nature portfolio

Peer Review File

Competing valence-related roles of dopamine in the tail of the striatum

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This manuscript has been previously submitted at another journal. This document only contains information relating to versions considered at Communications Biology.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The manuscript studies competing valence learning using a dual valence Pavlovian task, fiber photometry in striatum, and optogenetic manipulations. The approach is timely and the dataset is rich. Several analysis and presentation choices limit interpretability, mainly variability and potential non learners, across day averaging that masks learning dynamics, recording site heterogeneity, incomplete alignment between dopamine signals and behavior, and limited statistical information.

Issues and recommendations:

1. Variability and possible non learners in Fig. 2
Anticipatory measures show large inter animal variance across sessions, which suggests that some subjects may not have learned the task. Please pre specify an inclusion criterion for learners and apply it. For example, require a significant positive within subject slope of anticipatory licking during days 3 to 5, and or a minimum CS minus baseline effect size on late training. Report the fraction of animals excluded and repeat key analyses with and without non learners.

2. Correlation figures
For all correlation plots, place the exact statistics on the axes. Report r, the exact p value, and a 95 percent confidence interval. If feasible, add a bootstrap confidence band for the regression line. This avoids significance by legend and improves transparency.

3. Averaging across five training days for photometry
Pooling days 1 to 5 risks washing out learning related signals such as shifts from US locked to CS locked responses and day to day decay. Please add day resolved PSTHs or heatmaps for key dopamine signals and the matching behaviors, or at minimum early, middle, late bins within each phase. Keep the split between avoidance days 1 to 2 and adaptation days 3 to 5, but show the within phase time course instead of a single average.

4. Recording site heterogeneity
Supplemental placement maps include fibers in ventral striatum, septum adjacent regions, and dorsolateral striatum in addition to tail and anterior striatum. Treating these as one pool can mix functionally distinct territories. Please provide subregion specific analyses, at minimum posterior tail of striatum versus anterior tail versus anterior striatum versus other sites. Add a tail of striatum only sensitivity analysis for the main results and report counts per subregion. Given the gradient reported along the anterior to posterior axis, this is essential.

5. Show behavior alongside photometry
Wherever dopamine signals are analyzed, show the matching behavioral PSTH or summary metric from the same trials and sessions, blink or lick as appropriate. This will let readers align neural and behavioral changes in time and magnitude.

6. Test for temporal difference like backpropagation explicitly
If the narrative proposes a shift in prediction from US to CS, analyze it directly. Plot trial by trial acquisition curves for

behavior and dopamine on the same axis. Test whether US responses decrease as CS responses increase or retime early in training. A simple GLM with CS, US, expected value, and prediction error regressors, or a lagged regression of dopamine at trial t on behavior at trial t and behavior at trial t plus 1, would clarify directionality. This analysis also argues against pooling days 1 to 5.

7. Optogenetic effects in Fig. 7
Add behavioral PSTHs for blink and lick aligned to CS and US for control versus stimulation, ideally split into early, middle, and late training. Show per animal slopes in a supplement for transparency. This will reveal whether manipulations change the timing or shape of responses, not only their overall magnitude.

Overall evaluation
The study tackles an important question with a strong task and valuable recordings. Addressing variability and learner status, analyzing regions separately, avoiding across day pooling, aligning behavior with photometry, and enhancing statistical transparency will substantially improve interpretability. The suggested additions are feasible within the current dataset and will make the claims more precise without changing the overall story.

Reviewer #2

(Remarks to the Author)
In this manuscript, Tsuruga et al., investigated how the dopaminergic activities in the tale of the striatum control the competition between aversive and appetitive learning processes. To tackle this question, they investigated how these two learning processes develop in their 2-VCC task and found animal's gradual adaptation to aversive air puff-predicting cue as well as co-occurrence of conditioned appetitive licking behavior. Next, they recorded dopamine release using GRAB sensor and showed larger response of TS-DA to air puff CS compared to that of appetitive water CS, and the decline of dopaminergic response of TS depending on the conditioning process. They then focused on the possible regulatory role of US-evoked TS-DA response in the behavioral desensitization to air puff CS. By combining optogenetic manipulation of SNL DANs and recording of TS-DA, they mimicked natural aversive response of TS-DA and found this optogenetic excitation during air puff US period was sufficient to prevent development of the desensitization to air puff CS suggesting the decline of US-evoked TS-DA causally triggers the adaptive leaning.
Methodology and statistics are sound. The results are somewhat interesting. However, there are several issues that largely limit the conceptual advancement of this study. Therefore, I would not support this manuscript for publication in Communications Biology. The details are as follows.

Major issues:
(1) The results and conclusions are mostly replications of previous results about TS-DA. As the authors mentioned, it has been already reported that aversive CS and US evoked the increase of TS-DA [1,2]. Also, the ablation of TS-DA prevents adaptation to initially aversive novel object [3] and abolished the avoidance from aversive stimulus in the conflicting context between appetitive and avoidance behaviors [4] also suggesting the regulatory role of TS-DA in avoidance behavior under the context of reward-punishment competition. These results largely limit the conceptual advancement of the current study.

(2) Related to (1), the rationale, objective and conclusion of this study are vague. The authors need to clearly explain what has been done already, and what they have found for the first time and why it is important in the manuscript.

(3) The authors tried to investigate the competition of parallel learning processes (i.e. aversive learning during Day1-2 and adaptation to puff/appetitive learning during Day 3-5). However, they did not show whether each learning process was actually the result of the competition between aversive and appetitive learning. It is necessary to show that (i) increase or decrease of the intensity of air puff and/or the reward size facilitate or slowdown each learning process, (2) or the proportion of the number of rewarding, aversive and null trials (currently it's 3:1:3) flexibly impacts on the development of each learning process.

(4) In addition to optogenetic gain-of-function experiment, loss of function experiment such as ablation of TS-DA is necessary to support their conclusions.

(5) There are a lot of redundancies in the current version of manuscript. For example, I do not think Fig.1D,E,F,G and Fig.3B,C,E,F are necessary because the authors eventually showed that behaviors and dopamine responses could change following multiple training sessions. They might want to move these figures to supplement.

(6) The manuscript lacks important analyses.
(i) Dynamics of TS-DA during null trials should be analyzed and discussed like Fig.4
(ii) In addition to Fig.7G,H,I,J, PSTHs and trial-by-trial change of blinking and licking should be analyzed like Fig.2.
(iii) The authors analyzed regression slope of behavioral and dopamine change, but I prefer and it is standard to analyze daily changes of those indexes. Actually, when they analyzed regression slope, there are no changes of TS-DA during appetitive CS and US (Fig.4 F-H) but according to PSTHs (Fig.4E), there are apparently the day-by-day reductions of TS-DA during both CS and US periods.

(7) I do not think lines 82-83 is correct because appetitive learning is not supported by statistics (Fig.1G).

Minor issues:
(1) There is a typo in the title of Fig.S1. It should be anticipator"y" licking
(2) In the abstract (line 3-4), "Understanding how the brain evaluates aversion and appetition to guide behavior remains an open question." sounds like lack of respect to previous studies addressing this theme. This sentence should be modified.
(3) Overall, the texts in the figures are very small. The authors should make them lager.
(4) The authors use small icons to indicate each trial conditions (e.g. Fig.1D-G) but I do not think this is reader-friendly. They should use letters (i.e. Water, (Air) Puff, Null).
(5) The number of animals used are relatively small.

References
[1] Menegas, W., Babayan, B. M., Uchida, N. & Watabe-Uchida, M. Opposite initialization to novel cues in dopamine signaling in ventral and posterior striatum in mice. eLife 6,785 e21886 (2017).
[2] Zafiri, D., Salinas-Hernández, X. I., De Biasi, E. S., Rebelo, L. & Duvarci, S. Dopamine prediction error signaling in a unique nigrostriatal circuit is critical for associative fear learning. Nat. Commun. 16, 3066 (2025).
[3] Menegas, W., Akiti, K., Amo, R., Uchida, N. & Watabe-Uchida, M. Dopamine neurons projecting to the posterior striatum reinforce avoidance of threatening stimuli. Nat. Neurosci. 21, 1421–1430 (2018).
[4] Tsutsui-Kimura, I. et al. Dopamine in the tail of the striatum facilitates avoidance in threat–reward conflicts. Nat. Neurosci. 28, 795–810 (2025).

Reviewer #3

(Remarks to the Author)
The manuscript seeks to understand the role of the tail of the striatum in modifying behavior as animals learn to discriminate between conditioned aversive and appetitive stimuli. Specifically, the authors utilized a head-fixed paradigm where animals experienced unique odors associated with either a puff of air to the eye, delivery of water via solenoid, or no outcome. During the task, a subset of animals were also fixed to record dopamine dynamics in the tail of the striatum with additional animals also prepared for driving dopamine neurons projecting to the tail of the striatum. Animals exhibited an initial strong response to cues associated with the air puff but not to the water. Across days, the anticipatory responses to the puff decreased while the anticipatory licking for the water increased. Dopamine responses to CS and US aversive conditions decreased over days in line with behavior. However, if driven in a way that matched signal intensity due to actual puffs, the anticipatory blinking did not decrease across days. This suggests that disruption of normal dopamine signaling is sufficient to disrupt adaptations that typically occur to this aversive signal across days. In contrast, dopamine signaling in the tail of the striatum was not strongly modulated by the appetitive CS or US although it does appear to show significant response on the first subset of trials on a given day. Taken together, these results suggest that disruption of normal dopamine signaling in the tail of the striatum results in disrupted adaptive processes in response to repeated aversive, but not appetitive CS/US pairings.
Overall, the paper demonstrates an interesting approach to try to determine the specifics of dopamine dynamics in the tail of the striatum. Prior work has strongly linked this area to avoidance learning although the role in appetitive learning has remained uncertain. In this regard, the present manuscript adds important additional information to understanding the role of this subregion of the striatum in learning more generally. However, there are several concerns I have with the methodology and statistical approach to the data. These are outlined below:
1. Several of the box plots do include outliers as indicated by the whiskers not extending to them. How were outliers defined and were they used in statistical analysis? If not, what was the basis for exclusion or transformation?
2. In Figure 3, please remove the white boxes on panels A and D. It would allow a more accurate assessment of placement of the fiber in relation to the viral expression.
3. I am somewhat confused by the outcome variables in Figure 2 I-J. Specifically, what constitutes the datapoints on the graphs. I see nine points on each of the graphs but I'm unsure where those regression slopes come from. Please expand the explanation of these derived measures as it isn't clear if these are per animal, per day, or how they were calculated.
4. In general, I am curious why regression slope was chosen more generally as a derived measure for an outcome variable across the experiments. Lick rate and change in eye area would seem to be a sufficient measure to look at behavior as it changes across days. I am curious about the justification for using the regression slope as my intuition is that they don't meet many of the normal assumptions of statistical analysis. What, for instance, would be the normal distribution for regression slopes? Further, it seems to not really capture between day learning because it would be susceptible to the starting point more than just the average lick rate within a day or similar for the eye area. Wouldn't a within day average of the raw datapoints be a more accurate measure of the amount of anticipatory behavior rather than the change in the behavior within that day? Furthering this point, the authors seem to use various ways to describe the meaning of the measure that don't fully overlap. For example, in the figure legend for figure 7, it is described as, "Anticipatory licking was developed in the control…" for a significant difference. Where at other times it is described as "dynamically changing" or other terms.
5. It is unclear what is meant on lines 216 to 221 in regards to the CSpuff response. None of the measures in the supplemental figure indicated significant differences across any measured periods. There is certainly a trend but to be accurate, it should be described in the text as a non-significant trend rather than a "modest increase."
6. In figure 7 it appears that there is anticipatory eye blink behavior occurring in control animals. In figure 2 there is a significant decrease in the area measure that remains across all days of the task, although it reduces. Why is it that the animals in G and H don't differ from 0? This would seem to be concerning suggesting that even on day one they are not exhibiting an eyeblink response. In addition, it is unclear why the authors switch between lick rate, the area, and regression slope in these figures as well. If this is justified, it should be included in the text.
In summary, my main concerns are related to the behavioral measures and how they relate to day-to-day learning given the seemingly random difference in starting rate on a given day. In addition, it appears that there are differences in behavior

between rats that underwent injections for optogenetic manipulation and control, and those in the prior experiments that just had dopamine measured in the striatum.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The Authors addressed all of my concerns.

Reviewer #2

(Remarks to the Author)
The authors have carefully addressed my previous comments with substantial additional experiments and analyses. The manuscript has significantly improved and now meets the standard for publication in Communications Biology.

Reviewer #3

(Remarks to the Author)
The authors did an admirable job in addressing the comments raised by all three reviewers. In many cases, the comments were satisfactorily addressed resulting in a much-improved manuscript. However, several responses did not fully address concerns and result in remaining important unresolved issues. These are outlined below:

1. The response to the question regarding non-learners from reviewer one, combined with the question from reviewer three regarding the optogenetic experimental controls raises an important additional question. Specifically, it isn't clear that the same learner analysis was applied to the optogenetic experiment. Based on the data in 5N, it would appear that the majority of those animals would be considered non-learners, even with the relatively conservative cutoff of <0 applied by the authors. A one-way t-test of both groups would likely show, as groups, that both controls and opto animals are not significantly responding to the CS in day 1 or in the case of controls, in days 3-5. This makes this group, in particular, difficult to assess when it is clear animals generally are not performing the task. In fact, the same could likely be said with anticipatory licking as well. A one-way t-test might indeed show that as a group, they would not differ from 0 Hz (the exception being the 2 mice showing a rather high lick rate. The fact that the additional analysis of the DA ablation shows that animals were again showing responses on day one suggests something majorly confounding is going on in the procedures related to the optogenetic experiment.

2. Related to this, with the more complete explanation of animals used in the various experiments which utilized the task, one possibility for the difference in anticipatory blinking in the initial vs. optogentetic experiments is a possible difference between male and female mice in the task. The initial task only includes one female while the subsequent experiments utilized more balanced groups. Female mice have been found to show increased sensitivity to eye-blink conditioning. (PMID: 19272397) This would stand in contrast to the present finding with the largely male group showing increased sensitivity, but it could stand to reason that sex differences may be at play and that is one explanation for the present differences. However, this would be contradicted by the ablation experiment. Across groups, an analysis of sex differences in the task should be performed to account for this. Noted sex differences should also be mentioned in the limitations/discussion.

3. On a more minor note, I would argue that animals that did not adapt by reducing eye-blink measures on days 3-5 should still be counted as learners. They clearly demonstrate an association between the CS and US which by any definition would be considered learning. It could be the case that some animals are more sensitive than others to the air puff and thus are less likely to adapt to it for any number of reasons but have still learned the association.

4. The additional analysis of the data without non-learners should be contextualized in the manuscript. Specifically, it shouldn't be left to the reader to sort out whether any differences in results occurred because of not including non-learners in the analysis.

5. As mentioned as well, the definition of learners is very conservative in the subsequent analysis. Perhaps a better comparison would be in relation to the null trials. That way, random licks or blinks that would make the slopes greater or less than 0 would be accounted for in relation to meaningful CS experiences. This would seem to be a less arbitrary definition. It would also be helpful to see null responses in behavior figures to be able visualize learning in the meaningful CS experiences. Finally, it could support sentences claiming evidence of learning in lines 79-89 to show significant behavior vs. the null trials.

6. When comparing behavior in the ablation study, please utilize statistical analysis in the description of the results (lines 210 – 225). A direct comparison of change in eye area and lick rate between groups would determine whether learning was significantly different. This analysis should be done in addition to the analysis on slope included in the manuscript. However, one concern here is that striatal dopamine is closely associated with behavioral selection and coordination more generally. It

should be noted in the text that at least in the case of licking, however, ablation animals do eventually achieve similar behavior. However, the tail of the striatum has been associated with saccade behavior and thus might have a larger impact on eye blink as well (PMID: 22875934).

7. In your discussion of the optogenetic experiments, the lack of behavior in control animals shown in figures 5 and S7 are not accounted for beyond the limitations section. Any interpretation of these results must account for the fact that the control animals in this experiment did not behave similarly to other animals in the rest of the study. This makes comparisons between the optogenetic experiments and the other two experiments impossible to make.

In summary, the concerns that remain, in addition to several raised when accounting for prior concerns, result in a recommendation for further revision prior to publication. Specifically, using raw data reveals additional insight into animal behaviors beyond what the slope of learning could account for. It also reveals concerns about the optogenetic controls that make comparisons to other experiments invalid based on different baseline behavior exhibited by controls. Finally, additional straightforward analysis of behaviors will better account for learning and an understanding of how it proceeds across the experiment.

Version 2:

Reviewer comments:

Reviewer #3

(Remarks to the Author)
I would like to thank the authors for carefully addressing the additional concerns. While the learning measures remain modest in the manuscript, the additional analyses justify their validity. I now agree that this manuscript meets the criteria for publication.

We greatly appreciate the reviewers' insightful and constructive comments. To fully address the previous concerns, we conducted the following new experiments and analyses:

1) Loss of function of dopamine signaling in the tail of the striatum (TS) with bilateral 6-hydroxy dopamine (6-OHDA) injection.
2) Examining the effects of decreased air-puff intensity on the level of aversive and appetitive learning.
3) Within day average of behavioral and neural data.
4) Analyses without non-learners.
5) Reporting r and p values and a 95 percent confidence interval to each correlation plot.
6) Subregion specific analyses.
7) Behavioral and neural PSTH of anticipatory behavior for the optogenetic experiment.
8) Dynamics of TS dopamine during null trials.

In addition, we re-organized the manuscript and figures, and modified the text to reflect reviewers' advice. Importantly, we have now expanded our discussion and introduction to clarify the rationale, objective and conclusion of this study. We believe that this revision has greatly improved our manuscript and has addressed all the previous concerns. We sincerely hope that the reviewers think that this version is now acceptable for publication at *Communications Biology*.

Reviewers' Comments:

Reviewer #1

The manuscript studies competing valence learning using a dual valence Pavlovian task, fiber photometry in striatum, and optogenetic manipulations. The approach is timely and the dataset is rich. Several analysis and presentation choices limit interpretability, mainly

variability and potential non learners, across day averaging that masks learning dynamics, recording site heterogeneity, incomplete alignment between dopamine signals and behavior, and limited statistical information.

Thank you for recognizing our dataset is rich and raising the issues and recommendations.

Issues and recommendations:

1. Variability and possible non learners in Fig. 2
Anticipatory measures show large inter animal variance across sessions, which suggests that some subjects may not have learned the task. Please pre specify an inclusion criterion for learners and apply it. For example, require a significant positive within subject slope of anticipatory licking during days 3 to 5, and or a minimum CS minus baseline effect size on late training. Report the fraction of animals excluded and repeat key analyses with and without non learners.

Thank you for the comment. In the previous manuscript, we included all animals who experienced the behavioral task into the data analysis. According to the suggestion from the reviewer, we set a criterion for the learners as a within subject slope of anticipatory blinking < 0 in days 1 to 2 for the avoidance learning, and anticipatory blinking > 0 and anticipatory licking > 0 in days 3 to 5 for the adaptation and appetitive learning, respectively. We found that 1 animal who did not learn avoidance, 1 animal who did not learn adaptation, and 1 animal who did not learn both, total 3 out of 9 non-leaners for the aversive learning. Behavioral and neural anticipatory responses without non-learners were shown in Fig. S2. In addition, we found 2 out of 9 non-learners for the appetitive learning (regression slope of anticipatory licking during adaptation phase < 0). The results without those non-learners were shown in the Supplemental Figure S3 and in the text as follows:

Fig. S2,

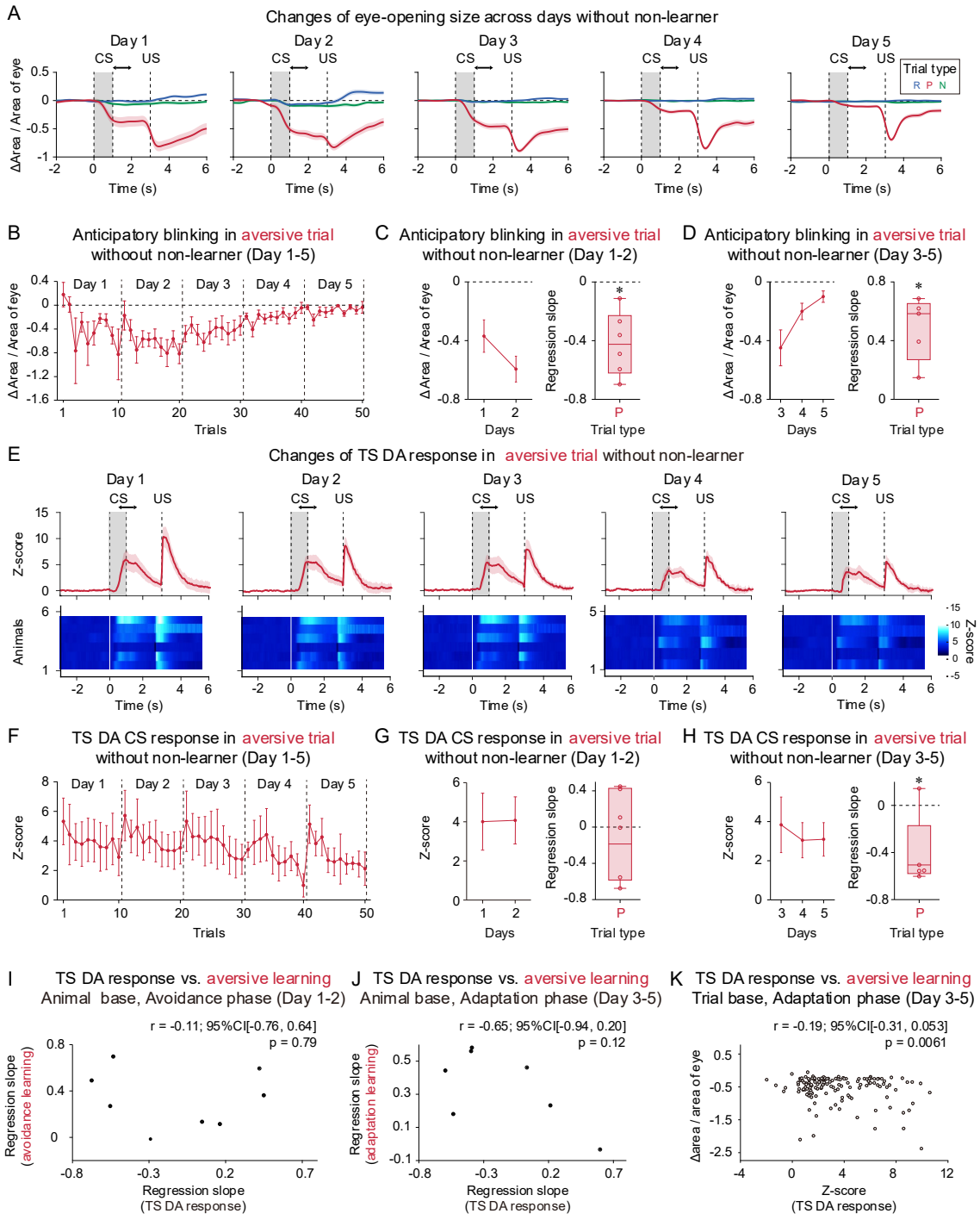
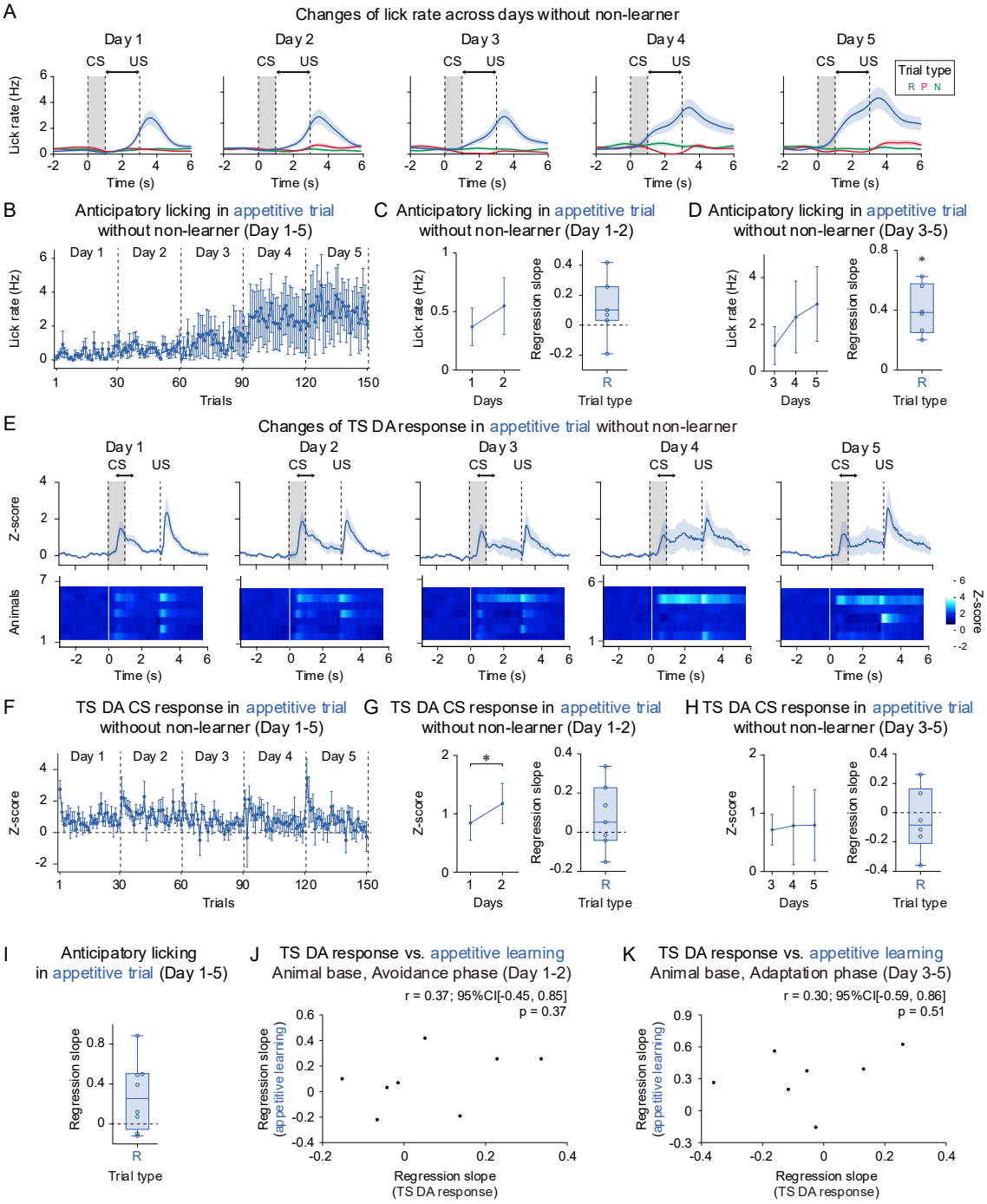

A
Changes of eye-opening size across days without non-learner
Day 1
Day 2
Day 3
Day 4
Day 5
CS
US
Trial type
R P N
ΔArea / Area of eye
Time (s)
B
Anticipatory blinking in aversive trial
without non-learner (Day 1-5)
Trials
C
Anticipatory blinking in aversive trial
without non-learner (Day 1-2)
Days
Regression slope
Trial type
D
Anticipatory blinking in aversive trial
without non-learner (Day 3-5)
E
Changes of TS DA response in aversive trial without non-learner
Z-score
Animals
F
TS DA CS response in aversive trial
without non-learner (Day 1-5)
G
TS DA CS response in aversive trial
without non-learner (Day 1-2)
H
TS DA CS response in aversive trial
without non-learner (Day 3-5)
I
TS DA response vs. aversive learning
Animal base, Avoidance phase (Day 1-2)
r = -0.11; 95%CI[-0.76, 0.64]
p = 0.79
Regression slope
(avoidance learning)
Regression slope
(TS DA response)
J
TS DA response vs. aversive learning
Animal base, Adaptation phase (Day 3-5)
r = -0.65; 95%CI[-0.94, 0.20]
p = 0.12
Regression slope
(adaptation learning)
K
TS DA response vs. aversive learning
Trial base, Adaptation phase (Day 3-5)
r = -0.19; 95%CI[-0.31, 0.053]
p = 0.0061
Δarea / area of eye
Z-score
(TS DA response)

Fig. S3,



Line 133-136,

There were 3 out of 9 mice who did not learn avoidance (regression slope of anticipatory blinking during avoidance phase > 0) and/or adaptation (regression slope of anticipatory blinking during adaptation phase < 0). Behavioral and neural anticipatory responses without non-learners were shown in Fig. S2.

Line 165-168,

There were 2 out of 9 mice who did not learn to approach (regression slope of anticipatory licking during adaptation phase < 0) to rewarding stimuli. Behavioral and neural anticipatory responses without non-learners were shown in Fig. S3.

2. Correlation figures

For all correlation plots, place the exact statistics on the axes. Report r, the exact p value, and a 95 percent confidence interval. If feasible, add a bootstrap confidence band for the regression line. This avoids significance by legend and improves transparency.

Thank you for the advice. We reported r and p values, and added 95% confidence interval in each correlation plot (Fig. 1E, 3I-J, S2I-K, S3J-K, and S5A).

Fig. 1E,

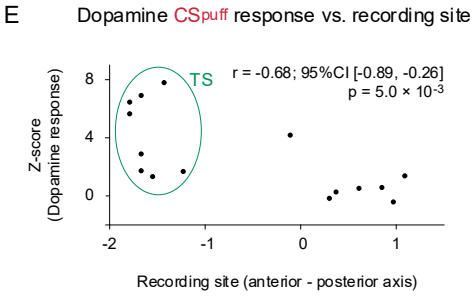


Fig. 3I-J,

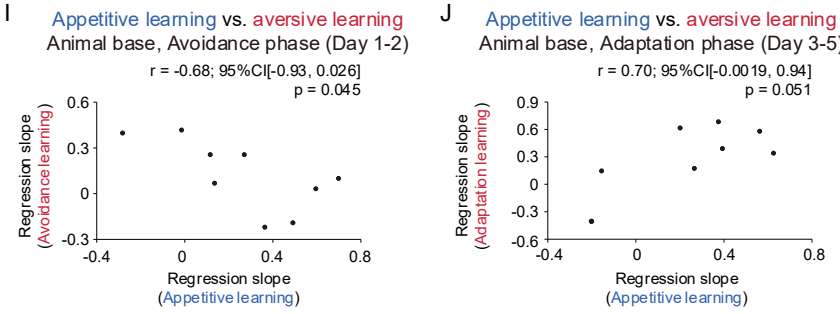


Fig. S2I-K,

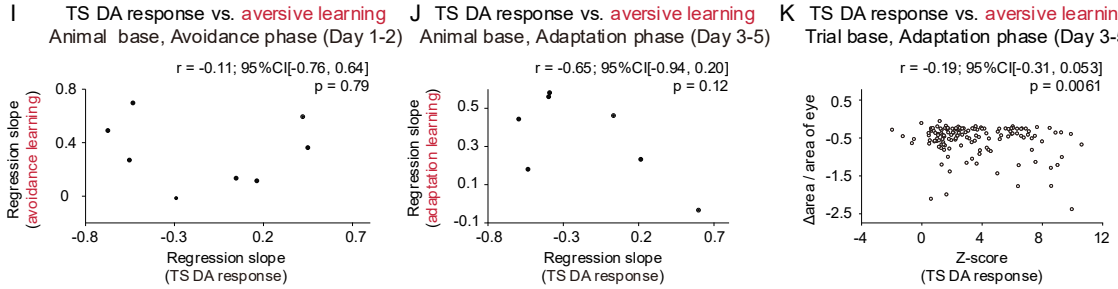


Fig. S3J-K,

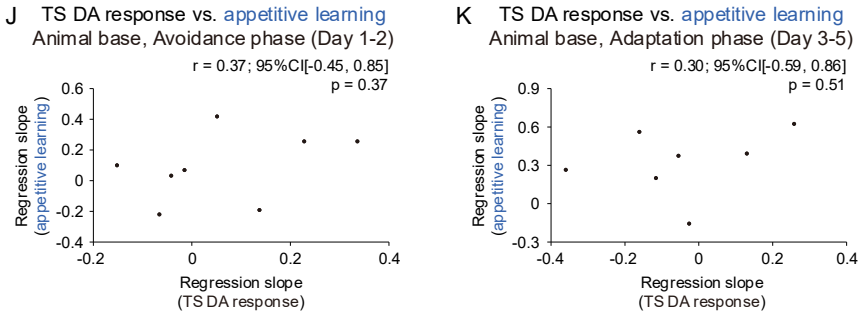
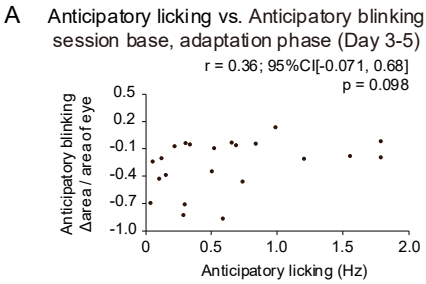


Fig. S5A,



3. Averaging across five training days for photometry

Pooling days 1 to 5 risks washing out learning related signals such as shifts from US locked to CS locked responses and day to day decay. Please add day resolved PSTHs or heatmaps for key dopamine signals and the matching behaviors, or at minimum early, middle, late bins within each phase. Keep the split between avoidance days 1 to 2 and adaptation days 3 to 5, but show the within phase time course instead of a single average.

We appreciate the reviewer's suggestions. In the current Fig. 2 and 3, we now show day resolved PSTH and the heatmap for the dopamine signals (Fig. 2E-H and 3E-H) and the matching behavior (Fig. 2A-D and Fig. 3A-D). Trial-based anticipatory behavior and dopamine CS response are also shown as below (Fig. 2B and 3B):

Fig. 2,

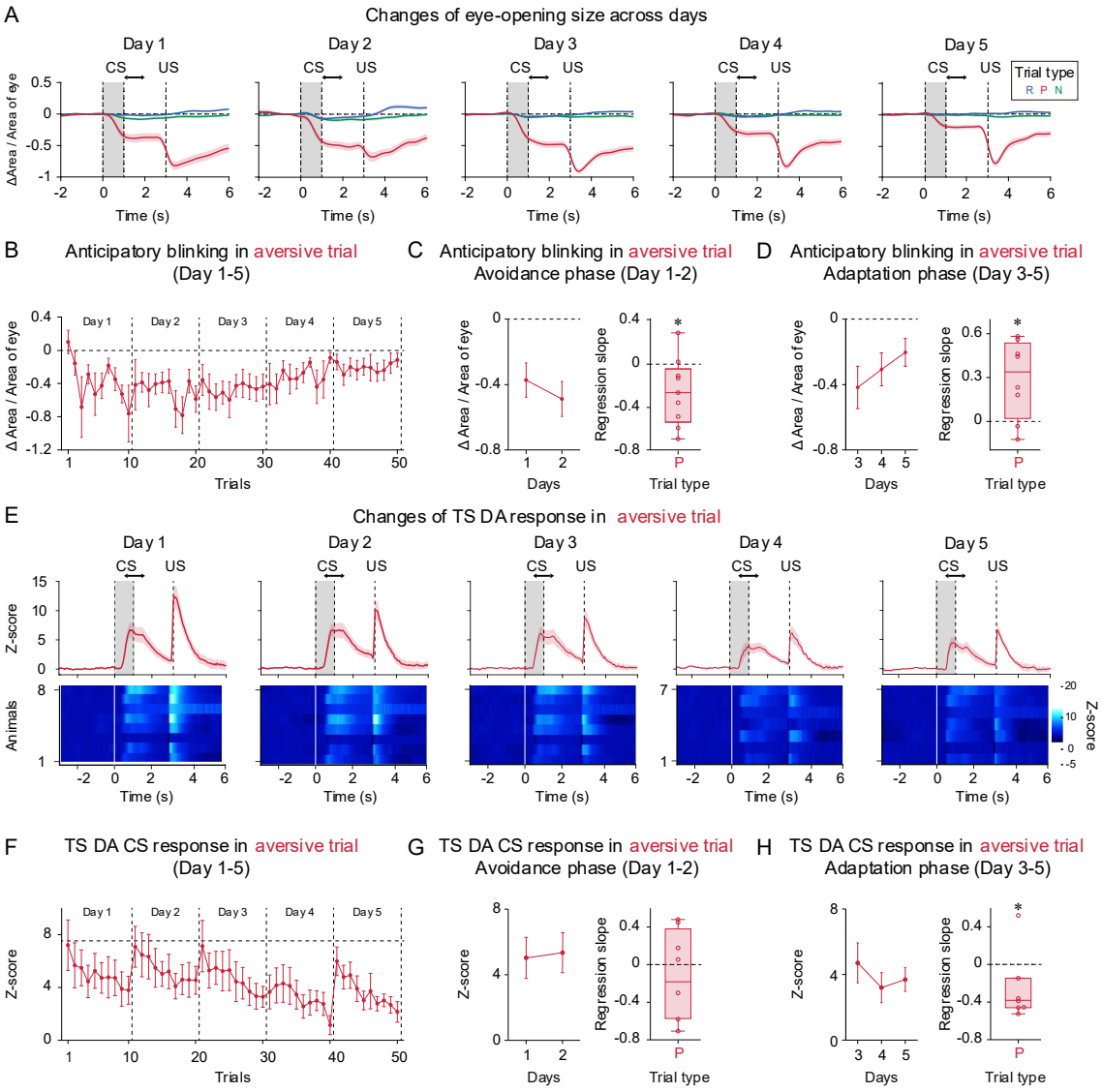
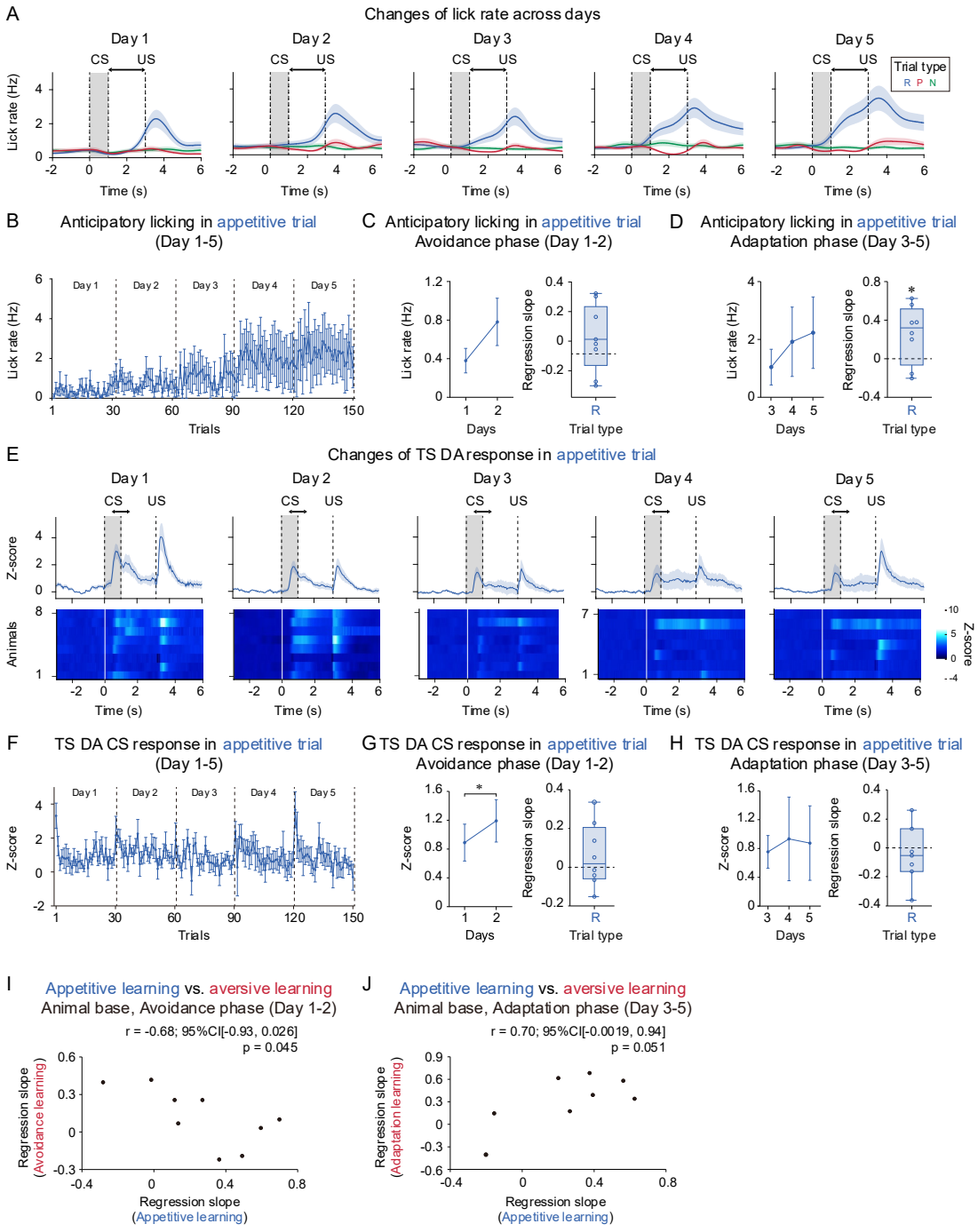

A
Changes of eye-opening size across days
Day 1
Day 2
Day 3
Day 4
Day 5
CS
US
Trial type
R P N
ΔArea / Area of eye
Time (s)
B
Anticipatory blinking in aversive trial
(Day 1-5)
Δ Area / Area of eye
Trials
C
Anticipatory blinking in aversive trial
Avoidance phase (Day 1-2)
Days
Regression slope
Trial type
D
Anticipatory blinking in aversive trial
Adaptation phase (Day 3-5)
E
Changes of TS DA response in aversive trial
Z-score
Animals
F
TS DA CS response in aversive trial
(Day 1-5)
G
TS DA CS response in aversive trial
Avoidance phase (Day 1-2)
H
TS DA CS response in aversive trial
Adaptation phase (Day 3-5)

Fig. 3,



4. Recording site heterogeneity

Supplemental placement maps include fibers in ventral striatum, septum adjacent regions, and dorsolateral striatum in addition to tail and anterior striatum. Treating these as one pool can mix functionally distinct territories. Please provide subregion specific analyses, at minimum posterior tail of striatum versus anterior tail versus anterior striatum versus

other sites. Add a tail of striatum only sensitivity analysis for the main results and report counts per subregion. Given the gradient reported along the anterior to posterior axis, this is essential.

Thank you for the suggestion. We added the PSTHs of TS (posterior than bregma -1.5 mm), anterior TS (posterior than bregma -0.5 and anterior than bregma -1.5), anterior dorsal striatum (anterior than bregma -0.5), and others (lateral septum and ventral striatum) in Fig. S1G-H.

Fig. S1G,

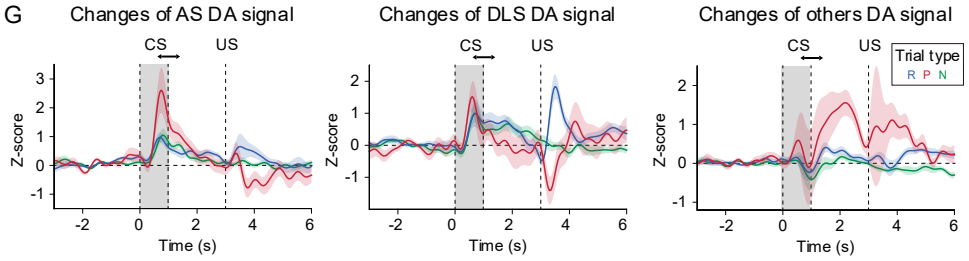
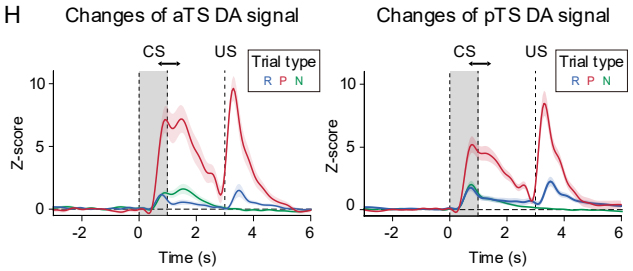
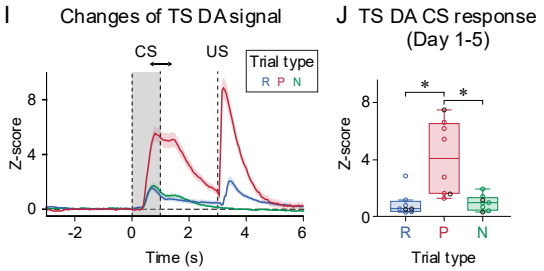


Fig. S1H,



As the dopamine dynamics of the anterior TS resemble to it's in the posterior TS, and anterior TS dopamine responded most to CSpuff as shown in black circles in the below panel, which is similar to posterior TS dopamine (Fig. S1I-J), we combined anterior and posterior TS data and handled as TS in the main results.

Fig. S1I-J,



5. Show behavior alongside photometry

Wherever dopamine signals are analyzed, show the matching behavioral PSTH or summary metric from the same trials and sessions, blink or lick as appropriate. This will let readers align neural and behavioral changes in time and magnitude.

We agree with reviewer's comment. We re-organized our figures and now show the behavioral data aligned with the neural data in Fig. 2 and 3.

Fig. 2,

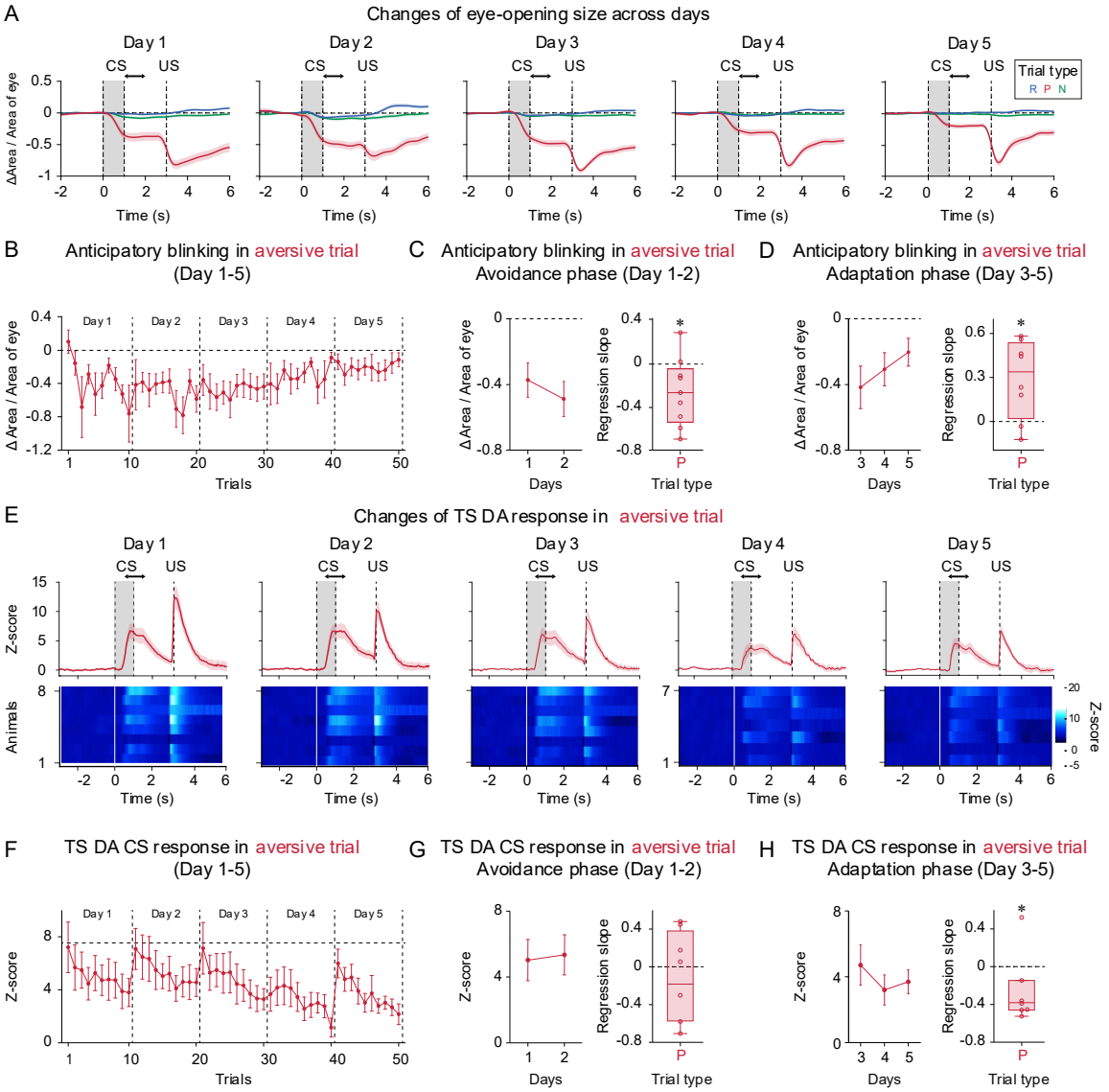
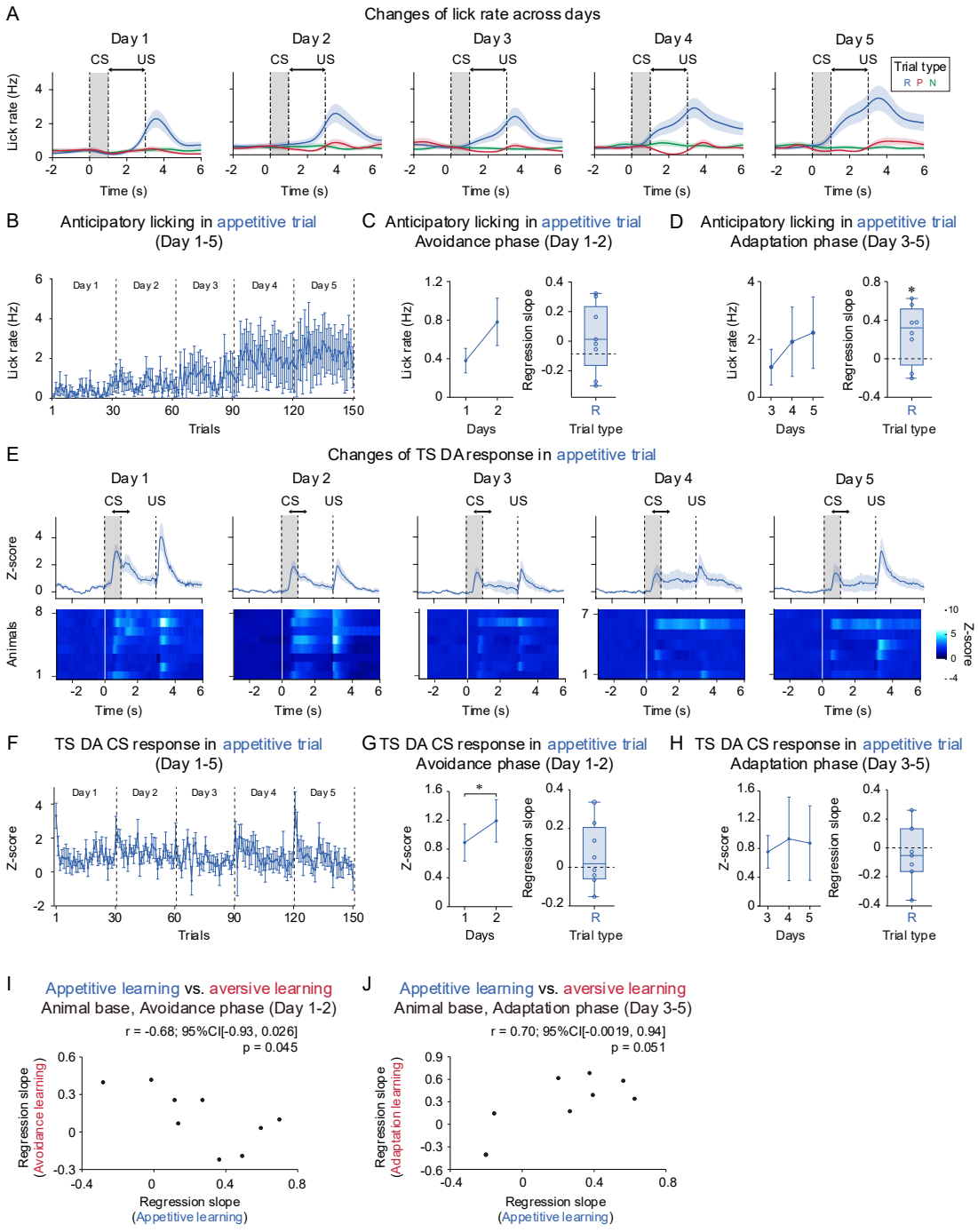


Fig. 3,



6. Test for temporal difference like backpropagation explicitly

If the narrative proposes a shift in prediction from US to CS, analyze it directly. Plot trial by trial acquisition curves for behavior and dopamine on the same axis. Test whether US responses decrease as CS responses increase or retime early in training. A simple GLM with CS, US, expected value, and prediction error regressors, or a lagged regression of

dopamine at trial t on behavior at trial t and behavior at trial t plus 1, would clarify directionality. This analysis also argues against pooling days 1 to 5.

Thank you for the insightful comment. Unlike the conventional dopamine signaling in the other subareas of striatum (e.g., Amo et al., 2022)[1], TS dopamine response did not show a gradual shift from US to CS (Fig. 2E). Rather, response of TS dopamine against CS was highest at the first trial and it was gradually decayed over trials regardless of trial types (Fig. 2E, Fig. 3E and Fig. S4A), suggesting that TS dopamine signals novelty or saliency of CS rather than prediction error of upcoming US. Such observation has been reported from multiple studies[2–4], unless the response to CS is habituated before the conditioning[5,6]. These characteristics of TS dopamine suggest that it does not perfectly follow canonical reinforcement learning theory, but instead transmits teaching signals to the striatum in its own unique way, such as the novelty of sensory information, novelty bonuses, and action prediction errors[3,4,7,8]. This discussion has been included in the current manuscript.

Fig. 2E,

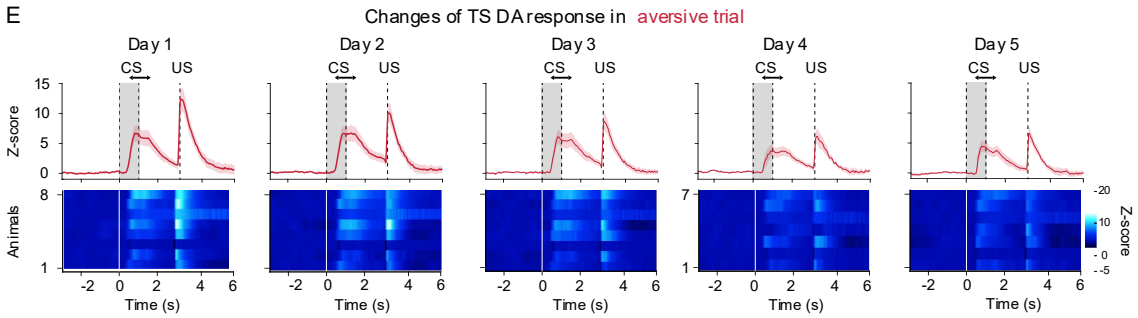


Fig. 3E,

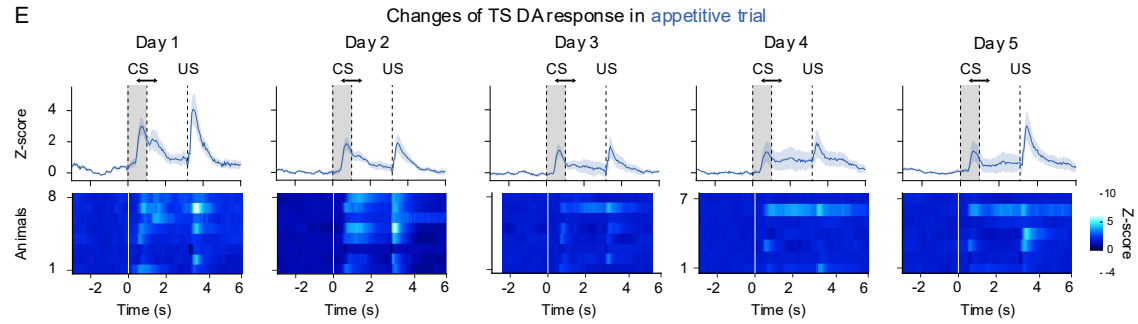
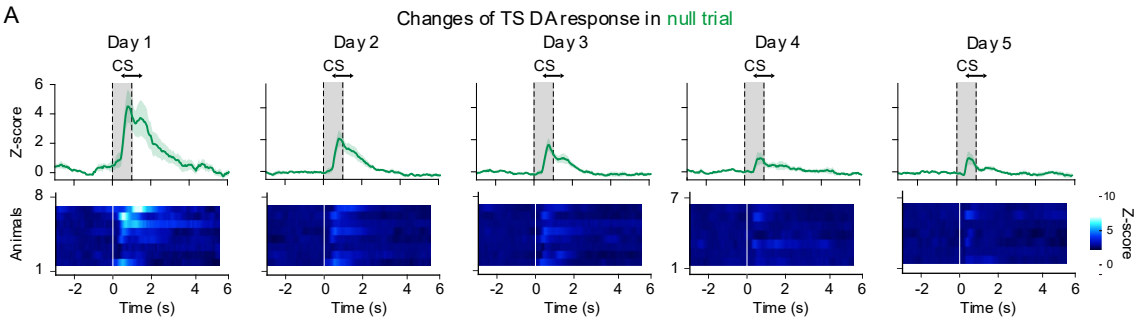


Fig. S4A,



Line 318-329,

TS dopamine in aversive learning and adaptation

Unlike the conventional dopamine signaling in the other subareas of striatum[16], TS dopamine response did not show a gradual shift from USpuff to CSpuff (Fig 2E). Rather, response of TS dopamine against CS was highest at the first trial and it was gradually decayed over trials regardless of trial types (Fig. 2E, Fig. 3E and Fig. S4), suggesting that TS dopamine signals novelty or saliency of CS. Such observation has been reported from multiple studies[9,13,20], unless the response to CS is habituated before the conditioning[12,20]. These characteristics of TS dopamine suggest that it does not perfectly follow canonical reinforcement learning theory, but instead transmits teaching signals to the striatum in its own unique way, such as the novelty of sensory information, novelty bonuses, and action prediction errors[9,11,20,21].

7. Optogenetic effects in Fig. 7

Add behavioral PSTHs for blink and lick aligned to CS and US for control versus stimulation, ideally split into early, middle, and late training. Show per animal slopes in a supplement for transparency. This will reveal whether manipulations change the timing or shape of responses, not only their overall magnitude.

We appreciate the reviewer's comment. We added the behavioral PSTH aligned to CS onset for control and manipulation in each day. Regression slopes and within day average of anticipatory behaviors were also included in Fig. S7. We found that optogenetic activation facilitated amplitude of anticipatory blinking (Fig. 5N left) while regression slope was comparable to control during adaptation phase (Fig. 5N right). In addition, optogenetic activation decreased regression slope of appetitive learning (Fig. 5P right) while amplitude of lick rate was comparable to control group during adaptation phase (Fig. 5P left). These findings suggest that optogenetic activation of SNL dopamine

prompted defensive behavior and facilitated appetitive learning in adaptation phase. We included the additional data and rephrased texts in the revised manuscript.

Fig. 5N-P,

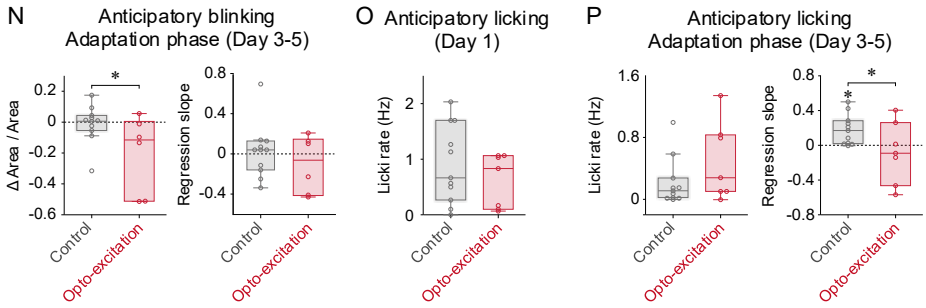
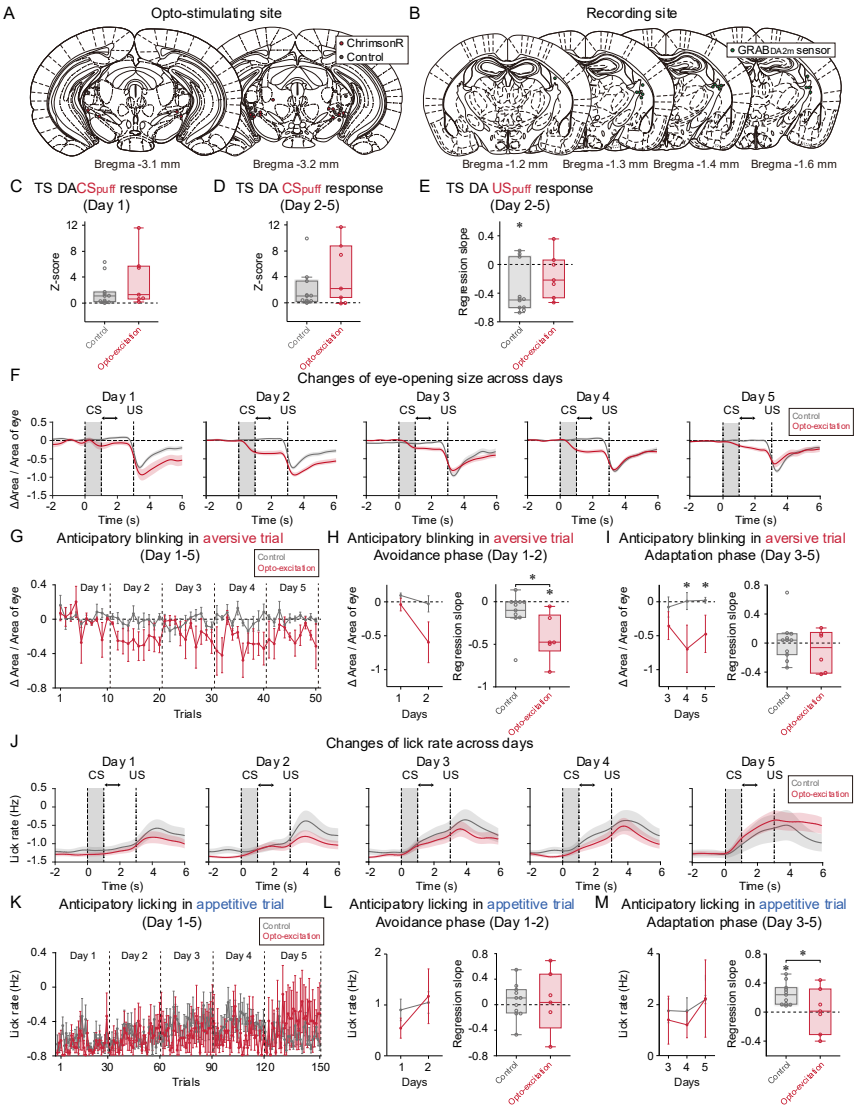


Figure S7,



Line 274-283,

To investigate the effects of optogenetic manipulation on aversive learning, we compared the amplitude of defensive-like anticipatory blinking between opto-excitation and control groups during aversive trials (Fig. 5M–N, S7F-I). On the first day, with no optogenetic manipulation, anticipatory blinking did not differ significantly between the groups (Fig. 5M). However, during the adaptation phase, the opto-excitation group exhibited significantly greater anticipatory blinking amplitude than the control group while regression slope was comparable (Fig. 5N), indicating reduced adaptation to repeated aversive stimuli. These results suggest that the sustained TS dopamine response to USpuff impaired adaptation learning.

Line 285-294,

To assess the effects on appetitive learning, we analyzed anticipatory licking in appetitive trials (Fig. 5O–P, S7J-M). On the first day, there was no significant difference between groups in licking magnitude in the absence of optogenetic manipulation (Fig. 5O). During the adaptation phase, however, the opto-excitation group showed a significantly lower regression slope of anticipatory licking than the control group while the magnitude of lick rate was comparable (Fig. 5P), suggesting that impaired TS dopamine decay to CSpuff weakened appetitive learning. Together, these results suggest that TS dopamine dynamics are critical for the development of aversive adaptation and, consequently, the progression of appetitive learning.

Overall evaluation

The study tackles an important question with a strong task and valuable recordings. Addressing variability and learner status, analyzing regions separately, avoiding across day pooling, aligning behavior with photometry, and enhancing statistical transparency will substantially improve interpretability. The suggested additions are feasible within the current dataset and will make the claims more precise without changing the overall story.

Thank you for the insightful/instructive suggestions and for the encouraging comments. We believe that this revision has greatly improved our manuscript and the current version is now acceptable.

Reviewer #2

In this manuscript, Tsuruga et al., investigated how the dopaminergic activities in the tale of the striatum control the competition between aversive and appetitive learning processes. To tackle this question, they investigated how these two learning processes develop in their 2-VCC task and found animal's gradual adaptation to aversive air puff-predicting cue as well as co-occurrence of conditioned appetitive licking behavior. Next, they recorded dopamine release using GRAB sensor and showed larger response of TS-DA to air puff CS compared to that of appetitive water CS, and the decline of dopaminergic response of TS depending on the conditioning process. They then focused on the possible regulatory role of US-evoked TS-DA response in the behavioral desensitization to air puff CS. By combining optogenetic manipulation of SNL DANs and recording of TS-DA, they mimicked natural aversive response of TS-DA and found this optogenetic excitation during air puff US period was sufficient to prevent development of the desensitization to air puff CS suggesting the decline of US-evoked TS-DA causally triggers the adaptive leaning.
Methodology and statistics are sound. The results are somewhat interesting. However, there are several issues that largely limit the conceptual advancement of this study. Therefore, I would not support this manuscript for publication in Communications Biology. The details are as follows.

Thank you for recognizing our results are interesting and raising the important issues.

Major issues:
(1) The results and conclusions are mostly replications of previous results about TS-DA. As the authors mentioned, it has been already reported that aversive CS and US evoked the increase of TS-DA [1,2]. Also, the ablation of TS-DA prevents adaptation to initially aversive novel object [3] and abolished the avoidance from aversive stimulus in the conflicting context between appetitive and avoidance behaviors [4] also suggesting the regulatory role of TS-DA in avoidance behavior under the context of reward-punishment competition. These results largely limit the conceptual advancement of the current study.

We appreciate the reviewer's comment. As the reviewer pointed out, previous studies have demonstrated that TS dopamine is evoked by aversive CS and US[3,5], and ablation of TS dopamine reduces avoidance of the novel object[7,9] and decreased avoidance in conflicting situations[8]. However, these studies could not investigate how TS dopamine

influences appetitive learning or its role when appetitive and aversive learning interfere. This is because in the previous study using a conflict task, the aversive stimulus was presented only after appetitive learning was already acquired[8]. The current study conceptually differs from the previous work by focusing on how the dynamics of individual learning processes change and how they interfere when both types of learning can occur simultaneously. This point was included in the Introduction of the revised manuscript.

Line 33-48,

Dopamine dynamics within the mesolimbic pathway play a central role in appetitive learning and approach behavior[5,6]. Recent studies have identified a distinct subpopulation of dopamine neurons projecting to the tail of the striatum (TS), which receive unique presynaptic inputs[7] and exhibit specialized activity. These neurons respond to novel objects, multiple sensory modalities, and threat-associated cues[8–12], but do not consistently signal reward in rodents and monkeys[10,13]. Ablation of dopamine signals in the TS reduces avoidance of aversive or threatening stimuli[8,10] in conflicting situations[11], suggesting that TS dopamine promotes avoidance of negative events when both appetition and aversion are predicted. However, these studies could not investigate how TS dopamine influences appetitive learning or its role when appetitive and aversive learning interfere. This is because in the previous study using a conflict task, the aversive stimulus was presented only after appetitive learning was already accomplished[11]. Therefore, clarifying the precise roles of TS dopamine in processing aversive and appetitive stimuli is critical for understanding the neural basis of competing valences.

(2) Related to (1), the rationale, objective and conclusion of this study are vague. The authors need to clearly explain what has been done already, and what they have found for the first time and why it is important in the manuscript.

We thank the reviewer for raising this important issue. We agree that the rationale and conclusion of our previous manuscript was vague. We added more explanation about the rational and objective of our current study in the Introduction as described above (Line 33-48). The current study demonstrated three findings: 1) under conditions where two types of learning can be developed simultaneously, aversive learning takes precedence; 2) aversive learning suppresses acquisition of appetitive learning; and 3) TS dopamine indirectly modulates appetitive learning by contributing to promoting avoidance learning and adaptation learning. The possibility that aversive learning can interfere with

appetitive learning has been proposed primarily in psychological research field[10–13]. However, the underlying brain mechanisms remained largely unclear. While previous studies have repeatedly reported that TS dopamine contributes to aversive learning, this study proposes for the first time that it impedes appetitive learning under conflicting conditions. This represents the discovery of a new function for TS dopamine. We highlighted this point in the revised manuscript.

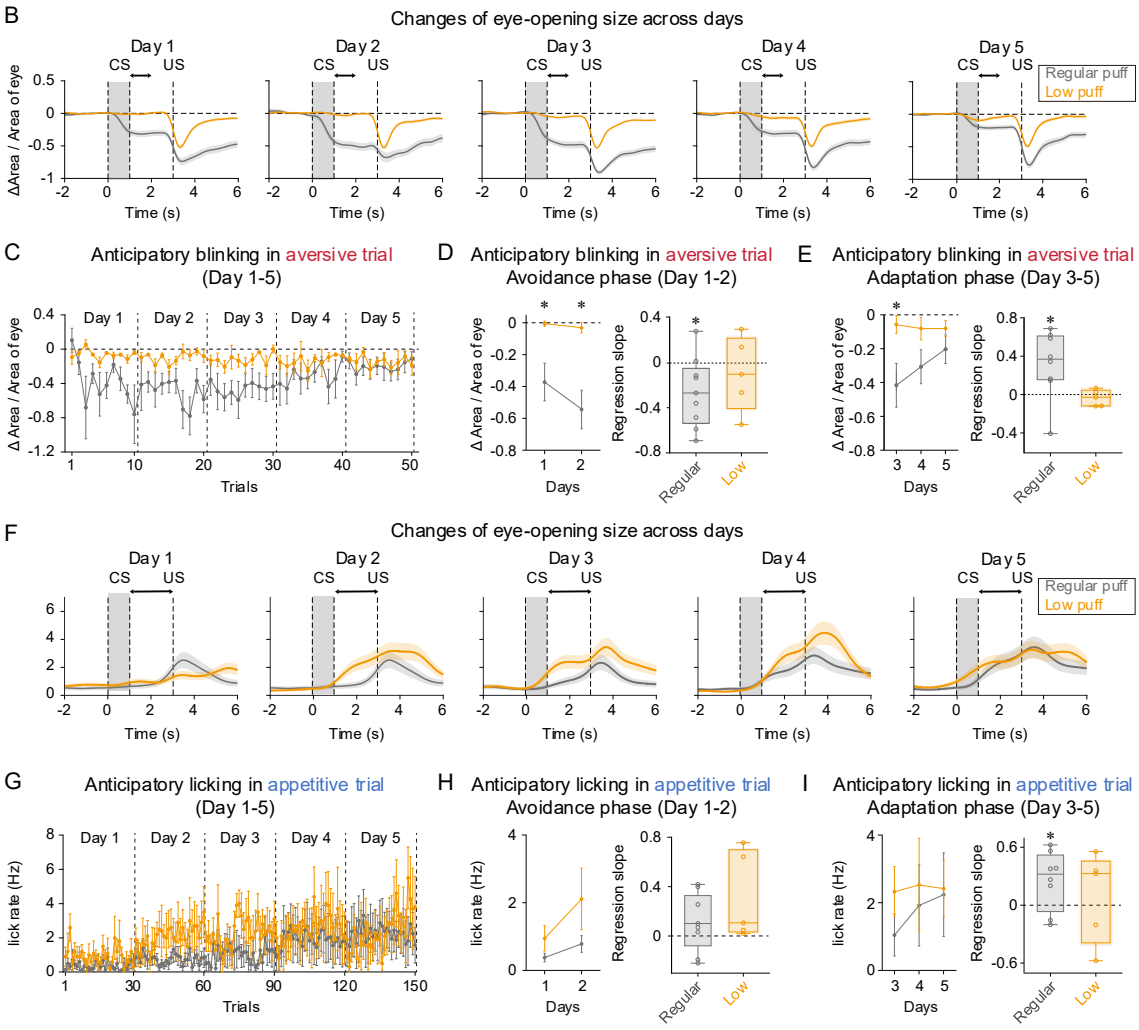
Line 426-435,
Using a dual valence-driven classical conditioning task, we demonstrated three findings: under conditions where two types of learning can be developed simultaneously, aversive learning takes precedence; aversive learning suppresses acquisition of appetitive learning; and TS dopamine indirectly modulates appetitive learning by promoting aversive learning. While previous studies have repeatedly reported that TS dopamine contributes to aversive learning, this study proposes for the first time that it impedes appetitive learning under conflicting conditions. This represents the discovery of a new function for TS dopamine. Future studies should further clarify how downstream targets of TS dopamine regulate aversive and appetitive behaviors.

(3) The authors tried to investigate the competition of parallel learning processes (i.e. aversive learning during Day1-2 and adaptation to puff/appetitive learning during Day 3-5). However, they did not show whether each learning process was actually the result of the competition between aversive and appetitive learning. It is necessary to show that (i) increase or decrease of the intensity of air puff and/or the reward size facilitate or slowdown each learning process, (2) or the proportion of the number of rewarding, aversive and null trials (currently it's 3:1:3) flexibly impacts on the development of each learning process.

Thank you for raising this issue. We indeed examined correlations between adaptive learning and appetitive learning within animal (previous Figure 2I-J and S2B, current Figure 3I-J and S5A) and it revealed a significant correlation between two learnings (mice with more adaptation to aversion developed more appetitive learning), implying an interference between competing valence. However, as the reviewer pointed out, we did not test the interference directly. To that end, we employed a decreased intensity of air-puff (5 psi) and examined how it affects each learning processes though we could run only a small number of animals (n=5, Fig. S5). While regular intensity (15 – 20 psi) group acquired anticipatory blinking during the avoidance phase and adapted to it during the

adaptation phase, mice with low intensity air-puff did not show avoidance nor adaptation (Fig. S5D-E), indicating an impaired aversive learning in the low air-puff group. We then compared licking behavior between two groups (Fig. S5F-I). There was a significant main effect of group in within day average of anticipatory licking in Day 1-2 (Two-way ANOVA for mixed design, main effect of group, $F(1,12) = 5.6$, $p = 0.043$, Fig. S5H) though post hoc comparison did not detect significant group difference in either Day 1 ($p=0.10$) or Day 2 ($p=0.096$). In later trials, regular air-puff group showed significant development of anticipatory licking which did not observed in the low air-puff group (Fig. S5I), suggesting a saturation of appetitive learning. These results supported the competition between aversive and appetitive valence. We included the new results and discussion in the revised manuscript.

Fig. S5B-I,



Line 178-203,

Competition between parallel learning processes

As appetitive learning developed only during adaptation to aversion, we next investigated whether the paradelle learning processes interfere in the 2-VCC task. We first examined correlations between aversive and appetitive learning intensities. We found that their regression slopes were negatively correlated during the avoidance phase but positively correlated during the adaptation phase (Fig. 3I–J and S5A). These results suggest that appetitive learning was suppressed when mice developed defensive behavior in anticipation of aversive stimuli but enhanced when they adapted to repeated aversive exposure.

We next examined the interference of two competing valence more directly with applying low intensity of aversive stimulation (Fig. S5). While regular intensity air-puff (15 – 20 psi) group acquired anticipatory blinking during the avoidance phase and adapted to it during the adaptation phase, mice with low intensity air-puff (5 psi) did not show avoidance nor adaptation (Fig. S5D–E), indicating an impaired aversive learning in the low air-puff group. We then compared licking behavior between two groups (Fig. S5F–I). Compared to regular air-puff group, low air-puff group showed non-significant trend of higher magnitude of anticipatory licking in Day 1-2 (Fig. S5H). In later trials, regular air-puff group showed significant gradual increase of anticipatory licking which did not observed in the low air-puff group (Fig. S5I), suggesting a saturation of appetitive learning. These results suggest that suppression of aversive learning tend to accelerate appetitive learning.

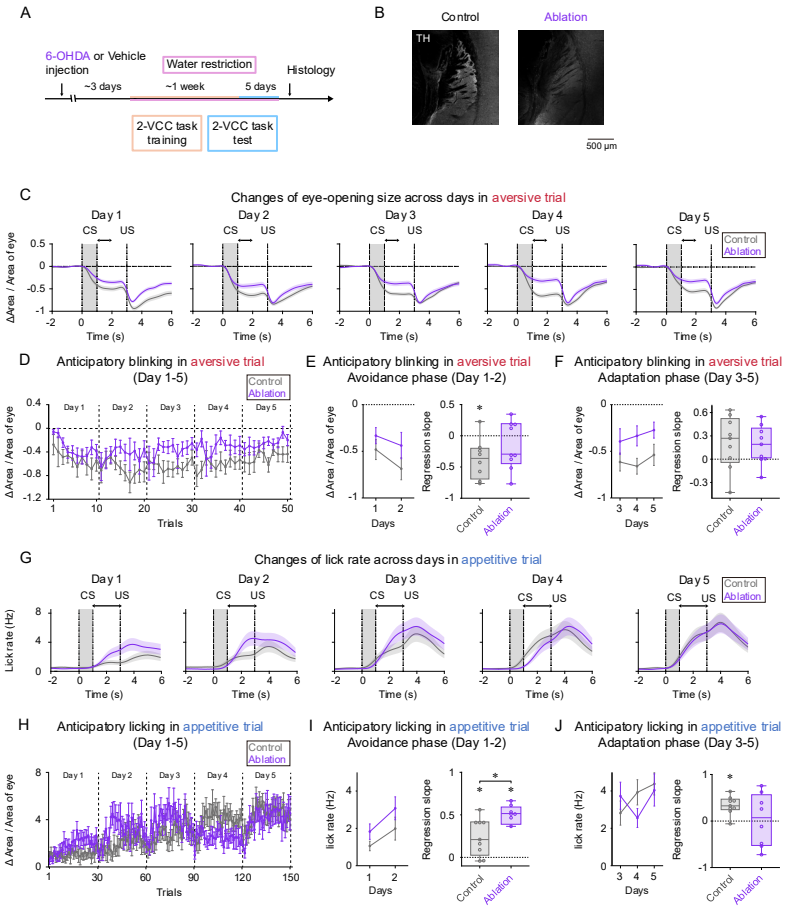
The results from correlation analysis and manipulation of magnitude of aversive stimuli revealed a competition of parallel learning processes in the 2-VCC task.

(4) In addition to optogenetic gain-of-function experiment, loss of function experiment such as ablation of TS-DA is necessary to support their conclusions.

We agree with the reviewer's comment. We bilaterally ablated dopamine signaling in the TS by using 6-OHDA before the 2-valence classical conditioning task. At a glance, removing TS dopamine decreased anticipatory blinking throughout 5 days (Fig. 4C-D). While control group showed significant development of anticipatory blinking during avoidance phase, such development was diminished in ablation group (Fig. 4E). Weakened anticipatory blinking in ablation group was maintained in adaptation phase

(Fig. 4F). We then analyzed licking behavior in ablation mice (Fig. 4G-I). Control group showed gradual increase of anticipatory licking across trials and days (Fig. 4H-J). Surprisingly, we found a significantly greater increase of anticipatory licking during avoidance phase (Fig. 4I), while no significant changes during adaptation phase (Fig. 4J) in ablation mice. These results indicate that ablation of TS dopamine suppressed avoidance learning and accelerated appetitive learning in early phase of conditioning, which was similar to the results of low intensity air-puff. In addition to our previous conclusion that TS dopamine dynamics are essential for behavioral adaptation to aversive stimuli, which indirectly facilitates appetitive learning, the current results highlight an essential role of TS dopamine in avoidance learning, which suppresses approach behavior in early phase of learning processes. Based on the loss-of-function and gain-of-function experiments, it is demonstrated that dopamine signaling in the TS is essential for both avoiding and adapting to aversive events and, in turn, suppressing and promoting appetitive learning in corresponding learning stages. We included the new results and rephrased sentences in the revised manuscript (Fig. 4 and line 12-17, 56-64, 205-225, 309-316, 426-435, and 559-579).

Fig. 4,



Line 12-17 Abstract,

Ablation of TS dopamine inhibited avoidance learning and accelerated appetitive learning. Optogenetic disruption of dopaminergic decline suppressed adaptation and hindered appetitive learning. These findings demonstrate that TS dopamine dynamics are essential for avoidance and adaptation to aversive stimuli, which indirectly modulates appetitive learning, underscoring a regulatory mechanism for shifting between defensive and reward-seeking behaviors.

Line 56-64 Introduction,

Removing TS dopamine suppressed avoidance learning and accelerated appetitive learning. Because dopaminergic decline correlated with adaptation to the USpuff, we optogenetically prevented the decay of the USpuff response to examine behavioral effects. Stimulation inhibited adaptation to the aversive stimulus and disrupted appetitive learning during the adaptation phase. These findings indicate that dopamine signaling in the TS is essential for avoiding and adapting to aversive events and, in turn, suppressing and promoting appetitive learning in corresponding learning phases.

Line 205-225 Results,

Ablation of TS dopamine impaired aversive learning and accelerated appetitive learning

We next questioned whether and how TS dopamine modulates the two competing learning processes. To tackle this question, we removed TS dopamine by injecting 6-hydroxy dopamine (6-OHDA) into the bilateral TS (Fig. 4A–B). At a glance, removing TS dopamine decreased anticipatory blinking throughout 5 days (Fig. 4C–D). While control group showed significant gradual increase of anticipatory blinking during avoidance phase, such behavioral change was diminished in ablation group (Fig. 4E). Weakened anticipatory blinking in ablation group was maintained in adaptation phase (Fig. 4F). These data suggest that ablation of TS dopamine impaired avoidance learning and flattened the changes of defensive behavior across days.

To address the effects of decreased aversive learning on development of appetitive learning, we analyzed licking behavior in ablation mice (Fig. 4G–J). Control group showed gradual increase of anticipatory licking across trials and days (Fig. 4H). Surprisingly, we found a significantly greater increase of anticipatory licking during avoidance phase (Fig. 4I), while no significant changes during adaptation phase (Fig. 4J) in ablation mice. These data suggest that although TS dopamine does not encode

reward value (Fig. 3), removing TS dopamine signals caused an acceleration of acquiring appetitive learning.

Line 309-316 Discussion,

Ablation of TS dopamine inhibited acquiring avoidance behavior and accelerated appetitive learning. We counteracted dopaminergic decay using optogenetic stimulation of TS-projecting dopamine neurons, which inhibited both adaptation and appetitive learning. These findings suggest that TS dopamine signaling plays a key role in acquiring avoidance and accelerating habituation to aversive or threatening stimuli, thereby inhibiting and facilitating the development of appetitive learning in corresponding learning stages.

Line 426-435 Discussion,

Using a dual valence-driven classical conditioning task, we demonstrated three findings: under conditions where two types of learning can be developed simultaneously, aversive learning takes precedence; aversive learning suppresses acquisition of appetitive learning; and TS dopamine indirectly modulates appetitive learning by promoting aversive learning. While previous studies have repeatedly reported that TS dopamine contributes to aversive learning, this study proposes for the first time that it impedes appetitive learning under conflicting conditions. This represents the discovery of a new function for TS dopamine. Future studies should further clarify how downstream targets of TS dopamine regulate aversive and appetitive behaviors.

Line 559-579 Methods,

Ablation of dopamine neurons

To bilaterally ablate dopamine neurons projecting to the striatum, we largely followed an existing protocol[11]. We prepared a solution of 2.5 mg ml^{-1} desipramine HCl and 0.5 mg ml^{-1} pargyline: 28.5 mg desipramine (D3900, Sigma-Aldrich) and 6.2 mg pargyline (P8013, Sigma-Aldrich) were dissolved in 10 ml sterile saline (0.9% NaCl), frozen in −20 °C and thawed each time before use. This cocktail prevents uptake of 6-OHDA by noradrenaline neurons (although noradrenalin projections are not detectable in TS) and degradation of 6-OHDA. The solution was intraperitoneally injected to animals at 10 ml kg^{-1} before surgery. After injection, the mice were anesthetized as described above for injection of 6-OHDA into the brain. We then prepared a solution of 0.2% (2 mg ml^{-1}) ascorbic acid: 10 mg ascorbic acid (1043003, Sigma-Aldrich) was dissolved in 5 ml sterile saline, frozen in −20 °C and thawed each time before use.

10 mg ml^{-1} 6-OHDA solution was freshly prepared before each surgery: 5 mg 6-OHDA.HBr (H116, Sigma-Aldrich) was dissolved in 0.5 ml of 0.2% ascorbic acid solution. The ascorbic acid in this solution prevents 6-OHDA from breaking down. The control animals were injected with 0.2% ascorbic acid solution (vehicle). To further prevent 6-OHDA from breaking down, we kept the solution on ice, wrapped in aluminum foil. The 6-OHDA (or vehicle) was injected bilaterally into TS (100 nl per side). The mice were given several days of resting to recover and to allow sufficient cell death to occur.

(5) There are a lot of redundancies in the current version of manuscript. For example, I do not think Fig.1D,E,F,G and Fig.3B,C,E,F are necessary because the authors eventually showed that behaviors and dopamine responses could change following multiple training sessions. They might want to move these figures to supplement.

Thank you for the comment. As the reviewer #1 also raised a potential risk for pooling days 1 to 5, we moved the combined behavioral and neural data to supplemental information (Fig. S1A-E and I-J) in the revised version.

Fig. S1A-E,

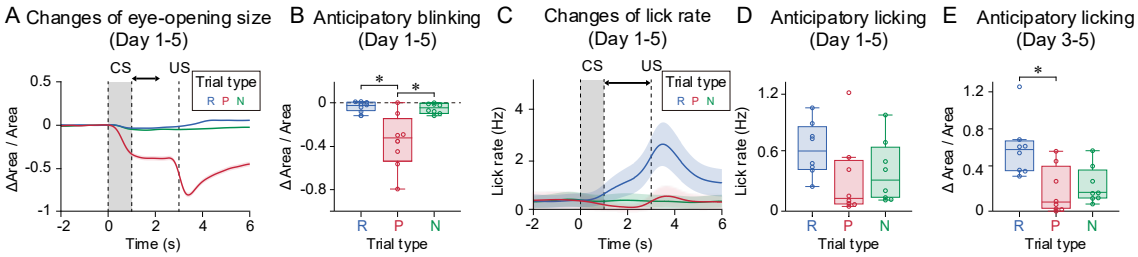
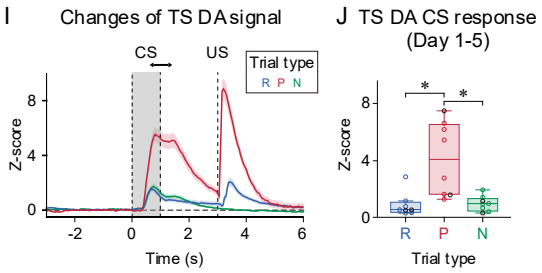


Fig. S1I-J,

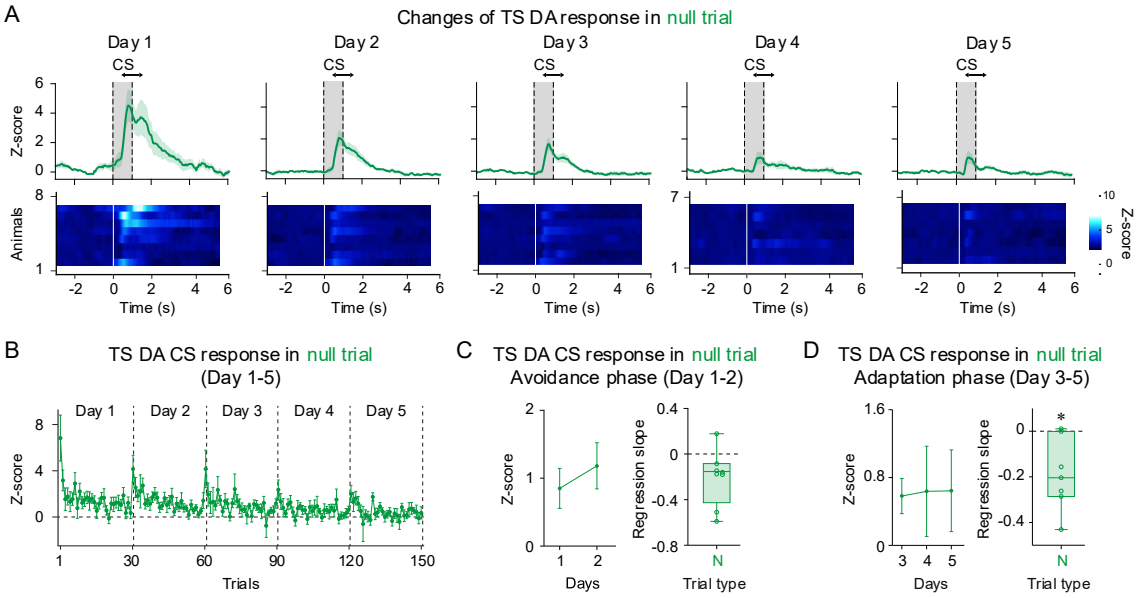


(6) The manuscript lacks important analyses.

(i) Dynamics of TS-DA during null trials should be analyzed and discussed like Fig.4

Thank you for raising the important issue. We added the dynamics of TS dopamine in the null trials in Fig. S4. In null trials, Ts dopamine showed weak response to CS and it gradually decayed across sessions. We included these data in the revised manuscript.

Fig. S4,



Line 171-172,

TS dopamine showed moderate response to CSnull and it gradually decayed across days (Fig. S4).

(ii) In addition to Fig.7G,H,I,J, PSTHs and trial-by-trial change of blinking and licking should be analyzed like Fig.2.

Thank you for the comment. The manuscript now includes the PSTH and trial-based data of anticipatory behaviors. Throughout the additional analysis, we found that optogenetic activation facilitated amplitude of anticipatory blinking (Fig. 5N left) while regression slope was comparable to control during adaptation phase (Fig. 5N right). In addition, optogenetic activation decreased regression slope of appetitive learning (Fig. 5P right) while amplitude of lick rate was comparable to control group during adaptation phase (Fig. 5P left). These findings suggest that optogenetic activation of SNL dopamine

prompted defensive behavior and facilitated appetitive learning in adaptation phase. We included the additional data and rephrased texts in the revised manuscript.

Fig. 5N-P,

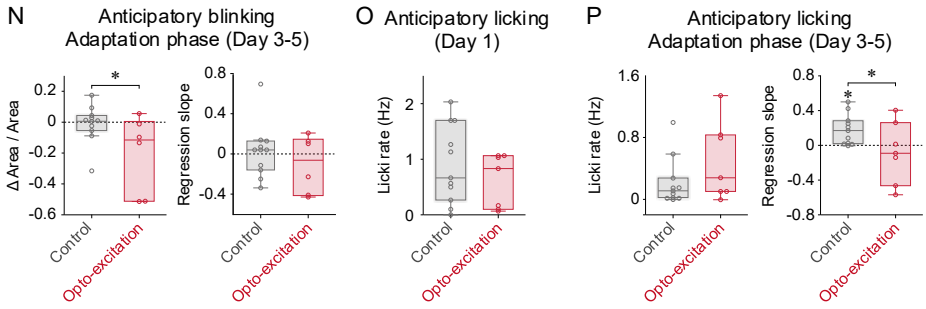
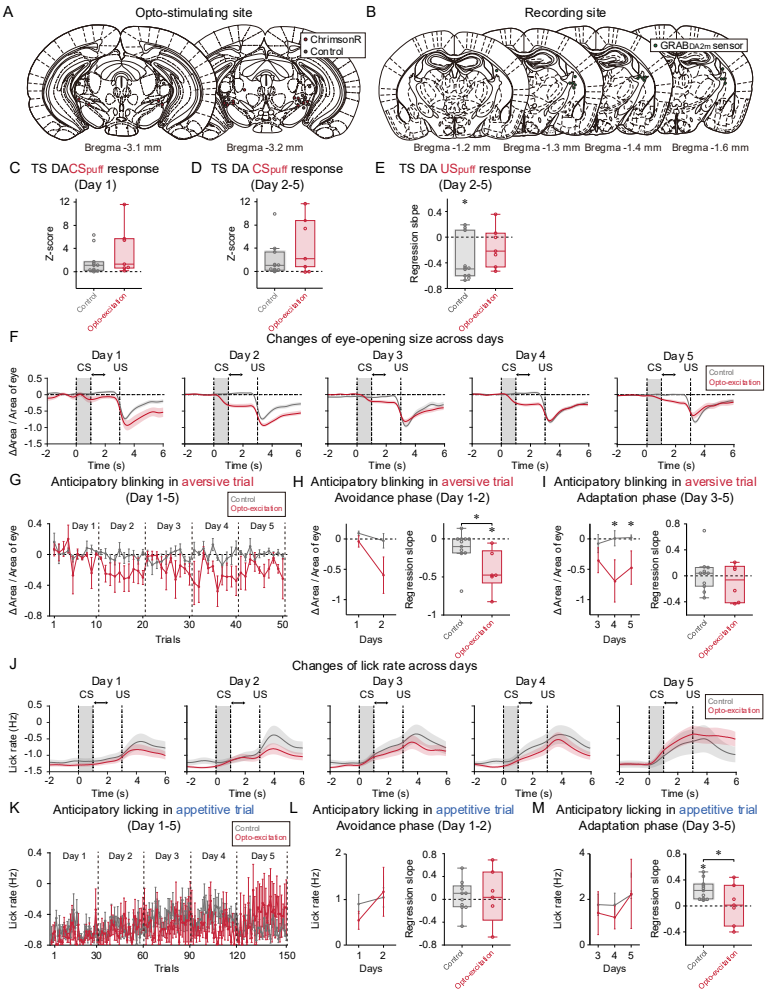


Fig. S7,



Line 274-283,

To investigate the effects of optogenetic manipulation on aversive learning, we compared the amplitude of defensive-like anticipatory blinking between opto-excitation and control groups during aversive trials (Fig. 5M–N, S7F-I). On the first day, with no optogenetic manipulation, anticipatory blinking did not differ significantly between the groups (Fig. 5M). However, during the adaptation phase, the opto-excitation group exhibited significantly greater anticipatory blinking amplitude than the control group while regression slope was comparable (Fig. 5N), indicating reduced adaptation to repeated aversive stimuli. These results suggest that the sustained TS dopamine response to USpuff impaired adaptation learning.

Line 285-294,

To assess the effects on appetitive learning, we analyzed anticipatory licking in appetitive trials (Fig. 5O–P, S7J-M). On the first day, there was no significant difference between groups in licking magnitude in the absence of optogenetic manipulation (Fig. 5O). During the adaptation phase, however, the opto-excitation group showed a significantly lower regression slope of anticipatory licking than the control group while the magnitude of lick rate was comparable (Fig. 5P), suggesting that impaired TS dopamine decay to CSpuff weakened appetitive learning. Together, these results suggest that TS dopamine dynamics are critical for the development of aversive adaptation and, consequently, the progression of appetitive learning.

(iii) The authors analyzed regression slope of behavioral and dopamine change, but I prefer and it is standard to analyze daily changes of those indexes. Actually, when they analyzed regression slope, there are no changes of TS-DA during appetitive CS and US (Fig.4 F-H) but according to PSTHs (Fig.4E), there are apparently the day-by-day reductions of TS-DA during both CS and US periods.

We appreciate the important comment. Because both behavioral and neural data exhibited gradual changes across trials within a day (e.g., Fig. 2B, 2F, 3B, and 3F), we thought that a trial-based linear regression would be a feasible way to capture the trial-by-trial changes. But we agree with the reviewer's comment that it is important to show between day changes using a within day average. Then, we performed and added the average magnitude of each anticipatory behavior and TS dopamine response in Fig. 2C-D, 2G-H, 3C-D, and 3G-H. Although the sensitivity to detect the differences was relatively low, day-by-day average data showed similar trend than the data derived from linear regression,

except for an increase of CSwater response in Day 2 (Fig. 3G) though the magnitude was much smaller compared to CSpuff response (Fig. 2G). These data suggest that the behavioral and neural changes obtained in the current study would be more significant in trial-based variability. We added the new analysis and results in the revised manuscript.

Fig. 2C-D,

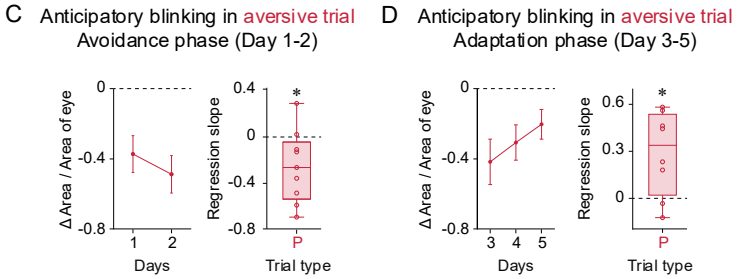


Fig. 2G-H,

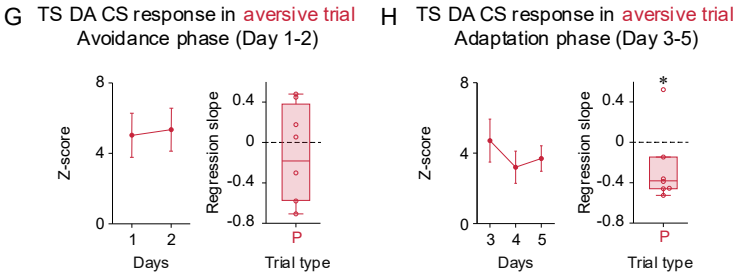


Fig. 3C-D,

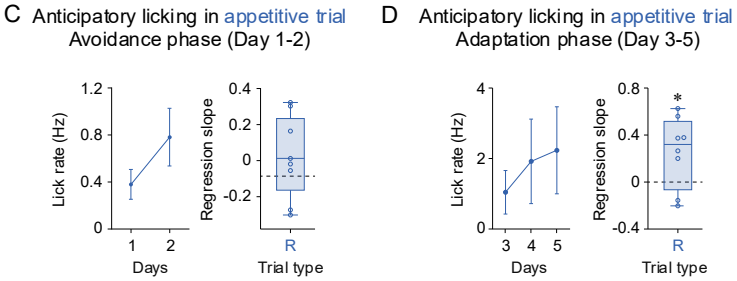
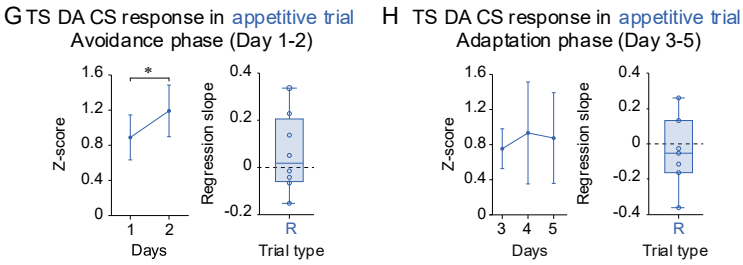


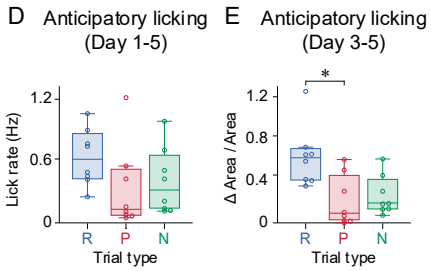
Fig. 3G-H,



(7) I do not think lines 82-83 is correct because appetitive learning is not supported by statistics (Fig.1G).

Thank you for the indication. As the reviewer indicated, our data do not support the accomplishment of appetitive learning when the analysis includes overall days (day 1 to 5, Fig. S1D), but appetitive learning indeed increased during later days of training (day 3 to 5, Fig. S1E). We clarify this point in the revised manuscript.

Fig. S1D and E,



Line 85-88,

This behavior became more pronounced in later days (Day 3–5 and Fig. S1E), suggesting that in the later days, mice developed anticipatory licking as a prospective approach behavior toward the expected appetitive stimulus[14,16].

Minor issues:

(1) There is a typo in the title of Fig.S1. It should be anticipator"y" licking

Thank you. Now the title of S1 is revised as "Anticipatory behavior and dopaminergic responses in the 2-VCC task".

(2) In the abstract (line 3-4), "Understanding how the brain evaluates aversion and appetition to guide behavior remains an open question." sounds like lack of respect to previous studies addressing this theme. This sentence should be modified.

Thank you for raising the important issue. We revised this sentence as "Understanding how the brain evaluates aversion and appetition to guide behavior is an important question." (Line 3-4).

(3) Overall, the texts in the figures are very small. The authors should make them lager.

We made the texts in figures larger, currently more than 8 pt.

(4) The authors use small icons to indicate each trial conditions (e.g. Fig.1D-G) but I do not think this is reader-friendly. They should use letters (i.e. Water, (Air) Puff, Null).

We revised them throughout all figures. Thank you.

(5) The number of animals used are relatively small.

Thank you for the comments. Although each experimental condition includes more than 5 animals which allows conducting statistical analysis, it should be notified in the manuscript. We added the notification as a limitation of the current study in the current manuscript.

Line 417-420,

Limitation

The number of animals used was relatively small, though each experimental condition includes more than 5 animals which allows conducting statistical analysis.

References

[1] Menegas, W., Babayan, B. M., Uchida, N. & Watabe-Uchida, M. Opposite initialization to novel cues in dopamine signaling in ventral and posterior striatum in mice. eLife 6,785 e21886 (2017).

[2] Zafiri, D., Salinas-Hernández, X. I., De Biasi, E. S., Rebelo, L. & Duvarci, S. Dopamine prediction error signaling in a unique nigrostriatal circuit is critical for associative fear learning. Nat. Commun. 16, 3066 (2025).

[3] Menegas, W., Akiti, K., Amo, R., Uchida, N. & Watabe-Uchida, M. Dopamine neurons projecting to the posterior striatum reinforce avoidance of threatening stimuli. Nat. Neurosci. 21, 1421–1430 (2018).

[4] Tsutsui-Kimura, I. et al. Dopamine in the tail of the striatum facilitates avoidance in threat–reward conflicts. Nat. Neurosci. 28, 795–810 (2025).

Reviewer #3

The manuscript seeks to understand the role of the tail of the striatum in modifying behavior as animals learn to discriminate between conditioned aversive and appetitive stimuli. Specifically, the authors utilized a head-fixed paradigm where animals experienced unique odors associated with either a puff of air to the eye, delivery of water via solenoid, or no outcome. During the task, a subset of animals were also fixed to record dopamine dynamics in the tail of the striatum with additional animals also prepared for driving dopamine neurons projecting to the tail of the striatum. Animals exhibited an initial strong response to cues associated with the air puff but not to the water. Across days, the anticipatory responses to the puff decreased while the anticipatory licking for the water increased. Dopamine responses to CS and US aversive conditions decreased over days in line with behavior. However, if driven in a way that matched signal intensity due to actual puffs, the anticipatory blinking did not decrease across days. This suggests that disruption of normal dopamine signaling is sufficient to disrupt adaptations that typically occur to this aversive signal across days. In contrast, dopamine signaling in the tail of the striatum was not strongly modulated by the appetitive CS or US although it does appear to show significant response on the first subset of trials on a given day. Taken together, these results suggest that disruption of normal dopamine signaling in the tail of the striatum results in disrupted adaptive processes in response to repeated aversive, but not appetitive CS/US pairings.
Overall, the paper demonstrates an interesting approach to try to determine the specifics of dopamine dynamics in the tail of the striatum. Prior work has strongly linked this area to avoidance learning although the role in appetitive learning has remained uncertain. In this regard, the present manuscript adds important additional information to understanding the role of this subregion of the striatum in learning more generally. However, there are several concerns I have with the methodology and statistical approach to the data. These are outlined below:

Thank you for recognizing our experimental approach as interesting.

1. Several of the box plots do include outliers as indicated by the whiskers not extending to them. How were outliers defined and were they used in statistical analysis? If not, what was the basis for exclusion or transformation?

Thank you for the important notification. In the boxplots, the edges of the boxes are the

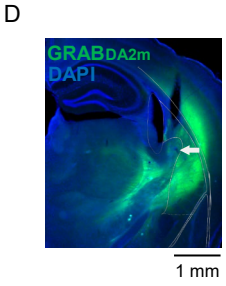
25th and 75th percentiles, and the whiskers extend to the most extreme data points not considered outliers. We included outliers in the statistical analysis. We added the explanation to clarify how we analyzed data (Line 667-670).

Line 667-670,
In the boxplots, the edges of the boxes are the 25th and 75th percentiles, and the whiskers extend to the most extreme data points not considered outliers. Outliers were included in the statistical analysis.

2. In Figure 3, please remove the white boxes on panels A and D. It would allow a more accurate assessment of placement of the fiber in relation to the viral expression.

Thank you for the suggestion. We removed the white box, currently Fig. 1D.

Fig. 1D,



3. I am somewhat confused by the outcome variables in Figure 2 I-J. Specifically, what constitutes the datapoints on the graphs. I see nine points on each of the graphs but I'm unsure where those regression slopes come from. Please expand the explanation of these derived measures as it isn't clear if these are per animal, per day, or how they were calculated.

Thank you for the important indication. We added the explanation where the data derived from either animal base or session base in each correlation panel (Fig. 3I-J, S2I-K, S3J-K, and S5A).

Fig. 3I-J,

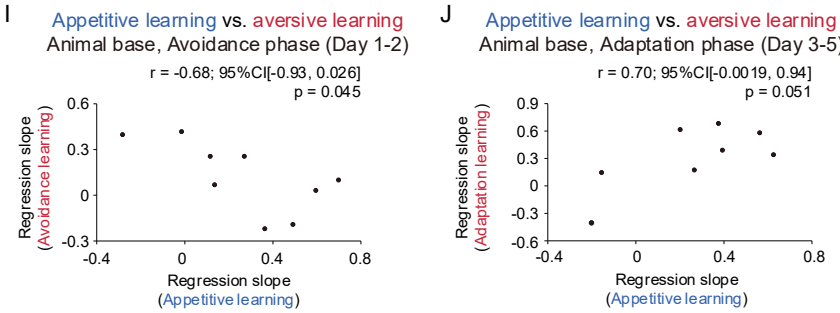


Fig. S2I-K,

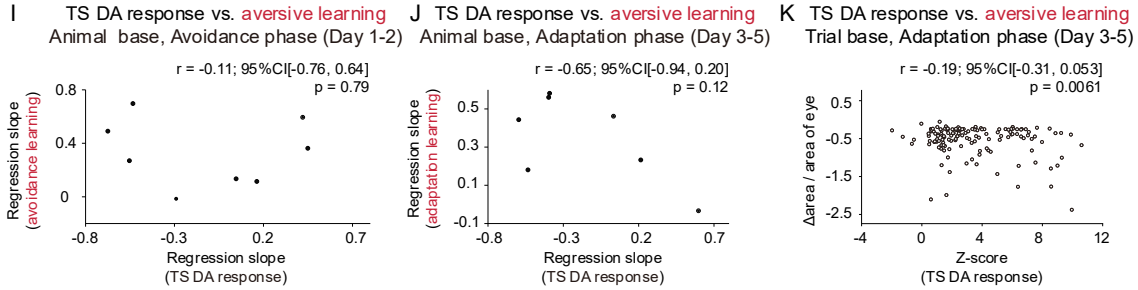


Fig. S3J-K,

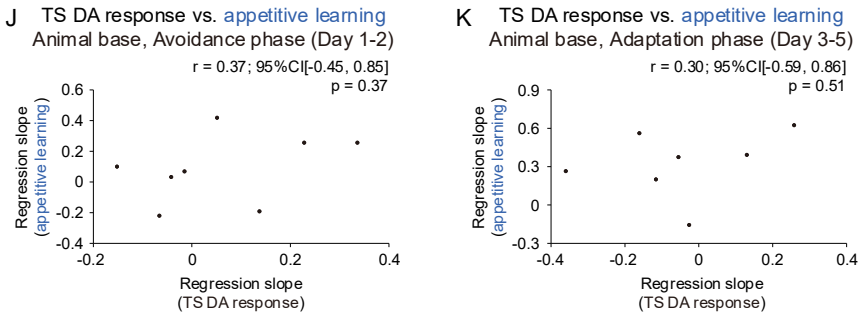
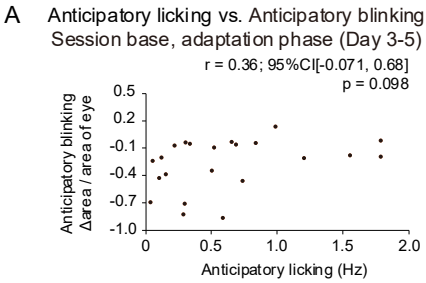


Fig. S5A,



4. In general, I am curious why regression slope was chosen more generally as a derived measure for an outcome variable across the experiments. Lick rate and change in eye area

would seem to be a sufficient measure to look at behavior as it changes across days. I am curious about the justification for using the regression slope as my intuition is that they don't meet many of the normal assumptions of statistical analysis. What, for instance, would be the normal distribution for regression slopes? Further, it seems to not really capture between day learning because it would be susceptible to the starting point more than just the average lick rate within a day or similar for the eye area. Wouldn't a within day average of the raw datapoints be a more accurate measure of the amount of anticipatory behavior rather than the change in the behavior within that day? Furthering this point, the authors seem to use various ways to describe the meaning of the measure that don't fully overlap. For example, in the figure legend for figure 7, it is described as, "Anticipatory licking was developed in the control…" for a significant difference. Where at other times it is described as "dynamically changing" or other terms.

Thank you for raising this issue. Because both behavioral and neural data exhibited gradual changes across trials within a day (e.g., Fig. 2B, 2F, 3B, and 3F), we thought that a trial-based linear regression would be a feasible way to capture the trial-by-trial changes. However, we agree with the comment from reviewers #2 and #3 that a within day average would be more common way to look at behavioral and neural activity changes. Therefore, we added the within day average analysis for anticipatory blink and lick, and TS dopamine dynamics in the revised manuscript Fig. 2C-D, 2G-H, 3C-D, and 3G-H). Although the sensitivity to detect the differences was relatively low, day-by-day average data showed similar trend than the data derived from linear regression, except for an increase of CSwater response in Day2 (Fig. 3G) though the magnitude was much smaller compared to CSpuff response (Fig. 2G). These results suggest that the behavioral and neural changes obtained in the current study would be more significant in trial-based processes. We added the new analysis and results in the revised manuscript.

Fig. 2C-D,

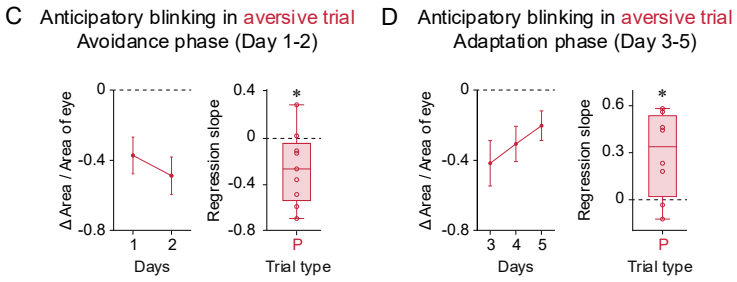


Fig. 2G-H,

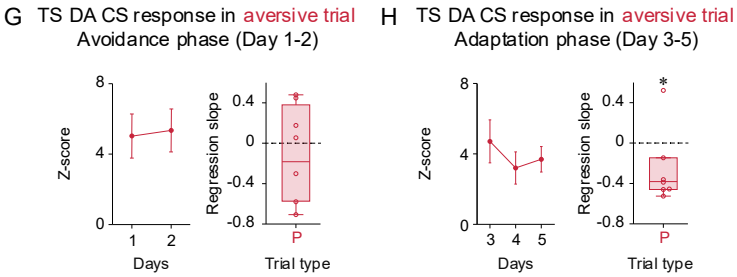


Fig. 3C-D,

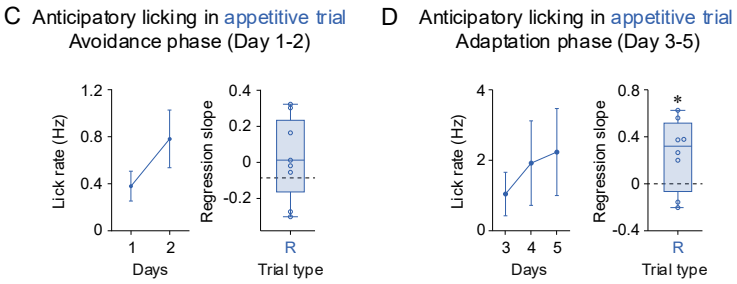
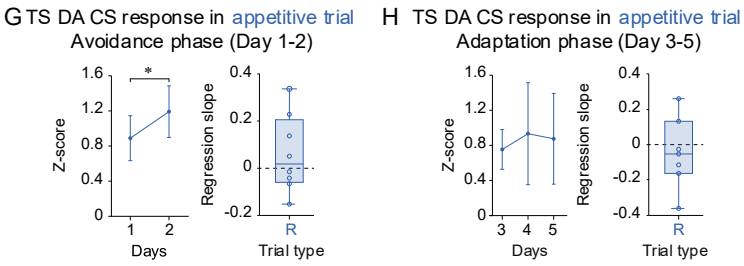


Fig. 3G-H,



Finally, we revised our manuscript not to use various terms to express the results. We consistently used "increase" or "decrease" instead of "dynamical change" or "developed" in Results and Figure legends (Line, 213 and 861).

Line 211-213,

While control group showed significant gradual increase of anticipatory blinking during avoidance phase, such behavioral change was diminished in ablation group (Fig. 4E).

Line 856-861,

Anticipatory licking was increased across Day 3-5 in control (One-sampled t-test, $p = 8.9\times10^{-3}$, $n = 11$) but not in ChrimsonR group (One-sampled t-test, $p = 0.56$, $n = 7$; two-sided t-test, $p = 0.049$, control group, $n = 11$, ChrimsonR group, $n = 7$).

5. It is unclear what is meant on lines 216 to 221 in regards to the CSpuff response. None of the measures in the supplemental figure indicated significant differences across any measured periods. There is certainly a trend but to be accurate, it should be described in the text as a non-significant trend rather than a "modest increase."

Thank you for the notification. According to the comment from reviewer, we rephrased the sentence as follows;

Line 268-270,
We then assessed the effects of optogenetic manipulation on TS dopamine response to CSpuff and found a non-significant trend of increased TS dopamine response (Fig. S7C–E).

6. In figure 7 it appears that there is anticipatory eye blink behavior occurring in control animals. In figure 2 there is a significant decrease in the area measure that remains across all days of the task, although it reduces. Why is it that the animals in G and H don't differ from 0? This would seem to be concerning suggesting that even on day one they are not exhibiting an eyeblink response. In addition, it is unclear why the authors switch between lick rate, the area, and regression slope in these figures as well. If this is justified, it should be included in the text.

We appreciate the important comment from the reviewer. According to the review's requirement, now the manuscript includes between day averaged eye area and lick rate in addition to regression slope (Fig. 5N and O). Throughout the additional analysis, we found that optogenetic activation facilitated amplitude of anticipatory blinking (Fig. 5N left) while regression slope was comparable to control during adaptation phase (Fig. 5N right). In addition, optogenetic activation decreased regression slope of appetitive learning (Fig. 5P right) while amplitude of lick rate was comparable to control group during adaptation phase (Fig. 5P left). These findings suggest that optogenetic activation of SNL dopamine prompted defensive behavior and facilitated appetitive learning in adaptation phase. We included the additional data and rephrased texts in the revised manuscript.

Fig. 5N and O,

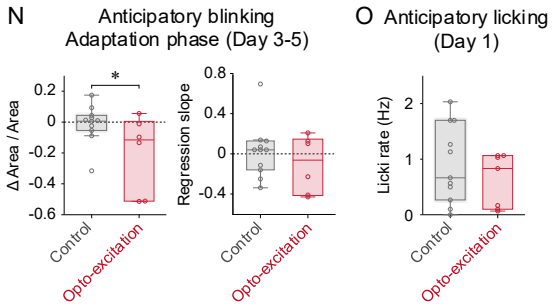
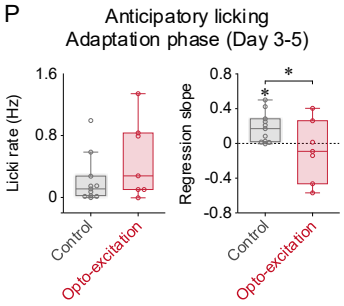
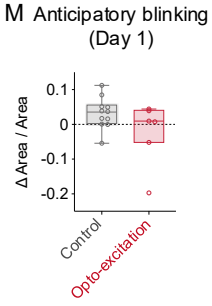


Fig. 5P,



However, As the reviewer pointed out, the magnitude of anticipatory blinking in control group of optogenetic experiment were lower compared to the mice with photometry experiment (Fig. 5M). This difference maybe induced by unknown effects of light stimulation upon the head and/or additional implantations of optical fibers and virus injections into the midbrain. We included this explanation as limitation of current study in the revised manuscript.

Fig. 5M,



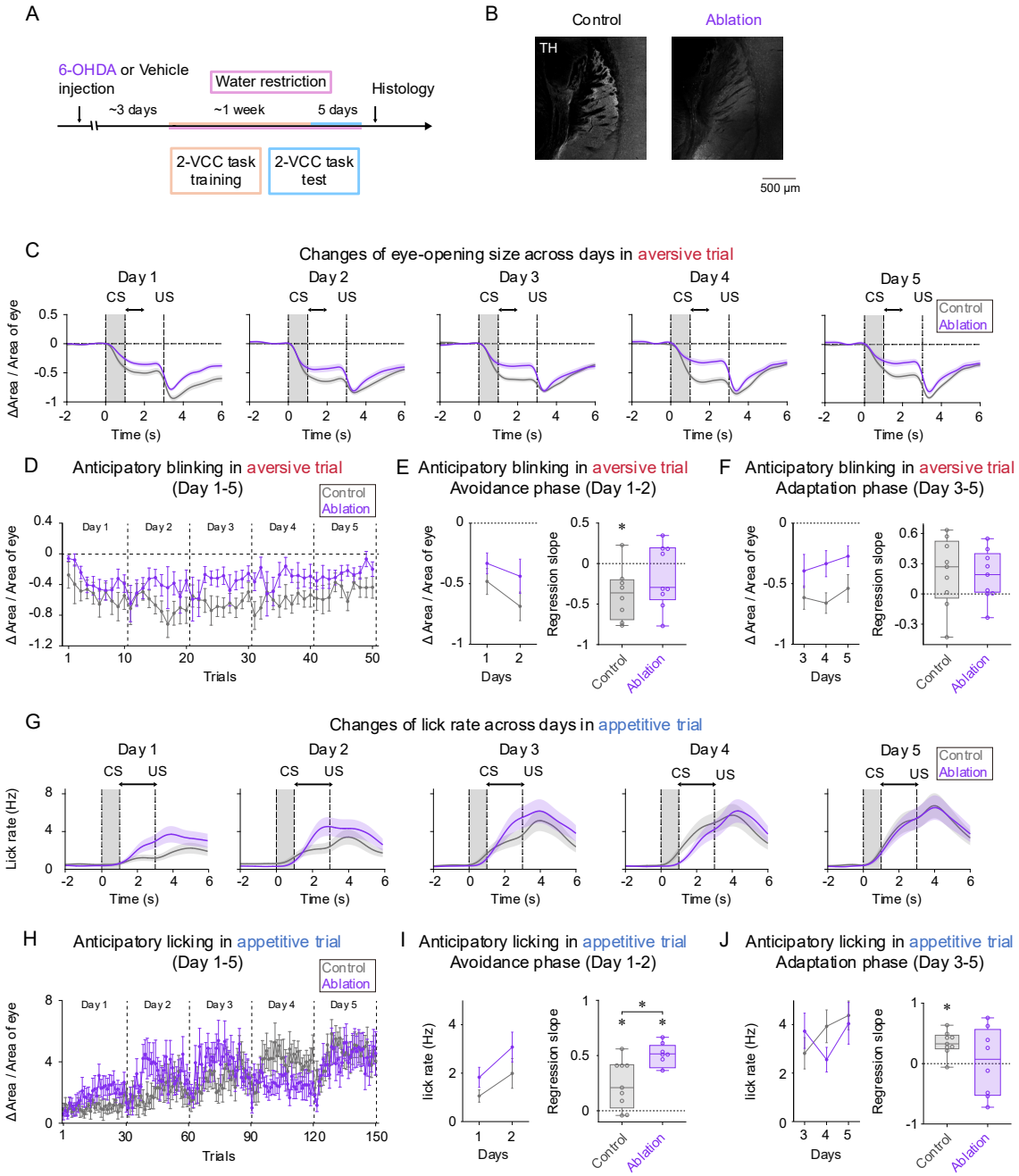
Line 417-424,

Limitations

The number of animals used was relatively small, though each experimental condition includes more than 5 animals which allows conducting statistical analysis. Control mice in optogenetic experiment showed lower magnitude of anticipatory blinking (Fig. 5M), compared to mice in the prior photometry experiment (Fig. 2). This difference maybe induced by unknown effects of light stimulation upon the head and/or additional implantations of optical fibers and virus injections into the midbrain.

To elucidate the roles of TS dopamine in aversive learning, we performed loss-of-function experiment during the revision and revealed that TS dopamine ablation inhibited aversive learning and accelerated appetitive learning (please also see our reply to Comment #4, Reviewer #2). These findings supported the previous our argument that TS dopamine dynamics are essential for avoidance learning, which indirectly modulates appetitive learning, underscoring a regulatory mechanism for shifting between defensive and reward-seeking behaviors. We now included these data and discussion in the current manuscript.

Fig. 4,



In summary, my main concerns are related to the behavioral measures and how they relate to day-to-day learning given the seemingly random difference in starting rate on a given day. In addition, it appears that there are differences in behavior between rats that underwent injections for optogenetic manipulation and control, and those in the prior experiments that just had dopamine measured in the striatum.

Thank you for the insightful comments. The additional analysis regarding day-by-day changes of behavior revealed that the behavioral and neural changes were more obvious within day (i.e., across trial) in the current study. Behavioral differences across controls were notified in the manuscript as limitation of current study. We believe that this revision has greatly improved our manuscript and the current version is now acceptable.

Reference list

1. Amo, R. *et al.* A gradual temporal shift of dopamine responses mirrors the progression of temporal difference error in machine learning. *Nat. Neurosci.* **25**, 1082–1092 (2022).
2. Kim, H. F., Ghazizadeh, A. & Hikosaka, O. Dopamine Neurons Encoding Long-Term Memory of Object Value for Habitual Behavior. *Cell* **163**, 1165–1175 (2015).
3. Menegas, W., Babayan, B. M., Uchida, N. & Watabe-Uchida, M. Opposite initialization to novel cues in dopamine signaling in ventral and posterior striatum in mice. *eLife* **6**, e21886 (2017).
4. Greenstreet, F. *et al.* Dopaminergic action prediction errors serve as a value-free teaching signal. *Nature* **643**, 1333–1342 (2025).
5. Zafiri, D., Salinas-Hernández, X. I., De Biasi, E. S., Rebelo, L. & Duvarci, S. Dopamine prediction error signaling in a unique nigrostriatal circuit is critical for associative fear learning. *Nat. Commun.* **16**, 3066 (2025).
6. Chen, A. P. F. *et al.* Nigrostriatal dopamine pathway regulates auditory discrimination behavior. *Nat. Commun.* **13**, 5942 (2022).
7. Akiti, K. *et al.* Striatal dopamine explains novelty-induced behavioral dynamics and individual variability in threat prediction. *Neuron* **110**, 3789-3804.e9 (2022).
8. Tsutsui-Kimura, I. *et al.* Dopamine in the tail of the striatum facilitates avoidance in

threat–reward conflicts. *Nat. Neurosci.* **28**, 795–810 (2025).

9. Menegas, W., Akiti, K., Amo, R., Uchida, N. & Watabe-Uchida, M. Dopamine neurons projecting to the posterior striatum reinforce avoidance of threatening stimuli. *Nat. Neurosci.* **21**, 1421–1430 (2018).

10. Goodman, J., McClay, M. & Dunsmoor, J. E. Threat-induced modulation of hippocampal and striatal memory systems during navigation of a virtual environment. *Neurobiol. Learn. Mem.* **168**, 107160 (2020).

11. Silston, B., Ochsner, K. N. & Aly, M. Threat impairs flexible use of a cognitive map. *Motiv. Emot.* **47**, 908–927 (2023).

12. Walters, C. J., Jubran, J., Sheehan, A., Erickson, M. T. & Redish, A. D. Avoid-approach conflict behaviors differentially affected by anxiolytics: implications for a computational model of risky decision-making. *Psychopharmacology (Berl.)* **236**, 2513–2525 (2019).

13. Thornton, I. M., Tagu, J., Zdravković, S. & Kristjánsson, Á. The Predation Game: Does dividing attention affect patterns of human foraging? *Cogn. Res. Princ. Implic.* **6**, 35 (2021).

We greatly appreciate the reviewers' insightful and constructive comments. To fully address the previous concerns, we conducted new analyses and rephrasing the text. We sincerely hope that the reviewers think that this version is now acceptable for publication at Communications Biology.

Reviewers' Comments:

Reviewer #1 (Remarks to the Author):

The Authors addressed all of my concerns.

Reviewer #2 (Remarks to the Author):

The authors have carefully addressed my previous comments with substantial additional experiments and analyses. The manuscript has significantly improved and now meets the standard for publication in Communications Biology.

Reviewer #3 (Remarks to the Author):

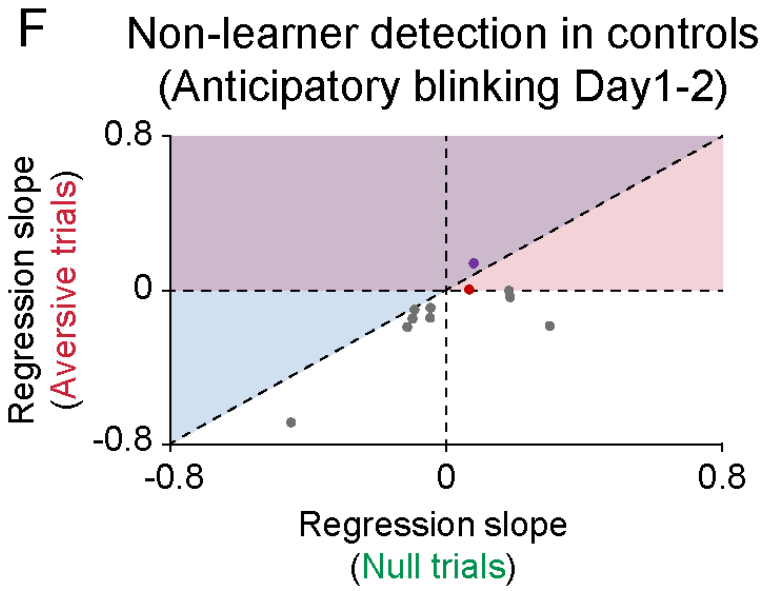
The authors did an admirable job in addressing the comments raised by all three reviewers. In many cases, the comments were satisfactorily addressed resulting in a much-improved manuscript. However, several responses did not fully address concerns and result in remaining important unresolved issues. These are outlined below:

1. The response to the question regarding non-learners from reviewer one, combined with the question from reviewer three regarding the optogenetic experimental controls raises an important additional question. Specifically, it isn't clear that the same learner analysis was applied to the optogenetic experiment. Based on the data in 5N, it would appear that the majority of those animals would be considered non-learners, even with the relatively conservative cutoff of <0 applied by the authors. A one-way t-test of both groups would likely show, as groups, that both controls and opto animals are not significantly responding to the CS in day 1 or in the case of controls, in days 3-5. This makes this group,

in particular, difficult to assess when it is clear animals generally are not performing the task. In fact, the same could likely be said with anticipatory licking as well. A one-way t-test might indeed show that as a group, they would not differ from 0 Hz (the exception being the 2 mice showing a rather high lick rate. The fact that the additional analysis of the DA ablation shows that animals were again showing responses on day one suggests something majorly confounding is going on in the procedures related to the optogenetic experiment.

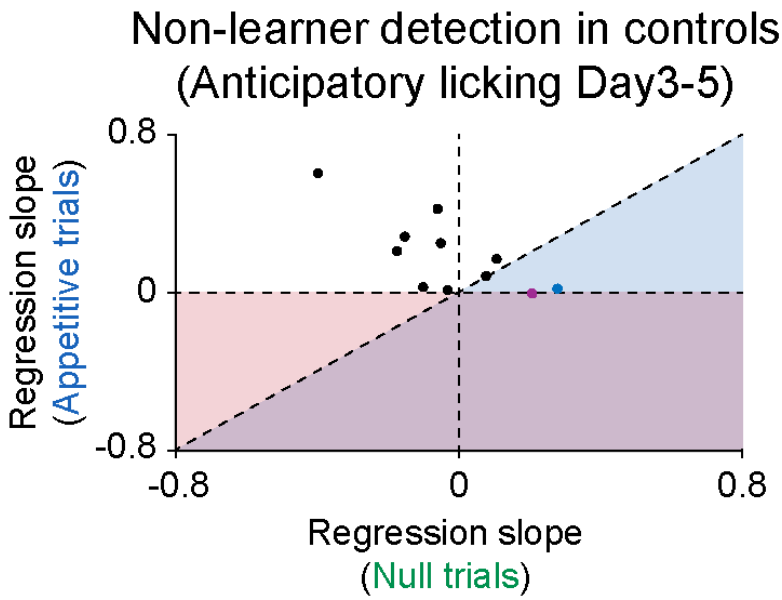
Thank you for your insightful comments. As well as the initial experiment (Figure 2), we included all animals who performed the 2-VCC task in the optogenetic experiment. As the reviewer pointed out, the control animals in the optogenetic experiment showed relatively small changes in amplitude of anticipatory blinking (Fig. S7H), however, it was revealed that 9/11 mice learned to avoid the air-puff during avoidance phase when we used the cutoff of regression slope >0 (red and purple in Supplement Figure S7F). We also applied the comparison between null trials to determine non-learners as the reviewer#3 suggested in the comment#4, and found that 10/11 mice learned to avoid (purple in Supplement Figure S7F). Considering that 7/9 (cutoff of regression slope <0) and 7/9 (in relation to the null trials) learners were detected in the initial experiment (please see Rebuttal Figure 3), the control group in the optogenetic experiment did include appropriate number of avoidance learners.

Supplemental Figure S7F,



Regression slope of anticipatory blinking in optogenetic controls. Non-learners were shown in red, blue or purple circles, and learners were shown in gray circles. Red shading, regression slope of anticipatory blinking during avoidance phase > 0. Blue shading, regression slope of anticipatory blinking during avoidance phase > that in null trials. Purple shading, meets with both definitions.

Regarding appetitive learning, the controls in the optogenetic experiment learned to show significant anticipatory licking during adaptation phase (day 3-5) as shown in Figure 5P right, consistent with the initial experiment (Figure 3D). We also found that only 1/11(cutoff of regression slope <0) and 2/11 (in relation to the null trials) mouse can be considered as non-learners as shown in below Rebuttal Figure 1. These data show that controls in the optogenetic experiment learned to approach reward in the task.



Rebuttal Figure 1. Regression slope of anticipatory licking during adaptation phase of the optogenetic controls. Non-learners were shown in red, blue or purple, and learners were shown in black circles. Red shading, regression slope of anticipatory blinking during avoidance phase < 0. Blue shading, regression slope of anticipatory blinking during avoidance phase < that in null trials. Purple shading, meets with both definitions.

Together, the previous analysis and the additional analysis indicate that the controls in the optogenetic analysis showed avoidance and appetitive learning. We included these arguments in the revised manuscript.

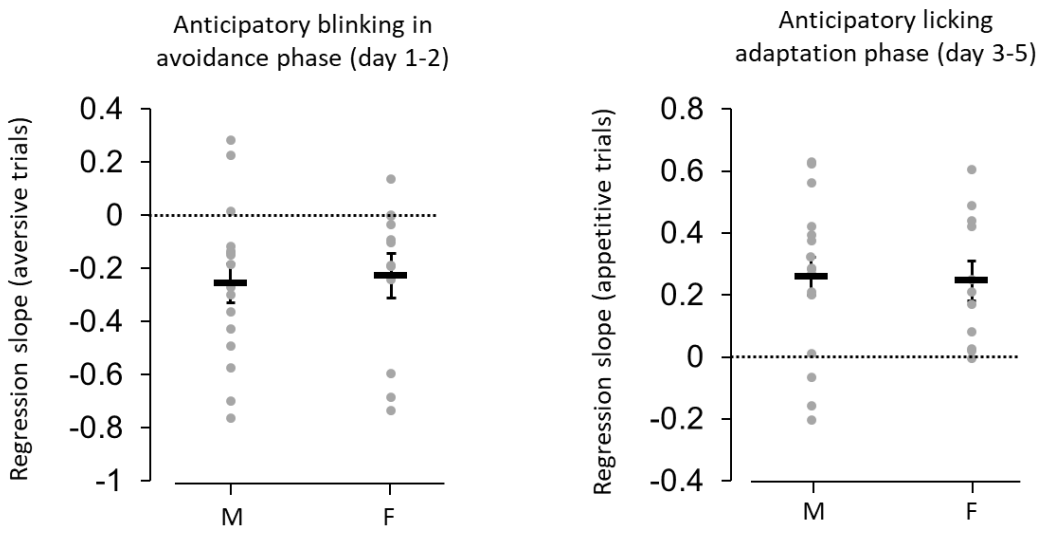
Line 277-279,
Although the amplitude of anticipatory blinking was relatively low (Fig. 5M and N), we confirmed that most of control animals learned to avoid air-puff (10 out of 11) in the task (Fig. S7F).

Line 290-291,
Control animals increased anticipatory licking during adaptation phase (Fig. 5P, right), indicating that they learned to approach reward in the task.

Line 428-431,
Control mice in the optogenetic experiment showed relatively lower magnitude of anticipatory blinking (Fig. 5M and N), though most of them successfully learned to avoid air-puff (Fig. S7F), compared to mice in the prior photometry experiment (Fig. 2).

2. Related to this, with the more complete explanation of animals used in the various experiments which utilized the task, one possibility for the difference in anticipatory blinking in the initial vs. optogentetic experiments is a possible difference between male and female mice in the task. The initial task only includes one female while the subsequent experiments utilized more balanced groups. Female mice have been found to show increased sensitivity to eye-blink conditioning. (PMID: 19272397) This would stand in contrast to the present finding with the largely male group showing increased sensitivity, but it could stand to reason that sex differences may be at play and that is one explanation for the present differences. However, this would be contradicted by the ablation experiment. Across groups, an analysis of sex differences in the task should be performed to account for this. Noted sex differences should also be mentioned in the limitations/discussion.

Thank you for raising this important issue. We compared males and females of all control animals including initial, ablation, and optogenetic experiment regarding aversive and appetitive learning, and found no clear difference between sex (Rebuttal Figure 2). However, as the reviewer pointed out, it is true that the gender ratio in the current study was not well controlled as shown in Methods section (Line 450-458). We noted this point and the potential sex differences in eye blink response in the Limitation section in the revised manuscript. We also submitted the numerical source data for all experiments which include sex information in the current revision.



Rebuttal Figure 2. Comparison of males (n=17) and females (n=12) controls used in the initial, ablation, and optogenetic experiments regarding aversive and appetitive learning. Regression slope of anticipatory blinking (left) and licking (right) during avoidance and adaptation phase, respectively. No gender difference was observed in aversive (p=0.80, Student's t-test) or appetitive learning (p=0.87, Student's t-test).

Line 466-468,

As gender difference in the sensitivity of eye blink response has been reported[68], difference in gender balance between experiments may also account for the behavioral differences.

3. On a more minor note, I would argue that animals that did not adapt by reducing eye-blink measures on days 3-5 should still be counted as learners. They clearly demonstrate an association between the CS and US which by any definition would be considered learning. It could be the case that some animals are more sensitive than others to the air puff and thus are less likely to adapt to it for any number of reasons but have still learned the association.

We agree with this comment. In the previous manuscript, we did not clearly describe the number of non-learners in avoidance and adaptation, which gave an impression that adaptation non-learners were included as avoidance non-learners. In the revised manuscript, we made clear this point as below:

Line 133-135,

There were 3 out of 9 mice who did not learn avoidance (regression slope of anticipatory blinking during avoidance phase > 0, n=2) and/or adaptation (regression slope of anticipatory blinking during adaptation phase < 0, n=1).

4. The additional analysis of the data without non-learners should be contextualized in the manuscript. Specifically, it shouldn't be left to the reader to sort out whether any differences in results occurred because of not including non-learners in the analysis.

Thank you for the notification. All data except for Figure S2, and S3 include both learner and non-learner. We labeled "without non-learner" in each panel which does not include non-learner in Figure S2 and S3.

Figure S2

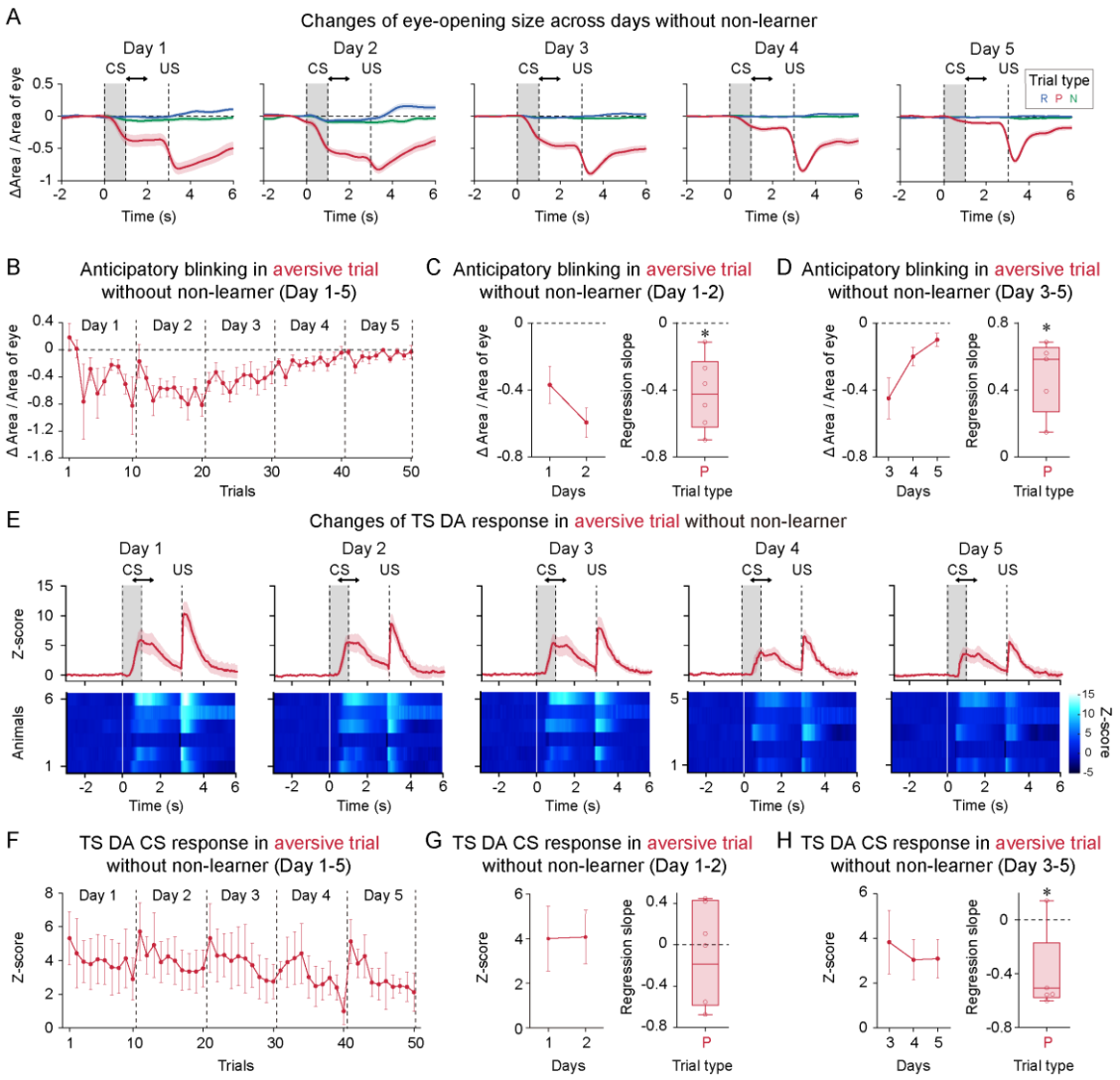
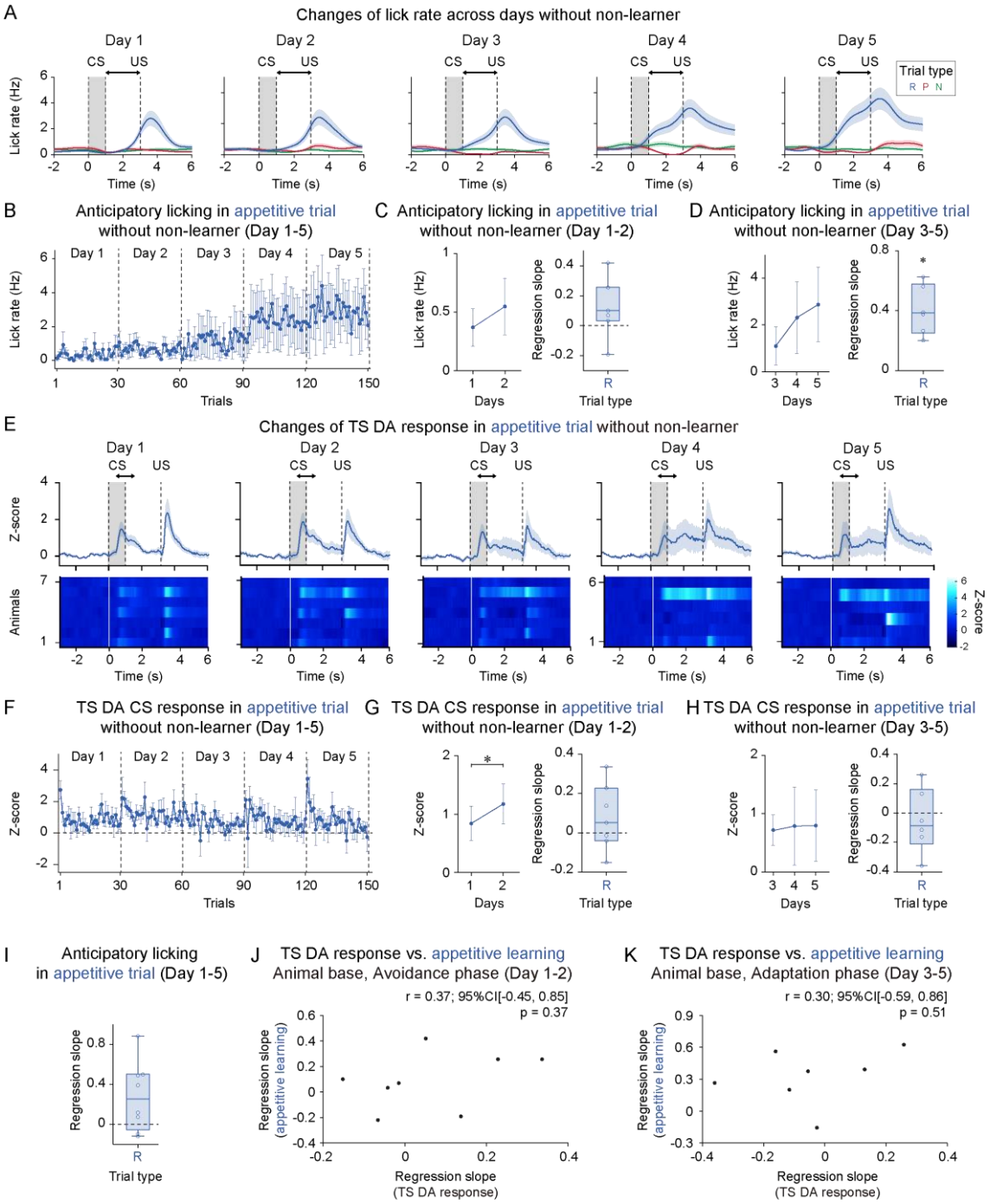


Figure S3

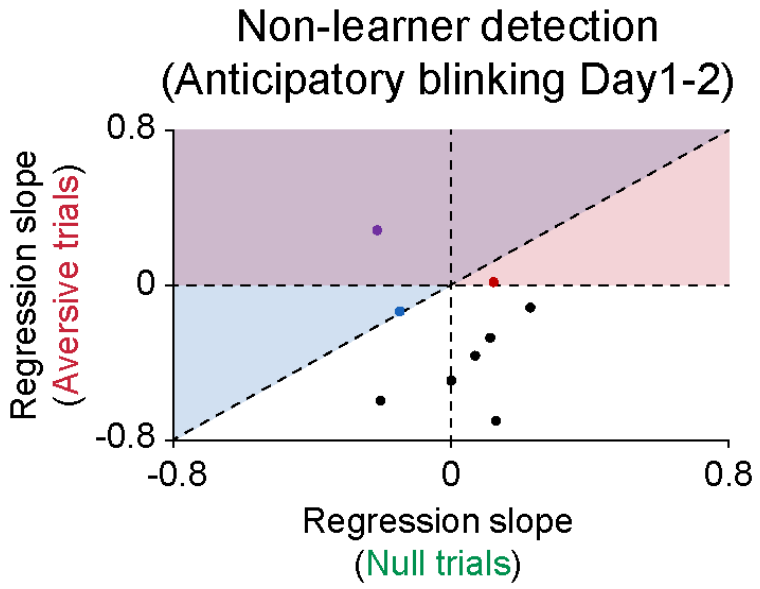


5. As mentioned as well, the definition of learners is very conservative in the subsequent analysis. Perhaps a better comparison would be in relation to the null trials. That way, random licks or blinks that would make the slopes greater or less than 0 would be accounted for in relation to meaningful CS experiences. This would seem to be a less

arbitrary definition. It would also be helpful to see null responses in behavior figures to be able visualize learning in the meaningful CS experiences. Finally, it could support sentences claiming evidence of learning in lines 79-89 to show significant behavior vs. the null trials.

Thank you for the insightful comments. We agreed that there would be a possibility that random licks or blinks make the slope greater or less which would hamper the determination of learners. Then, we tested this possibility by analyzing regression slopes of anticipatory blinking and licking in null trials and comparing with those of aversive or appetitive trials in the initial experiment.

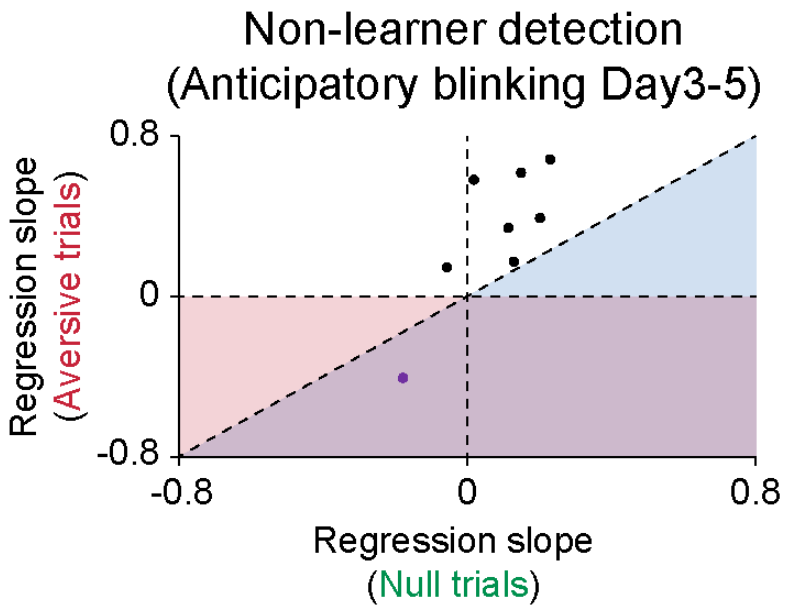
Anticipatory blinking in avoidance phase:
As described in the previous version of the manuscript, 2 out of 9 mice were determined as non-learner with the regression slope cut off >0 (shown in red or purple below). By comparing the regression slope of null trials, 2 out of 9 mice were determined as non-learners (shown in blue or purple below).



Rebuttal Figure 3. Regression slope of anticipatory blinking in the initial experiment. Non-learners were shown in red, blue or purple circles, and learners were shown in black circles. Red shading, regression slope of anticipatory blinking during avoidance phase > 0. Blue shading, regression slope of anticipatory blinking during avoidance phase > that in null trials. Purple shading, meets with both definitions.

Anticipatory blinking in adaptation phase:

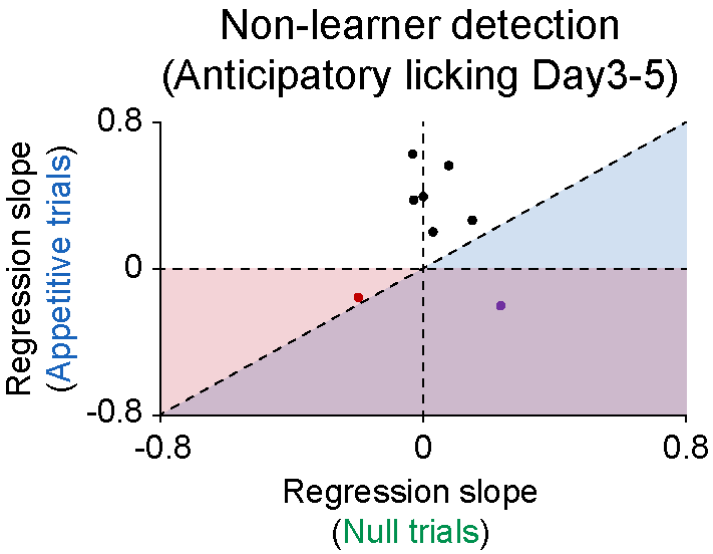
As described in the previous manuscript, 1 out of 9 mice was determined as non-learner with the regression slope cut off <0 (shown in purple below). By comparing the regression slope of null trials, same mouse was determined as non-learner (shown in purple below).



Rebuttal Figure 4. Regression slope of anticipatory blinking in during adaptation phase of the initial experiment. Non-learners were shown in red, blue or purple, and learners were shown in black circles. Red shading, regression slope of anticipatory blinking during avoidance phase < 0. Blue shading, regression slope of anticipatory blinking during avoidance phase < that in null trials. Purple shading, meets with both definitions.

Anticipatory licking in adaptation phase:

As described in the previous manuscript, 2 out of 9 mice were determined as non-learner with the regression slope cut off <0 (shown in red or purple below). By comparing the regression slope of null trials, 1 out of 9 mouse was determined as non-learner (shown in purple below).



Rebuttal Figure 5. Regression slope of anticipatory licking during adaptation phase of the initial experiment. Non-learners were shown in red, blue or purple, and learners were shown in black circles. Red shading, regression slope of anticipatory blinking during avoidance phase < 0. Blue shading, regression slope of anticipatory blinking during avoidance phase < that in null trials. Purple shading, meets with both definitions.

Overall, comparable numbers of animals were determined as non-learner when we applied the comparison between null trials. These results suggest that random lick or blink made little impacts on categorizing learner and non-learner, and the cutoff off < or > 0 criteria was able to properly detect non-learner in the current study.

We have visually showed null responses in anticipatory blinking and licking in the revised manuscript (Figure 2A and 3A) and have compared the appetitive and aversive behaviors vs. null trials (Figure S1A-E). As shown in below graphs, the frequency of occurrence of anticipatory behaviors in the null trials (green) was much lower compared to behaviors in the appetitive (blue) or aversive (red) trials.

Figure 2A

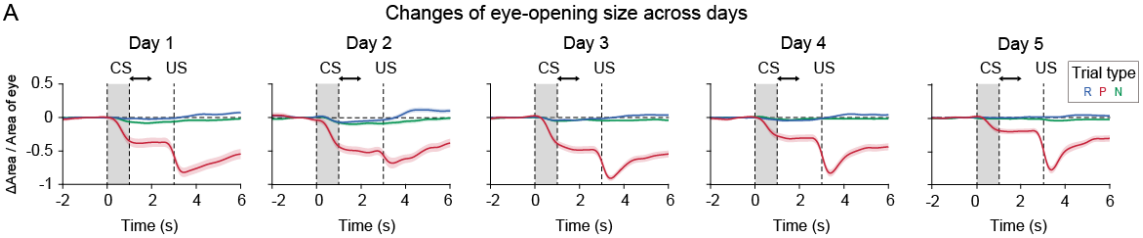
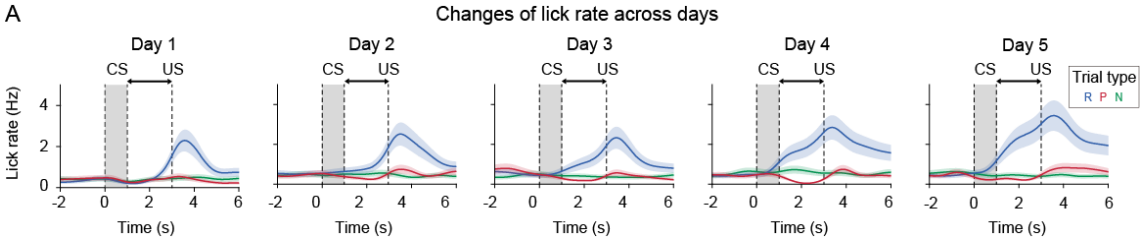


Figure 3A



6. When comparing behavior in the ablation study, please utilize statistical analysis in the description of the results (lines 210 – 225). A direct comparison of change in eye area and lick rate between groups would determine whether learning was significantly different. This analysis should be done in addition to the analysis on slope included in the manuscript. However, one concern here is that striatal dopamine is closely associated with behavioral selection and coordination more generally. It should be noted in the text that at least in the case of licking, however, ablation animals do eventually achieve similar behavior. However, the tail of the striatum has been associated with saccade behavior and thus might have a larger impact on eye blink as well (PMID: 22875934).

Thank you for the comment. We have compared the amplitude of anticipatory blinking in aversive trials (Figure 4E and 4F) and anticipatory licking in appetitive trials (Figure 4I and 4J) in control vs. ablation groups, and found no significant difference between groups in any of comparisons. This is probably because the anticipatory responses were dramatically changes across trials, averaging them over in a session would mask behavioral changes. Indeed, the session-based analysis could not capture the behavioral dynamics in the initial experiment (Figure 2C, 2D, 3C, and 3D).

As the reviewer pointed out, ablation animals eventually achieved similar level of anticipatory licking (Figure 4J). However, it is possible that ablation directly affected to eye blink response. We noted this point in the revised manuscript.

Line 215-219,

These data suggest that ablation of TS dopamine impaired avoidance learning and flattened the changes of defensive behavior across days although there was a possibility that ablation of TS dopamine directly affected the eye blink response[17].

7. In your discussion of the optogenetic experiments, the lack of behavior in control animals shown in figures 5 and S7 are not accounted for beyond the limitations section. Any interpretation of these results must account for the fact that the control animals in this experiment did not behave similarly to other animals in the rest of the study. This makes comparisons between the optogenetic experiments and the other two experiments impossible to make.

Thank you for the comment. We agree that it is important to clearly state whether the controls in the optogenetic experiment learned the task or not. As we mentioned above, most of the optogenetic controls did acquire anticipatory blinking and licking in the task (Fig. S7F, Figure 5P, and Rebuttal Figure 1). We clearly described this point in the revised manuscript and kept the discussion about the lower amplitude of anticipatory blinking in the limitation section.

Line 277-279,
Although the amplitude of anticipatory blinking was relatively low (Fig. 5M and N), we confirmed that most of control animals learned to avoid air-puff (10 out of 11) in the task (Fig. S7F).

Line 290-291,
Control animals increased anticipatory licking during adaptation phase (Fig. 5P, right), indicating that they learned to approach reward in the task.

Reviewers' Comments:

Reviewer #3 (Remarks to the Author):

I would like to thank the authors for carefully addressing the additional concerns. While the learning measures remain modest in the manuscript, the additional analyses justify their validity. I now agree that this manuscript meets the criteria for publication.

Thank you for the insightful and instructive comments. We sincerely appreciate the efforts of the reviewers to improve our manuscript. We believe that the manuscript has been improved dramatically and now meets the criteria for the publication.