Supplemental materials for:

A causal link between prediction errors, dopamine neurons and learning

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Figure S1

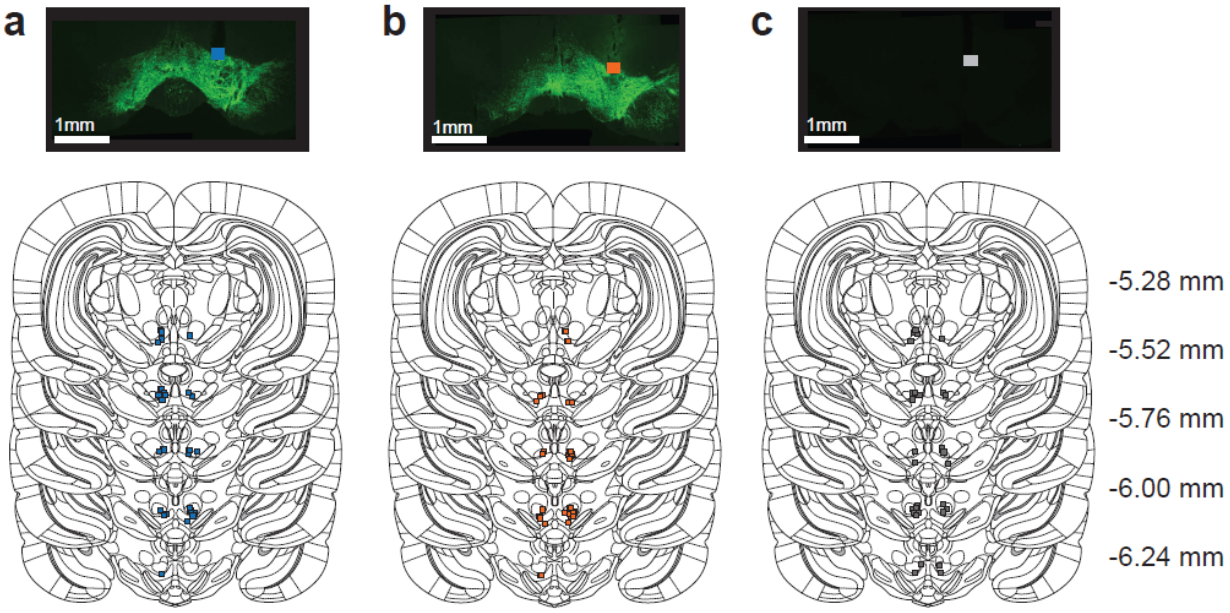


Fig. S1. Histological reconstruction of optical fiber placements for subjects in all studies. (**a**) Top, ChR2-YFP expression for a representative PairedCre+ rat. Blue line denotes the location of the optical fiber tip in this rat. Note that virus injection and optical fiber placements were unilateral in this and all other studies. Bottom, optical fiber tip placement for all rats in this group (n=37). (**b**) Top, ChR2-YFP expression for a representative UnpairedCre+ rat. Orange line denotes optical fiber tip location in this rat. Bottom, optical fiber tip placement for all rats in this group (n=36). (**c**) Top, ChR2-YFP expression for a representative PairedCre– rat. Grey line denotes optical fiber tip location in this rat. Bottom, optical fiber tip placement for all rats in this group (n=41). Distance is posterior relative to bregma.

Figure S2

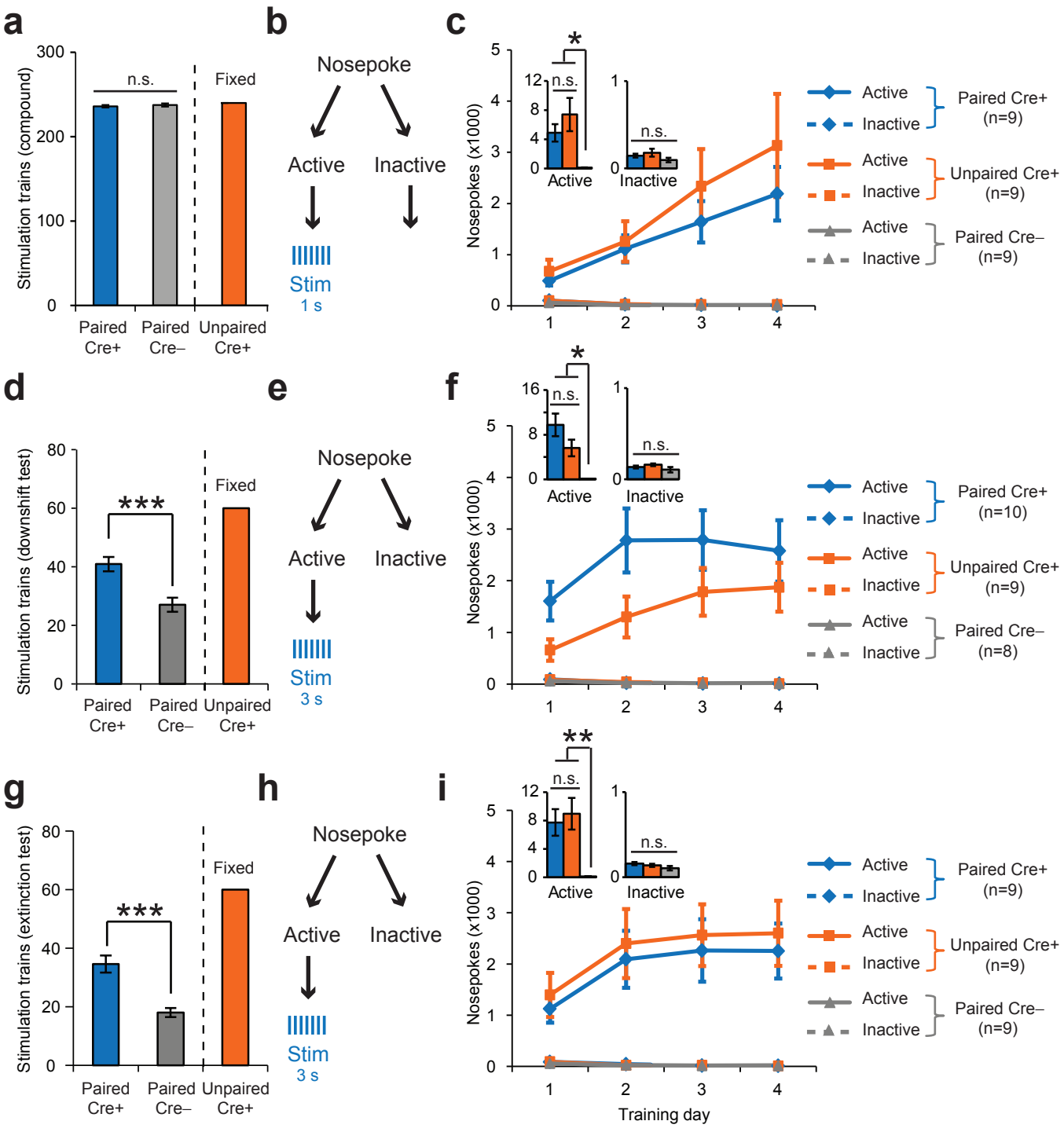

a
b
c
Stimulation trains (compound)
300
200
100
0
n.s.
Fixed
Paired Cre+
Paired Cre–
Unpaired Cre+
Nosepoke
Active
Inactive
Stim
1 s
Nosepokes (x1000)
*
n.s.
Active
Inactive
Paired Cre+ (n=9)
Unpaired Cre+ (n=9)
Paired Cre– (n=9)
d
e
f
Stimulation trains (downshift test)
***
Stim
3 s
Paired Cre+ (n=10)
Paired Cre– (n=8)
g
h
i
Stimulation trains (extinction test)
**
Training day

Fig. S2. Optical activation of dopamine neurons is equally reinforcing for Cre+ groups in Blocking, Downshift and Extinction studies. (**a**) Stimulation trains delivered to all groups across four days of compound training in the Blocking study (Fig. 2). PairedCre+ and PairedCre– rats received equivalent amounts of optical stimulation (t-test, $p=0.723$) because of equivalent behavioral performance during these sessions. UnpairedCre+ rats received a fixed number of stimulation trains during the ITIs, set at the maximum number of trains that could be earned by Paired groups. (**b**) Schematic of intracranial self-stimulation (ICSS) task for rats in the Blocking study (see Methods). Stimulation parameters were 1s train, 5ms pulse, 20 Hz to match those used in the Blocking experiment. (**c**) Responding across four daily ICSS sessions for rats in the Blocking study. PairedCre+ and UnpairedCre+ groups made significantly more active nosepoke responses than PairedCre– controls (two-way RM ANOVA, main effect of group and group x day interaction $F>4.589$, $p<0.008$; SNK post-hoc test PairedCre+ vs. PairedCre– $p=0.018$, UnpairedCre+ vs. Paired Cre–$p=0.005$) but did not differ from each other ($p=0.355$). There were no group differences in inactive nosepoke responding (two-way RM ANOVA, main effect of group and group x day interaction, $F<2.17$, $p>0.056$). Inset, summed responding across all 4 sessions. (**d**) Stimulation trains delivered to all groups during the downshift test session (Fig. 3). PairedCre+ rats earned more stimulation trains than PairedCre– rats (t-test, $p<0.001$) because of differences in behavioral performance during this test. (**e**) Schematic of ICSS task for rats in Downshift study. Stimulation parameters were 3s train, 5ms pulse, 20 Hz to match those used in the Downshift/Extinction experiments. (**f**) As in C, but for rats in the Downshift study. PairedCre+ and UnpairedCre+ groups made significantly more active nosepoke responses than PairedCre– controls (two-way RM ANOVA, main effect of group and group x day interaction $F>3.711$, $p<0.003$; SNK post-hoc test PairedCre+ vs. PairedCre– $p<0.001$, UnpairedCre+ vs. PairedCre– $p=0.025$) but did not differ from each other ($p=0.068$). There were no group differences in inactive nosepoke responding (two-way RM ANOVA, main effect of group and group x day interaction, $F<1.7$, $p>0.154$). (**g**) Stimulation trains delivered to all groups during the extinction test session (Fig. 4). PairedCre+ rats earned more stimulation trains than PairedCre– rats (t-test, $p<0.001$) because of differences in behavioral performance during this test. (**h**) Schematic of ICSS task for rats in Extinction study. Stimulation parameters were 3s train, 5ms pulse, 20 Hz to match those used in the Downshift/Extinction experiments. (**i**) As in C, but for rats in the Extinction study. PairedCre+ and UnpairedCre+ groups made significantly more active nosepoke responses than PairedCre– controls (two-way RM ANOVA, main effect of group and group x day interaction $F>3.041$, $p<0.01$; SNK post-hoc test PairedCre+ vs. PairedCre– $p=0.003$, UnpairedCre+ vs. PairedCre– $p=0.004$) but did not differ from each other ($p=0.612$). There were no group differences in inactive nosepoke responding (two-way RM ANOVA, main effect of group and group x day interaction, $F<2.187$, $p>0.054$). Note that the same behavioral data was used in F and I, but data from Cre+ rats was sorted differentially in each case according to each subject's group assignment in Downshift and Extinction studies, as the same rats were used for both experiments. *$p<0.05$; ***$p<0.001$.

Figure S3

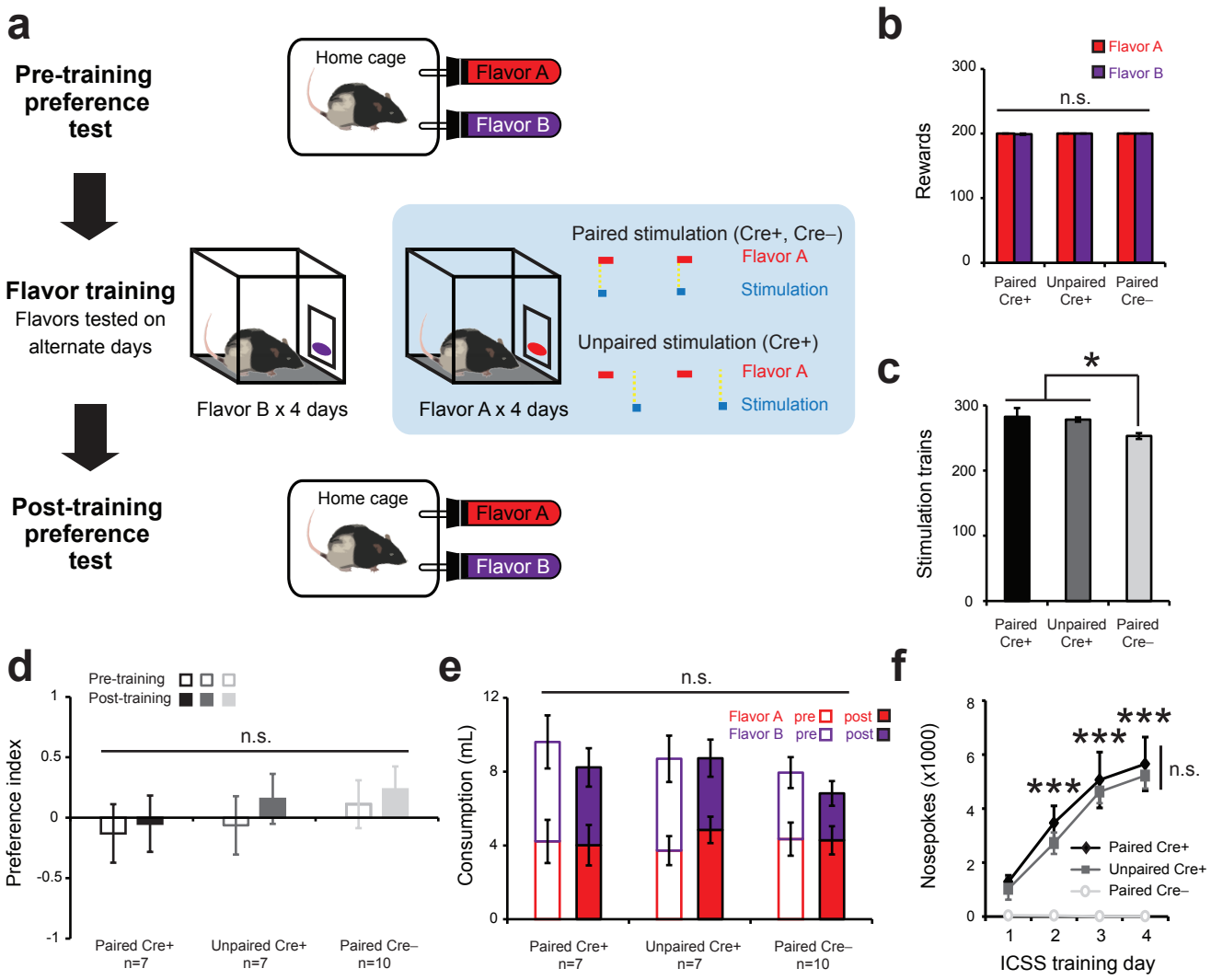

a
Pre-training preference test
Home cage
Flavor A
Flavor B
Flavor training
Flavors tested on alternate days
Flavor B x 4 days
Flavor A x 4 days
Paired stimulation (Cre+, Cre–)
Flavor A
Stimulation
Unpaired stimulation (Cre+)
Flavor A
Stimulation
Post-training preference test
Home cage
Flavor A
Flavor B
b
Flavor A
Flavor B
n.s.
Rewards
300
200
100
0
Paired Cre+
Unpaired Cre+
Paired Cre–
c
*
Stimulation trains
300
200
100
0
Paired Cre+
Unpaired Cre+
Paired Cre–
d
Pre-training
Post-training
n.s.
Preference index
1
0.5
0
-0.5
-1
Paired Cre+ n=7
Unpaired Cre+ n=7
Paired Cre– n=10
e
n.s.
Flavor A pre post
Flavor B pre post
Consumption (mL)
12
8
4
0
Paired Cre+ n=7
Unpaired Cre+ n=7
Paired Cre– n=10
f
***
***
***
n.s.
Nosepokes (x1000)
8
6
4
2
0
Paired Cre+
Unpaired Cre+
Paired Cre–
1
2
3
4
ICSS training day

Fig. S3. Dopamine neuron activation does not alter preference for a paired natural reward. (**a**) Experimental design for flavor preference study. Cre+ rats, or their Cre– littermates, were exposed to distinctly-flavored sucrose solutions in their home cage to quantify initial preference between the two flavors during a brief (10 min) pre-training test. The next day, flavor training commenced. Flavor training sessions were conducted in operant chambers; subjects received 50 reward deliveries on a VI-30s schedule. Only one flavor was available per day; flavor A and flavor B training days were interleaved. On days where flavor A was available, rats also received optical stimulation (1s train, 5ms pulse, 20 Hz) of VTA DA neurons either paired or unpaired with reward delivery. 24 hours after the last flavor training session, subjects were again simultaneously exposed to both flavors during a brief (10 min) post-training test in their home cage to assess flavor preference. (**b**) Total number of rewards (flavor A and flavor B) consumed by each group during flavor training. There were no differences between groups or between flavors (two-way RM ANOVA, main effect of group, flavor and group x flavor interaction $F<1.296$, $p>0.268$). (**c**) Total number of stimulation trains delivered during flavor A training sessions. Note similarity to stimulation parameters used in the blocking experiment (Fig. S2A). PairedCre+ and UnpairedCre+ rats received more stimulation trains than PairedCre– rats (one-way ANOVA, main effect of group $F_{2,21}=4.869$, $p=0.018$; SNK post-hoc test PairedCre+ vs. PairedCre– $p=0.027$, UnpairedCre+ vs. PairedCre– p-0.026) because of slight differences in behavior during training sessions. (**d**) Pre-training and post-training flavor preference quantified using a preference index which was calculated as follows: (mL flavor A – mL flavor B) ÷ (mL flavor A + mL flavor B). Positive values indicate a preference for flavor A, and negative values indicate a preference for flavor B. There were no group differences in flavor preference before or after flavor training (two-way RM ANOVA, main effect of group, time and group x time interaction $F<0.815$, $p>0.453$). (**e**) Total consumption of flavor A and flavor B during 10 min home cage tests. There were no group differences in the consumption of either flavor before or after flavor training (flavor A; two-way RM ANOVA, main effect of group, time and group x time interaction $F<0.137$, $p>0.715$; flavor B; two-way RM ANOVA, main effect of group, time and group x time interaction $F<2.061$, $p>0.152$). (**f**) Although no group differences were observed in the flavor preference study, subsequent ICSS training revealed that optical stimulation was highly reinforcing in the Cre+ rats used in these experiments (two-way RM ANOVA, main effect of group and group x day interaction $F>14.738$, $p<0.001$; SNK post-hoc test PairedCre+ vs. PairedCre–, $p<0.001$; UnpairedCre+ vs. PairedCre–, $p<0.001$; PairedCre+ vs. UnpairedCre+, $p=0.406$). *$p<0.05$; ***$p<0.001$.

Figure S4

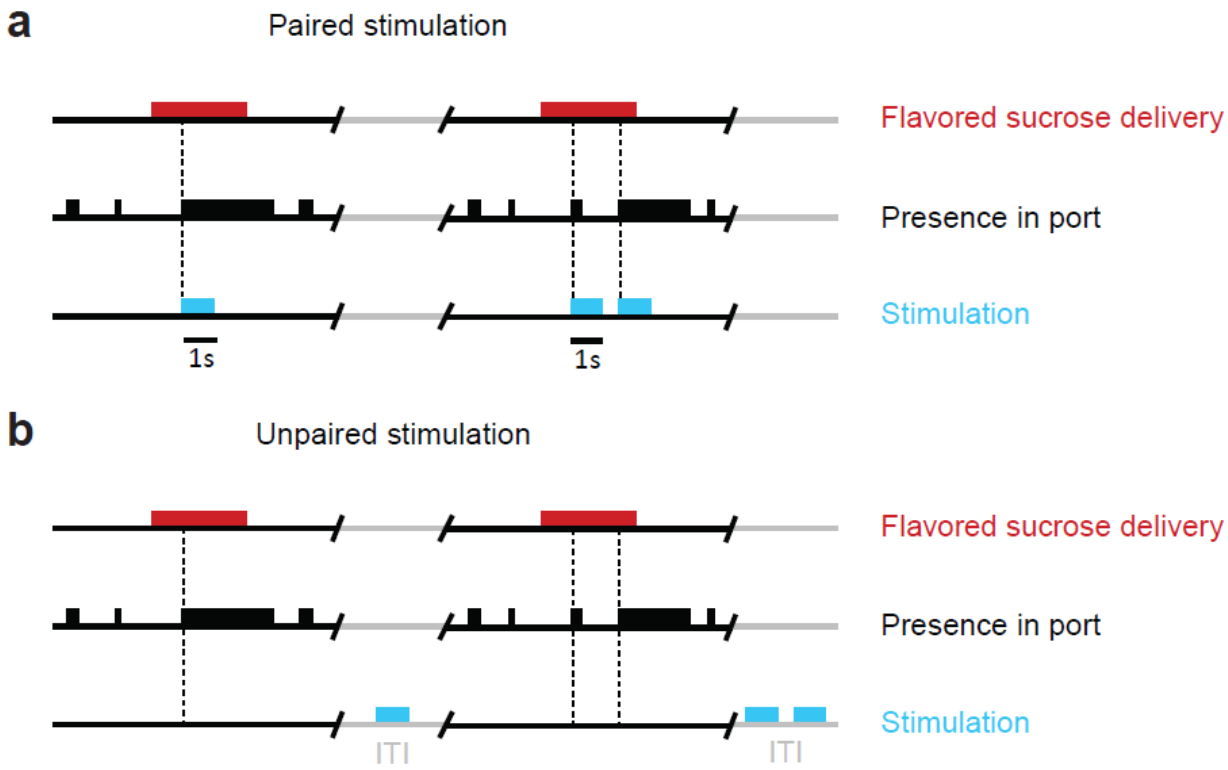
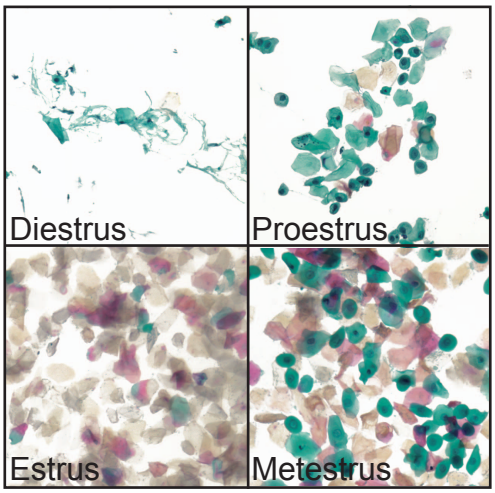


Fig. S4. Relative timing of reward delivery and optical stimulation for the flavor preference study. Relative timing of flavored sucrose delivery, the rats' presence in the reward port, and optical stimulation for two example trials. (**a**) For rats assigned to Paired groups, optical stimulation is delivered the first time the rat enters the reward port when a new reward is available. If the rat does not maintain presence in the port for 1s or longer, indicating that the rat has not fully consumed the reward, subsequent port entries will trigger additional stimulation trains until the 1s requirement is met (second trial example). This is done to ensure that reward consumption and optical stimulation are always experienced coincidently. Because of this, more stimulation trains were delivered than rewards. (**b**) To ensure that UnpairedCre+ rats received equivalent amounts of stimulation as their PairedCre+ counterparts, the number of stimulation trains delivered to the UnpairedCre+ rats was determined using the same criteria, but instead of being delivered during flavored sucrose consumption, the stimulation trains were delivered during the ITI (grey line), when no sucrose solution was present.

Figure S5

a

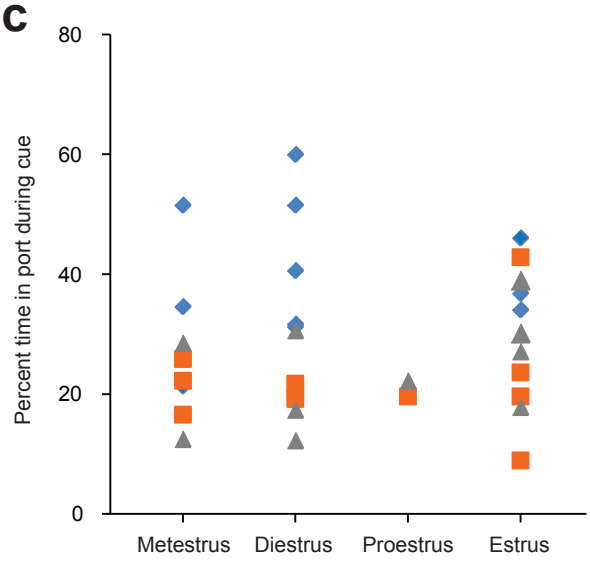


b

	Paired Cre+	Unpaired Cre+	Paired Cre–
Diestrus	5	2	3
Proestrus	0	1	1
Estrus	3	4	4
Metestrus	3	3	2

Cycling	8	10	8
Irregular/ Noncycling	3	0	2

c



d

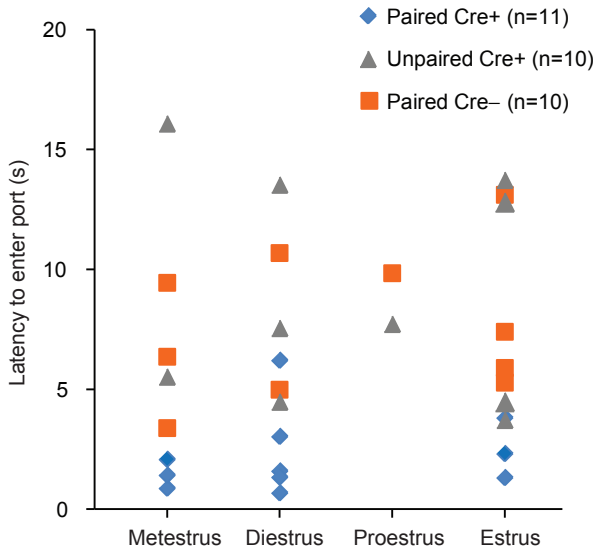


Fig. S5. Estrus cycle stage is not related to behavioral performance during a test session with dopamine neuron activation. (**a**) Typical cytology observed during diestrus, proestrus, estrus and metestrus from cell samples collected from the vaginal wall. Stages were classified according to established criteria (see Methods). (**b**) Vaginal cytology results from all rats in the Downshift experiment during the downshift test when optical stimulation was delivered. Assessments of cycle regularity were based on an examination of cytology samples over five consecutive days. Estrus cycle stage was determined by identifying cellular morphology characteristic to each phase (diestrus, proestrus, estrus, metestrus) according to previously described criteria[48]. Rats were considered to be cycling if consecutive daily samples represented three stages out of four. If morphology consistent with diestrus, metestrus, or a combination of both was observed for four consecutive days, the rat was considered to be non-cycling. If estrus was observed but further samples did not indicate progression through successive stages, the rat's cycle was considered to be irregular. (**c**) Scatterplot of behavioral performance and estrus cycle stage for all rats during the downshift test. A one-way ANOVA revealed no main effect of estrus cycle stage on performance (pooled data from all subjects; main effect of stage, $F_{3,27}$=0.502, p=0.684). Behavioral performance was measured as percent time spent in the reward port during the cue. (**d**). As in C, but with behavioral performance measured as the latency to enter the reward port after cue onset. A one-way ANOVA revealed no main effect of estrus cycle stage on performance (pooled data from all subjects; main effect of stage, $F_{3,27}$=0.41, p=0.747).