VIS} neurons are selectively recruited after error trials during
329 5CSRTT (Norman et al., 2021b), we next examined ACA_{VIS} neuron activity in relation to the past
330 trial outcomes in 2CSRTT. In contrast to 5CSRTT (Norman et al., 2021b), ACA_{VIS} neuron activity
331 during 2CSRTT trials did not differ between trials following errors or after correct trials (**Fig.3E**).
332 When trials during 2CSRTT were classified into four groups based on the combined outcome of the
333 previous and current trials and showed that overall ACA_{VIS} activity also did not correlate with
334 improved performance after an error was made (**Fig.3-1**). However, when we compared the change
335 in ACA_{VIS} neuron signaling between correct and error trials between 2CSRTT and 5CSRTT, ACA_{VIS}

336 neuron activity showed a less change during the 2CSRTT than the 5CSRTT (**Fig.3F**). This result
337 suggests that ACA_{VIS} activity may not provide an error monitoring signal when task demand is low.
338 Collectively, the data reveal a task demand-dependent recruitment of ACA_{VIS} projection neurons
339 during the visual attention task following error trials.

340

341 **Discussion**

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

348

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

358

359 One limitation of the current study is that all mice in optogenetics and fiber photometry
360 experiments performed the 5CSRTT prior to the 2CSRTT. However, it is unlikely that the prior
361 5CSRTT training impacted the subsequent 2CSRTT performance because we showed that
362 2CSRTT performance outcomes were indistinguishable regardless of the prior 5CSRTT training
363 (Fig.1-2). Future studies are warranted to fully control for the task order by randomizing order in
364 which mice were trained on each of the task. Another limitation is that the 5CSRTT and 2CSRTT
365 are limited by only including relevant target stimuli (Young et al., 2012; Young and Geyer, 2015).
366 The ability to filter irrelevant sensory stimuli is another key component of attentional behavior. The
367 five-choice continuous performance task (5C-CPT) integrates aspects of the 5CSRTT and with the
368 continuous performance task (CPT), a gold standard of attention task in human patients(Young et
369 al., 2009), that includes nontarget stimuli which require response inhibition. To date, it is unknown
370 whether varying degrees of task demand alter neural recruitment during the 5C-CPT, which
371 includes a response inhibition component, as was observed in the current study using the 5CSRTT.
372
373 Our study provides insight into the neural mechanisms underlying cognitive behavior
374 deficits in psychiatric disease. In low attentional load conditions, attention function while ignoring
375 visual distractors does not differ between patients with schizophrenia and healthy controls but those
376 with schizophrenia performed worse during high load conditions, suggesting that attentional deficits
377 only emerge under more challenging tasks (Ducato et al., 2008). Our study may ultimately inspire
378 consideration of task demand when designing and interpreting the results of interventions
379 specifically targeting top-down frontal-sensory circuits to improve attention.
380
381

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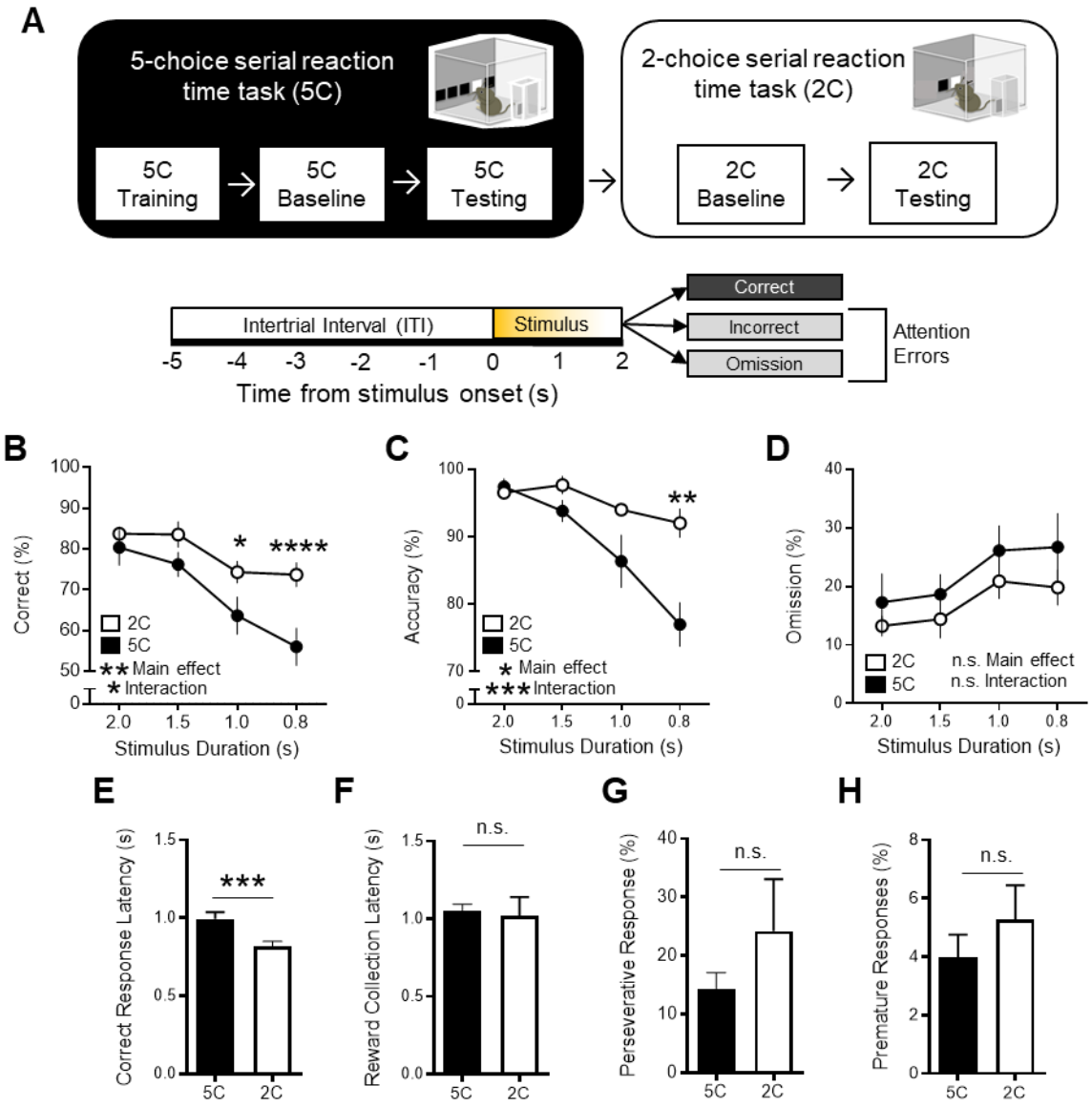
Author Contributions

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, PM, CC, and KC performed behavioral training. HM supervised the research.

406 **Figure Legends**



407

408 **Figure 1. 2CSRTT is less task demanding than the 5CSRTT. A)** Experimental timeline: Mice
409 were first trained on the 5CSRTT before undergoing 5CSRTT testing. During 5CSRTT testing,
410 with a fixed 5s ITI and pseudorandomized stimulus duration (2.0, 1.5, 1.0, or 0.8s), (8 mice;
411 2,161 total trials). The same mice were then baselined with 2CSRTT before undergoing 2CSRTT
412 testing (2,354 total trials). **B)** Mice had a significantly increased correct trials during the 2CSRTT
413 compared to the 5CSRTT(%, two-way repeated measures analysis of variance (RM ANOVA),

414 Main effect: $F_{1,7}$ =15.26, ***P*=0.0059, Interaction: $F_{3,21}$ =3.626, **P*=0.0298, Holm-Sidak multiple
415 comparisons at 2.0, 1.5, 1.0, 0.8 s stimulus duration, *P*=0.7623, 0.1197, *0.0121, ****<0.0001,
416 n=8 mice). **C)** Mice had a significantly increased accuracy during the 2CSRTT compared to the
417 5CSRTT (%, two-way RM ANOVA, Main effect: $F_{1,7}$ =11.40, **P*=0.0118, Interaction: $F_{3,21}$ =10.64,
418 ****P*=0.0002, Holm-Sidak multiple comparisons at 2.0, 1.5, 1.0, 0.8 s stimulus duration,
419 *P*=0.9781, 0.2574, 0.3198, **0.0017, n=8 mice). **D)** Mice had no difference in omissions during
420 the 2CSRTT compared to the 5CSRTT(%, two-way RM ANOVA, Main effect: $F_{1,7}$ =3.246,
421 *P*=0.1146, Interaction: $F_{3,21}$ =0.2317, *P*=0.8733, n=8 mice). **E)** The 2CSRTT had a significantly
422 reduced correct response latency compared to the 5CSRTT (t_7=7.193, ****P*=0.0002). **F-H)** No
423 difference in **F**) reward collection latency (t_7=0.3012, *P*=0.7720), **G**) premature responses
424 (t_7=1.066, *P*=0.3218), and **H)** perseverative responses (t_7=1.291, *P*=0.2377) was observed
425 between 2CSRTT and 5CSRTT testing. Error bars indicate mean ± s.e.m. n.s. = non significant.
426 * indicates *P*<0.05, ** indicates *P*<0.01, *** indicates *P*<0.001, **** indicates *P*<0.0001.

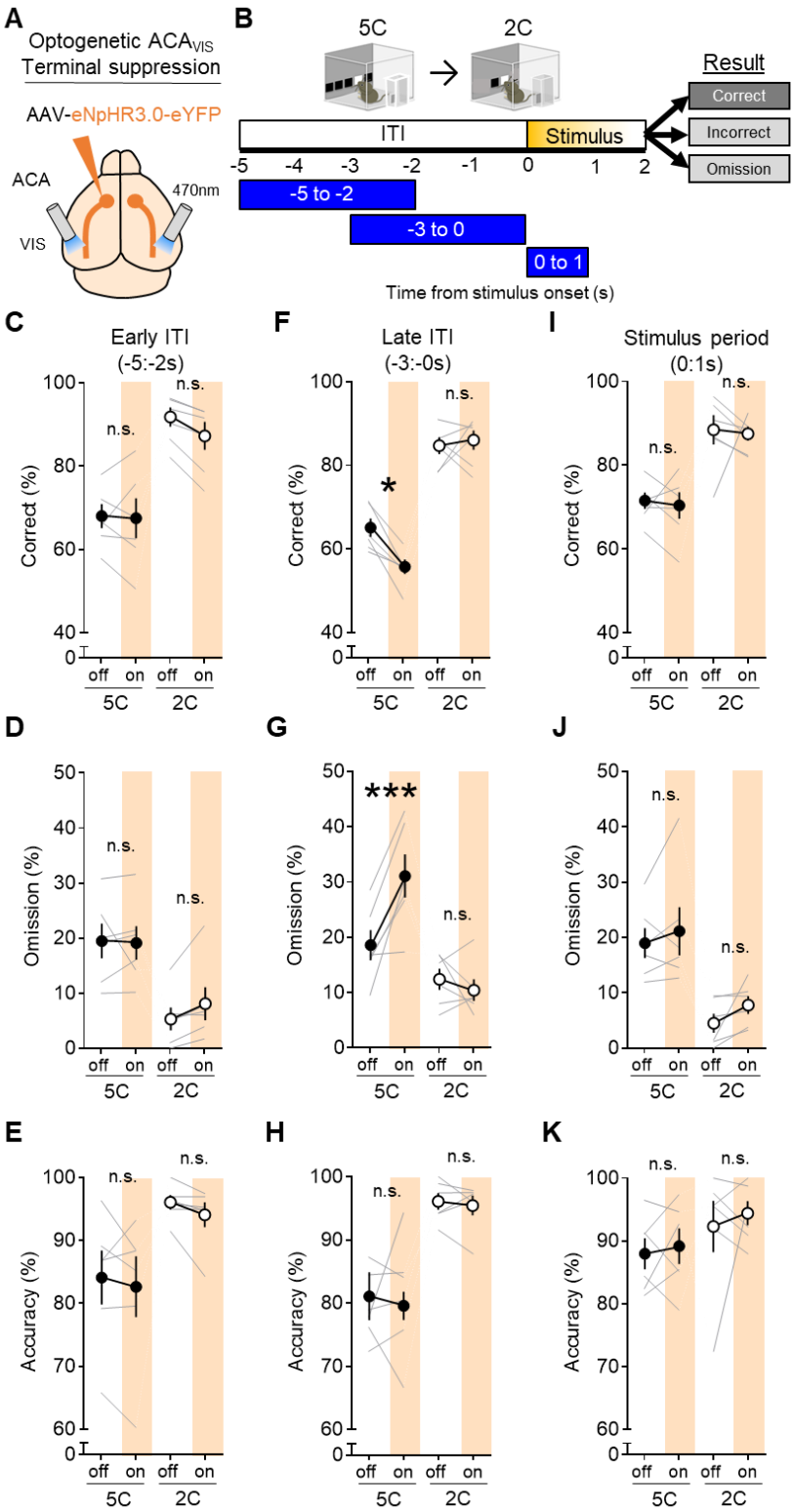


Figure 2. ACAVIS projections, while required for 5CSRTT, are dispensable for 2CSRTT. A) Viral strategy: To express light-gated chloride channel eNpHR3.0 in excitatory ACA neurons, eNpHR3.0

430 encoding AAV under the CaMKII promoter was injected in the ACA. An optic fiber was implanted at
431 the VIS to optically suppress ACA_{VIS} projection terminals with temporal precision during the
432 5CSRTT. **B)** Experimental timeline: Mice were first trained on the 5CSRTT, before viral injection.
433 After allowing three weeks for maximal viral expression, mice underwent 5CSRTT testing. During
434 5CSRTT testing, with a fixed 5s ITI and pseudorandomized stimulus duration (2.0 or 1.0s), 470nm
435 LED was illuminated during early ITI (-5:-2s), late ITI (-3:0s), or during period of stimulus
436 presentation (0:1s) during 50% of trials (6 mice; 2,694 total trials). One timing was tested per day.
437 The effect of optogenetic suppression during early ITI, late ITI, or stimulus period on behavioral
438 performance (correct %, omission %, and accuracy %) at each light timing tested. The same mice
439 were then baselined with 2CSRTT before undergoing the same optogenetic manipulation protocol
440 with the 2CSRTT testing (6 mice; 2,844 total trials). **C-E)** Continuous 470nm illumination during ITI
441 -5:-2s had no effect on correct trials (%, general linear mixed model (GLMM), effect of light: P =
442 0.1010, effect of task: $P < 0.0001$, interaction: $P = 0.1412$), omissions (GLMM, effect of light P =
443 0.2873, effect of task: $P < 0.0001$, interaction: $P = 0.2214$), or accuracy (GLMM, effect of treatment
444 (light on vs. off): $P = 0.1796$). **F-H)** Continuous 470nm illumination during ITI -3:0s had an effect on
445 correct trials (GLMM, effect of light: $P = 0.1274$, effect of task: $P < 0.0001$, interaction: $P = 0.0305$)
446 and omissions (GLMM, effect of light $P = 0.0470$, effect of task: $P < 0.0001$, interaction: $P = 0.0008$).
447 Light disrupted correct trials during the 5C task (*P = 0.0114), but not during the 2C task (P =
448 0.9784). Light disrupted omissions during the 5C task (***P = 0.0001), but not during the 2C task (P
449 = 0.8267). Continuous 470nm illumination during ITI -3:0s had no effect on accuracy (GLMM, effect
450 of light: $P = 0.6049$, effect of task: $P < 0.0001$, interaction: $P = 0.6068$). **I-K)** Continuous 470nm
451 illumination during the stimulus period had no effect on correct trials (GLMM, effect of light: P =
452 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 $P<0.001$.

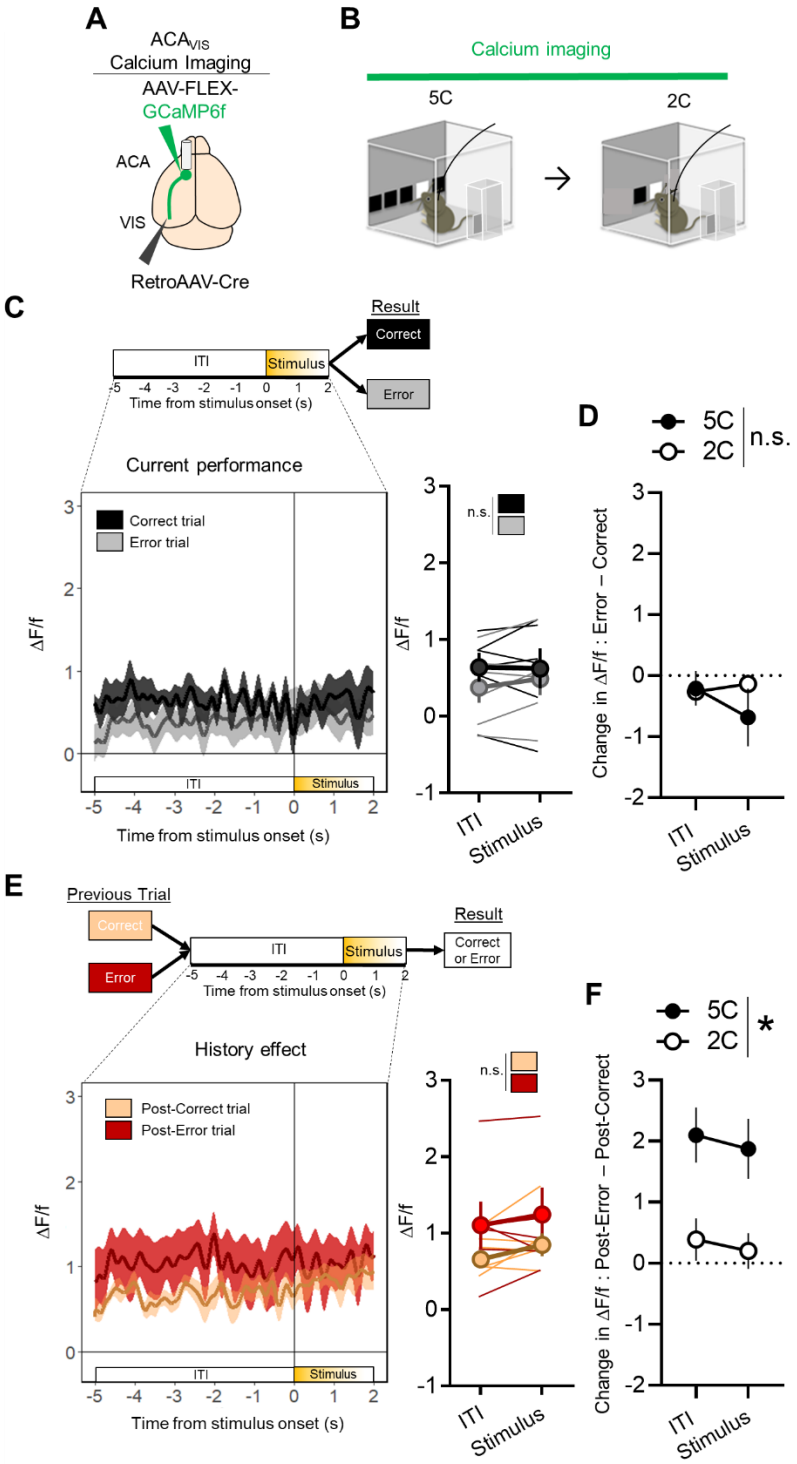


Figure 3. ACAVIS projection neurons, recruited after errors during 5CSRTT, are not recruited during 2CSRTT. A) Intersectional viral strategy: To selectively express GCaMP6f calcium indicator in ACAVIS neurons, Cre-dependent GCaMP6f and retrograde-Cre encoding AAVs were injected

463 bilaterally into the ACA and visual cortex (VIS), respectively. An optic fiber was implanted at the
464 ACA for calcium dependent recordings of ACA_{VIS} neurons using fiber photometry. **B)** Mice (n=6
465 mice) were first trained on the 5CSRTT, before viral injection. After allowing three weeks for maximal
466 viral expression, fiber photometry recordings of ACA_{VIS} neurons were conducted while mice
467 performed two days of 5CSRTT (6 mice; 753 total trials) testing with a fixed 5s intertrial interval (ITI)
468 and pseudorandomized stimulus duration (2.0, 1.5, 1.0, or 0.8s). After baselining with 2CSRTT,
469 fiber photometry recordings of ACA_{VIS} neurons during 2CSRTT was conducted (6 mice; 668 total
470 trials). **C)** During 2CSRTT testing, there was no difference in averaged ACA_{VIS} activity between
471 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
472 during the stimulus period (0:2s). **D)** There was no difference between the change in ACA_{VIS} activity
473 (error – correct) between mice performing the 5CSRTT and 2CSRTT (two-way RM ANOVA, Main
474 effect: $F_{1,10}$ =0.5005, P=0.4955, Interaction: $F_{1,10}$ =1.952, P=0.1926, n=6 mice)**. E)** During 2CSRTT
475 testing, there was no difference in averaged ACA_{VIS} activity between trials following correct and
476 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
477 stimulus period. **F)** ACA_{VIS} activity was higher (previous error – previous correct) during the 5CSRTT
478 compared to the 2CSRTT (two-way RM ANOVA, Main effect: $F_{1,10}$ = 9.227, *P=0.0125, Interaction:
479 $F_{1,10}$ = 0.02314, P=0.8821, n=6 mice; posthoc analyses,ITI: P=0.0141, stimulus period, P=0.0163).
480 Error bars indicate mean ± s.e.m. n.s. = non significant. * indicates P<0.05.
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Extended Data and Figure Legends

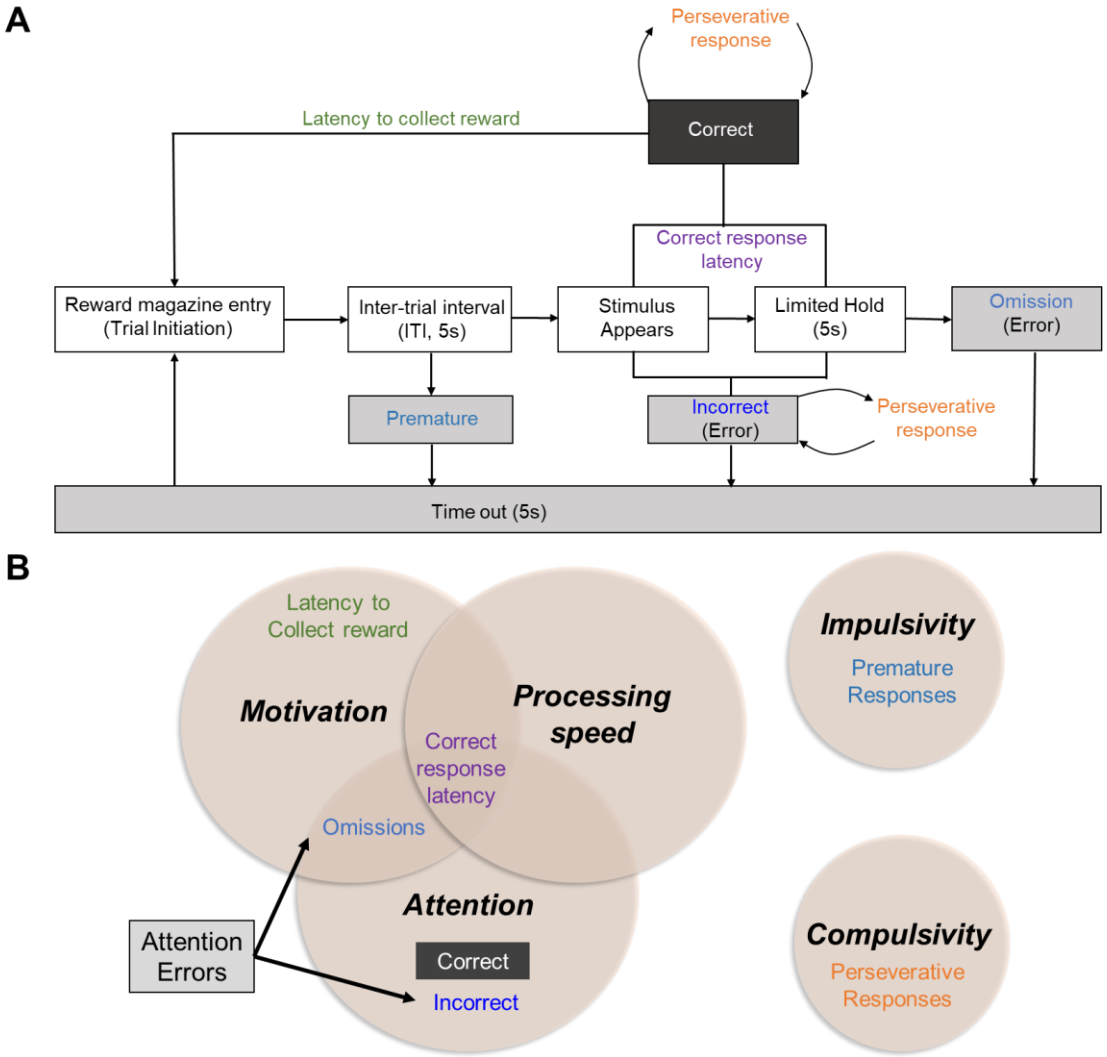

Figure 1-1. Overview of 5CSRTT and 2CSRTT protocol. A) Schematic of 5CSRTT and 2CSRTT testing. A trial begins when the reward magazine is entered. Upon exiting the reward magazine, a 5 second intertrial interval (ITI) begins. After the ITI, a white square stimulus will randomly appear at 1 of 5 touchscreens for either 2.0, 1.5, 1.0, or 0.8 s (2.0 or 1.0 s for optogenetic experiments). If one of the touchscreens are touched during the ITI, it's considered a premature response and a 5s timeout is initiated. If the correct touchscreen is touched when the stimulus is present or during the following 5s (limited hold) a milk reward is dispensed in the reward magazine. However, if the incorrect location is touched, a 5s timeout is initiated. If none of the locations are touched, a 5s timeout is initiated (omission). If the touchscreen is touched again after either a correct or incorrect response, it's considered a perseverative response. Correct response latency refers to the time from stimulus presentation to correct response. Latency to collect reward refers to the time from correct response to reward tray entrance. **B)** Overview of cognitive functions that contribute to various actions during 5CSRTT or 2CSRTT performance. While there is some overlap in measurements used to assess attention, each measure has a specific purpose and represents various levels and

specificity of attention capacity. Correct response rate is a broad and overall index of attention capacity which reflects what is captured by both accuracy and omission, two readouts which are used to assess attention level in previous studies. Placing ‘Omissions’ at the intersection of attention and motivation in the Venn diagram emphasizes that omission probability is sensitive to both. However, another readout, the latency to collect reward following a correct response, serves as an indicator of motivation to assess if omission change reflects attention or motivation level. Premature responses that occur prior to the stimulus appearing on the touchscreen are excluded from error trials in our analysis because premature trials are rather interpreted as errors of impulsivity as the mice are unable to wait or inhibit a response during the 5s ITI (Robbins, 2002).

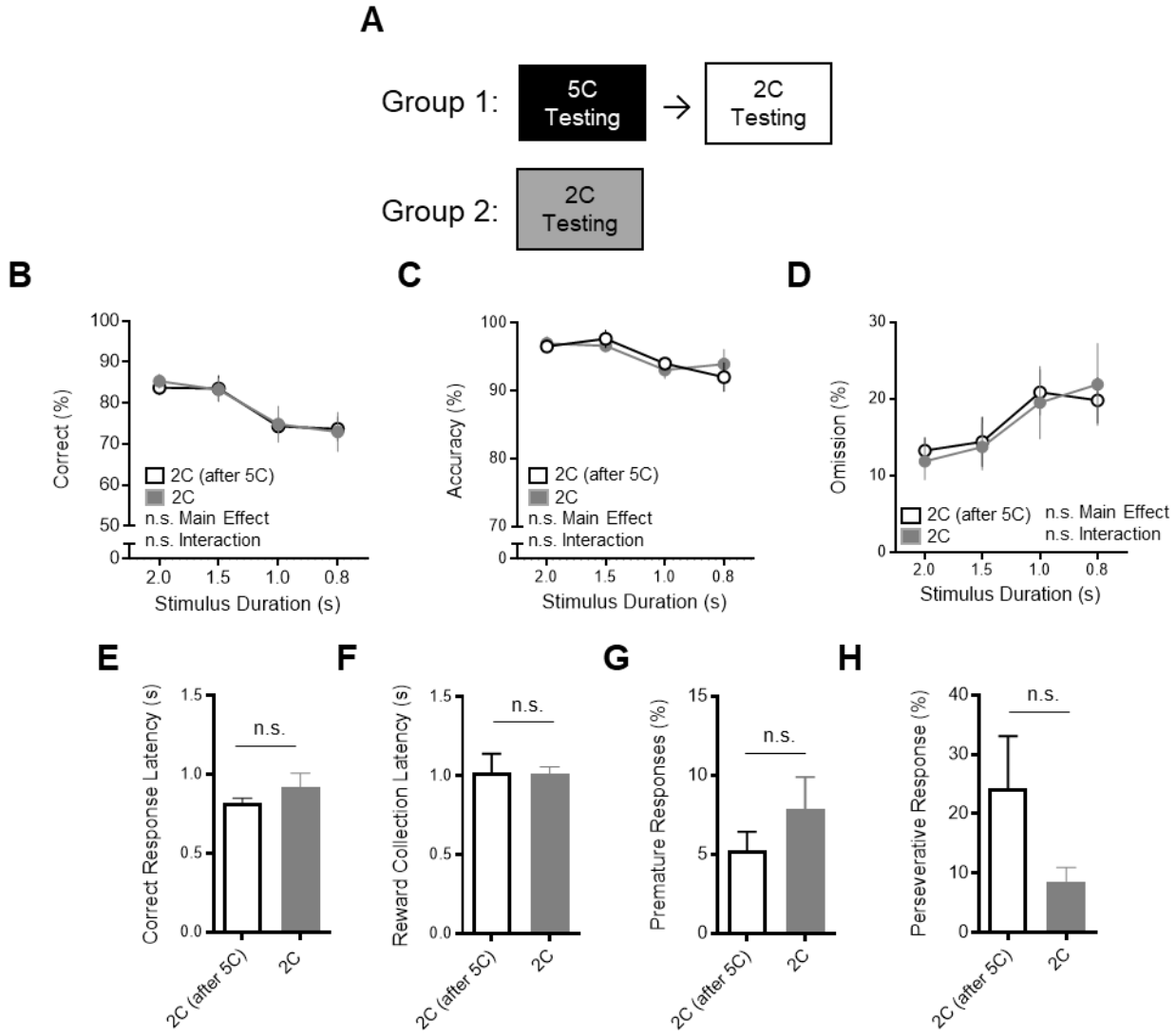

Figure 1-2. Prior 5CSRTT performance has no effect on 2CSRTT performance. A) The impact of prior 5CSRTT testing on 2CSRTT testing was examined. Group 1 trained and tested on the 5CSRTT and then next performed the 2CSRTT testing. Group 2 trained and tested the 2CSRTT, without prior 5CSRTT testing. **B)** There was no difference in correct % during the 2CSRTT between mice with prior 5CSRTT testing (n=8 mice) and mice without 5CSRTT testing (n=8 mice, two-way RM ANOVA, Main effect: $F_{1,14}$ =0.006283, *P*=0.9379, Interaction: $F_{3,42}$ =0.1039, *P*=0.9573). **C)** There was no difference in accuracy % during the 2CSRTT between mice with prior 5CSRTT testing and mice without 5CSRTT testing (two-way RM ANOVA, Main effect: $F_{1,14}$ =0.004251, *P*=0.9489, Interaction: $F_{3,42}$ =0.5455, *P*=0.6539). **D)** There was no difference in omission % during the 2CSRTT between mice with prior 5CSRTT testing and mice without 5CSRTT testing (two-way RM ANOVA, Main effect: $F_{1,14}$ =0.006206, *P*=0.9383, Interaction: $F_{3,42}$ =0.3126, *P*=0.8162). **E-H)** No difference in E) correct response latency (t_{14}=1.049, *P*=0.3119), **F**) reward collection latency (t_{14}=0.08138, *P*=0.9363), **G**) premature responses (t_{14}=1.097, *P*=0.2913), and **H)** perseverative responses (t_{14}=1.701, *P*=0.1111) was observed between the two groups. Error bars indicate mean ± s.e.m. n.s. = non significant.

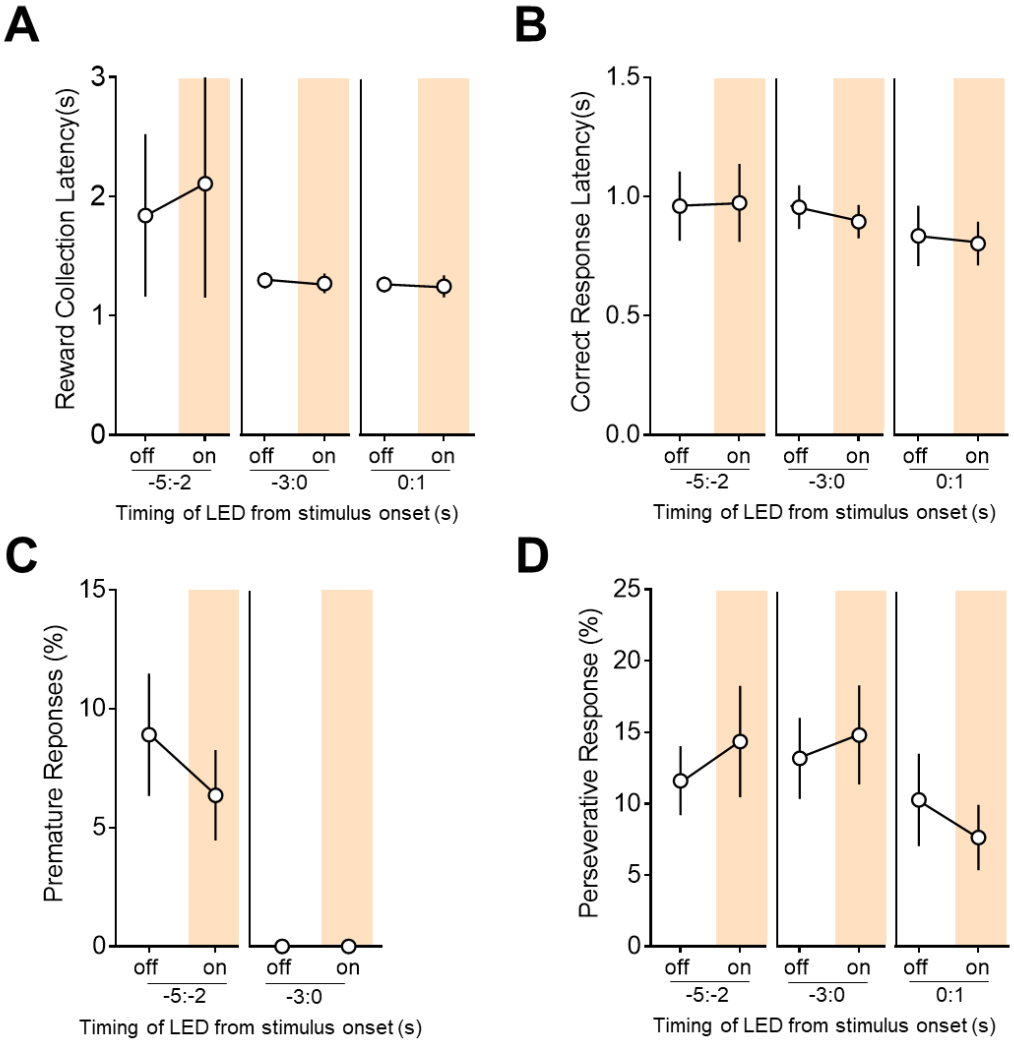

Figure 2-1. Optogenetic suppression of ACA$_{VIS}$ projections during 2CSRTT. **A)** Reward collection latency was not affected by ACA$_{VIS}$ suppression during ITI -5:-2s (t_5=0.9526, P=0.3846, n=6 mice), ITI -3:0s (t_5=0.2985, P=0.7773, n=6 mice) or stimulus period (t_5=0.1226, P=0.9072, n=6 mice) compared to light off trials. **B)** Correct response latency was not affected by ACA$_{VIS}$ suppression during ITI -5:-2s (t_5=0.4064, P=0.7012, n=6 mice), ITI -3:0s (t_5=1.280, P=0.2568, n=6 mice) or during stimulus period (t_5=0.3198, P=0.7620, n=6 mice) compared to light off trials. **C)** Premature responses were not affected by ACA$_{VIS}$ suppression during ITI -5:-2s (t_5=2.014, P=0.1002, n=6 mice) or ITI -3:0s compared to light off trials. The effect of suppression during stimulus period on premature responses was not analyzed, because premature responses occur before the stimulus appears. **D)** Perseverative responses were not affected by ACA$_{VIS}$ suppression during -5:-2s (t_5=0.6252, P=0.5593, n=6 mice), ITI -3:0s (t_5=0.6316, P=0.5554, n=6 mice) or stimulus period (t_5=1.224, P=0.2756, n=6 mice) compared to light off trials.

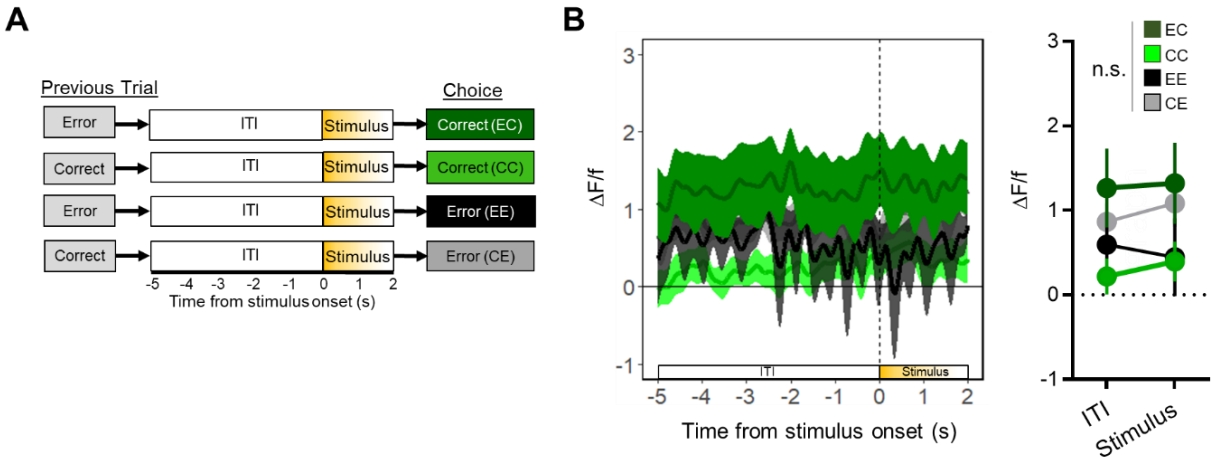

Figure 3-1. Fiber photometry imaging of ACA$_{VIS}$ activity during 2CSRTT based on trial type. A) Four possible trial types based on previous and current trial result. **B)** There was no significant difference between trial types (two-way RM ANOVA, $F_{2.038,10.19}$=1.611, **P=0.2467, n=6 mice). Error bars indicate mean ± s.e.m., n.s. = non significant.

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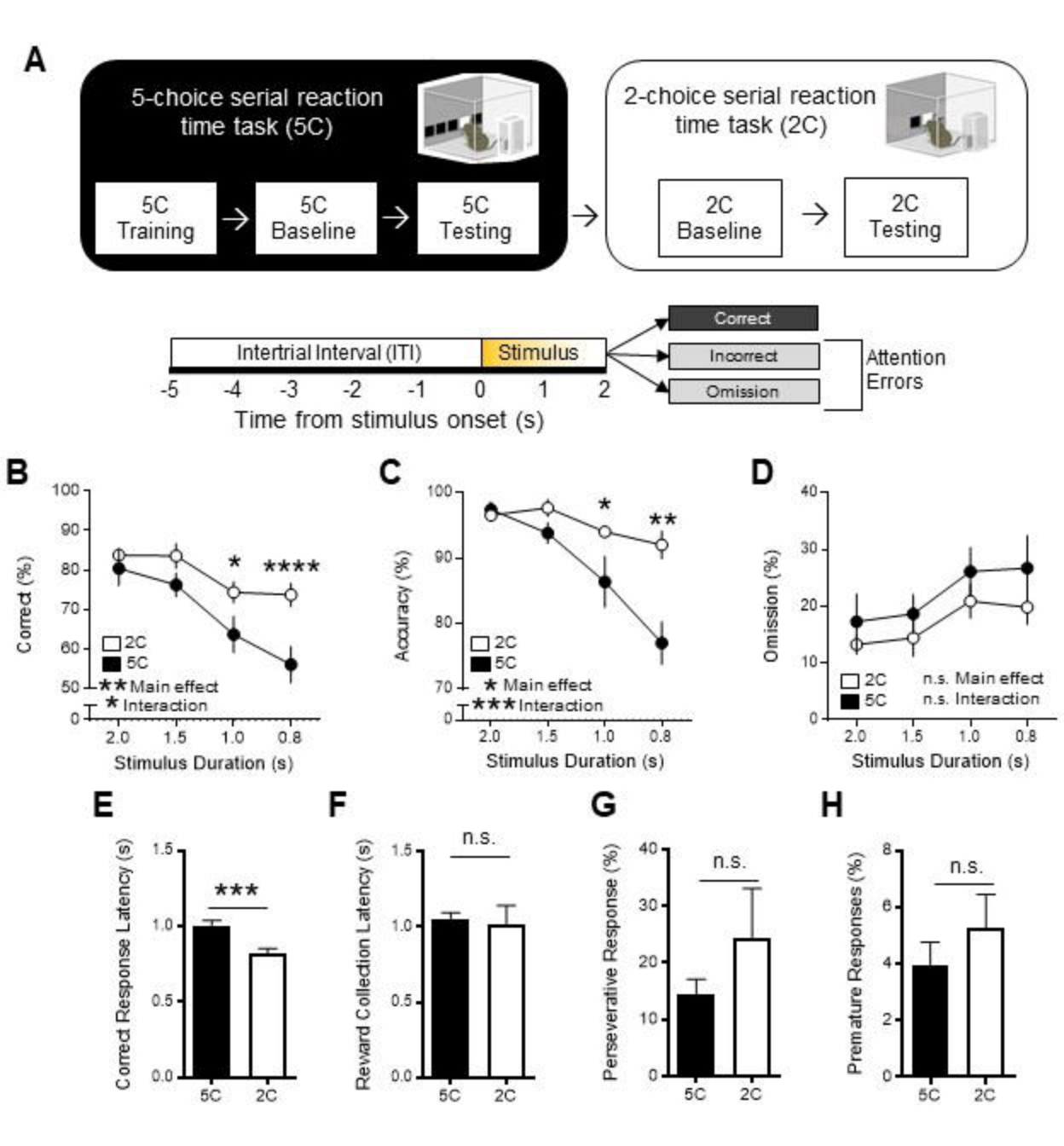
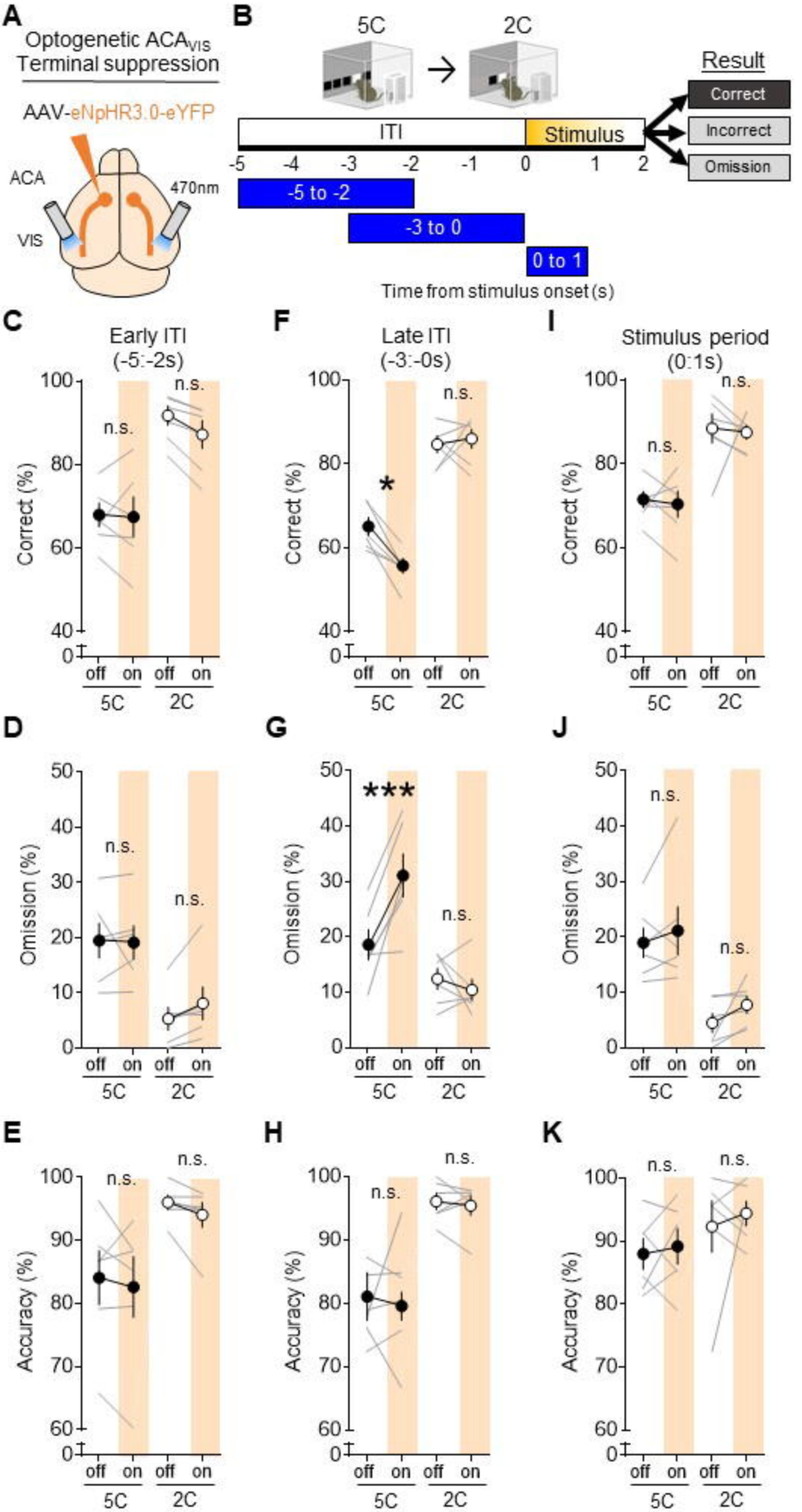

A
5-choice serial reaction time task (5C)
5C Training
5C Baseline
5C Testing
2-choice serial reaction time task (2C)
2C Baseline
2C Testing
Intertrial Interval (ITI)
Stimulus
-5 -4 -3 -2 -1 0 1 2
Time from stimulus onset (s)
Correct
Incorrect
Omission
Attention Errors
B
Correct (%)
2C
5C
** Main effect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


A
Optogenetic ACA$_{VIS}$ Terminal suppression
AAV-eNpHR3.0-eYFP
ACA
470nm
VIS
B
5C
2C
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)
I
Stimulus period (0:1s)
Correct (%)
n.s.
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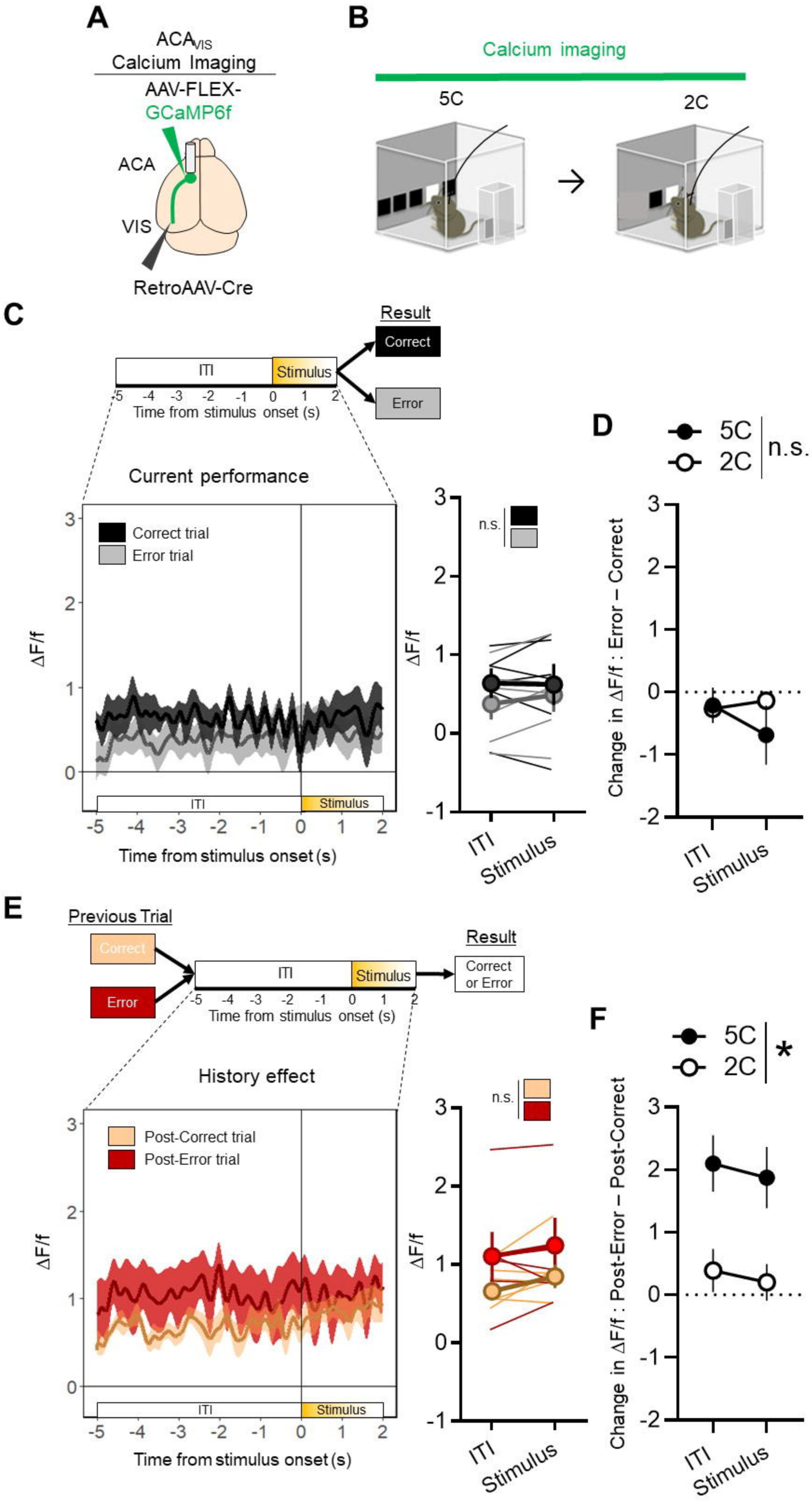
off on off on
5C 2C
D
G
J
Omission (%)
***
E
H
K
Accuracy (%)


A
ACA$_{VIS}$
Calcium Imaging
AAV-FLEX-
GCaMP6f
ACA
VIS
RetroAAV-Cre
B
Calcium imaging
5C
2C
C
Result
Correct
Error
ITI
Stimulus
Time from stimulus onset (s)
Current performance
Correct trial
Error trial
ΔF/f
Time from stimulus onset (s)
n.s.
ITI
Stimulus
D
5C
2C
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
*
Change in ΔF/f : Post-Error – Post-Correct