Sex-Specific Effects of Psychedelic Drug Exposure on Central Amygdala Reactivity and Behavioral Responding

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ABSTRACT

Psilocybin, and its active metabolite psilocin, have been shown to elicit rapid and long-lasting symptom improvements in a variety of affective psychiatric illnesses. However, the region-specific alterations underlying these therapeutic effects remain relatively unknown. The central amygdala (CeA) is a primary output region within the extended amygdala that is dysregulated in affective psychiatric disorders. Here, we measured CeA activity using the activity marker *c-Fos* and CeA reactivity using fiber photometry paired with an aversive air-puff stimulus. We found that psilocin administration acutely increased CeA activity in both males and females and increased stimulus-specific CeA reactivity in females, but not males. In contrast, psilocin produced time-dependent decreases in reactivity in males, but not females as early as 2-days and lasting to 28-days post administration. We also measured behavioral responses to the air-puff stimulus and found sex-dependent changes in threat responding but not exploratory behavior or general locomotion. Repeated presentations of the auditory component of the air-puff were also performed and sex-specific effects of psilocin on CeA reactivity to the auditory-alone stimulus were also observed. This study provides new evidence that a single dose of psilocin produces sex-specific, time-dependent, and enduring changes in CeA reactivity and behavioral responding to specific components of an aversive stimulus.

INTRODUCTION

Clinical studies suggest that psilocybin, and active metabolite psilocin [1-3], produce rapid, long-lasting improvements in psychiatric disorders including depression, anxiety, and substance use disorder [4-12]. Sustained improvements in symptoms were seen as long as 4 years following administration [13]. Research on psychedelic compounds has focused primarily on human imaging studies and receptor pharmacology. Human fMRI studies have shown that psychedelics produce robust alterations in network connectivity [8, 14-19]. Receptor studies have demonstrated that these compounds promote synaptogenesis [20-22] and identified the serotonin 5-hydroxytryptamine $(5\text{-HT})_{2A}$ receptor to be instrumental in mediating the hallucinogenic actions of psychedelic compounds including psilocybin and lysergic acid diethylamide (LSD) [16, 23-28]. Despite the observed clinical outcomes in psychedelic studies, the underlying neural correlates of these therapeutic effects remain relatively unknown.

The central amygdala (CeA) is a primary output region within the extended amygdala that receives input regarding internal and external arousal state and coordinates appropriate behavioral responses [29, 30]. CeA dysregulation has been implicated in anxiety, depression, and PTSD [29, 31-36]. Preclinical studies demonstrate that the psychedelic compounds LSD and 2,5-dimethoxy-4-iodoamphetamine (DOI) increase Fos expression in the amygdala [37, 38] and that systemic and local DOI administration in the amygdala promotes suppression of fear responses [38]. Psilocybin produces alterations in reactivity and connectivity of the amygdala that correlate with positive therapeutic outcome [12, 15, 17, 39-41]. One clinical imaging study reported significantly decreased amygdala reactivity 1-week following psilocybin administration, with lasting changes in affect at one-month follow up [41].

The current study utilized a preclinical approach to determine how the active compound, psilocin, alters CeA activity, reactivity, and behavioral responding to an aversive stimulus in male and female Sprague Dawley rats. Psilocin was used as it is the active metabolite of psilocybin in the central nervous system (CNS), mediating effects of psilocybin at 5-HT_{2AR} [2, 28]. We focused on the CeA as the primary output region of the amygdala and as amygdala hyperreactivity is associated with many psychiatric disorders that psilocybin shows promise

in treating [42-45]. We first measured expression of *c-Fos*, an immediate early gene that is upregulated following neuronal activity, in the CeA of male and female rats administered psilocin (2 mg/kg) or vehicle. Next, we measured CeA reactivity using an air-puff stimulus involving a burst of air directed towards the face. The air-puff stimulus has been validated as an aversive stimulus in passive avoidance tests [46] and, due to known CeA involvement in pain modulation [47, 48], it was essential to utilize a stimulus that would evoke a response without the addition of potential confounding effects of pain. Utilizing fiber photometry in conjunction with the aversive air-puff stimulus, we were able to demonstrate a robust and reliable CeA reactivity response. We then used this model to determine how administration of psilocin altered CeA reactivity during acute exposure and at more prolonged timepoints. We observed sex-dependent effects of psilocin on CeA activity as measured by *c-Fos* expression and in-vivo reactivity and behavioral responding. Additionally, we found that psilocin elicited long-term decreases in CeA reactivity in males that were not seen in females or in the vehicle control. To test whether these effects would contribute to the development of a conditioned response to the sound of the air-puff, additional assays were performed utilizing an auditory-only air-puff stimulus, wherein an air-puff was administered with the nozzle facing outside of the box to isolate the auditory component of the air-puff. In doing so, we show differential reactivity of the CeA suggesting that the observed changes were stimulus-specific and dynamic in nature.

METHODS

Animals/Stereotaxic Surgery

Adult male and female Sprague Dawley rats (200-400 g, 6-weeks old at arrival, Envigo, Indianapolis, IN) were group-housed in a humidity- and temperature-controlled (22°C) vivarium on a 12hr light/dark cycle with *ad libitum* access to food and water. Following intracranial surgery, animals were single-housed in flat lid cages with waterspout access, and food bowls to avoid damage to implants. Bilateral infusions of pGP-AAV-syn-jGCaMP7f-WPRE (Addgene plasmid# 104488-AAV9) were performed at a rate of 100nl/minute into the CeA (AP:-2.0mm; ML:+/- 3.9mm; DV:-8.0mm). Directly following injection, Doric 200um NA 0.37 silica optic fiber

cannulas with a 9mm tip were inserted at DV -7.7mm and secured in place using Metabond (Parkell, Brentwood, NY).

Drug Administration

On the day of treatment, animals received a subcutaneous (s.c.) injection of either vehicle (0.9% Saline/2% glacial acetic acid) or a 2 mg/kg psilocin (Cayman Chemical, graciously provided by Dr. Bryan Roth) dissolved in 2% glacial acetic acid, as previously described [62]. Solutions were prepared fresh on the day of testing and were stored at 4ºC for 30min prior to first injection. Injections were given at a 1mg/ml volume 30min prior to the beginning of fiber photometry recordings. In the *c-Fos* cohort, injections were performed 2hr prior to perfusion.

Fiber Photometry Recording

Animals were habituated to testing room for 1 hour prior to recording sessions. Rats were individually placed in a clear plexiglass chamber [50cm (l)×50cm(w)×38cm(h)] in a red-light sound-attenuated behavioral cabinet and allowed to acclimate for 10min. Fiber placement and GCaMP injection were performed bilaterally, however, recordings were collected unilaterally. Baseline recordings were taken from both hemispheres and analyzed to determine which hemisphere to use for the remainder of the experiment. Ca^{2+} signals from subjects, with 200µm optic fibers placed .3mm above the injection site in the CeA, were recorded using a TDT RZ5 real time processor equipped with the TDT Synapse program (Tucker-Davis Technologies, Alachua, FL). For each recording, jGCaMP7f was excited using both a 465nm calcium-dependent signal and a 405nm isosbestic control. MATLAB script [63] was then used to analyze raw signal. Changes in fluorescence as a function of baseline (ΔF/F) showing fluctuations in fluorescence compared to the overall baseline fluorescence throughout the session were calculated through smoothing isosbestic control channel signal, scaling the isosbestic signal by regressing it onto smoothed GCaMP signal, and then generating a predicted 405nm from the regression to remove calcium-independent signaling from the raw GCaMP signal and control for artifact from movement, photo-bleaching, and fiber bending artifacts. Additionally baseline fluorescence was calculated through a least-squares linear fitting of the 405nm isosbestic control to the 465nm GCaMP signal to

create a resting GCaMP signal over the entire session not containing event-dependent fluctuations in GCaMP signal [64]. Peri-event plots were then generated by averaging changes in fluorescence (ΔF/F) across two different air-puff trials to generate a mean change in reactivity [63]. Area under curve (AUC) and peak point (PP) were used to capture characteristics of amplitude and duration of CeA response. All subjects were video recorded during stimulus presentations and behavior was scored using the Behavioral Observation Research Interactive Software (BORIS) program [65]. Behavioral analysis was performed by an experimenter blind to the experimental status of each animal.

Air-Puff Stimulus

An aversive air-puff stimulus was designed using house air supply (85psi) controlled by a Parker solenoid (Part #003-0868-900), powered by a custom made 12V electrical circuit box connected to the TDT RZ5 system to trigger 500ms openings of the solenoid and simultaneous timestamps through the Synapse program. Each session consisted of a 10min habituation period before recording sessions that consisted of two air-puff administrations with a 5min inter-stimulus interval (ISI) between them. Air-puffs were directed at the face and within close proximity of the animal to maintain similar conditions and level of aversion to the stimulus between subjects.

Perfusion/Tissue Collection

Rats were transcardially perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (w/*v*). Brains were extracted, stored in 4% paraformaldehyde for 24h, then transferred to 30% sucrose (*w/v*) and stored at 4ºC until sectioning. Using a freezing microtome, tissue was sliced into 40µm thick sections and stored in cryoprotectant (30% *v/v* ethylene glycol + 30% *w/v* sucrose in phosphate buffered saline) at 4ºC until use.

Immunofluorescence Staining

For site verification on the fiber photometry cohorts, 6 sections/subject anterior-posterior (AP) coordinates -1.75 to -2.75 from bregma were washed in phosphate buffered saline (PBS) prior to 1hr blocking in 5% normal donkey serum + 0.3% Triton X in PBS. Sections were then incubated in chicken anti-GFP (Abcam 13970; 1:1000) in 0.5% normal donkey serum/0.3% Triton X/PBS overnight at 4°C. Next, samples were triple-washed and incubated in blocking solution (1hr) followed by goat anti-chicken 488 (Jackson 703-545-155; 1:700) in 0.5% normal donkey serum/0.3% Triton X/PBS for 2hr at room temperature.

For the *c-Fos* cohort, sections AP coordinates -1.92mm to -2.16mm were washed in PBS, incubated in 50% methanol/PBS solution for 30min, washed in 3% hydrogen peroxide/PBS for 5min, and placed in blocking solution (0.3% Triton X-100; Thermo Fisher), 1% bovine serum albumin (BSA; Sigma) for 1hr, at room temperature (RT). Slices were then incubated with rabbit anti-*cFos* (1:3000, Millipore Sigma; ABE457) for 24hr at 4°C. Next, slices were washed with 0.1% Tween-20 in tris-buffered saline (TNT) before being transferred into TNB blocking buffer (Perkin-Elmer FP1012) for 30min. After blocking, slices were incubated in goat anti-rabbit horseradish peroxidase (HRP; 1:200, Abcam ab6721) for 2hr followed by another round of TNT washes. Finally, slices were incubated in tyramide conjugated fluorescein (1:50) in TSA amplification diluent (Akoya Biosciences, NEL741001KT) for 10min at RT. Slices were washed with TNT buffer, mounted, cover slipped with Vectashield ® HardSet™ Antifade Mounting Medium with DAPI (H1500, Vector Laboratories, Burlingame, CA) and stored at 4ºC before being imaged with the Keyence BZ-X800 fluorescence microscope. Images were then analyzed by an experimenter blinded to the animal's condition. Images were taken at 20X and the BZ-X800 Analyzer program was used to stitch images together. Randomly selected hemispheres were used, and the area of interest was outlined and manually counted using the ImageJ multi-point counter tool. Cell counts were averaged per animal.

Statistical Analysis

Raw data from the TDT RZ5 machine were imported and processed for artifact removal, down sampling, and detrending using MATLAB code [63]. Changes in fluorescence (ΔF/F) were then calculated and collected 5s

before and 10s following air-puff administration. The mean of two traces were averaged together for each subject and combined using custom MATLAB script (https://doi.org/10.5061/dryad.pnvx0k6q9) to sort individual data into groups. Group 465nm signal data were then converted into 500ms bins for analysis to quantify differences between experimental conditions. Area under the curve (AUC) analysis was calculated by summing all raw ΔF/F values following the air puff administration. This would correspond to the timepoints 0-20 on the binned analysis plots. This calculation was captured for each animals mean trace within each condition and then collapsed across subjects to determine group means in AUC following air puff. All statistical analyses were conducted using GraphPad Prism 7. Five subjects were removed from the study due to lack of signal/cannula removal. Repeated-measures one-way analysis of variance (ANOVA) with Dunnett's post hoc test was used to determine differences between conditions within each groups in the air-puff condition. Two-way ANOVAs with Šidák's post hoc tests were used to compare area under the curve, peak point, and time bins between groups and time points. Paired t-tests were used to determine differences within groups between time points in the binned trace and auditory-only analyses. Fisher's exact tests were used to compare percentages in darting behavior. Mixed-effects two-way ANOVA were used to compare time spent walking and rearing. All data including Matlab structures and scripts can be found at https://doi.org/10.5061/dryad.pnvx0k6q9.

RESULTS

Acute Changes in CeA Reactivity in Response to an Aversive Air-Puff Stimulus

Male and female Sprague Dawley rats received bilateral injection of pGP-AAV-syn-jGCaMP7f-WPRE into the CeA immediately followed by installation of fiber optic cannulae above the injection site (**Fig. 1A-B**). Following 3-weeks for virus incubation, animals underwent a series of fiber photometry experiments testing the effects of psilocin on fluctuations in calcium dynamics within the CeA time-locked to an aversive air-puff stimulus (**Fig. 1A**). For both baseline and treatment recording sessions, each animal received two air-puffs with an ISI of 5min and trace plots were generated showing change in fluorescence as a function of baseline (ΔF/F). Traces were analyzed 5s before and 10s following administration of air-puff (**Fig. 1C**).

Sub-Region-Specific Differences in CeA Basal Activity Following Psilocin Administration

To assess the effects of psilocin on basal activity in the CeA, male and female Sprague Dawley rats were injected with psilocin (2 mg/kg, s.c.) or vehicle (1 ml/kg, s.c.) 2hr prior to transcardial perfusion. Immunohistochemistry (IHC) assays were performed using CeA tissue collected within the anterior-posterior bregma range of -1.92mm to -2.16mm. In females, there was a significantly increased *c-Fos* expression in the psilocin group as compared to vehicle across regions ($F_{Interaction}(2,24)=1.597$, $p=0.22$; $F_{Region}(1,24)=30.84$, $p<0.0001$; $F_{Treatment}(1,24)=9.149$ $p=0.006$; **Fig.2A-B**) with greater increases in the capsular division of the central amygdala (CeC) (Šidák's: $p=0.02$). In males, there was also significantly increased *c-Fos* expression in the psilocin group across regions ($F_{Interaction}(2,27)=1.241$, $p=0.30$; $F_{Region}(2,27)=12.02$, $p=0.0002$; $F_{Treatment}(1,27)=5.543$, $p=0.02$; **Fig.2C-D**).

Acute Changes in CeA Reactivity in Response to an Aversive Air-Puff Stimulus Following Psilocin Administration

To test the acute effects of psilocin on CeA reactivity, we measured changes in CeA reactivity during time-locked stimuli presentation at baseline and 30min post administration of vehicle or psilocin (2 mg/kg, s.c.). Following a 10min habituation period, air-puffs were delivered as described above. Mean traces were taken from two separate air-puff responses within a session (**Fig. 2E, 2I**). These traces were then collapsed into 500ms bins for statistical analysis (**Fig. 2G, 2H, 2K, 2L**). To compare baseline to treatment, paired t-tests were performed comparing the individual time bins following the air-puff collapsed across subjects (Fig. **2F**, **2J**). In the female vehicle control group (n=9), there was a significant increase between baseline and treatment ($t(19)=2.780$, $p=0.01$; **Fig.2F-G**). In the female psilocin group (n=7), there was a significant increase between baseline and treatment ($t(19)=12.63$, $p<0.0001$; **Fig.2F, 2H**). Though changes were seen in both vehicle and psilocin, the increase in mean of differences in reactivity was greater in the psilocin group compared to the vehicle control (**Fig.2F**). In the male vehicle control group (n=10), there was an increase in reactivity between baseline and treatment, though the effect was small ($t(19)=4.452$, $p=0.0003$; **Fig.2J-K**). In the male psilocin

group (n=11), there was no significant difference between baseline and treatment (t(19)=0.6231, *p*=0.54; **Fig.2J, 2L**).

Prolonged Changes in CeA Reactivity in Response to an Aversive Air-Puff Stimulus Following Psilocin Administration

To assess long-term alterations in CeA reactivity following a single dose of psilocin, fiber photometry recordings were performed on days 2, 6, and 28 post-administration of vehicle or psilocin. On each follow up recording day, animals received two air-puffs with a 5-minute ISI. In females, there was no difference between vehicle and psilocin groups in the area under curve (AUC) of the group-averaged individual mean traces between baseline and the 28-day follow ($F_{Interaction}(1,14)=0.003383$, $p=0.95$; $F_{Treatment}(1,14)=0.09635$, $p=0.76$; $F_{Time}(1,14)=0.2126$, $p=0.65$; **Fig.3A-B**). Additionally, no differences were found between vehicle and psilocin groups at any of the follow-up timepoints ($F_{Interaction}(2,28)=1.437$, $p=0.25$; $F_{Treatment}(1,14)=0.0003490$, $p=0.99$; $F_{Time}(2,28)=3.067$, $p=0.06$; **Fig.3B**). However, in the vehicle control females, 1-way ANOVA found an effect of treatment (F(3,57)=50.02, $p<0.0001$; **Fig.3C**) and a significant increase in reactivity at the 2-day and 6-day follow up (Dunnett's: $p<0.0001$). In the female psilocin group (**Fig. 3A, 3D**), 1-way ANOVA found an effect of treatment (F(3,57)=54.13, $p<0.0001$; **Fig.3D**) with significant increases in reactivity at the 2-day (Dunnett's: $p<0.0001$) and 6-day follow up (Dunnett's: $p<0.0001$).

In the males, there was decreased reactivity between the vehicle and psilocin group from baseline to the 28-day follow up ($F_{Interaction}(1,19)=5.068$, $p=0.03$; $F_{Treatment}(1,19)=0.2503$, $p=0.62$; $F_{Time}(1,19)=1.361$, $p=0.26$; **Fig.3F**) with a significant reduction in reactivity at the 28-day follow up in the psilocin group (Šidák's: $p=0.04$). There were also reductions in the AUC at the follow up recordings in the psilocin group compared to the vehicle control ($F_{Interaction}(2,38)=0.2113$, $p=0.81$; $F_{Treatment}(1,19)=4.926$, $p=0.03$; $F_{Time}(2,38)=0.7906$, $p=0.46$; **Fig.3E-F**). In the male vehicle group, 1-way ANOVA found an effect of treatment (F(3,57)=5.554, $p=0.002$; **Fig.3G**) with a significant increase in reactivity at the 28-day follow up (Dunnett's: $p=0.01$). In the male psilocin group, 1-way ANOVA found a significant effect of treatment (F(3,57)=72.60, $p<0.0001$; **Fig.3H**) revealing significant decreases in reactivity at the 2-day, 6-day, and 28-day follow up (Dunnett's: $p<0.0001$). A 2-way ANOVA found

differential effects of treatment across time points between groups in females ($F_{Interaction}(4,152)=35.02$, $p<0.0001$; $F_{Treatment}(1,38)=0.9089$, $p=35$; $F_{Time}(2.343,89.04)=68.58$, $p<0.0001$; **Fig.3J**) and males ($F_{Interaction}(4,152)=38.35$, $p<0.0001$; $F_{Treatment}(1,38)=1.059$, $p=0.31$, $F_{Time}(2.603,98.90)=42.25$, $p<0.0001$; **Fig.3I**) There were increases in reactivity during treatment and reductions at the 2-day follow up in the female psilocin group compared to vehicle control (Šidák: $p=0.001$; **Fig.3J**). In males, there was a significant decrease in reactivity at the 28-day follow up in the psilocin group compared to vehicle control (Šidák: $p=0.01$; **Fig.3J**)

Acute and Prolonged Behavioral Effects of Psilocin

To determine if psilocin elicited changes in locomotion and exploratory behavior, video recordings were scored for total time spent walking and time spent rearing, respectively. In females, there were no differences in overall locomotion between vehicle and psilocin groups ($F_{Interaction}(3,42)=0.2118$, $p=0.89$; $F_{Treatment}(1,14)=2.238$, $p=0.16$; $F_{Time}(2.497,34.95)=0.6065$, $p=0.59$; **Fig.4A**). There were also no differences in rearing between female vehicle and psilocin groups ($F_{Interaction}(4,56)=1.345$, $p=0.26$; $F_{Treatment}(1,14)=4.433$, $p=0.05$; $F_{Time}(4,56)=2.486$, $p=0.05$; **Fig. 4B**). In the males, there were no differences in locomotion ($F_{Interaction}(3,57)=0.1054$, $p=0.96$; $F_{Treatment}(1,19)=2.563$, $p=0.12$; $F_{Time}(3,57)=1.255$, $p=0.29$; **Fig.4C**) or rearing ($F_{Interaction}(4,76)=0.5546$, $p=0.69$; $F_{Treatment}(1, 19)=0.7576$, $p=0.39$; $F_{Time}(4,76)=1.190$, $p=0.32$; **Fig.4D**) between vehicle and psilocin groups. Collectively, no significant alterations in locomotion were seen resulting from psilocin administration (**Fig.4A, 4C**), although females appeared to display increased locomotion overall as compared to males, regardless of treatment.

To probe potential differences in threat responding to the air-puff stimulus, we separated groups based on active or passive coping strategy. Subgroups were created containing animals that remained immobile (passive) following air-puff administration versus animals that exhibited darting behavior (active) following air-puff administration. Consistent with previous literature [49], females primarily employed an active darting response (**Fig.4E**), while active vs passive coping behavior was more evenly split in males (**Fig.4F**). In females, there were no differences in the percentage of animals that darted or that stayed immobile between groups at any of the time points (**Fig. 4E**). In contrast, the male psilocin group displayed nonsignificant

increases in darting and reductions in immobility in the vehicle control group that were not seen in the psilocin group under treatment conditions (*p*=0.08; **Fig. 4F**). At the 2-day follow up, there was a significantly higher percentage of darting in the vehicle control group compared to the psilocin group (*p*=0.03; **Fig.4F, left**) and a significantly higher percentage immobile in the psilocin group (*p*=0.03; **Fig.4F, right**). Though differences were not significant at every time point, psilocin seemed to prevent a trending increase in darting behavior seen in the male vehicle control group and increase in overall immobility in male but not female rats (**Fig.4F**).

To explore the CeA reactivity correlates to the observed behavioral differences in threat responding, fiber photometry traces were split into the corresponding active vs. passive responder groups. Since females primarily employed active coping strategies that were not affected by psilocin, we only examined CeA reactivity between the male active and passive responders. To assess changes in the amplitude of CeA response to the stimulus, comparisons looking at the peak point, or highest ΔF/F value following air-puff, were made. In the male passive responder, there were no significant differences in peak point in the vehicle ($F(4,20)=1.219$, $p=0.33$; **Fig.4G**) or psilocin group ($F(4,20)=1.276$, $p=0.31$; **Fig.4H**). In the active responder vehicle males, no differences were seen in peak point compared to baseline ($F(4,12)=1.046$, $p=0.42$; **Fig.4I**). However, in the active responder psilocin males, there was a main effect of treatment ($F(4,16)=6.566$, $p=0.003$; **Fig.4J**) with a significant decrease in peak point at day 2 compared to baseline ($p=0.003$), suggesting that psilocin-induced decreases in the amplitude of CeA reactivity were more pronounced in males employing an active vs. passive threat response at baseline.

Changes in CeA Reactivity in Response to an Auditory Stimulus Following Psilocin Administration

To test whether the animals developed a conditioned response to the tone associated with the air-puff stimulus, and whether psilocin treatment altered the response to the auditory stimulus alone, two separate auditory-only sessions were conducted immediately following baseline recordings and again at the 28-day recording session. These auditory only sessions followed the same parameters as the air-puff with the exceptions being that the ISI was reduced to 3min and the air-puff apparatus was facing outside of the box to maintain the same auditory tone without the physical sensation of the air-puff.

In vehicle control females (**Fig.5A**), there was a significant decrease in peak point reactivity from the initial to the final auditory condition (t(8)=3.934, *p*=0.004; **Fig.5B**) and a significant decrease in binned analysis from the initial auditory to the final auditory condition (t(19)=18.28, *p*<0.0001; **Fig.5C-D**). In psilocin females (**Fig.5E**), there were no significant differences between the initial and final auditory session in either peak point (t(6)=0.4346, *p*=0.67; **Fig.5F)** or binned analysis (t(19)=0.01624, *p*=0.98; **Fig.5G-H**).

In the vehicle males (**Fig.6A**), there were no significant differences in peak point between initial and final auditory condition (t(9)=2.136, *p*=0.06; **Fig. 6B**). However, there was a significant decrease in binned analysis of the final auditory session compared to the initial (t(19)=9.719, *p*<0.0001; **Fig.6C-D**). In the psilocin-treated males (**Fig.6E**), there was no difference in peak point from the initial to the final auditory session (t(10)=0.09932, *p*=0.92; **Fig.6F**). However, there was a significant decrease in binned analysis of the initial auditory and final auditory condition (t(19)=4.943, *p*<0.0001; **Fig.6G-H**). While there was a decrease in both the vehicle and psilocin groups, the decrease in peak point in the vehicle group was of greater overall magnitude (**Fig.6B, 6F**) and the estimation plots showed a much greater reduction in the vehicle (**Fig.6D**) as compared to the psilocin group (**Fig.6H**).

DISCUSSION

The present study explored the effects of the psychedelic compound psilocin on CeA activity using an immunohistological activity marker and on CeA reactivity to an aversive stimulus using *in-vivo* fiber photometry recordings during acute drug exposure and at 2- , 6- , and 28-days post drug administration. The study examined multi-level neurobiological and behavioral effects of psilocin by looking at acute drug effects, long-term alterations in activity following drug administration, and by probing for potential sex-dependent effects of both treatment and time. Psilocin administration produced increases in *c-Fos* expression in both males and females and sub-region analysis showed that increases in the female CeC were significantly greater in psilocin compared to the vehicle control (**Fig. 2B**). CeA reactivity in response to the air-puff stimulus following psilocin administration also displayed sex- and time-dependent effects. In females, psilocin increased CeA reactivity as

compared to vehicle controls (**Fig. 2E-H**). Interestingly, both vehicle and psilocin groups showed increases in reactivity at the 2- and 6-day follow up compared to baseline. In contrast, the male psilocin group did not show the same increased reactivity seen in the females (**Fig. 2H**) and instead displayed decreases in CeA reactivity at 2-day, 6-day, and 28-day follow-up recordings (**Fig. 3F, 3H**) that were not seen in vehicle control males (**Fig. 3G**).

Clinical studies have illustrated that psilocybin produces alterations in amygdala reactivity and connectivity [8, 12, 15, 17, 39-41]. Here, we utilized a preclinical approach to examine the actions of psilocin, the psychoactive metabolite of psilocybin, on central amygdala activity in a rodent model. As most clinical studies report a reduction in amygdala reactivity or connectivity that correlated with therapeutic improvements [8, 15, 17, 39-41], we hypothesized that psilocin would produce similar decreases in reactivity in our model. Here we found that a single dose of psilocin produced persistent reductions in CeA reactivity to an aversive stimulus in male but not female rats. These findings lend to recent studies in both clinical [11, 13, 50] and preclinical [51] literature showing persistent reductions in depressive behavior after a single dose of psilocybin. Although the mechanism behind these therapeutic effects remains unknown, this study provides evidence that changes in CeA activity may play a role in observed therapeutic effects.

We report sex-specific effects of psilocin on basal activity and stimulus-induced reactivity within the CeA. Changes in basal activity were measured by *c-Fos*, an immediate early gene shown to be upregulated following neuronal activation [52]. We observed an increase in *c-Fos* expression in both males and females following administration of psilocin, with region specific increases found the CeC subregion in females. CeA reactivity was assessed using fiber photometry to measure in-vivo stimulus-induced fluctuations in calcium signaling. In the female psilocin group, we observed an acute increase in reactivity to the air-puff stimulus while on the drug that was not seen in the males. Additionally, we saw increases in reactivity at 2- and 6-day follow up recording, while we saw reductions in CeA reactivity in the males at each of the follow up recording days (**Fig. 3B, 3D, 3E, 3G**). A recent study looking at cerebral blood flow and the expression of the serotonin transporter (5-HTT) showed that, in response to an aversive predator odor, there were increases in blood flow and *c-Fos* expression in the CeA that were related to 5-HTT expression in males but not females [53]. These

findings suggest potential sex-specific expression of serotonin receptors within the amygdala. Given the affinity of psilocin for the serotonin system, differential expression of serotonin receptors within the CeA or surrounding nuclei could explain the sex-specific effects seen in this study. Additionally, a previous study showed sex-specific differences in 5-HT_{2AR} expression at baseline, wherein females had lower expression in the orbitofrontal cortex (OFC) than males [54]. Another potential explanation for the differential effects of psilocin between males and females may be due to differences in CeA circuitry. For example, the OFC has been shown, in rodents and non-human primates, to have direct connections to the CeA as well as to the intercalated nuclei, a local GABAergic population within the amygdala complex [55, 56]. Lack of activation of this circuit due to a reduced expression of OFC 5-HT_{2AR} in females could prevent an inhibition of CeA firing, thus causing the increase in reactivity seen while on psilocin in females but not males. Future experiments are needed to examine the effects of psilocin on CeA circuits innervating distinct brain regions.

In the presence of a threatening stimulus, animals will employ either active or passive coping strategies. In this study, the active threat response was characterized by darting, or immediate fleeing from the air-puff stimulus, while the passive threat response was characterized by freezing, or immobility following the air-puff. Here, we show that females predominantly exhibited an active response, while males were more evenly split between active and passive responses. In male psilocin-treated animals who exhibited an active response at baseline, there was a significant decrease in the amplitude of CeA reactivity following exposure to the air-puff at the 2-day follow up, with no changes in vehicle control. This decrease in reactivity was not seen in the passive-responder vehicle or psilocin groups. While further interpretation regarding adaptive vs. maladaptive strategies in this context is outside the scope of the current study, these results suggest that there may be differences in CeA reactivity and threat responding strategy that are implicated in the efficacy of psychedelics.

To determine the effects of psilocin on associative learning, we utilized an auditory-only stimulus recording taken after the initial baseline recordings and then again after the 28-day recording. Interestingly, in both males and females the vehicle control group displayed reductions in CeA response to the auditory-only stimulus. In the psilocin group however, reductions in CeA reactivity to the auditory stimulus were not observed in females, while in males, reductions were of a much lesser degree than the vehicle control group (**Fig. 6**). Given the

reductions in reactivity seen with the standard air-puff stimulus, the sustained reactivity to the auditory stimulus in the psilocin group suggests that the decreases in reactivity we found under the air-puff conditions were not due to an overall blunting of CeA function. Instead, these data suggest a more dynamic alteration in CeA reactivity in response to different types of stimuli. One potential interpretation of these data is that psilocin may influence associative learning to an aversive stimulus. Several studies have shown that 5-HT_{2A} agonist psychedelics produce improvements in various dimensions of cognition, including associative learning [57-59]. Another potential interpretation of these findings is that psilocin created a more robust/meaningful memory of the air-puff. Many of the clinical studies cite participants rating their experience during a psilocybin treatment as one of the most meaningful experiences of their life [13, 60, 61]. Additionally, increases in plasticity could contribute to differences in memory reconsolidation during air-puff administration while on the drug.

Our findings demonstrate that the psychedelic compound, psilocin, produces dynamic, sex-specific changes in the CeA, a key nucleus within the amygdala complex. Here we showed that administration of psilocin produces increases in CeA *c-Fos* expression and that, in females, these increases were primarily driven by increases in expression in the CeC. We have also shown that there are sex-dependent changes in CeA reactivity while on psilocin. Specifically, we show that a single administration of psilocin produced acute increases in CeA reactivity in females, but not males. Additionally, we show that persistent changes in CeA reactivity are seen as early as 2 and as long as 28 days following administration of the drug in males, but not females. Given that dysregulation of the amygdala is a hallmark in many different psychiatric disorders, including anxiety, MDD, and PTSD [29, 31-36] and that psychedelic compounds show promise in treating many of these illnesses [4, 6-11], these findings provide important information on subregion-specific alterations in CeA function that may play a part in observed therapeutic effects of psychedelics. Additionally, the long-term reductions in amygdala reactivity seen with the air-puff stimulus do not seem to be the result of overall desensitization of the CeA, given the sustained response in the auditory-only stimulus response seen in the psilocin group for both males and females. Future work will be required to explore potential circuit-based mediators contributing to these observed alterations in CeA reactivity, but these findings provide important evidence for CeA alterations underlying the potential therapeutic effects of psilocin and psilocybin.

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COMPETING INTERESTS

The authors have no competing interests to report.

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Figure 1. (**A**) Experimental timeline. Animals arrived at 6-7 weeks old and were allowed to habituate for 1-week prior to surgeries. Bilateral injection of calcium sensor (jGCaMP7f) and implantation of fiber optic cannulas were conducted. After 3 weeks for viral incubation, animals underwent a series of fiber photometry experiments. (**B**) Representative coronal section showing fiber placement and GCaMP expression. (**C**) Mean air-puff traces showing consistency of CeA signal in response to the air-puff stimulus.

Figure 2. *Acute Changes in CeA Reactivity in Response to an Aversive Air-Puff Stimulus Following Psilocin Administration* **(A)** Females: representative images showing c-Fos+ cells tagged with a green fluorescence protein (GFP) in the CeA. **(B)** Female vehicle vs. psilocin: histogram showing the number of c-Fos+ cells in each subregion of the CeA. CeC = capsular CeA, CeM =. Medial CeA, CeL = lateral CeA. Data points correspond to the mean of 2-4 hemispheres for each individual subject. 2-way ANOVA: Treatment x time: F (2, 24) = 1.597, p = 0.22; Treatment: F (1, 24) = 9.149, p = 0.006; Time: F (1, 24) = 9.149, p = 0.006 **(C)** Males: representative images showing c-Fos+ cells tagged with a green fluorescence protein (GFP) in the CeA. **(D)** Male vehicle vs. psilocin: histogram showing the number of c-Fos+ cells in each subregion of the CeA. CeC = capsular CeA, CeM =. Medial CeA, CeL = lateral CeA. Data points correspond to the mean of 2-4 hemispheres for each individual subject. 2-way ANOVA: Treatment x time: F (2, 27) = 1.241, p = 0.30; Treatment: F (1, 27) = 5.543, p = 0.03; Time: F (2, 27) = 12.02, p = 0.0002; Dunnett's: CeC (p = 0.01) **(E)** Female psilocin group: representative raw ΔF/F traces showing an individual at baseline and while on psilocin. Blue line = air-puff onset, Grey lines = individual traces, Green line = mean trace, Std= standard deviation **(F)** Females: Estimation plots for binned trace plots comparing baseline to treatment. Each data point corresponds to a 500ms bin averaged across subjects +/- SEM, Vehicle: p = 0.01, t (19) =2.780, mean of differences = 0.34; Psilocin: p = 0.01, t (19) =2.780, mean of differences = 0.34 (**G)** Female vehicle group: air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M. **(H)** Female psilocin group: air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M. **(I)** Male psilocin group: representative raw ΔF/F traces showing an individual at baseline and while on psilocin. Blue line = air-puff onset, Grey lines = individual traces, Green line = mean trace, Std= standard deviation **(J)** Males: Estimation plots for binned trace plots comparing baseline to

treatment. Each data point corresponds to a 500ms bin averaged across subjects +/- SEM. Vehicle: p = 0.0003, t (19) =4.452, mean of differences = 0.33; Psilocin: p = 0.54, t (19) =0.6231, mean of differences = -0.06 **(K)** Male vehicle group: air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M. **(L)** Male psilocin group: air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M. *p<0.05, ***p<0.01, *** p<0.001, **** p<0.0001.*

Figure 3. *Prolonged Changes in CeA Reactivity in Response to an Aversive Air-Puff Stimulus Following Psilocin Administration* **(A)** Female psilocin group: representative raw ΔF/F traces showing an individual at baseline and at the 28-day follow up. Blue line = air-puff onset, Grey lines = individual traces, Green line = mean trace, Std= standard deviation **(B)** First graph: area under curve (AUC) histogram plots comparing baseline to the 28-day follow up. 2-way ANOVA: Treatment x Time: F (1, 14) = 0.003383, p = 0.95; Treatment: F (1, 14) = 0.09635, p = 0.76; Time: F (1, 14) = 0.2126, p = 0.65; Second graph: changes in AUC at each of the follow up time points between the vehicle and psilocin group. Mean AUC +/- S.E.M., 2-way ANOVA: Treatment x Time: F (2, 28) = 1.437, p = 0.25; Treatment: F (1, 14) = 0.0003490, p = 0.99; Time: F (2, 28) = 3.067, p = 0.06 **(C)** Female vehicle group: air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M., 1-way ANOVA: F (3, 57) = 50.02, p < 0.0001; Dunnett's: 2- day (p < 0.0001) and 6-day (p < 0.0001**).** **(D)** Female psilocin group: air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M., (3, 57) = 54.13, p < 0.0001; Dunnett's: 2-Day (p < 0.0001) and 6-day (p < 0.0001). **(E)** Male psilocin group: representative raw ΔF/F traces showing an individual at baseline and while on psilocin. Blue line = air-puff onset, Grey lines = individual traces, Green line = mean trace, Std= standard deviation **(F)** First graph: AUC histogram plots comparing baseline to the 28-day follow up between male vehicle and psilocin groups. 2-way ANOVA: Treatment x Time: F (1, 19) = 5.068, p = 0.03; Treatment: F (1, 19) = 0.2503, p = 0.62; Time: F (1, 19) = 1.361, p = 0.26, Šidák: 28-day (p = 0.04). Second graph: changes in AUC at each of the follow up time

points between the male vehicle and psilocin group. Mean AUC +/- S.E.M. 2-way ANOVA: Treatment x Time: F (2, 38) = 0.2113, p = 0.81; Treatment: F (1, 19) = 4.926, p = 0.03; Time: F (2, 38) = 0.7906, p =0.46 **(G)** Male vehicle group: air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M. 1-way ANOVA: F (3, 57) = 5.554, p = 0.002; Dunnett's: 28-day follow up (p = 0.01) **(H)** Male psilocin group: air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window. 1-way ANOVA: F (3, 57) = 72.60, p < 0.0001; Dunnett's: 2-day (p < 0.0001), 6-day (p < 0.0001), and 28-day (p < 0.0001) **(I)** Females: Summary histogram comparing the mean of all 20 time bins following air-puff administration. Each data point represents an individual time bin within that condition collapsed across all subjects. +/- S.E.M.; 2-way ANOVA: Treatment x time: F (4, 152) = 35.02, p < 0.0001; Treatment: **(J)** Males: Summary histogram comparing the mean of all 20 time bins following air-puff administration +/- S.E.M. Each data point represents an individual time bin within that condition collapsed across all subjects. +/- S.E.M. *p<0.05, ***p<0.01, *** p<0.001, **** p<0.0001.*

Figure 4. *Acute and Prolonged Behavioral Effects of Psilocin*. **(A)** Females: locomotion plots comparing vehicle and psilocin groups at each time point. Data points are mean time in seconds +/- SEM. 2-way ANOVA: Treatment x Time: F (3, 42) = 0.2118, p = 0.89; Treatment: F (1, 14) = 2.238, p = 0.16; Time: F (2.497, 34.95) = 0.6065, p = 0.59 **(B)** Females: time spent rearing comparing vehicle and psilocin groups at each time point. Data points are mean time in seconds +/- SEM. 2-way ANOVA: Treatment x Time: F (4, 56) = 1.345, p = 0.26; Treatment: F (1, 14) = 4.433, p = 0.05 Time: F (4, 56) = 2.486, p = 0.05 **(C)** Males: locomotion plots comparing vehicle and psilocin groups at each time point. Data points are mean time in seconds +/- SEM. 2-way ANOVA: Treatment x Time: F (3, 57) = 0.1054, p = 0.96; Treatment: F (1, 19) = 2.563, p = 0.12; Time: F (3, 57) = 1.255, p =0.29 **(D)** Males: time spent rearing comparing vehicle and psilocin groups at each time point. Data points are mean time in seconds +/- SEM. 2-way ANOVA: Treatment x Time: F (4, 76) = 0.5546, p = 0.69; Treatment: F (1, 19) = .7576, p = 0.39; Time: F (4, 76) = 1.190, p =0.32 **(E)** Females threat responding behavior. First figure is showing percentage of subjects that darted following air-puff administration. Second figure is showing the percentage of animals that remained immobile following air-puff. Third and fourth figure are pie charts

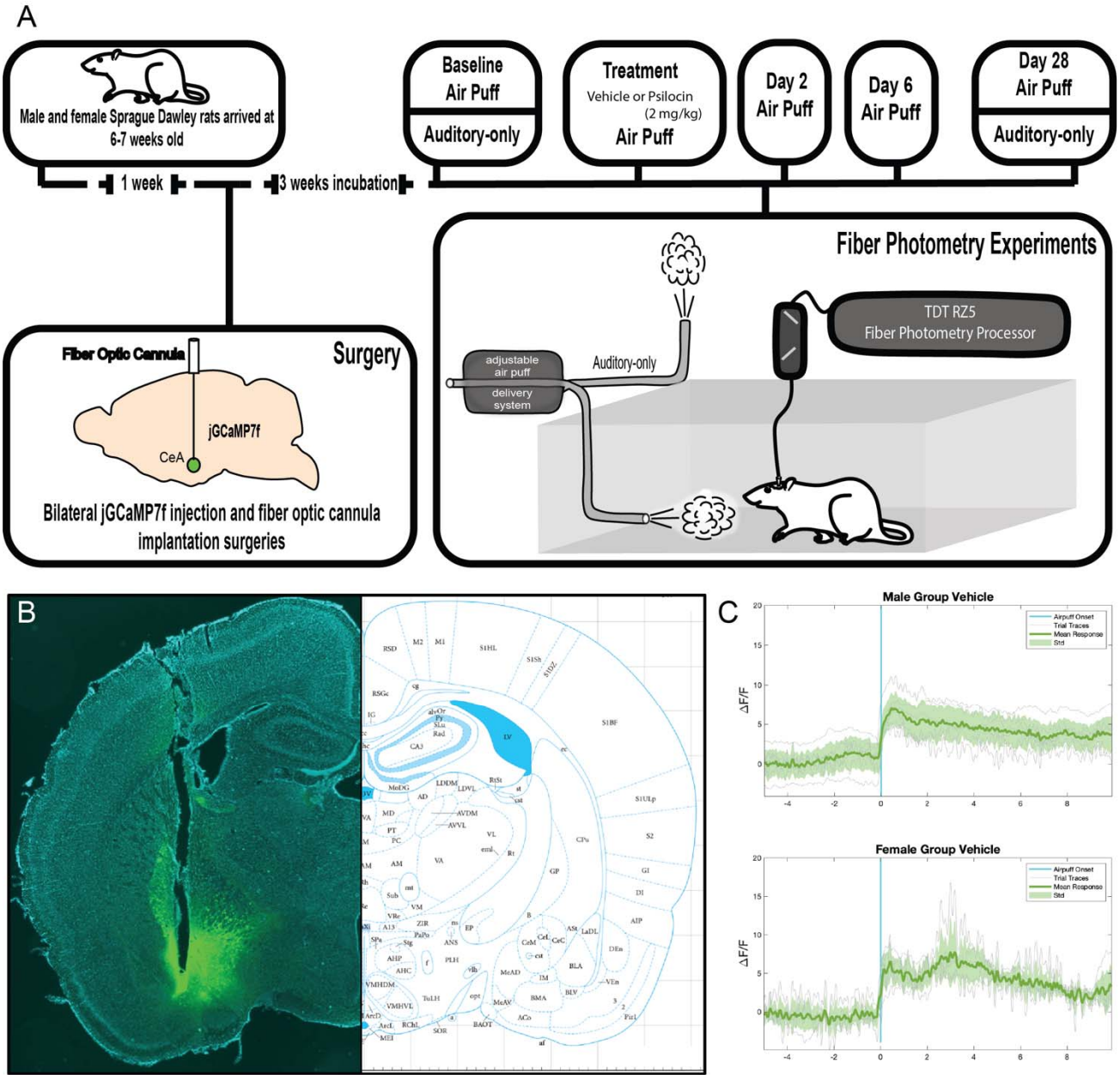
showing the percentage of animals that darted vs. remained immobile **(F)** Males threat responding behavior: First figure is showing percentage of subjects that darted following air-puff administration. Fisher's exact test: 2-day (p = 0.03); Second figure is showing the percentage of subject that remained immobile following air-puff. Fisher's exact test: 2-day (p = 0.03);; Third and fourth figure are pie charts showing the percentage of animals that darted vs. remained immobile **(G)** Male vehicle passive responders (remained immobile) group: First figure shows air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M.; Second figure is a histogram showing differences in the mean peak ΔF/F value for each group +/- S.E.M following air-puff administration. Data points reflect each individual subject within the corresponding subgroup. 1-way ANOVA: Treatment: F (4, 20) = 1.219, p = 0.33; **(H)** Male Psilocin passive responders (remained immobile) group: First figure shows air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M. Second figure is a histogram showing differences in the mean peak ΔF/F value for each group +/- S.E.M following air-puff administration. Data points reflect each individual subject within the corresponding subgroup. 1-way ANOVA: Treatment: F(4, 20) = 1.276, p = 0.31 **(I)** Male vehicle active responders (darted) group: First figure shows air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M. Second figure is a histogram showing differences in the mean peak ΔF/F value for each group +/- S.E.M following air-puff administration. Data points reflect each individual subject within the corresponding subgroup. 1-way ANOVA: Treatment: F (4, 12) = 1.046, p = 0.42 **(J)** Male Psilocin active responders (darted) group: First figure shows air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M. Second figure is a histogram showing differences in the mean peak ΔF/F value for each group +/- S.E.M following air-puff administration. Data points reflect each individual subject within the corresponding subgroup. 1-way ANOVA: Treatment: F (4, 16) = 6.566, p = 0.003

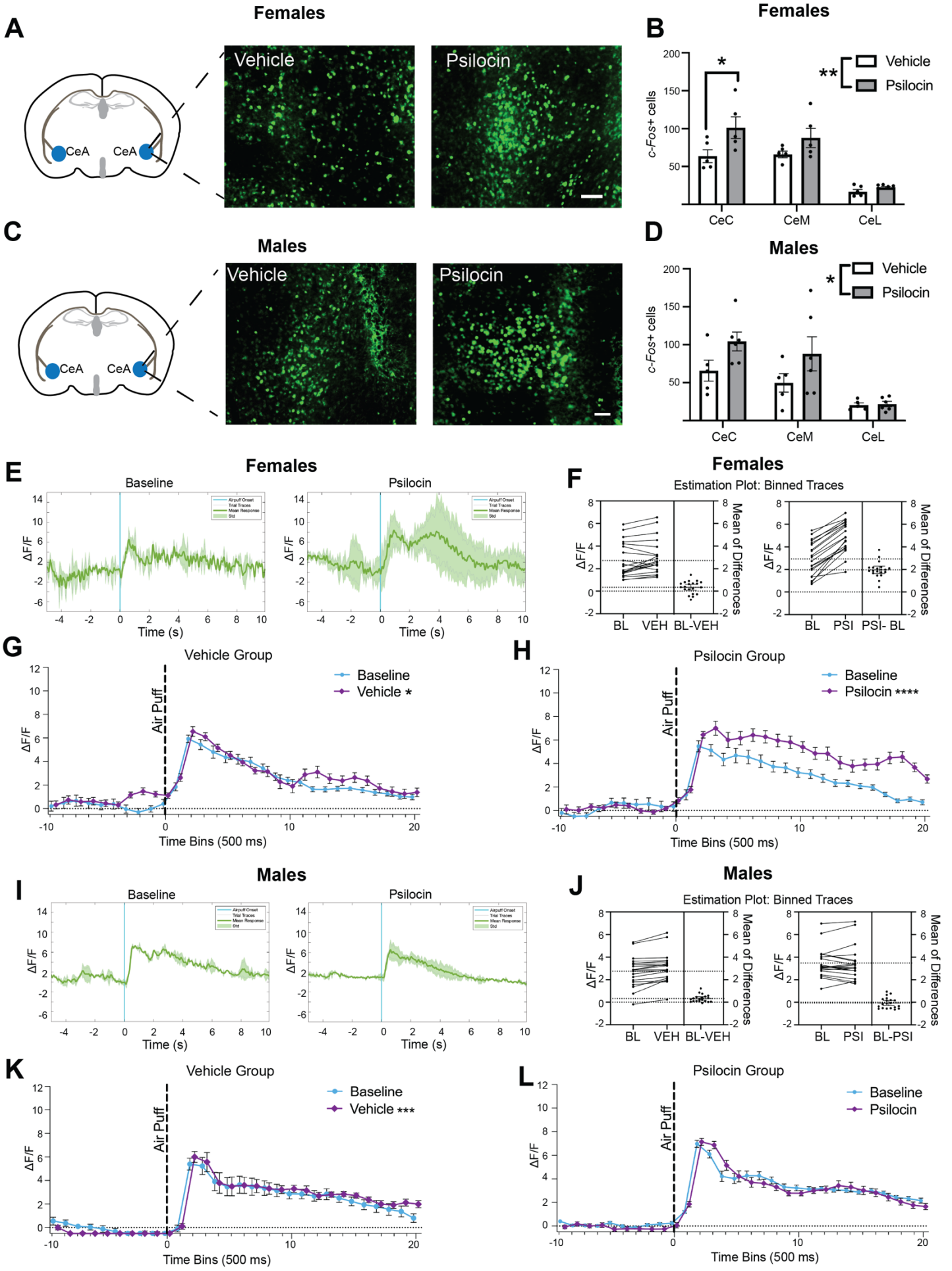
Figure 5. *Changes in CeA Reactivity in Response to an Auditory Stimulus Following Psilocin Administration in Female Subjects.* **(A)** Female vehicle group: representative mean ΔF/F traces showing an individual at initial

auditory compared to final auditory. Blue line = air-puff onset, Grey lines = individual traces, Green line = mean trace, Std= standard deviation **(B)** Female vehicle group: peak point of binned mean ΔF/F traces compared between initial and final auditory recordings. Data points represent each individual peak ΔF/F value +/- SEM. Paired t-test: p = 0.004, t (8) =3.934, mean of differences = -3.17; **(C)** Female vehicle group: air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M **(D)** Female vehicle group: Estimation plots for binned trace plots comparing baseline to treatment. Each data point corresponds to a 500ms bin averaged across subjects +/- SEM.; Paired t-test: p < 0.0001, t (19) =18.28, mean of differences = -2.30; **(E)** Female psilocin group: representative mean ΔF/F traces showing an individual at initial auditory compared to final auditory. Blue line = air-puff onset, Grey lines = individual traces, Green line = mean trace, Std= standard deviation **(F)** Female psilocin group: peak point of binned mean ΔF/F traces compared between initial and final auditory recordings. Data points represent individual peak ΔF/F value +/- SEM. Paired t-test: p = 0.67, t (6) =0.4346, mean of differences = -0.49; **(G)** Female psilocin group: air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M. **(H)** Female psilocin group: Estimation plots for binned trace plots comparing baseline to treatment. Each data point corresponds to a 500ms bin averaged across subjects +/- SEM. Paired t-test: p = 0.98, t (19) =0.01624, mean of differences = 0.002; *p<0.05, ***p<0.01, *** p<0.001, **** p<0.0001.*

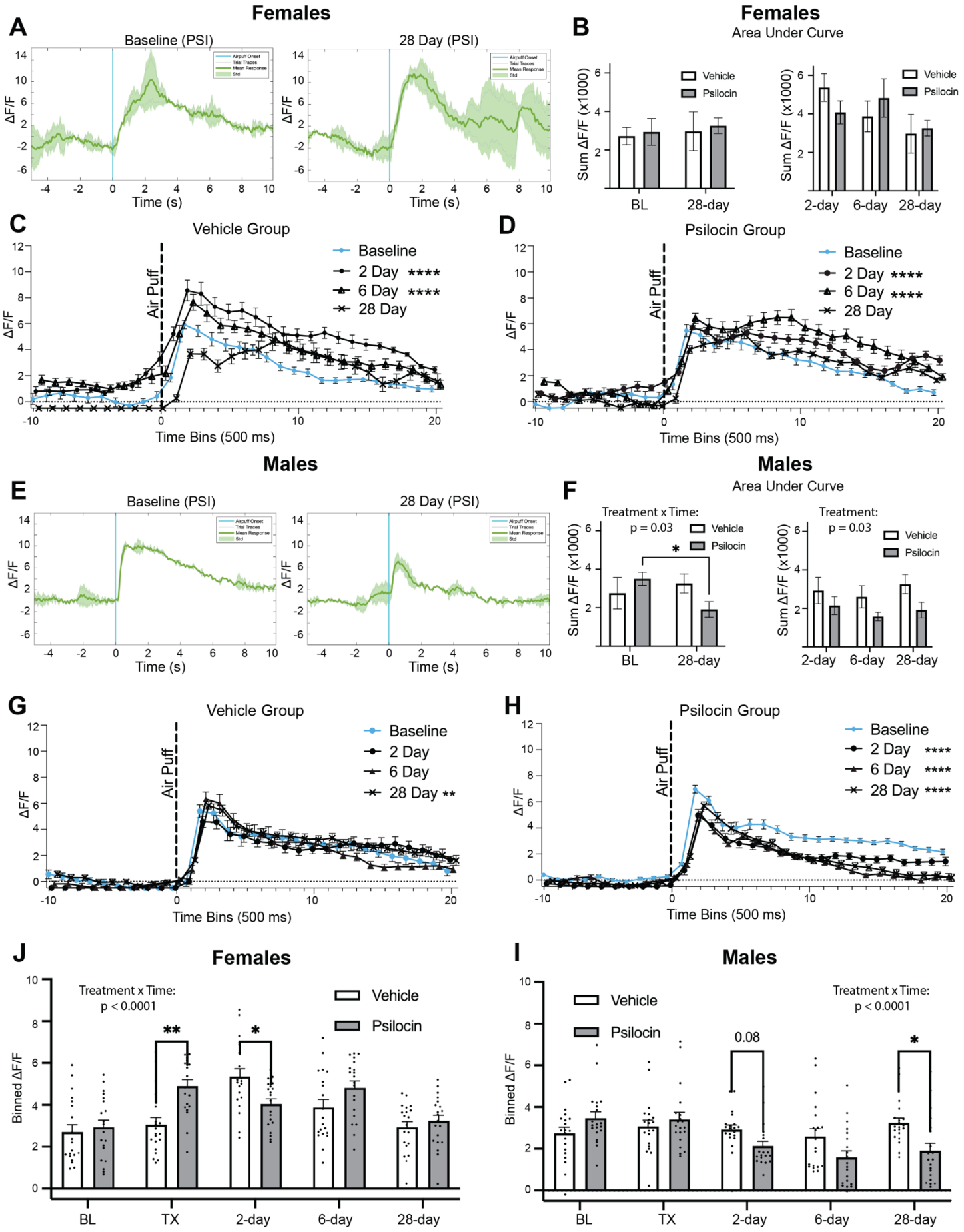
Figure 6. *Changes in CeA Reactivity in Response to an Auditory Stimulus Following Psilocin Administration in Male Subjects.* **(A)** Male vehicle group: representative mean ΔF/F traces showing an individual at initial auditory compared to final auditory. Blue line = air-puff onset, Grey lines = individual traces, Green line = mean trace, Std= standard deviation **(B)** Male vehicle group: peak point of binned mean ΔF/F traces compared between initial and final auditory recordings. Data points represent each individual peak ΔF/F value +/- SEM. Paired t-test: p = 0.06, t (9) =2.136, mean of differences = -1.58 **(C)** Male vehicle group: air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M. **(D)** Male vehicle group: Estimation plots for binned trace plots comparing baseline to treatment. Each data point corresponds to a 500ms bin averaged across subjects

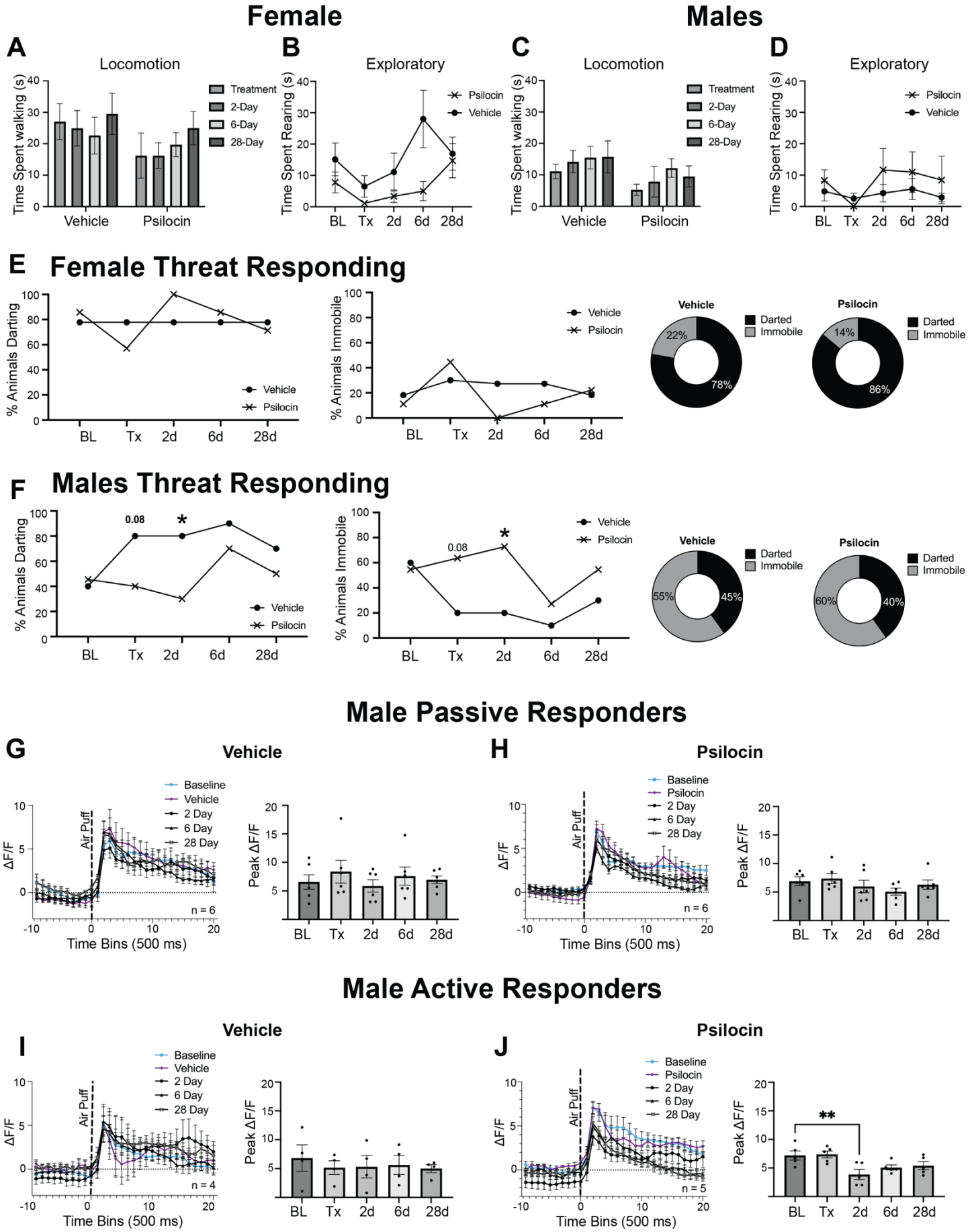
+/- SEM. Paired t-test: $p < 0.0001$, t (19) =9.719, mean of differences = -0.85 **(E)** Male psilocin group: representative mean ΔF/F traces showing an individual at initial auditory compared to final auditory. Blue line = air-puff onset, Grey lines = individual traces, Green line = mean trace, Std= standard deviation **(F)** Male psilocin group: peak point of binned mean ΔF/F traces compared between initial and final auditory recordings. Data points represent individual peak ΔF/F value +/- SEM. Paired t-test: $p = 0.92$, t (10) =0.09932, mean of differences = 0.09 **(G)** Male psilocin group: air-puff trace plots of changes in CeA fluorescence following exposure to a 500ms air-puff at 85 psi. Data points represent group averages within 500ms binned window +/- S.E.M. **(H)** Male psilocin group: Estimation plots for binned trace plots comparing baseline to treatment. Each data point corresponds to a 500ms bin averaged across subjects +/- SEM. Paired t-test: $p < 0.0001$, t (19) =4.943, mean of differences = -0.39; *$p<0.05$, ***$p<0.01$, *** $p<0.001$, **** $p<0.0001$.*

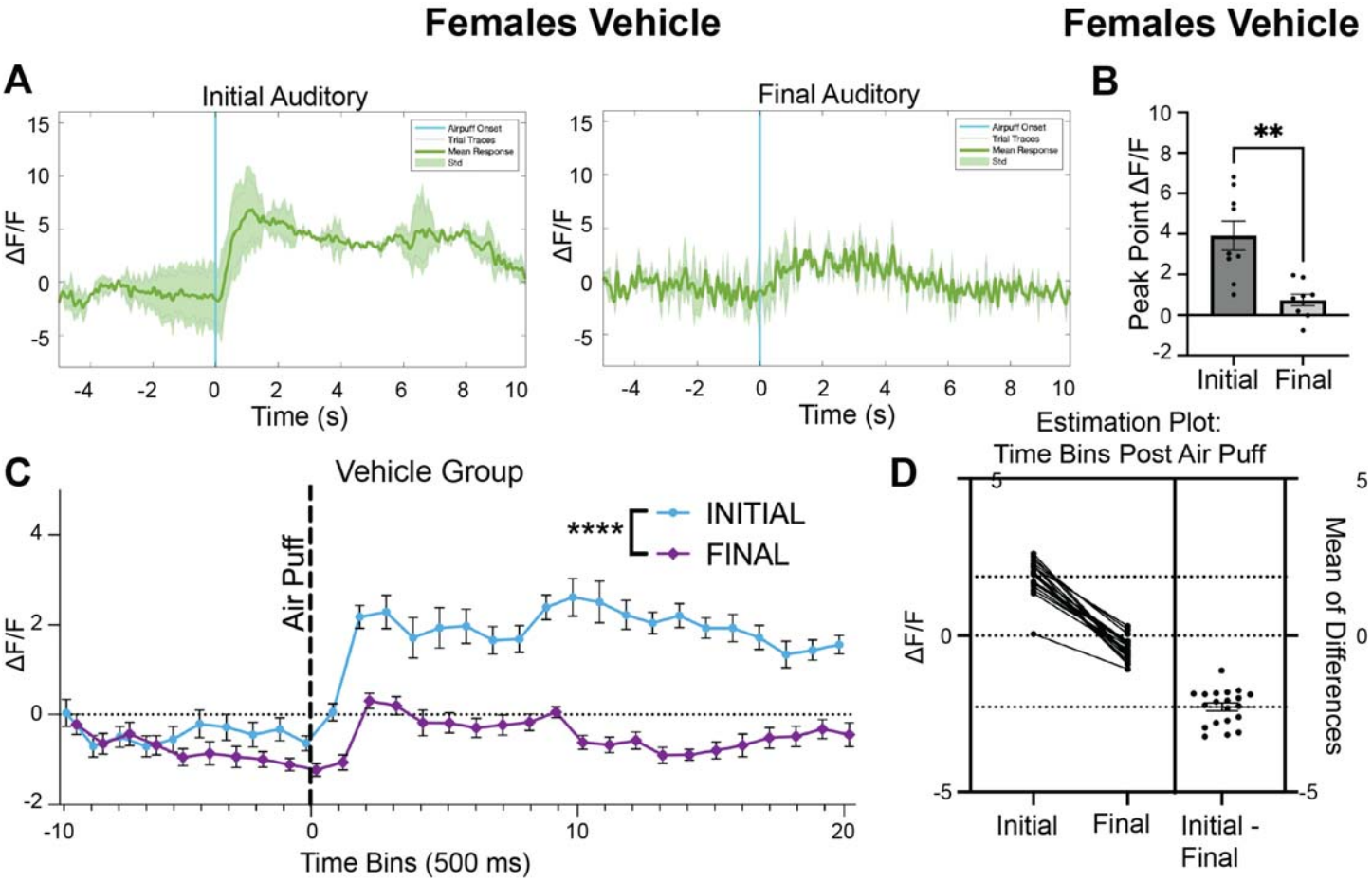

A
Male and female Sprague Dawley rats arrived at 6-7 weeks old
1 week
3 weeks incubation
Baseline Air Puff
Auditory-only
Treatment
Vehicle or Psilocin (2 mg/kg)
Air Puff
Day 2 Air Puff
Day 6 Air Puff
Day 28 Air Puff
Auditory-only
Fiber Optic Cannula
Surgery
jGCaMP7f
CeA
Bilateral jGCaMP7f injection and fiber optic cannula implantation surgeries
Fiber Photometry Experiments
TDT RZ5
Fiber Photometry Processor
adjustable air puff
delivery system
Auditory-only
B
C
Male Group Vehicle
Female Group Vehicle
ΔF/F
Airpuff Onset
Trial Traces
Mean Response
Std

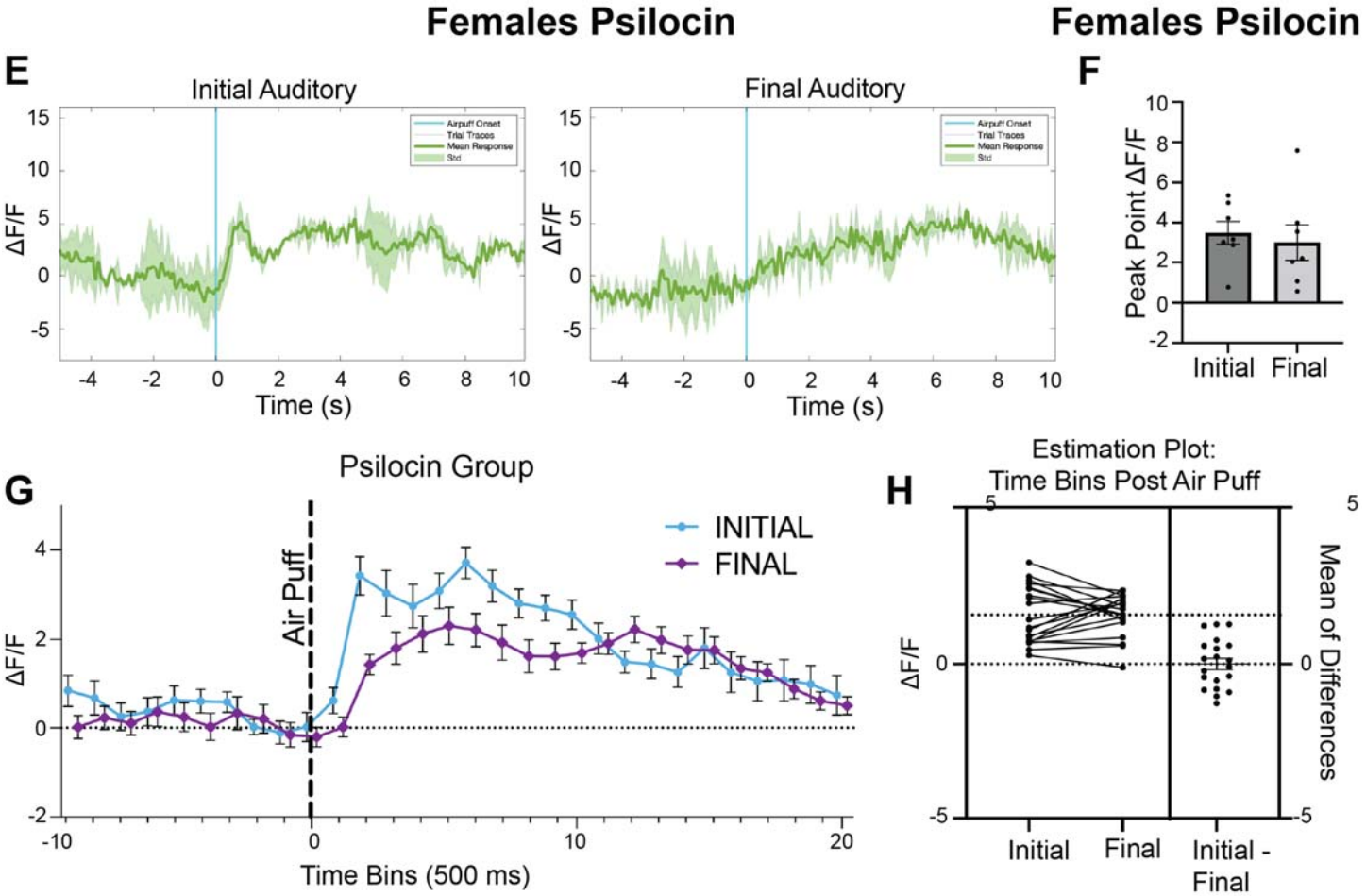

A
Females
CeA
CeA
Vehicle
Psilocin
B
Females
c-Fos+ cells
Vehicle
Psilocin
CeC
CeM
CeL
C
Males
Vehicle
Psilocin
D
Males
E
Females
Baseline
Psilocin
ΔF/F
Time (s)
F
Females
Estimation Plot: Binned Traces
Mean of Differences
BL
VEH
BL-VEH
PSI
PSI- BL
G
Vehicle Group
Baseline
Vehicle *
Air Puff
Time Bins (500 ms)
H
Psilocin Group
Psilocin ****
I
Males
J
Males
BL-PSI
K
Vehicle Group
Vehicle ***
L
Psilocin Group

1

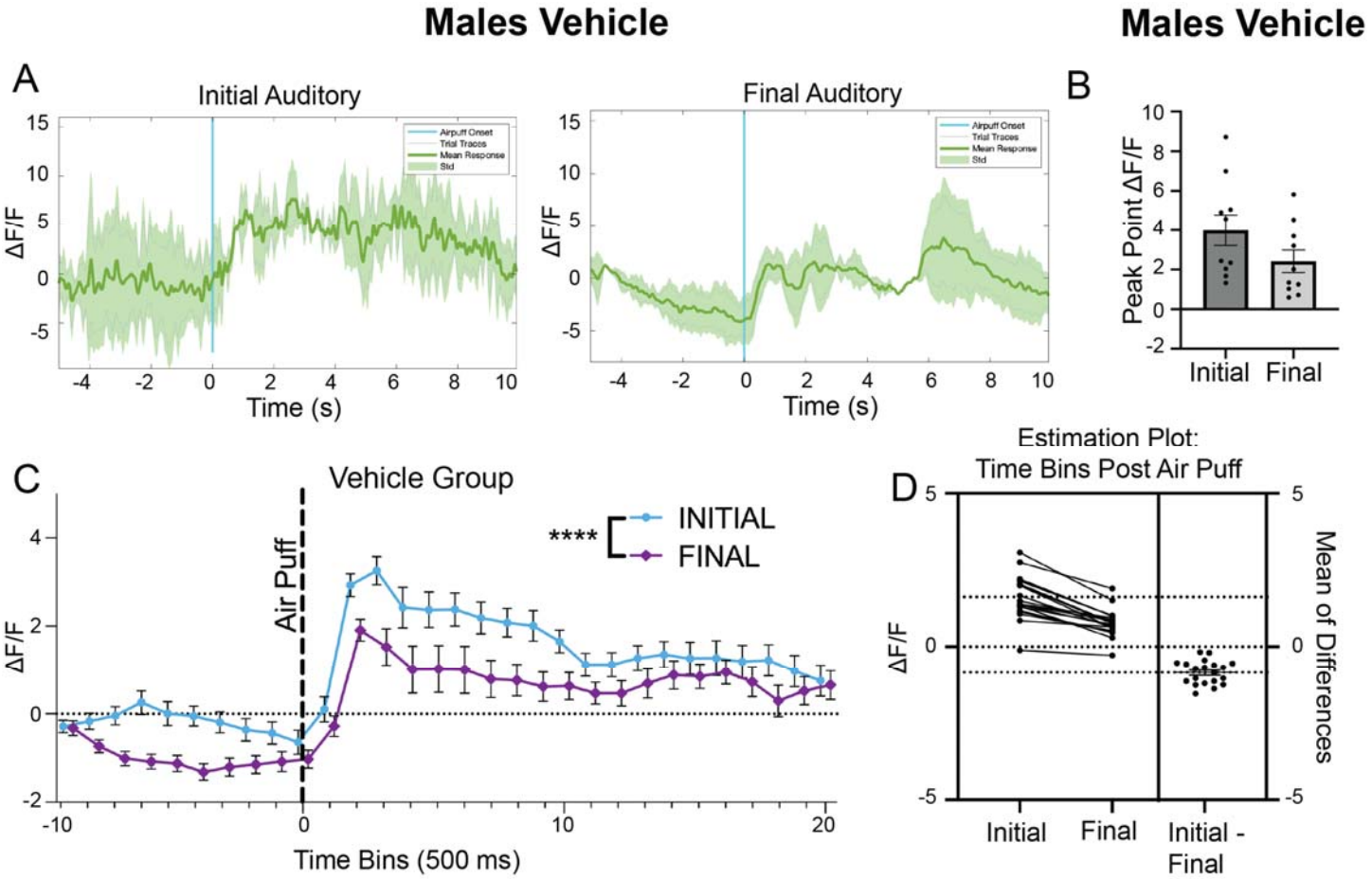

Females
A
Baseline (PSI)
28 Day (PSI)
ΔF/F
Time (s)
B
Females
Area Under Curve
Sum ΔF/F (x1000)
Vehicle
Psilocin
BL
28-day
2-day
6-day
C
Vehicle Group
Air Puff
Baseline
2 Day ****
6 Day ****
28 Day
Time Bins (500 ms)
D
Psilocin Group
Air Puff
Baseline
2 Day ****
6 Day ****
28 Day
Time Bins (500 ms)
Males
E
Baseline (PSI)
28 Day (PSI)
ΔF/F
Time (s)
F
Males
Area Under Curve
Treatment x Time:
p = 0.03
Treatment:
p = 0.03
Sum ΔF/F (x1000)
Vehicle
Psilocin
BL
28-day
2-day
6-day
G
Vehicle Group
Air Puff
Baseline
2 Day
6 Day
28 Day **
Time Bins (500 ms)
H
Psilocin Group
Air Puff
Baseline
2 Day ****
6 Day ****
28 Day ****
Time Bins (500 ms)
J
Females
Treatment x Time:
p < 0.0001
Vehicle
Psilocin
Binned ΔF/F
BL
TX
2-day
6-day
28-day
I
Males
Vehicle
Psilocin
Treatment x Time:
p < 0.0001
0.08
Binned ΔF/F
BL
TX
2-day
6-day
28-day

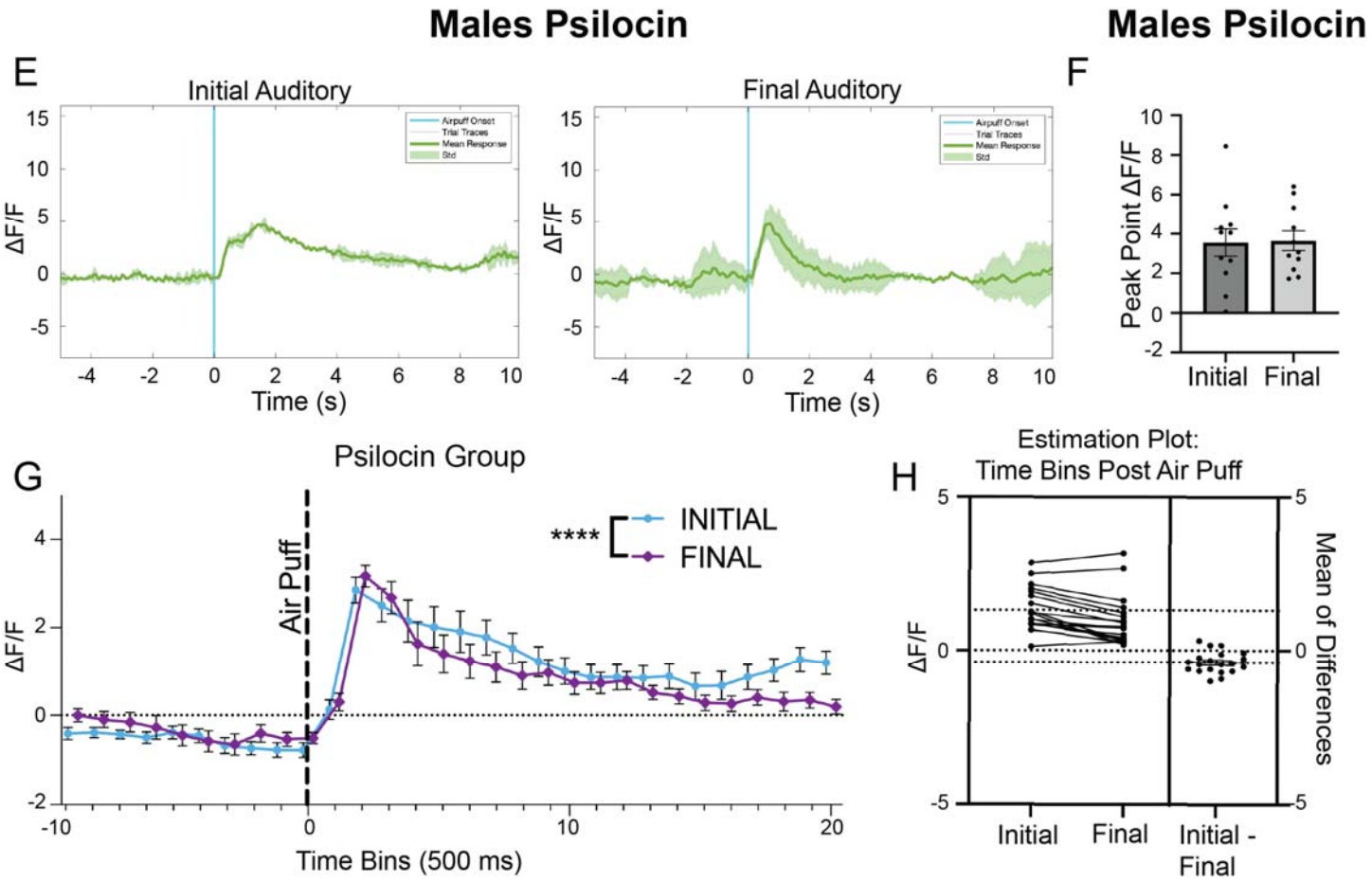

Female
Males
A
Locomotion
Time Spent walking (s)
Treatment
2-Day
6-Day
28-Day
Vehicle
Psilocin
B
Exploratory
Time Spent Rearing (s)
Psilocin
Vehicle
BL
Tx
2d
6d
28d
C
Locomotion
D
Exploratory
E
Female Threat Responding
% Animals Darting
% Animals Immobile
Vehicle
Psilocin
Darted
Immobile
22%
78%
14%
86%
F
Males Threat Responding
0.08
*
55%
45%
60%
40%
Male Passive Responders
G
Vehicle
H
Psilocin
Baseline
Vehicle
Psilocin
2 Day
6 Day
28 Day
Air Puff
ΔF/F
Peak ΔF/F
n = 6
Time Bins (500 ms)
Male Active Responders
I
J
n = 4
n = 5
**


Females Vehicle
Females Vehicle
A
Initial Auditory
Final Auditory
Airpuff Onset
Trial Traces
Mean Response
Std
ΔF/F
Time (s)
B
Peak Point ΔF/F
**
Initial
Final
C
Vehicle Group
Air Puff
****
INITIAL
FINAL
ΔF/F
Time Bins (500 ms)
D
Estimation Plot:
Time Bins Post Air Puff
ΔF/F
Mean of Differences
Initial
Final
Initial - Final


Females Psilocin
Females Psilocin
E
Initial Auditory
Final Auditory
Airpuff Onset
Trial Traces
Mean Response
Std
ΔF/F
Time (s)
F
Peak Point ΔF/F
Initial
Final
G
Psilocin Group
Air Puff
INITIAL
FINAL
ΔF/F
Time Bins (500 ms)
H
Estimation Plot:
Time Bins Post Air Puff
ΔF/F
Mean of Differences
Initial
Final
Initial - Final

1


Males Vehicle
Males Vehicle
A
Initial Auditory
Final Auditory
Airpuff Onset
Trial Traces
Mean Response
Std
ΔF/F
Time (s)
B
Peak Point ΔF/F
Initial
Final
C
Vehicle Group
Air Puff
****
INITIAL
FINAL
ΔF/F
Time Bins (500 ms)
D
Estimation Plot:
Time Bins Post Air Puff
ΔF/F
Mean of Differences
Initial
Final
Initial - Final


Males Psilocin
Males Psilocin
E
Initial Auditory
Final Auditory
Airpuff Onset
Trial Traces
Mean Response
Std
ΔF/F
Time (s)
F
Peak Point ΔF/F
Initial
Final
G
Psilocin Group
Air Puff
****
INITIAL
FINAL
ΔF/F
Time Bins (500 ms)
H
Estimation Plot:
Time Bins Post Air Puff
ΔF/F
Mean of Differences
Initial
Final
Initial - Final