Supplementary Material:

Materials and Methods

Animals

Male adult (8–13 weeks) C57BL/6J mice from The Jackson Laboratory were used for all experiments. All procedures were in accordance with the US National Institutes of Health (NIH) guidelines for the care and use of laboratory animals, and were approved by Stanford University's Administrative Panel on Laboratory Animal Care.

Surgery

All animal surgery was performed on 8–13 weeks old mice under ketamine and xylazine (100 mg Kg^{-1} and 10 mg Kg^{-1}, i.p.) anesthesia using a stereotaxic instrument (BenchMARK Digital, Lecia). For fiber photometry, optogenetic and electrophysiology experiments, a custom-made headplate was cemented onto the skull using dental cement (Lang Dental Manufacturing) after fiber, cannula or electrode implant.

A small volume of concentrated virus solution was injected into the posterior PVT (200 nL AAV, bregma -1.4 mm A/P; -0.1 mm M/L; -3.0 mm D/V, with a 4° angle toward the midline) with a pulled glass capillary at a slow rate (100 nL min^{-1}) using a pressure microinjector (Micro 4 system, World Precision Instruments). The injection needle was withdrawn 5 min after the end of the injection to allow for diffusion and reduced backflow. Mice for fiber photometry experiments were also implanted an optical fiber (200 μm, 0.39 NA, Thorlabs) with ceramic ferrules (1.25 mm OD) 100 μm above the virus injection site; mice for optogenetic experiments were also implanted single cannula or double cannula with inter-center-distance of 0.5 mm (Plastic one) 500 μm above the virus injection site. Then, the optical fiber or cannula was cemented onto the skull using dental cement (Lang Dental Manufacturing). Mice for *in vivo* electrophysiological recording were implanted a custom-made stereotrode into the PVT using the same coordinates as for virus injection. Three screws (small part) were implanted above the cerebellum to secure the electrode and to use as ground. Following surgery, a dummy was inserted to keep the guide cannula from getting clogged or a dust cap was used to keep

the optical fiber clean. Mice were allowed at least 3 weeks to recover and to express the virus before behavioral training. AAVs used in this study were produced by the University of Pennsylvania vector core or in-house: AAV1.hSyn.eGFP.WPRE.bGH, 1.76 X 10^{13} GC ml^{-1} titre; AAV1.hSynp.GCaMP6m.WPRE.hGH, 2.23 X 10^{13} GC ml^{-1} titre; AAV9.CAG.ArchT.GFP.WPRE.SV40, 4.0 X 10^{12} GC ml^{-1} titre, AAV1.hSynp.hChR2(H134R)-mCherry.WPRE.hGH, 3.35 X 10^{13} GC ml^{-1}; and in-house rAAV2-retro.hSynp.mTagBFP2-P2A-Cre.WPRE.hGH, 8.0 X 10^{12} GC ml^{-1} titre.

Behavioral task

All mice used in behavioral assays were allowed to recover from surgery for at least 2 weeks. Mice were trained to associate odors with reinforcing outcomes in head-fixed configuration using a custom-made apparatus. Delivery of odor, water, air puff and tail shock were controlled by a microcontroller (Arduino Due)-based behavioral system running on MATLAB (MathWorks). Licks were detected by a custom-made lickometer with a capacitive touch sensor (Sparkfun MPR121) and a microcontroller (Arduino). The odors used in this study were ethyl acetate, 2-pentanone, citral, 1-butanol, (R)-(+)-limonene and (R)-(-)-carvone, which have neutral value to mice. Odors were randomly chosen for each outcome for different mice. Each odor was diluted to 1% v/v in mineral oil.

Mice were water-deprived and habituated to the head-fixed recording configuration and to lick the water spout for 2-3 days. Once mice reliably licked free water, they were randomly delivered odors and water to record novelty response. After novelty responses extinguished, Pavlovian conditioning began. During training, odors were delivered for 1 s, followed by 2 s of delay period, then one of the outcomes were delivered. In the appetitive trials, 5 µl of water (small reward) or 15 µl of water (big reward) were delivered. In the neutral trials, nothing happened after cue delivery. In the aversive trials, an air puff (100 ms) was delivered to the eye of mice or a mild shock (0.125 mA, 250ms duration) was delivered to the tail. Each training session contained 50 trials. After each training, the mice were allowed to drink 1 mL water before return to their home cage where no water was available. The

anticipatory licking after delivery of water predicting cue reached a plateau after 5-6 training session, indicating that the mice had leant the association and were considered as well-trained animals. For reward omission, the water reward was omitted on a random 10% of the trials. Extinction training was performed in a different set of well-trained mice. CS-US association was tested for the first 10 trials, then the US omitted for the following 30 trials.

For go/no-go behavior test, the water-deprived mice were first trained to associate both odor cues with water reward in the head-fixed recording configuration. Once mice reliably lick during the odor cue (2-3 days), they were proceeded to go/no-go training protocol. During training, odors were delivered for 1 s, followed by 1 s of no stimulus, then a 1s response window. Licking within the response window on go trials was rewarded with a drop of water (15 µl). Licking during the no-go trials was punished with an air puff to the eye.

Optogenetic inhibition

Mice recovered from virus injection and cannula implantation surgery for at least 3 weeks, then the water deprivation, habituation and Pavlovian conditioning were performed. Two optical fibers were connected to the double cannula for constant illumination (532 nm, 3mW from tip of 200 µm fiber) of the PVT during the cue + delay period (Fig. 3, A, B, G, H, Fig. 6D, sFig. 8B) or after US delivery (Fig. 3, C and D) of each trial for the entire training session, or during the test (Fig. 3E, Fig. 6E, sFig. 8D). For sFig. 6, the photoinhibition for 3 s happened during the inter trial interval of entire training session.

Tail-immersion test

Mice were gently restrained, 2 cm of the tip of the tail was submerged into a 52°C water bath, and the latency to withdrawal the tail was recorded. Three tests were performed with constant optogenetic inhibition of the PVT during second test. Each test was separated by at least 1 hour to prevent noxious heat induced pain sensitization or stress induced analgesia.

Fiber photometry and data analysis

Fiber photometry experiments were performed at least 3 weeks after AAV-GCaMP6m or AAV-eGFP injection. In the head-fixed mice, the implanted fiber was connected to FiberOptoMeter (FOM-02M, C-Light, SooChow, China) through an optical fiber patch cord (200 μm, 0.39 NA, Thorlabs). To record fluorescence signals, a beam from a 470 LED was reflected with a dichroic mirror, focused with a lens coupled to a PMT. The LED power at the tip of the patch cord was less than 50 μW. The analog voltage signal was digitalized, filtered (200 Hz low-pass) and sampled at 3 kHz using a RZ5D processor (Tucker-Davis Technologies). Fiber photometry data were recorded using OpenEx software (Tucker-Davis Technologies) and analyzed in MATLAB (MathWorks). To record novel odor responses, 3 new odor were randomly delivered for 1 sec at each trials. To record novel sensory responses, odor (1%), LED light (RB-08E095, 520nm, 3-5 cd) and sound (5 kH, 85 dB) were randomly delivered for 1 sec. The fluorescence change (ΔF/F) was calculated as $(F-F_0)/F_0$, where F_0 is averaged fluorescence signals during one second baseline period in each trial. The area under the curve (AUC) was calculated as mean ΔF/F during a one second period (0.5 – 1.5 sec after CS onset for CS response and 0 – 1 sec after US onset for US response).

Electrophysiological recording and data analysis

To record the activity of PVT neurons, we used a custom-build micro-drive electrode array consisting of 12 microwire (Stablohm 675, California Fine Wire) twisted into stereotrodes or tetrodes. The electrode tips were electroplated with platinum (H_2PtCl_6, Sigma-Aldrich) to an impedance of ~300 kΩ. The electrodes were inserted into a screw-driven microdrive and mounted to allow vertical movement. The electrodes were moved 50 μm per day until they reached 100 μm above the PVT and then recording started. Each recording sent a TTL signal to trigger the initiation of behavioral test and synchronize the electrophysiological recording and the behavioral test. The signals were recorded using a RZ5D system (Tucker-Davis Technologies), filtered (0.3–8 kHz) and digitized at 25 kHz for offline spike detection and sorting with offline sorter (Plexon) based on principal component analysis. Recording locations were marked by passing a current (100 μA, 15 - 20 s) through one or two electrodes

at the end of the recording experiments. Firing rate was binned per 100ms, and converted to Z score based on $Z = (x-\mu)/\sigma$, where x and μ were firing rate and mean firing rate, respectively. σ was standard deviation of firing rate calculated during 1s pre-stimulus period (*18,23*). Neurons were considered responders when the maximum Z score exceeded 2 times of σ above baseline.

iDISCO and light-sheet imaging

Adult male mice were injected with 200nL AAV1.hSynp. DIO.hChR2(H134R)-mCherry unilaterally in the PVT and 150nL rAAV2-retro.hSynp.mTagBFP2-P2A-Cre in the central nucleus of amygdala (bregma -1.22 mm A/P; -2.92 mm M/L; -4.75 mm D/V). Virus was allowed to express for four weeks before the brains were extracted. Animals were euthanized with CO_2 and brains were fixed by intracardial perfusion with 4% paraformaldehyde (PFA) in PBS and remained in PFA overnight at 4°C in 5ml Eppendorf tubes. The following day brains were washed in PBS with 0.02% NaN_3 three times for 30 minutes then left in PBS with NaN_3 for two days at 4°C in new 5ml tubes. Brains were then dehydrated using a freshly prepared dilution series of methanol (MeOH) in PBS with NaN_3. Brains were washed in 20%, 40%, 60%, 80%, and 100% MeOH for 30 min to 3 hours followed by a final 30 min 100% MeOH wash in a new tube. Brains were then put on ice and transferred to a 5% H_2O_2 bleach solution, prepared by diluting 30% H_2O_2 in MeOH on ice, and stored at 4°C overnight. The following day brains were allowed to warm to room temperature before being rehydrated with another freshly mixed MeOH/PBS dilution series. Here, brains were washed in 80%, 60%, 40%, 20% MeOH in PBS with NaN_3 for 30 min to 3 hours. Brains were transferred to new tubes before a final 1 hour wash in PBS with NaN_3. Brains were then transferred to a new tube filled with permeabilization solution (400ml PBS/0.2% Triton/11.5g Glycine/100 ml DMSO/1μl NaN_3) for two days kept rotating at 37°C. Then brains were washed in PTwH (100ml 10x PBS/2ml Tween-20/1ml of 10mg/μl Heparin/1μl NaN_3/ddH_2O to 1L) two times for 5 min then transferred to a new tube filled with blocking solution (PBS with NaN_3/0.2% Triton/10% DMSO/6% donkey serum) and left rotating for 2 days at 37°C. Brains were then transferred to primary antibody

solution (PTwH/5%DMSO/3%donkey serum, 1:1000 Rockland rabbit anti-RFP) and left rotating for 7 days at 37°C. Brains were then removed from primary antibody and washed three times in PTwH for 5 min, then every few hours for the rest of the day. The following day brains were placed in secondary antibody solution (PTwH/3% donkey serum, 1:1000 Alexafluor donkey anti-rabbit 647) and kept rotating at 37°C for 7 days. Samples were exposed to minimal light from this step onward. After 7 days samples were washed in PTwH three times for 5 min then every few hours for the rest of the day and the following day. The next day samples were again dehydrated in a MeOH/ddH_2O series of 20%, 40%, 60%, 80%, and 100% MeOH for 30 min to 3 hours. Brains were transferred to a new tube for a final 1 hour 100% MeOH wash. Brains were then transferred to a solution comprised of 66% dichloromethane (DCM) and 33% MeOH and left washing overnight at room temperature. The following day, the brains were washed twice in 100% DCM for 15 minutes and then transferred to a new tube containing dibenzyl ether (DBE) and left to clear for three days before imaging.

Brains were imaged in coronal, sagittal, and horizontal orientations using a light-sheet microscope (Ultramicroscope II, LaVision Biotec) with a sCMOS camera (Andor Neo) and a 2x/0.5 objecive lens (MVLAPO 2x). Samples were imaged in the 640nm channel at 0.8x (coronal acquisition) and 0.63x (horizontal acquisition) using a 3μm step size.

Statistics

No statistics were used to predetermine sample size. However, our sample sizes were similar to those reported in previous publications. Statistical methods were indicated when it used, and statistical analysis of all figures are provided in Table S1. All analyses were performed using MATLAB (MathWorks) or Prism. No method of randomization was used in any of the experiments. For ANOVA analyses, the variances were similar as determined by Brown–Forsythe test. Experimenters were not blind to group allocation in behavioral experiments, but licks were measured automatically by custom software running on MATLAB (MathWorks). All animals that finished the entire behavioral training and testing were included in analysis. Data are presented as mean ± SEM.

Table S1. Statistical Analysis

Figure	n	Statistical Analysis
Fig. 1G	Big, small reward (n = 7, 7, 7) Big, small punishment (n = 6,6,5)	**Reward - CS:** One-way ANOVA: $F_{(2,18)} = 12.45$, $P < 0.001$ Post-hoc Tukey's test: $P_{(B.vs.S)} < 0.05$; $P_{(B.vs.N)} < 0.01$; $P_{(S.vs.N)} < 0.05$ **Reward - US:** One-way ANOVA: $F_{(2, 18)} = 14.07$, $P < 0.001$ Post-hoc Tukey's test: $P_{(B.vs.S)} < 0.05$; $P_{(B.vs.N)} < 0.01$; $P_{(S.vs.N)} < 0.01$ **Punishment - CS:** One-way ANOVA: $F_{(2, 14)} = 5.335$, $P < 0.05$ Post-hoc Tukey's test: $P_{(B.vs.S)} < 0.05$; $P_{(B.vs.N)} < 0.01$; $P_{(S.vs.N)} < 0.05$ **Punishment - US:** One-way ANOVA: $F_{(2, 14)} = 7.230$, $P < 0.01$ Post-hoc Tukey's test: $P_{(B.vs.S)} < 0.05$; $P_{(B.vs.N)} < 0.01$; $P_{(S.vs.N)} < 0.05$
Fig. 2B	CS, US responses across 5 training sessions	**CS-Responses:** Two-way ANOVA $F_{(15,60)} = 4.8$, $P < 0.0001$ Post-hoc Bonferroni test. * $P < 0.05$, *** $P < 0.001$ **US-Responses:** Two-way ANOVA $F_{(15,60)} = 15.03$, $P < 0.0001$ Post-hoc Bonferroni test. * $P < 0.05$,
Fig. 2D	Expected vs Unexpected Reward (n = 10) Expected vs Unexpected Punishment (n = 10)	Reward: Wilcoxon signed-rank test: $P = 0.32$ Punishment: Wilcoxon signed-rank test: $P = 0.49$
Fig. 2F	Expected vs Unexpected Reward (n = 31)	Reward: Wilcoxon signed-rank test: $P = 0.28$ Punishment: Wilcoxon signed-rank test: $P = 0.11$

	Expected vs Unexpected Punishment (n = 22)	
Fig. 3B	GFP group (n = 7) Arch group (n = 7)	Two-way ANOVA $F_{(1,72)} = 42.72$, $P < 0.001$ Post hoc Bonferroni test. $^{**}P < 0.01$
Fig. 3D	GFP group (n = 6) Arch group (n = 8)	Two-way ANOVA $F_{(1,72)} = 52.75$, $P < 0.05$ Post hoc Bonferroni test. $^{**}P < 0.01$
Fig.3E	Light On (n = 6) Light OFF (n = 6)	Wilcoxon signed-rank test: $P = 0.84$
Fig.3G	GFP group (n = 9)Arch group (n = 10)	Mann-Whitney U-test, $^{*}P < 0.05$
Fig.3H	GFP group (n = 9) Arch group (n = 10)	Two-way ANOVA $F_{(1,17)} = 18.01$, $P < 0.001$, Post hoc Bonferroni test, $^{**}P < 0.01$, $^{*}P < 0.05$
Fig.4C	Thirsty group (n = 7) Sated group (n = 7)	CS: Mann-Whitney U-test: $P < 0.001$, $P < 0.05$, $P = 0.57$ US: Mann-Whitney U-test: $P < 0.01$, $P = 0.055$, $P < 0.05$
Fig.5C	Air puff group (n = 6) Tail shock group (n = 6)	CS: Mann-Whitney U-test, $P < 0.01$ US: Mann-Whitney U-test, $P = 0.15$
Fig.5D	Air puff group (n = 6) Tail shock group (n = 6)	Air puff: One-way ANOVA $F_{(5,30)} = 0.67$, $P = 0.53$ Tail shock: One-way ANOVA $F_{(5,30)} = 4.7$, $P < 0.05$, Post-hoc Dunnett test: $P = 0.062$, 0.094, 0.031, 0.062, 0.031.
Fig.5G	CS: air puff (n = 85) vs tail shock (n = 62) US: air puff (n = 85) vs tail shock (n = 62)	CS: Mann-Whitney U-test, $P < 0.05$ US: Mann-Whitney U-test, $P < 0.01$
Fig.5H	Air puff group (n = 7) Shock group (n = 7)	Two-way ANOVA $F_{(1,60)} = 25.01$, $P < 0.001$ Post-hoc Bonferroni test. $^{**}P < 0.01$
Fig.6B	Expected reward: n = 10 Reward omission: n = 10	Wilcoxon signed-rank test, $P = 0.19$ Wilcoxon signed-rank test, $P < 0.01$
Fig.6D	GFP group (n = 6) Arch group (n = 10)	Mann-Whitney U-test, $P < 0.05$
Fig.6E	GFP group (n = 9) Arch group (n = 10)	Mann-Whitney U-test, $P < 0.05$
fig.S2A	Odor A group (n = 6) Odor B group (n = 6) Odor C group (n = 6)	Odor A: One-way ANOVA, $F_{(5, 55)} = 11.12$, $P < 0.001$ Odor B: One-way ANOVA, $F_{(5, 55)} = 3.57$, $P < 0.05$ Odor C: One-way ANOVA, $F_{(5, 55)} = 3.85$, $P < 0.05$ Post-hoc Dunnett test, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$
fig.S2B	D1 group (n = 8) D3 group (n = 8)	Day 1: One-way ANOVA, $F_{(7, 63)} = 9.68$ Day 3: One-way ANOVA, $F_{(7, 63)} = 4.83$ Post-hoc Dunnett, $^{*}P < 0.05$, $^{**}P < 0.01$

fig.S2C	Odor group (n = 6) Tone group (n = 6) Light group (n = 6)	Odor: One-way ANOVA, $F_{(9, 50)} = 2.66$, $P < 0.05$ Tone: One-way ANOVA, $F_{(9, 50)} = 2.18$, $P < 0.05$ Light: One-way ANOVA, $F_{(9, 55)} = 2.52$, $P < 0.05$ Post-hoc Dunnett test, * $P < 0.05$
fig.S3A	n = 7	Appetitive: One-way ANOVA, $F_{(4, 30)} = 15.67$, $P < 0.001$ Neutral: One-way ANOVA, $F_{(4, 30)} = 2.39$, $P = 0.07$ Aversive: One-way ANOVA, $F_{(4, 30)} = 1.29$, $P = 0.29$ Post-hoc Dunnett, *** $P < 0.001$
fig.S5B	n = 11	Wilcoxon signed-rank test, $P = 0.73$
fig.S5D	n = 20	Wilcoxon signed-rank test, $P = 0.43$
fig.S6A	GFP group (n = 5) Arch group (n = 6)	Two-way ANOVA, $F_{(5, 54)} = 0.24$, $P = 0.92$
fig.S6B	GFP group (n = 6) Arch group (n = 6)	Mann-Whitney U-test, $P = 0.59$
fig.S8C	GFP group (n = 6) Arch group (n = 6)	Two-way ANOVA $F_{(1, 60)} = 25.07$, $P < 0.0001$, Post-hoc Bonferroni test, ** $P < 0.01$.
fig.S8C Inset	n = 8	Light OFF: Mann-Whitney U-test, $P = 0.18$ Light ON: Mann-Whitney U-test, $P = 0.72$ Light OFF: Mann-Whitney U-test, $P = 0.16$
fig.S8E	GFP group (n = 7) Arch group (n = 8)	Two-way ANOVA $F_{(1,78)} = 1.06$, $P = 0.31$.

Supplementary Figure Legends

Fig S1. Estimated sites for fiber photometry, in vivo single-unit recoding and ArchT expression in the PVT.

(A) Example image (left) and estimated recording sites (right). Dash line: fiber track. **(B)** Example image (left) and estimated *in vivo* single-unit recording site (right). Red arrow head: the electrical lesion made at the end of the recording. **(C)** Example image (left) and estimated areas of ArchT expression for photoinhibition experiments (right). Dash line: cannula track. Scale bar: 500 μm.

Fig S2. Repetition suppression in the PVT is specific and persistent.

(A) PVT responses to ten presentations of odor A (blue) followed by ten presentations of odor B (green), then odor C (red). Top, Representative photometric traces. Scale bar, 10% ΔF/F, 3 s. Bottom, Quantification of odor evoked ΔF/F over 10 repetitions. n = 6. Odor A: One-way ANOVA, $F_{(5, 55)} = 11.12$, $P < 0.001$, Post-hoc Dunnett, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$; Odor B: One-way ANOVA, $F_{(5, 55)} = 3.57$, $P < 0.05$, Post-hoc Dunnett, *$P < 0.05$; Odor C: One-way ANOVA, $F_{(5, 55)} = 3.85$, $P < 0.05$, Post-hoc Dunnett, **$P < 0.01$, *$P < 0.05$. **(B)** PVT responses to ten presentations of the same odor separate by two days. n = 8. Day 1: One-way ANOVA, $F_{(7, 63)} = 9.68$, $P < 0.01$, Post-hoc Dunnett, **$P < 0.01$, *$P < 0.05$; Day 3: One-way ANOVA, $F_{(7, 63)} = 4.83$, $P < 0.01$, Post-hoc Dunnett, *$P < 0.05$; (**C**) PVT responses to ten presentations of odor (blue) followed by ten presentations of tone (green), then LED light (red). Top, Representative photometric traces. Scale bar, 10% ΔF/F, 3 s. Bottom, Quantification of odor evoked ΔF/F over 10 repetitions. n = 6. Odor: One-way ANOVA, $F_{(9, 50)} = 2.66$, $P < 0.05$, Post-hoc Dunnett, **$P < 0.01$, *$P < 0.05$; Tone: One-way ANOVA, $F_{(9, 50)} = 2.18$, $P < 0.05$, Post-hoc Dunnett, *$P < 0.05$; Light: One-way ANOVA, $F_{(5, 50)} = 2.52$, $P < 0.05$, Post-hoc Dunnett, *$P < 0.05$. Error Bars, SEM.

Fig S3. Pavlovian conditioning and GFP control for fiber photometry.

(A) Representative lick raster plots (top) and histograms (bottom) across 5 conditioning sessions (D1 to D5, n = 7). Back lines indicate the start and end time for odor delivery, respectively. Red line indicates water delivery. The thirsty mice were trained in head-fixed configuration to associate odors with either water reward, nothing, or air puff. The mice gradually showed anticipatory licking following reward prediction cue over 5 training sessions. Reward: One-way ANOVA, $F_{(4, 30)} = 15.67$, $P < 0.001$, Post-hoc Dunnett, ***$P < 0.001$;

Nothing: One-way ANOVA, $F_{(4, 30)} = 2.39$, $P = 0.07$; Punishment: One-way ANOVA, $F_{(4, 30)} = 1.29$, $P = 0.29$. Data are means ± SEM. **(B)** GFP control for fiber photometry recording (n = 5). Shade: SEM across mice. The mice were stereotaxically injected with AAV-eGFP and were implanted with optical fiber into the PVT. After 3 weeks of recovery from surgery and GFP expression, the mice were trained with 5 sessions of Pavlovian conditioning to associate odor with water reward. We then recorded PVT activity of these mice with fiber photometry while they performed reward-seeking task. Note minimal fluorescent change was observed when animals were actively licking.

Fig S4. No licking-related spike activity in the PVT during behavior.

(A) Left: Representative lick raster plots (top) and histogram (bottom). Right: spikes raster plots (top) and peristimulus time histograms of spikes (bottom). For each cell we computed the Spearman correlation coefficient between single-trial spike activity and licking activity. Mean (CC) was averaged correlation coefficient across 30 trials. Gray bar: 1s of CS delivery; Vertical dash line: US delivery. **(B)** Histogram denotes distribution of mean (CC) of 147 neurons from 12 mice (red). Neurons that have significant correlation ($P < 0.01$) between spikes and licks was shown in blue. **(C)** Mean spikes probability when align spike trains to each lick. Note no significant change of spike probability around each lick.

Fig S5. PVT doesn't encode reward prediction error (related to Fig.2, E and F).

Neurons in Fig. 2, E and F were divided into two subgroups based on whether or not its CS responses retune to baseline during the delay period. **(A)** Mean Z score of the PVT neurons' responses to expected and unexpected delivery of reward from 11 neurons which their CS responses retune to baseline during delay period. **(B)** Quantification of Z score in **A**. Wilcoxon signed-rank test: $P = 0.73$, n = 11. **(C)** Mean Z score of the PVT neurons' responses to expected and unexpected delivery of reward from 20 neurons with persistent CS responses during delay period. **(D)** Quantification of Z score in **C**. Wilcoxon signed-rank test: $P = 0.43$, n = 20. Gray bar: 1s of CS delivery; Vertical dash line: US delivery; Scale bar: 1s in **A, C**. Data are means ± SEM. Shade, SEM across mice in **A, C**.

Fig S6. Inhibiting PVT activity during inter-trial interval (ITI) has no effect on reward learning and extinction learning.

(A) Laser delivered for 3 sec during ITI has no effect on anticipatory licks across 5 conditioning sessions and the NL test during Pavlovian reward learning from PVT :: GFP (n = 5) and PVT :: ArchT (n = 6) mice. NL, non-laser. Two-way ANOVA, $F_{(5, 54)} = 0.24$, $P = 0.92$. **(B)** Representative anticipatory licks in 30 extinction trials from PVT :: GFP (left) and PVT :: ArchT (right) mice with laser delivered for 3 sec during ITI. Licks are normalized to Pre-extinction trials. Inset, histogram shows the mean time constants (τ) of extinction from PVT :: GFP (white, n = 6) and PVT :: ArchT (green, n = 6) mice. Mann-Whitney U-test, $P = 0.59$.

Fig S7. Example lick raster plots for GO/No-go task.

(A) Representative lick raster plots (top) and histograms (bottom) from PVT :: GFP mice in go (left) and no-go (right) trials across 4 days of conditioning sessions (D1-D4) and the NL test. **(B)** Representative lick raster plots (top) and histograms (bottom) from PVT :: ArchT mice in go (left) and no-go (right) trials across 4 days of conditioning sessions (D1-D4) and the NL test. NL, non-laser. Laser was delivered during CS + delay period (green shade).

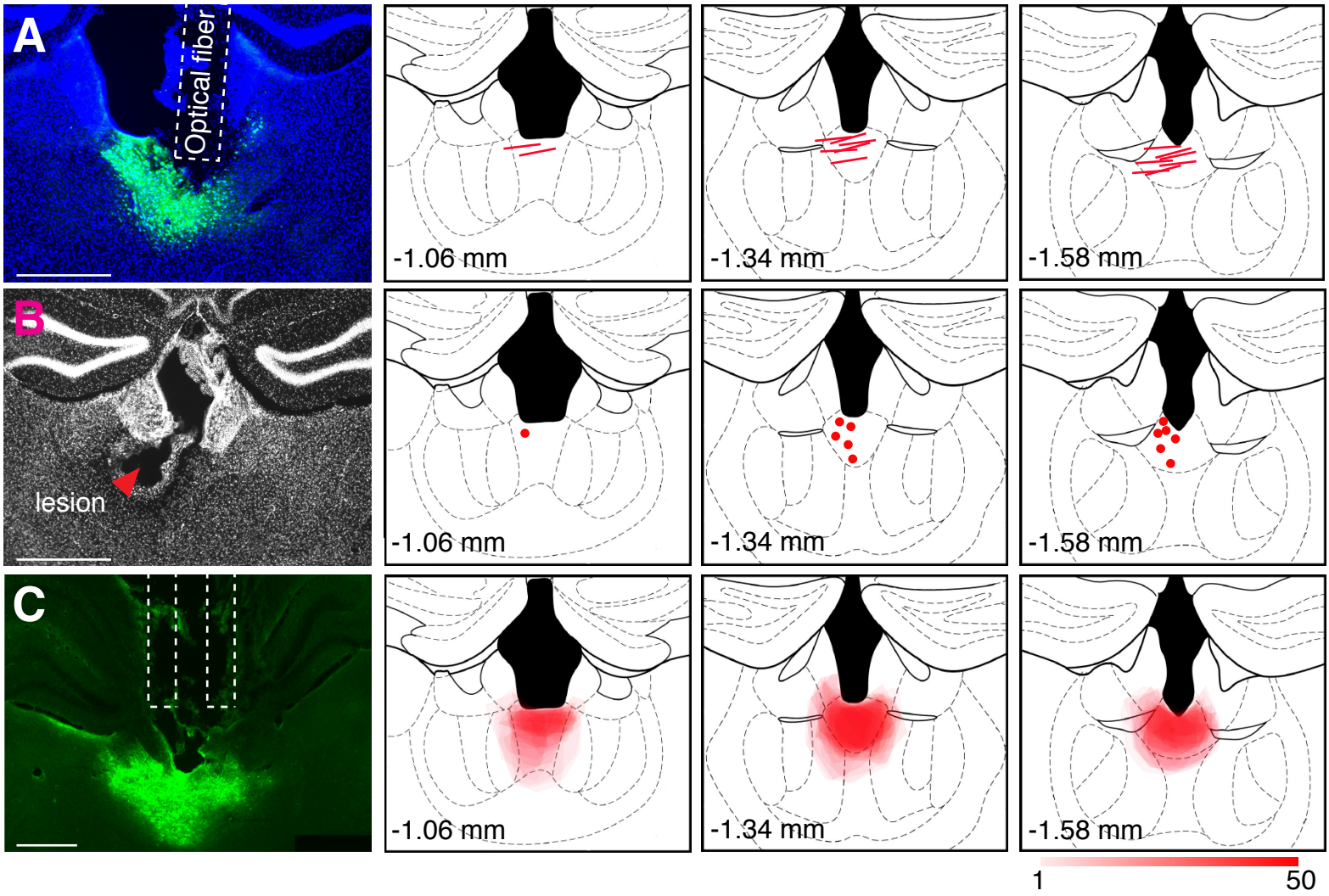
Fig S8. Effects of suppressing PVT activity on aversive learning.

(A) Two phases Pavlovian aversive conditioning procedure. **(B)** Representative lick raster plots (top) and histograms (bottom) from PVT :: GFP (left) and PVT :: ArchT mice (right) across 5 phase 2 conditioning sessions (D1-D5) and the NL test. NL, non-laser. Laser was delivered during CS + delay period (green shade). **(C)** Quantification of anticipating licks in phase 2. PVT :: GFP mice, n= 6; PVT :: ArchT mice, n= 6. Two-way ANOVA $F_{(10,60)} = 25.07$, $P < 0.0001$, Post-hoc Bonferroni test, ** $P < 0.01$. Inset, Quantification of tail flick latency from PVT :: ArchT mice with laser off and on. n = 8, water temperature 52.5 °C. Mann-Whitney U-test, $P = 0.18$ (left, off), $P = 0.72$ (middle, on), $P = 0.16$ (right, off). **(D)** Representative lick raster plots (top) and histograms (bottom) from PVT :: GFP (left) and PVT :: ArchT mice (right) across 5 phase 2 conditioning sessions (D1-D5) and the NL test. NL, non-laser. Laser was delivered after US period (green shade). **(E)** Quantification of anticipating licks in phase 2. PVT :: GFP mice, n = 7; PVT :: ArchT mice, n = 8. Two-way ANOVA $F_{(1,78)} = 1.06$, $P = 0.31$.

Fig S9. Extensive axonal collateralization in the PVT.

(A) Schematics for visualizing the axonal collateralization in the PVT. The mice were injected with 200nL AAV-DIO-ChR2-mCherry unilaterally in the PVT and 150nL rAAV2-retro-Cre in the central nucleus of amygdala (CeA). This intersectional strategy specifically labels PVT neurons that innervate the CeA with the ChR2-mCherry fusion protein. 4 weeks later, brains

of these mice were collected and subjected to iDISCO clearance process and whole mount immunostaining against mCherry. The brains were imaged using light-sheet microscope and reconstructed for 3D visualization. **(B)** Representative images show extensive collateralization of axons from PVT-CeA projecting neurons that also innervate ACC, PFC, NAc, OT, IC, COA, LH, DMH. ACC, anterior cingulate cortex; PFC, prefrontal cortex; OT, olfactory tubercle; IC, insular cortex; COA, cortical amygdala; LH, lateral hypothalamus; DMH, dorsomedial hypothalamus. Scale bar, 1 mm.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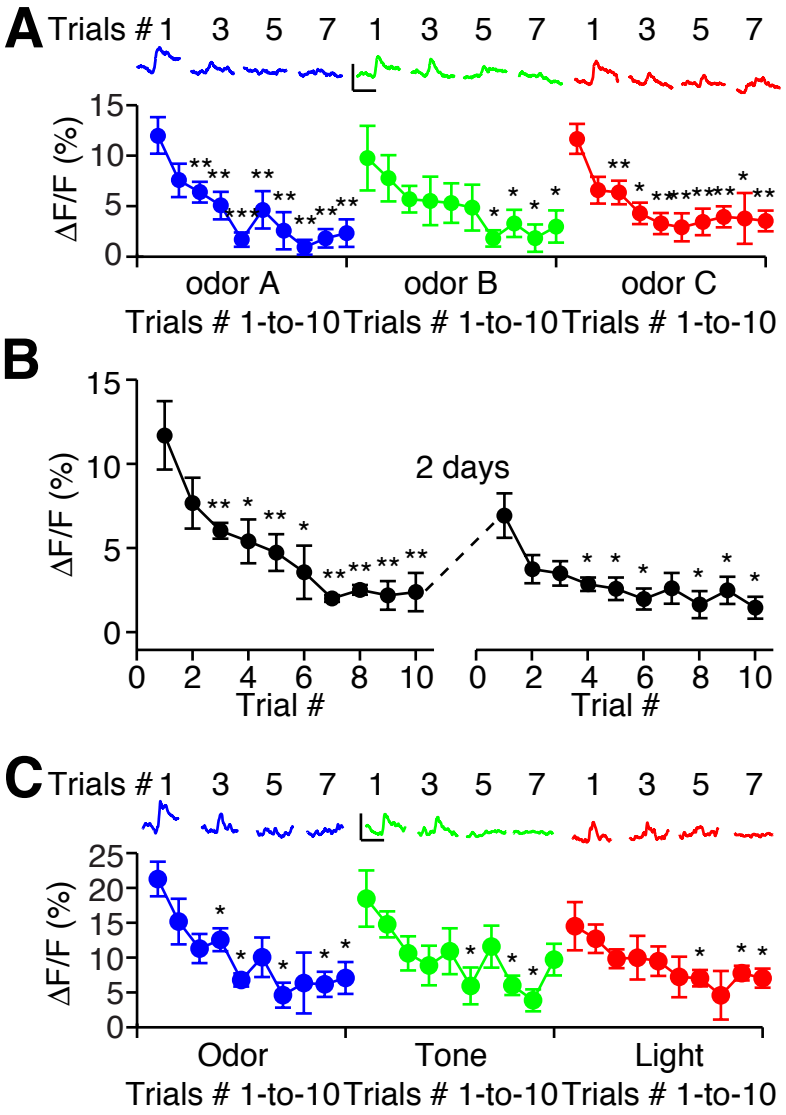
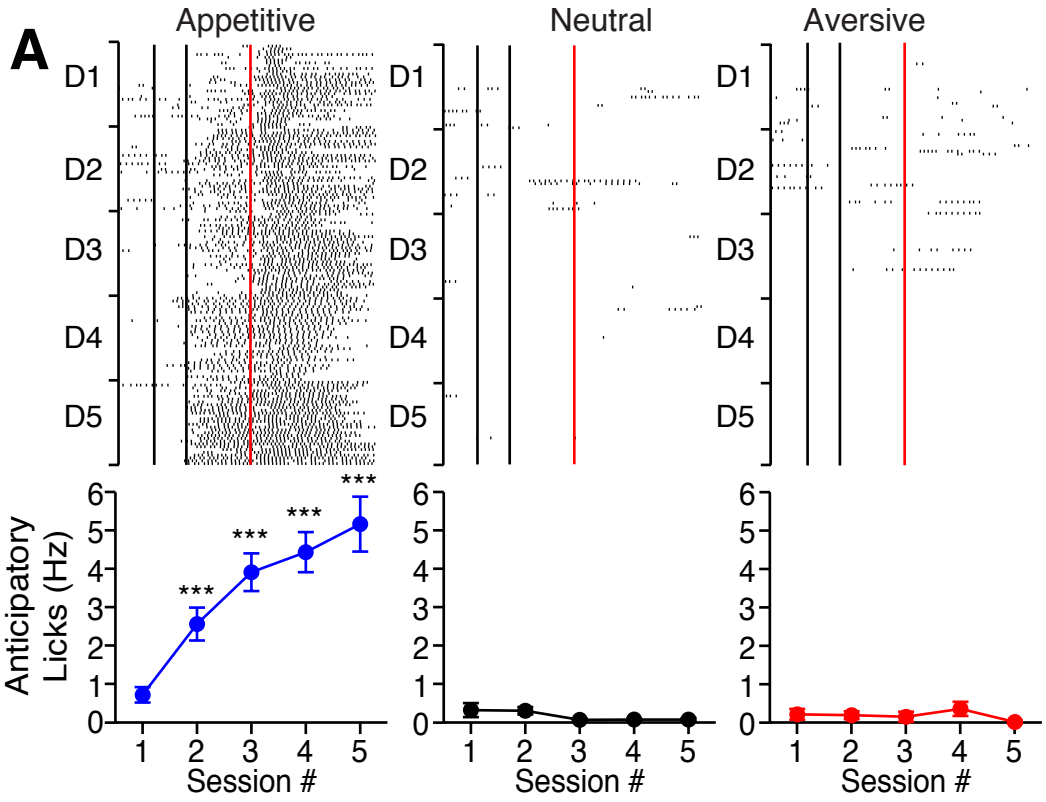
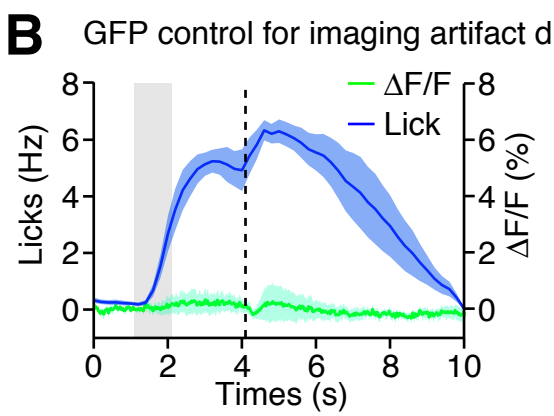
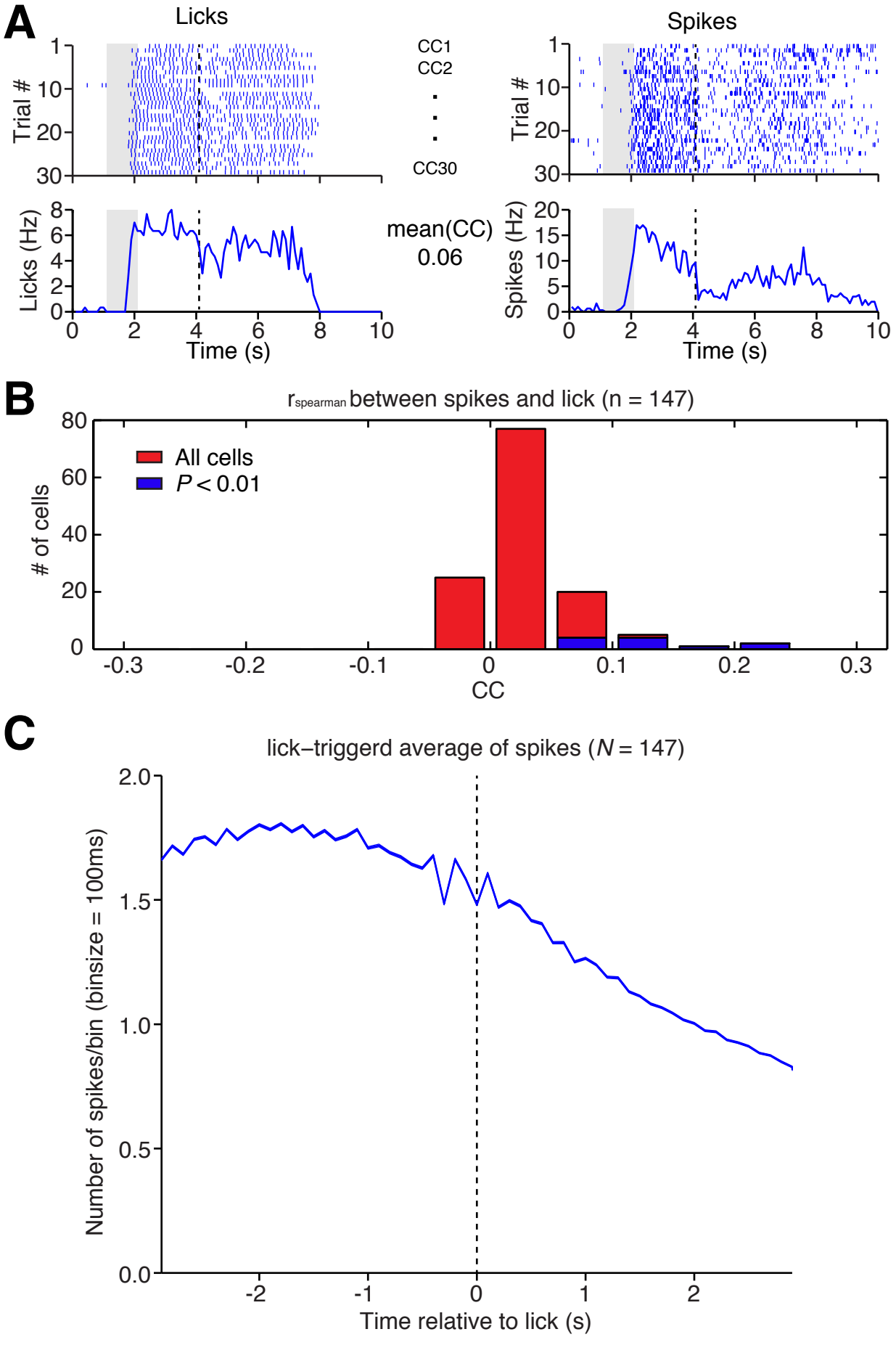
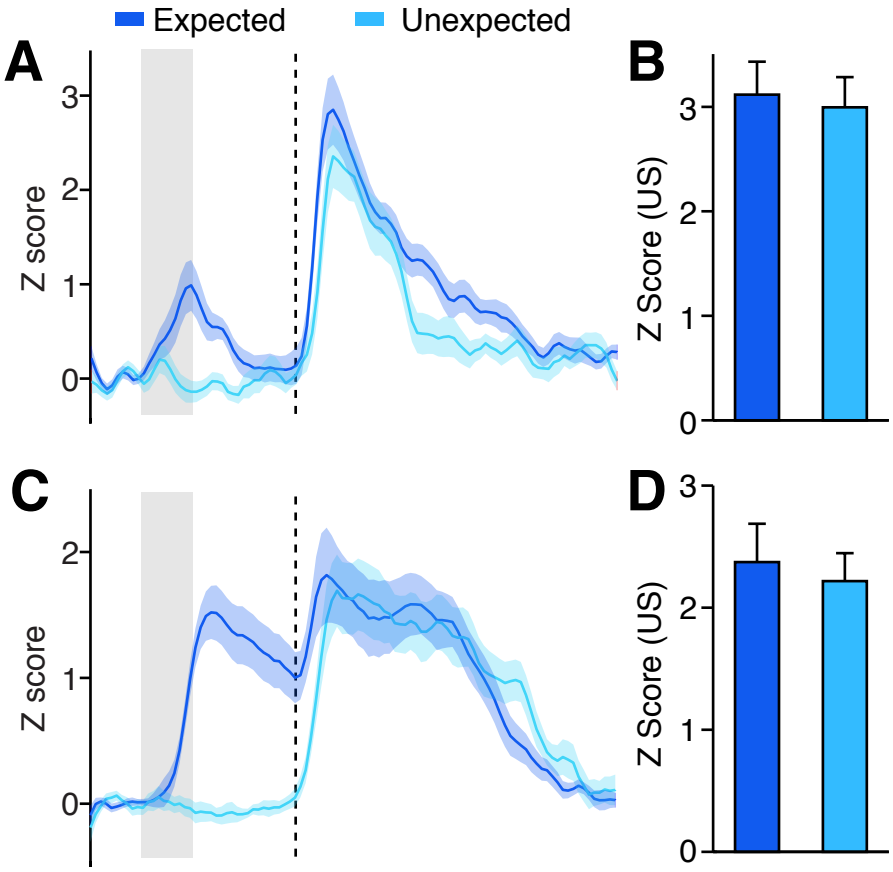
1
50

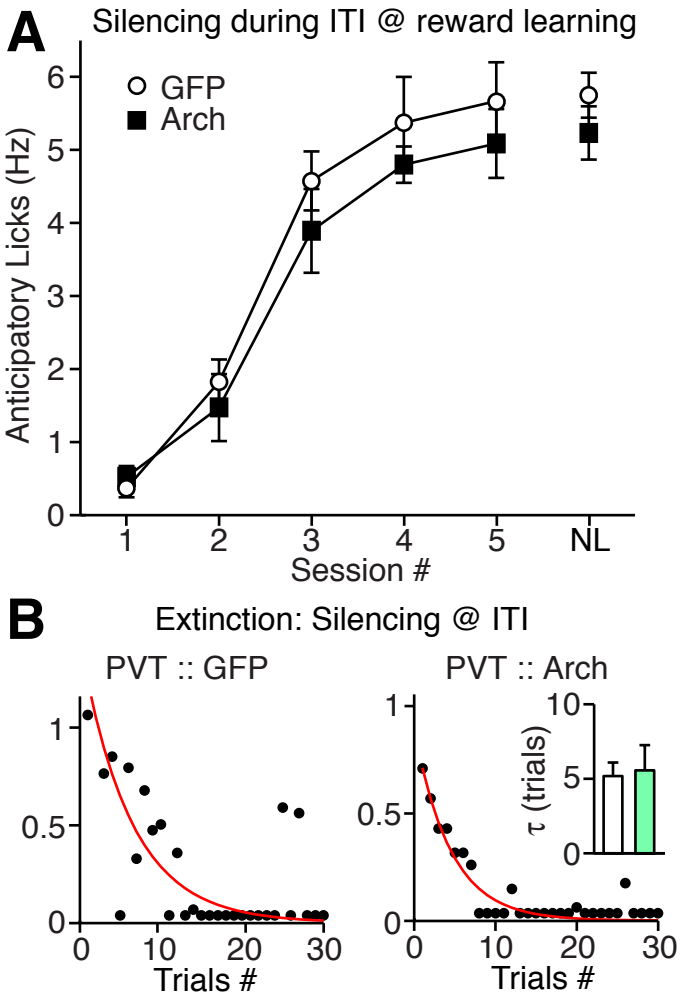

A
Trials # 1 3 5 7 1 3 5 7 1 3 5 7
ΔF/F (%)
15
10
5
0
odor A
odor B
odor C
Trials # 1-to-10 Trials # 1-to-10 Trials # 1-to-10
B
ΔF/F (%)
15
10
5
0
2 days
0 2 4 6 8 10
Trial #
0 2 4 6 8 10
Trial #
C
Trials # 1 3 5 7 1 3 5 7 1 3 5 7
ΔF/F (%)
25
20
15
10
5
0
Odor
Tone
Light
Trials # 1-to-10 Trials # 1-to-10 Trials # 1-to-10


A
Appetitive
Neutral
Aversive
D1
D2
D3
D4
D5
Anticipatory Licks (Hz)
***
Session #


B
GFP control for imaging artifact during licking
ΔF/F
Lick
Licks (Hz)
ΔF/F (%)
Times (s)

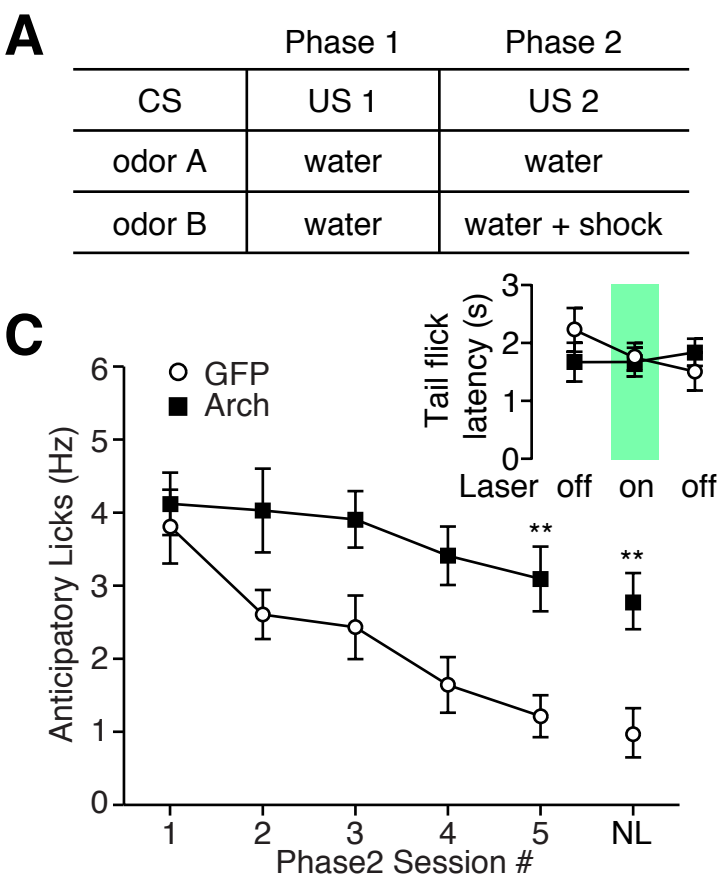

A
Licks
Spikes
Trial #
CC1
CC2
CC30
mean(CC)
0.06
Licks (Hz)
Spikes (Hz)
Time (s)
B
$r_{spearman}$ between spikes and lick (n = 147)
All cells
P < 0.01
# of cells
CC
C
lick–triggerd average of spikes (N = 147)
Number of spikes/bin (binsize = 100ms)
Time relative to lick (s)

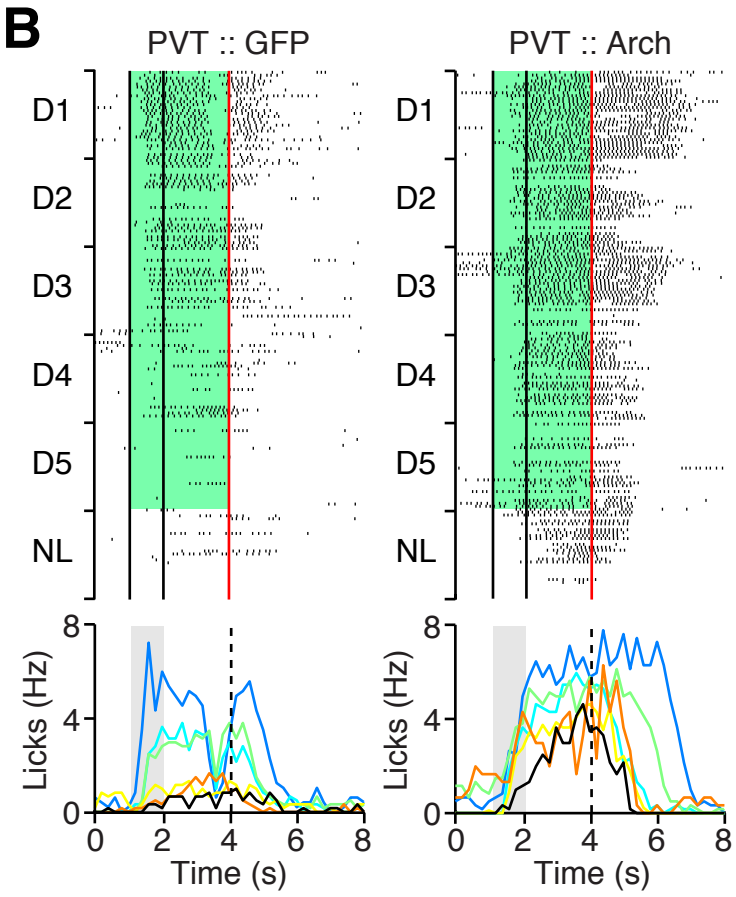

Expected
Unexpected
A
Z score
3
2
1
0
B
Z Score (US)
3
2
1
0
C
Z score
2
1
0
D
Z Score (US)
3
2
1
0

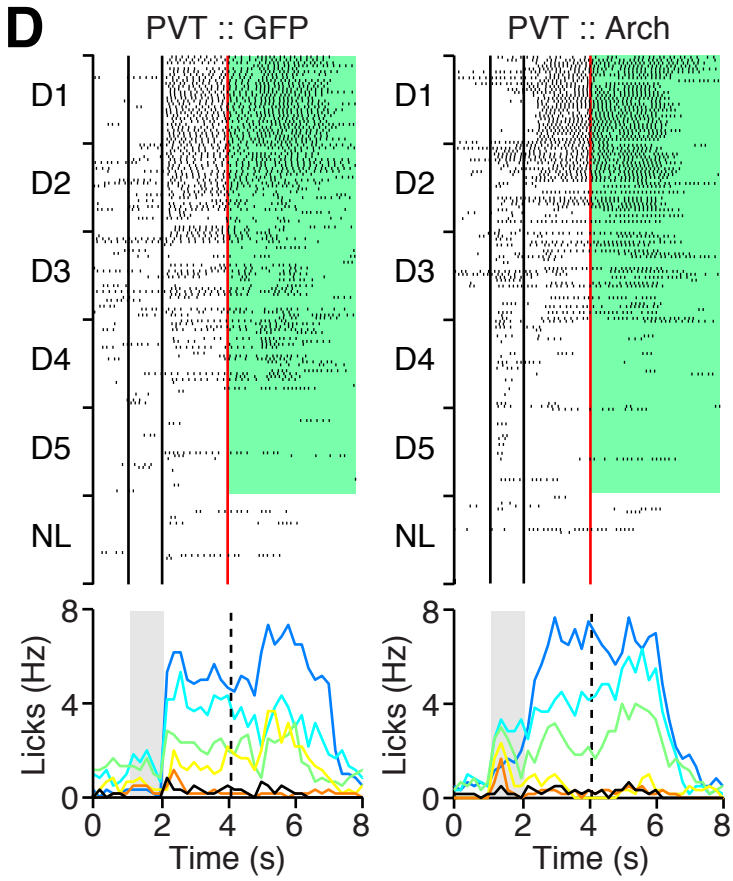

A
Silencing during ITI @ reward learning
GFP
Arch
Anticipatory Licks (Hz)
0
1
2
3
4
5
6
1
2
3
4
5
NL
Session #
B
Extinction: Silencing @ ITI
PVT :: GFP
PVT :: Arch
0
0.5
1
0
10
20
30
Trials #
τ (trials)
0
5
10

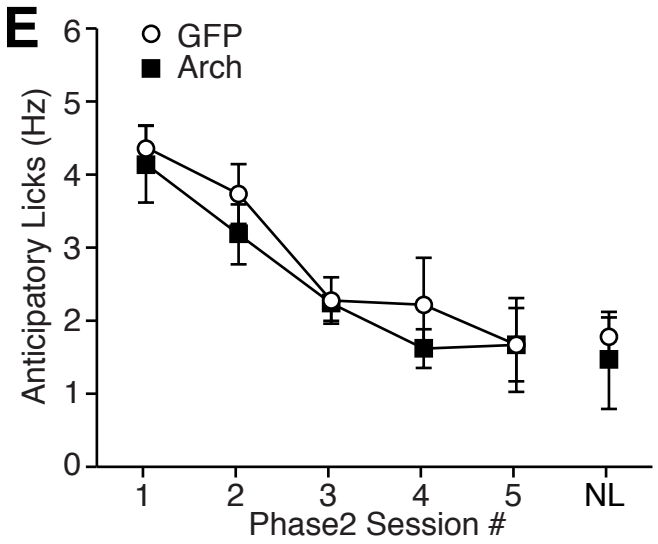
A

	Phase 1	Phase 2
CS	US 1	US 2
odor A	water	water
odor B	water	water + shock









DIO-ChR2-mCherry

retro-AAV-cre