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Thalamic Regulation of Sucrose Seeking during Unexpected Reward Omission

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SUMMARY

The paraventricular nucleus of the thalamus (PVT) is thought to regulate behavioral responses under emotionally arousing conditions. Reward-associated cues activate PVT neurons; however, the specific PVT efferents regulating reward seeking remain elusive. Using a cued sucrose-seeking task, we manipulated PVT activity under two emotionally distinct conditions: (1) when reward was available during the cue as expected or (2) when reward was unexpectedly omitted during the cue. Pharmacological inactivation of the anterior PVT (aPVT), but not the posterior PVT, increased sucrose seeking only when reward was omitted. Consistent with this, photoactivation of aPVT neurons abolished sucrose seeking, and the firing of aPVT neurons differentiated reward availability. Photoinhibition of aPVT projections to the nucleus accumbens or to the amygdala increased or decreased, respectively, sucrose seeking only when reward was omitted. Our findings suggest that PVT bidirectionally modulates sucrose seeking under the negative (frustrative) conditions of reward omission.

INTRODUCTION

Cues in the environment that are associated with rewarding or aversive outcomes induce changes in emotional states (Flagel et al., 2011; Namburi et al., 2015; Robinson and Berridge, 2013). While the neural encoding of such changes has long been attributed to the amygdala (Esber et al., 2015; Madarasz et al., 2016; Peck and Salzman, 2014; Sears et al., 2014; Stillman et al., 2015; Tye et al., 2008), emerging evidence suggests that the paraventricular nucleus of the thalamus (PVT) contributes to the regulation of emotional responses (Choi and McNally, 2017; Haight and Flagel, 2014; Hsu et al., 2014; Kirouac, 2015). PVT neurons are activated by contexts/cues associated with reward (Choi et al., 2010; Igelstrom et al., 2010; Li et al., 2016; Matzeu et al., 2017; Schiltz et al., 2007) or aversion (Beck and Fibiger, 1995; Do-Monte et al., 2015b; Penzo et al., 2015; Yasoshima et al., 2007; Zhu et al., 2016). This pattern of activation to stimuli with opposing valence suggests that distinct PVT circuits are recruited to modulate different responses. PVT is broadly connected with regions implicated in motivation, including the prefrontal cortex, the nucleus accumbens (NAc), and the amygdala, and receives extensive hypothalamic projections related to feeding (Lee et al., 2015; Li and Kirouac, 2008, 2012; Moga et al., 1995; Vertes and Hoover, 2008). These connections place PVT in a unique position to integrate positive and negative emotional states in response to cues (for a review, see Do Monte et al., 2016).

Findings from previous studies investigating the role of PVT in reward seeking have been inconclusive. Increased food seeking has been reported following PVT lesions (Haight et al., 2015) or PVT excitation (Barson et al., 2015; Labouèbe et al., 2016), and differing effects on food consumption have been described depending on whether the manipulations were made in the anterior PVT (aPVT) or posterior PVT (pPVT; Bhatnagar and Dallman, 1999; Nakahara et al., 2004; Stratford and Wirtshafter, 2013). These discrepancies may reflect antero-posterior differences, as well as different functions of distinct PVT efferents; however, the projections of PVT regulating reward seeking remain to be determined.

Here, we used a cued sucrose-seeking task to assess the role of PVT and its efferents in reward seeking under conditions of opposing emotional valence: (1) when reward was available during the cue as expected (positive outcome) or (2) when reward was unexpectedly omitted during the cue (negative outcome). Using pharmacological inactivation, unit recording, and optogenetic manipulation of PVT and its outputs, we identified a specific role of aPVT and its projections to the nucleus accumbens and the amygdala in the regulation of reward seeking, specifically during negative outcomes.

RESULTS

Unexpected Reward Omission Increases Sucrose Seeking and Induces Anxiety

Rats previously trained to press a bar for sucrose pellets on a variable reward schedule were given 3 days of cued sucrose seeking in which the availability of reward was signaled by a light (30 s)

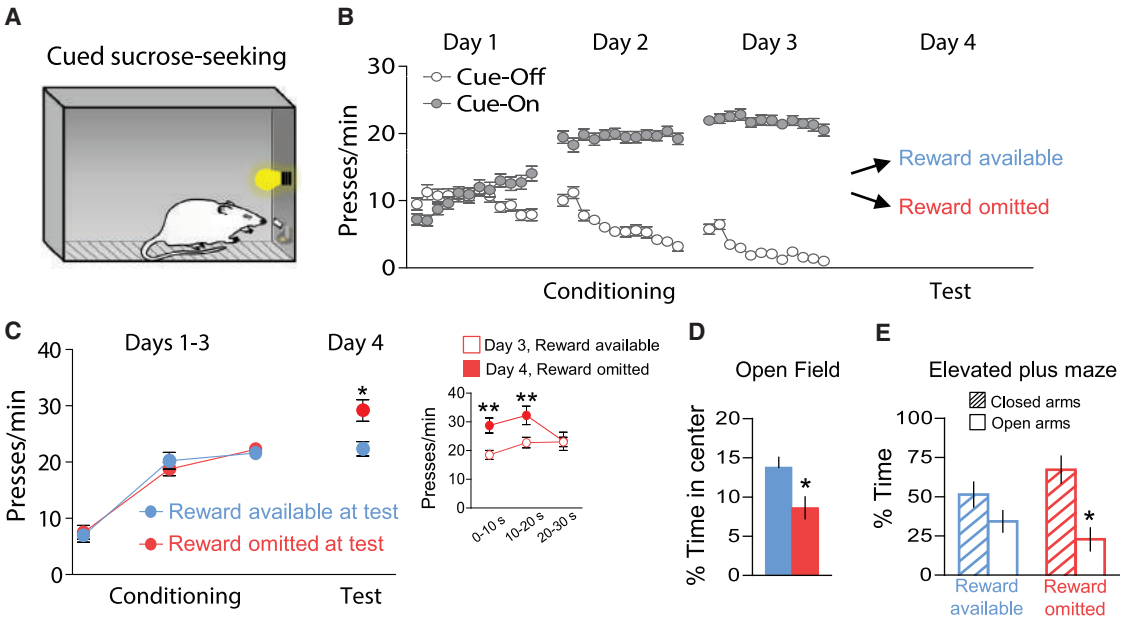

Figure 1. Unexpected Omission of Sucrose Reward Increases Pressing and Induces Anxiety-like Behavior

(A) Schematic of cued sucrose-seeking model. Rats were trained to press a bar for sucrose in the presence of a 30 s light cue, receiving one sucrose pellet per press.

(B) Rate of bar pressing (presses/min) during the conditioning phase (days 1–3). After 3 days of training, press rates increased during the cue-on period (gray dots) compared to the cue-off period (white dots, blocks of 2, n = 48). On day 4, rats were exposed to a test session in which reward was either available or omitted during the light cue.

(C) Cued reward conditioning. Unexpected omission of reward at day 4 test (red group, n = 24) significantly increased rats' press rate compared to the previous day in the presence of reward (paired t test, *p < 0.01, t = 3.73, single trials) or across groups on day 4 (blue group, n = 24, unpaired t test *p < 0.01, t = 2.95). Inset: reward-omitted group pressed significantly more during the first 10 s of the reward-omitted session (day 4, red dots) compared to the previous day in which reward was available (day 3, white dots, ANOVA repeated measures $F_{(1,46)} = 9.04$, p = 0.0042; Duncan post hoc test, **p < 0.01).

(D) A subset of rats were placed in an open field immediately following the test. Rats experiencing reward omission at test (red bar, n = 8) spent less time in the center of the open field (blue bar; n = 7, unpaired t test *p = 0.018, t = 2.67).

(E) A subset of rats were tested in an elevated plus maze immediately following the reward available (blue bars, n = 10) or reward omitted (red bars, n = 10) test. Rats experiencing reward omission spent less time in the open arms (solid white) compared to the closed arms (striped unpaired t test *p = 0.0010, t = 3.88). Data shown as mean ± SEM. *p < 0.05, **p < 0.01.

located above the bar. Each press in the presence of the cue delivered one sucrose pellet to a nearby dish (Figure 1A). After 3 days of reward conditioning, rats learned to limit pressing to the cue-on periods (Figure 1B). The following day (day 4), rats were randomly assigned to two groups: (1) those receiving sucrose during the cue as expected and (2) those for which sucrose would be unexpectedly omitted during the cue. Rats in the reward-omitted group increased their press rate compared to the previous day in the presence of reward or compared to the reward-available group (Figure 1C). Rats in this group exhibited a greater number of press bursts (≥3 presses/s, 1.2 ± 0.36 bursts/min, n = 24) compared to the reward-available group (0.11 ± 0.11 bursts/min, n = 24; unpaired t test, *p = 0.010, t = 2.68). This is consistent with previous studies showing that omission of an expected reward increases reward-seeking responses (Burokas et al., 2012; Dudley and Papini, 1997; Stout et al., 2002), a phenomenon initially described as a "reinforcement-omission effect" or "frustration effect" (Amsel and Roussel, 1952; Jensen and Fallon, 1973).

Omission of expected reward has aversive properties (Amsel, 1958; Huston et al., 2013; Papini, 2003), which increase stress (Dantzer et al., 1980; Zimmerman and Koene, 1998) and anxiety-like behaviors (Komorowski et al., 2012; Manzo et al., 2014) in both experimental animals and humans (Henna et al., 2008; Papini, 2003; Yu et al., 2014). Consistent with this, rats in the reward-omitted group spent less time in the center of an open field (Figure 1D) or in the open arms of an elevated plus maze (Figure 1E) when tested immediately following the day 4 test. This suggests that unexpected omission of reward is anxiogenic in our task.

Pharmacological Inactivation of Anterior PVT Increases Sucrose Seeking during Reward Omission

To assess the role of PVT in cued sucrose seeking under distinct emotional states, we used the $GABA_A$ (γ-aminobutyric acid type A) receptor agonist muscimol (MUS) to inactivate aPVT or pPVT neurons during the reward-available or reward-omitted tests (day 4). Pharmacological inactivation of aPVT had no effect when reward was available (Figures 2A and 2B). When reward was omitted, inactivation of aPVT did not eliminate the increased pressing induced by omission but augmented it even further (Figure 2C). In contrast, inactivation of pPVT had no effect in either condition (Figure S1). Inactivation of either area did not alter consumption of sucrose pellets available ad libitum (aPVT: SAL: 5.0 ± 0.9 g; MUS: 4.8 ± 0.9 g, unpaired t test, *p = 0.89,

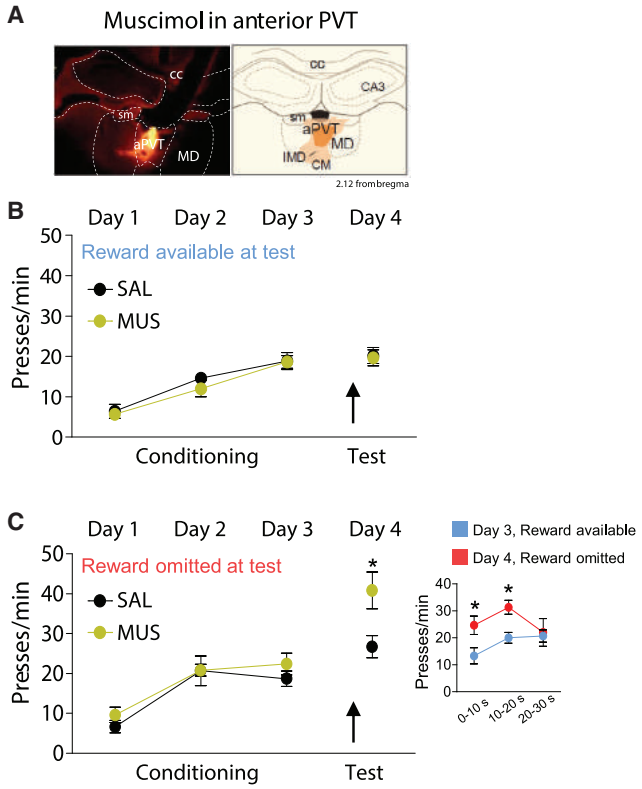

Figure 2. Pharmacological Inactivation of Anterior PVT Increases Sucrose Seeking during Reward Omission

(A) Left: representative micrograph showing the site of fluorescent muscimol (MUS) injection into the anterior PVT (aPVT). Right: orange areas represent the minimum (dark) and the maximum (light) spread of MUS into aPVT. cc, corpus callosum; IMD, intermediodorsal nucleus of the thalamus; CM, central medial nucleus of the thalamus; MD, mediodorsal thalamus; sm, stria medullaris; CA3, hippocampal CA3 subregion.

(B) Press rate in rats infused with saline (SAL, black, n = 9) or MUS (olive, n = 6) 30 min before a reward-available test performed on day 4 (black arrow). MUS inactivation of aPVT had no effect on sucrose seeking when reward was available (unpaired t test, p = 0.79, t = 0.27).

(C) Press rate in rats infused with saline (n = 9) or MUS (n = 5) 30 min before a reward-omitted test performed at day 4. MUS inactivation of aPVT increased sucrose seeking when reward was unexpectedly omitted during the test (unpaired t test, *p = 0.015, t = 2.80). Inset: SAL rats pressed significantly more during the first 10 s of the reward-omitted session (day 4, red dots) when compared to the previous day in which reward was available (day 3, blue dots, ANOVA repeated measures $F_{(1,16)} = 6.19$, p = 0.024; Duncan post hoc test, block 1–10 s, *p = 0.013; block 10–20 s, *p = 0.014). Data shown as mean ± SEM, first trial of each day. *p < 0.05. See also Figure S1.

t = 0.14; pPVT: SAL: 4.5 ± 1.0 g; MUS: 4.4 ± 1.0 g, unpaired t test, *p = 0.97, t = 0.03). Together, these findings suggest that activity in aPVT opposes reward seeking when rats are experiencing a negative emotion (e.g., unexpected omission of reward).

Photoactivation of aPVT Neurons Reduces Reward-Seeking Behavior

Because pharmacological inactivation of aPVT increased reward seeking, we hypothesized that optogenetic activation of aPVT neurons with the light-activated cation channel channelrhodopsin (ChR2) would decrease reward seeking. Accordingly, aPVT was infused with an adeno-associated viral vector (AAV-5) to express ChR2 combined with enhanced yellow fluorescent protein (eYFP) under the control of a CaMKIIa promoter (AAV5:CaMKIIa::hChR2-eYFP). Laser illumination of aPVT somata increased the expression of the neuronal activity marker cFos in aPVT (Figures 3A–3C). Consistent with our prediction, photoactivation of aPVT neurons at cue onset abolished pressing during both reward-available and reward-omitted conditions (Figure 3D; Movie S1). Pressing was also reduced when aPVT was photoactivated in the middle of the cue or in rats that were trained to press a bar on a variable interval (60 s) schedule of reinforcement (Figure S2). Such effects were not accompanied by changes in locomotion or anxiety assessed in the open field task (Figure S2). Thus, activation of aPVT neurons is sufficient to interrupt both cued and uncued sucrose seeking. Interestingly, however, photoactivation of aPVT had no effect on consumption of sucrose in an ad libitium test (Figure S2), suggesting that aPVT regulates the foraging, rather than the consumption, of sucrose.

aPVT Neurons Signal Reward Omission

Rats implanted with unit-recording electrodes in aPVT underwent cued conditioning sessions as described above. A small number of aPVT neurons showed either inhibitory or excitatory responses to the cue (Figure S3), and there were no differences between reward-available and reward-omitted trials because the cue preceded omission. aPVT neurons also signaled bar presses, with inhibitory responses mainly observed during the reward-available trials (Figure S4). However, the greatest changes in aPVT activity occurred when the rat's head entered the sucrose dish to discover the presence or absence of reward (Figures 4A–4C). Two types of responses were observed. Cells showing inhibitory responses when reward was available no longer showed those responses when reward was omitted (Figures 4D and 4E), and cells showing excitatory responses when reward was omitted no longer showed those responses when reward was available (Figures 4F and 4G). Although the percentage of neurons showing excitatory responses during reward omission was relatively low (9%), the magnitude of the observed response was substantial (Z score average > 6; Figure 4G). Despite the observed differences, the normalized baseline firing rate of neurons showing excitatory versus inhibitory responses across the sessions did not differ significantly (excitatory: 13.9 ± 1.4, inhibitory: 6.36 ± 1.28, unpaired t test, p = 0.10, t = 1.71). Thus, aPVT activity distinguished reward availability from reward omission in this task.

Photoinhibition of aPVT Projections to NAc Increases Sucrose Seeking during Reward Omission

PVT is the main source of glutamatergic inputs to the nucleus accumbens (Li and Kirouac, 2008; Moga et al., 1995; Vertes and Hoover, 2008), a region known to play a crucial role in reward-seeking behavior (for a review, see Baldo and Kelley, 2007; Salamone et al., 2003; Urstadt and Stanley, 2015). We therefore investigated whether projections from aPVT to the NAc are involved in the regulation of cued sucrose seeking. Rats were infused with AAV-5 expressing the light-sensitive chloride pump halorhodopsin (AAV5:CaMKIIa::eNpHR3.0-eYFP)

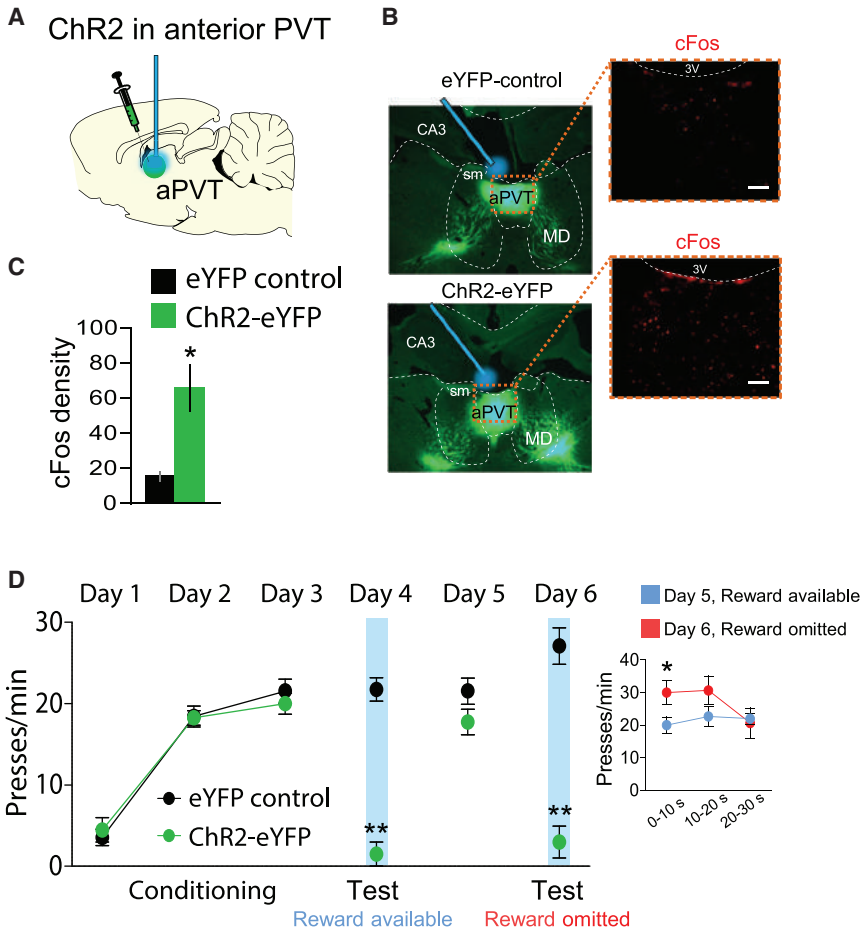

Figure 3. Photoactivation of Anterior PVT Abolishes Sucrose Seeking

(A) Diagram showing ChR2-eYFP expression and fiber optic placement in the aPVT.

(B) Left: representative micrograph showing the expression of eYFP-control or ChR2-eYFP within aPVT and (right) the expression of cFos within the aPVT after photoactivation of aPVT neurons. Scale bar, 100 μm. MD, mediodorsal thalamus; sm, stria medullaris; CA3, hippocampal CA3 subregion; 3V, third ventricle.

(C) Photoactivation of aPVT increased the number of cFos-positive neurons (per 0.1 mm^2) in aPVT of ChR2-eYFP group (green, n = 4) compared to eYFP-control group (black, n = 3; unpaired t test, p = 0.024, t = 3.00).

(D) Rats expressing eYFP (control, black dots, n = 9) or ChR2-eYFP (green dots, n = 8) in aPVT were trained in cued sucrose seeking (days 1–3). ChR2-activation of aPVT at cue onset (blue bar, 20 Hz, 30 s) abolished sucrose seeking when reward was available (day 4, unpaired t test, $p < 0.001$, t = 4.20) or when reward was omitted (day 6, unpaired t test, $p < 0.01$, t = 4.09). Inset: eYFP-control rats pressed significantly more during the first 10 s of the reward omitted session (day 6, red dots) when compared to the previous day in which reward was available (day 5, blue dots, ANOVA repeated measures $F_{(1,16)} = 4.96$, p = 0.040; Duncan post hoc test, *p = 0.042). Data shown as mean ± SEM, first trial of each day. *$p < 0.05$; **$p < 0.01$. See also Figure S2 and Movie S1.

into aPVT and implanted with optical fibers aimed mainly at the shell portion of the NAc. In anesthetized rats, we observed that photoinhibiton of aPVT terminals within the NAc either reduced the firing rates (6 out of 68 tested, 9%) or increased the firing rates (17 out of 68 tested, 25%) of NAc neurons (Figures 5A–5C). Because PVT is largely devoid of GABAergic neurons (Frassoni et al., 1997; Ottersen and Storm-Mathisen, 1984), the excitatory responses observed in the NAc neurons following photoinhibition of aPVT fibers suggest that at least a fraction of aPVT inputs induce feed-forward inhibition of NAc neurons. This could be mediated by activation of local inhibitory circuits in the NAc (Meredith and Wouterlood, 1990; Zhu et al., 2016) or by direct activation of dopaminergic synapses onto NAc neurons, independent of dopamine cell firing (Parsons et al., 2007; Pinto et al., 2003). Considering that medium spiny neurons, which account for 95% of NAc neurons (Graveland and DiFiglia, 1985), can be hyperpolarized under anesthesia (Kirouac and Ciriello, 1997), the possibility exists that our recordings were biased to local interneurons and somewhat overestimated the percentage of neurons with excitatory responses.

We next assessed the effects of aPVT→ NAc photoinhibition on sucrose seeking. Similar to pharmacological inactivation of aPVT (see above), photoinhibition of aPVT→ NAc projections had no effect on pressing when reward was available but increased pressing when reward was omitted (Figures 5D and 5E). This effect was not observed in rats trained under a variable interval schedule of reinforcement (Figure S5), suggesting that aPVT→ NAc projections are recruited specifically during omission of an expected reward. In further support of this, photoinhibition of aPVT→ NAc projections did not affect sucrose consumption during an ad libitium test (Figure S5). Neither did photoinhibition of this pathway affect locomotion or anxiety in an open field (Figure S5) or extinction of the reward-associated cue (Figure S6). Thus, activity in aPVT efferents to the NAc negatively regulates reward seeking under the frustrative state induced by reward omission.

Photoactivation of aPVT Projections to NAc Reduces Sucrose Seeking and Induces Place Aversion

Using a different set of rats, we next demonstrated that photoactivation of aPVT→ NAc projections with channelrhodopsin (AAV5:CaMKIIa::hChR2-eYFP) reduced cue-induced sucrose seeking at frequencies of 10 Hz and 20 Hz (all p values < 0.05), but not at 1 Hz or 5 Hz (all p values > 0.05; Figures 6A and 6B). In addition, photoactivation of PVT→ NAc projections (at 10 Hz) reduced the time spent on the side of the chamber paired with laser stimulation in a real-time place preference

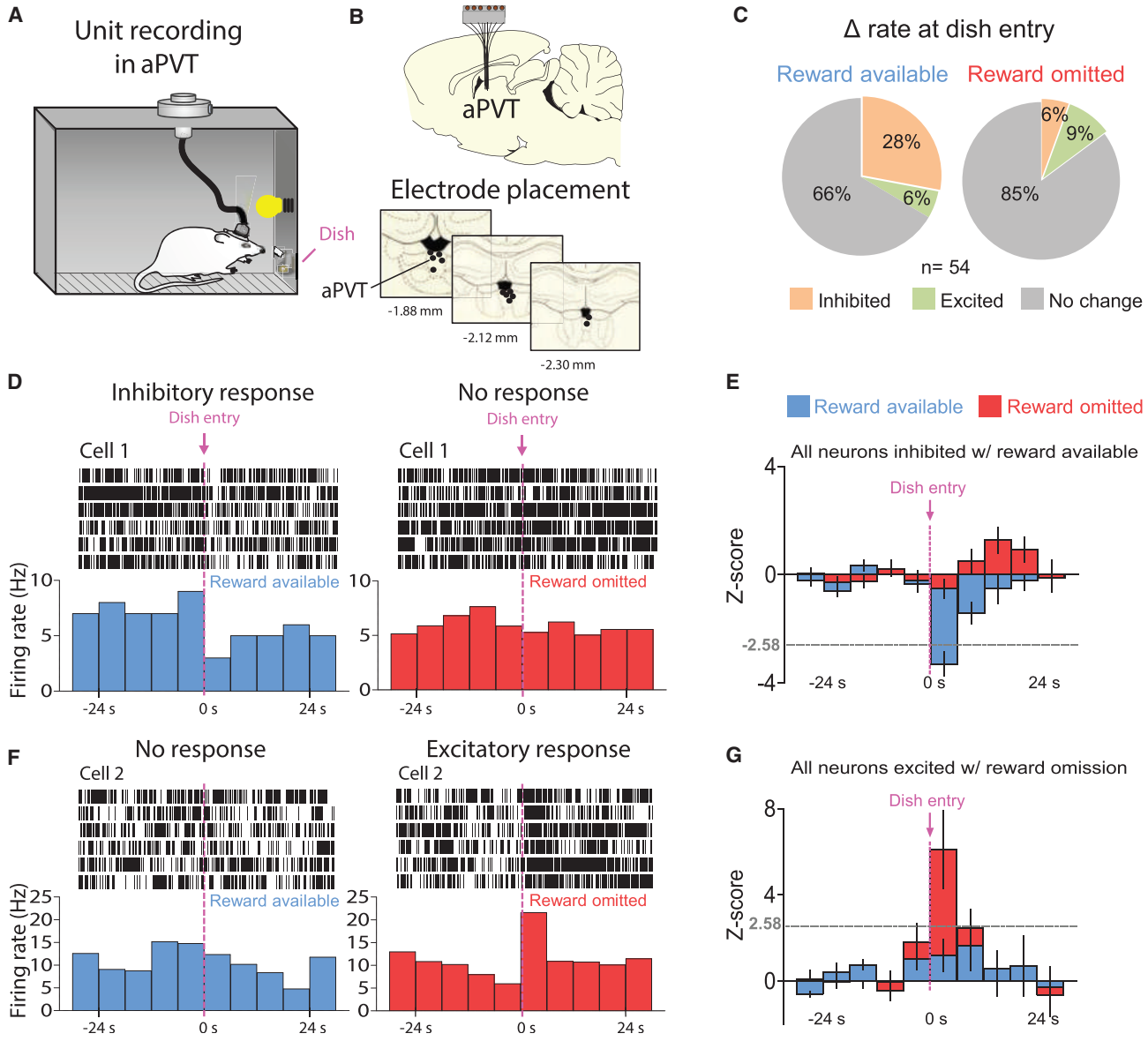

Figure 4. aPVT Neurons Signal Reward Omission

(A) aPVT neurons were recorded from rats undergoing cued sucrose seeking.

(B) Diagram of the electrode placements in the aPVT (coordinates from bregma).

(C) Pie charts summarizing changes in aPVT firing rate at dish entry; 28% inhibited, 6% excited, and 66% did not change when reward was available, whereas 6% inhibited, 9% excited, and 85% did not change when reward was omitted (Fisher exact test, inhibition with reward available versus excitation with reward available, $p = 0.014$; inhibition with reward available versus inhibition with reward omitted, $p = 0.014$, $n = 54$ neurons from 13 rats; bins of 6 s, unpaired t test, all p values < 0.05).

(D) Left: raster plot and peristimulus time histogram (PSTH) of a representative aPVT neuron showing inhibitory response at dish entry when reward was available (blue). Right: same neuron showing no response when reward was omitted (red).

(E) Average PSTH of all aPVT neurons showing inhibitory responses when reward was available.

(F) Left: raster plot and PSTH of a representative aPVT neuron showing no response at dish entries when reward was available. Right: same neuron showing excitatory response when reward was omitted.

(G) Average PSTH of all aPVT neurons showing excitatory responses when reward was omitted. See also Figures S3 and S4.

task (Figure 6C) without affecting locomotion and anxiety in an open field (distance traveled in meters: eYFP-control: 12.4 ± 2.0, ChR2-eYFP: 8.9 ± 1.0, unpaired t test, $p = 0.16$, $t = 1.50$; percentage of time in center: eYFP-control: 11.8 ± 5.9, ChR2-eYFP: 00.0 ± 0.0, unpaired t test, $p = 0.07$, $t = 2.00$, $n = 6$ per group). These results suggest that activity in the aPVT→ NAc pathway is sufficient to induce aversive states and reduce reward-seeking behavior.

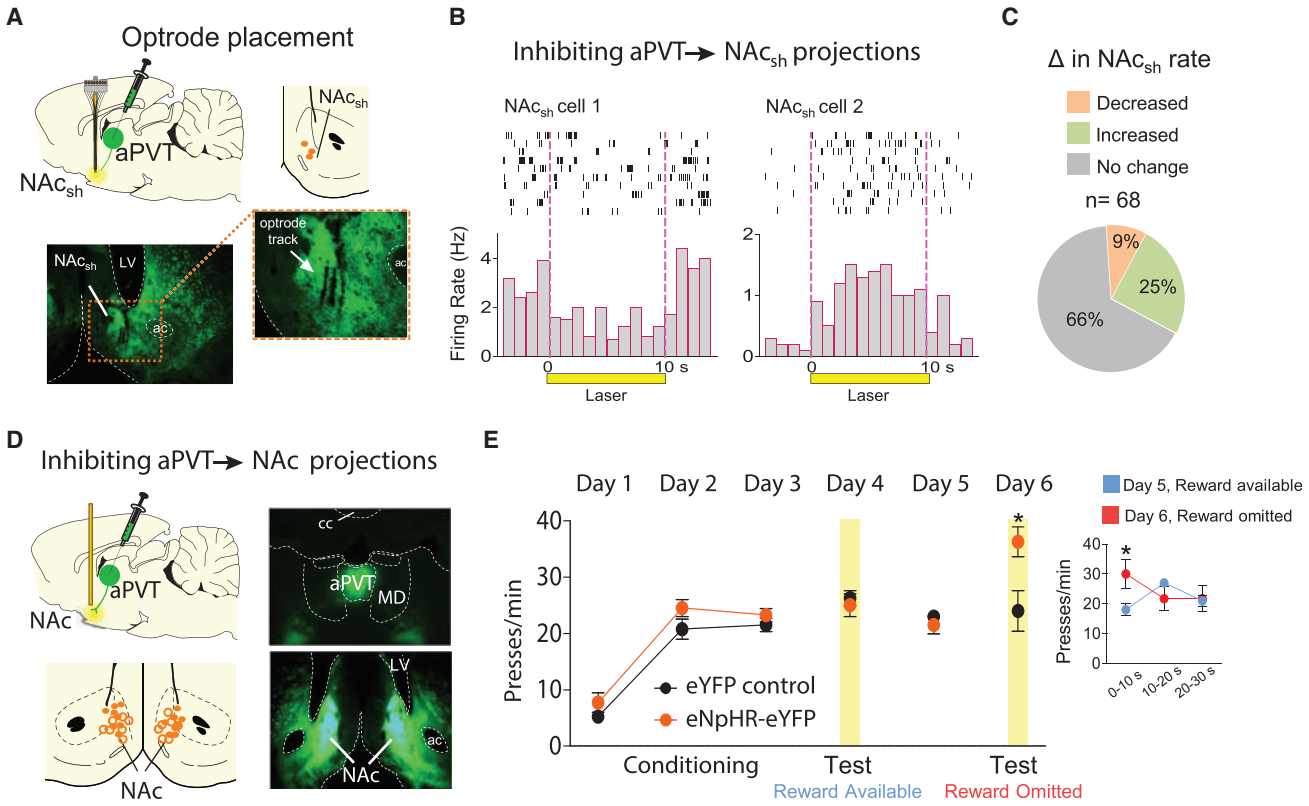

Figure 5. Photoinhibition of aPVT to NAc Projections Increases Pressing When Reward Is Omitted

(A) Diagram of recording placement and representative micrograph showing optrode tracks in the nucleus accumbens shell (NAc_{sh}) following infusion of eNpHR-eYFP into the aPVT. LV, lateral ventricle; ac, anterior commissure.

(B) Raster plot and PSTH of two representative NAc_{sh} neurons responding to photoinhibition (yellow bar) of aPVT inputs showing either inhibition (left) or excitation (right).

(C) Pie chart summarizing changes in NAc_{sh} firing rate with photoinhibition of aPVT terminals in the NAc (9% decreased, 25% increased, 66% did not change, unpaired t test, all p values < 0.05 as compared with laser-off period, n = 68 neurons from 3 rats).

(D) Diagram of optical fiber placement and representative micrograph showing eNpHR-eYFP expression in aPVT and fiber location in the NAc. cc, corpus callosum; MD, mediodorsal thalamus.

(E) Rats expressing eYFP-control (black dots, n = 8) or eNpHR-eYFP (orange dots, n = 8) in aPVT and implanted with optical fibers in the NAc were trained in cued sucrose seeking (days 1–3). Photoinhibition of aPVT→ NAc projections (yellow vertical bar) had no effect on sucrose seeking when reward was available during the test (day 4, unpaired t test, p = 0.61, t = 0.52) but increased sucrose seeking when reward was unexpectedly omitted (day 6, unpaired t test, p = 0.016, t = 2.71). Inset: eYFP-control rats pressed significantly more during the first 10 s of the reward-omitted session (day 6, red dots) compared to the previous day in which reward was available (day 5, blue dots, ANOVA repeated measures $F_{(2,28)} = 4.31$, p = 0.023; Duncan post hoc test, *p = 0.016). Data shown as mean ± SEM, first trial of each day. *p < 0.05. See also Figures S5, S6, and S8.

Photoinhibition of aPVT Projections to the Amygdala Decreases Sucrose Seeking during Reward Omission

In addition to its projections to the NAc, aPVT also sends dense projections to the amygdala central (CeA) and basolateral (BLA) nuclei (Li and Kirouac, 2008; Moga et al., 1995; Vertes and Hoover, 2008), regions known to regulate reward seeking (Ambroggi et al., 2008; Knapska et al., 2013; Mahler and Berridge, 2009; Robinson et al., 2014; Tye and Janak, 2007). In anesthetized rats, we first demonstrated that photoinhibiton of aPVT terminals within the amygdala either reduced the firing rates (5 out of 44 tested, 11%) or increased the firing rates (14 out of 44 tested, 32%) of CeA neurons (Figures 7A–7C). Similar to NAc, the higher proportion of excitatory responses suggests that aPVT inputs induce feed-forward inhibition of CeA neurons, as previously demonstrated (Penzo et al., 2015). Photoinhibition of aPVT→ amygdala projections (with optical fibers aimed at the CeA portion) did not affect pressing when reward was present but *decreased* pressing when reward was omitted (Figures 7D and 7E). These effects were not observed when the optical fibers were aimed at the BLA (Figure S7); however, due to the dense spread of aPVT fibers and the diffuse propagation of light to both amygdalar subregions, we cannot exclude the participation of the aPVT→ BLA pathway in such effects. Our results suggest that activity in aPVT efferents to the amygdala has the opposite effect of aPVT efferents to the NAc, increasing sucrose seeking during frustrative outcomes. This bidirectional regulation of reward seeking by PVT is supported by our observation that most NAc-projecting neurons do not overlap with CeA-projecting neurons in the aPVT (Figure S8).

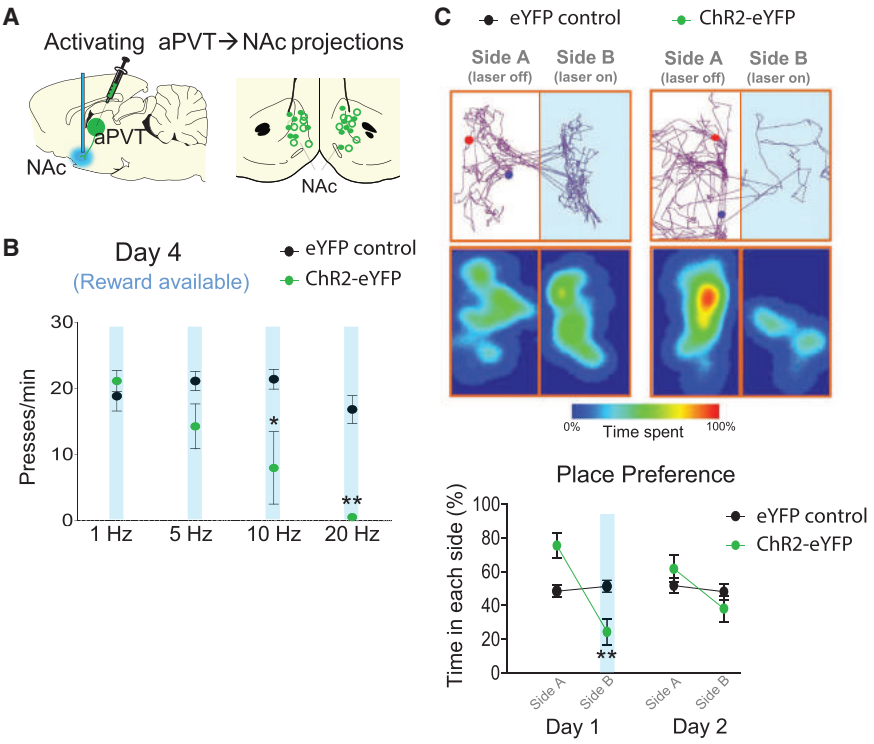

Figure 6. Photoactivation of aPVT to NAc Projections Inhibits Sucrose Seeking and Induces Place Aversion

(A) Diagram of optical fiber placement in the nucleus accumbens (NAc) of rats expressing ChR2-eYFP in the aPVT.

(B) Rats expressing eYFP (control, black dots, n = 7) or ChR2-eYFP (green dots, n = 7) in aPVT and implanted with optical fibers aimed at the NAc were trained in cued sucrose seeking (days 1–3, data not shown). Photoactivation of aPVT→ NAc projections on day 4 (reward available) reduced cued sucrose seeking at frequencies of 10 Hz (unpaired t test, *p = 0.035, t = 2.35) and 20 Hz (unpaired t test, **p < 0.01, t = 5.51), but not at 1 Hz and 5 Hz (unpaired t test, all p values > 0.05).

(C) Top: representative real-time place preference tracks and heatmaps showing laser-evoked behavioral aversion (at 10 Hz) in ChR2-eYFP group (right, n = 6), but not in eYFP-control group (left, n = 6). Bottom: quantification of laser-evoked behavioral aversion. Photoactivation of PVT→ NAc projections reduced the time spent on the side of the chamber paired with laser stimulation (day 1, unpaired t test, **p < 0.01, t = 3.19) but had no effect on the following day without photoactivation (day 2, unpaired t test, p = 0.29, t = 1.10). Data shown as mean ± SEM. *p < 0.05, **p < 0.01.

Photoactivation of aPVT Projections to the Amygdala Reduces Sucrose Seeking and Induces Place Aversion

Because photoinactivation of aPVT→ amygdala projections reduces sucrose seeking during reward omission, we sought to determine whether photoactivation of this same pathway would increase reward seeking. Surprisingly, we found that photoactivation of aPVT→ amygdala projections reduced reward seeking at frequencies of 10 Hz and 20 Hz (all p values < 0.01), but not at 1 Hz or 5 Hz (all p values > 0.05; Figures 8A and 8B). In addition, photoactivation of PVT→ amygdala projections (at 10 Hz) decreased the time spent on the side of the chamber paired with laser stimulation (Figure 8C). This effect persisted the following day when the animals were re-tested in the same chamber without laser illumination, suggesting a role of PVT→ amygdala projections in aversive learning. Photoactivation of PVT→ amygdala projections was also sufficient to impair locomotion and increase anxiety in an open field (distance traveled in meters: eYFP-control: 13.5 ± 1.5, ChR2-eYFP: 7.5 ± 1.1, unpaired t test, p = 0.011, t = 3.05; percentage of time in center: eYFP-control: 20.2 ± 6.0, ChR2-eYFP: 3.6 ± 2.7, unpaired t test, p = 0.031, t = 2.47, n = 6 per group). These results suggest that activity in the aPVT→ amygdala pathway reduces reward-seeking behavior, induces aversive and anxiogenic states, and promotes aversive learning.

DISCUSSION

We examined the role of PVT and its outputs to the NAc or amygdala in the modulation of sucrose-seeking behavior in rats. Remarkably, PVT appears to modulate sucrose seeking only when reward is unexpectedly omitted. Under this condition, photoinhibition of aPVT→ NAc projections increases reward seeking, whereas photoinhibition of aPVT→ amygdala projections reduces reward seeking. Furthermore, aPVT activity distinguishes reward availability from reward omission. These results suggest that different populations of aPVT neurons are recruited to balance foraging during frustrative conditions (e.g., when a cue-reward association is violated).

Our observation that omission of an expected reward increased bar pressing and induced anxiety-like behavior agrees with prior studies suggesting that unexpected reward omission is aversive (Amsel and Roussel, 1952; Burokas et al., 2012; Dudley and Papini, 1997; Jensen and Fallon, 1973; Komorowski et al., 2012; Manzo et al., 2014; Stout et al., 2002). Recruitment of aPVT neurons during aversive outcomes may serve to adjust reward-seeking responses, resembling previously demonstrated PVT modulation of autonomic, neuroendocrine, and behavioral responses to stress (for review, see Hsu et al., 2014). The lack of effect of aPVT inactivation on sucrose consumption ad libitum reinforces the idea that aPVT neurons are necessary for sucrose seeking rather than sucrose consumption and suggests that aPVT operates as a switch for regulating foraging under aversive conditions.

Reduction of sucrose seeking by activation of aPVT→ NAc projections is consistent with previous observations that nucleus accumbens shell (NAc_{sh}) activity and glutamate levels are decreased during feeding (Krause et al., 2010; Rada et al., 1997; Saulskaya and Mikhailova, 2002), and blockade of AMPA receptors in the NAc increases food consumption (Reynolds and Berridge, 2003; Stratford et al., 1998). Considering

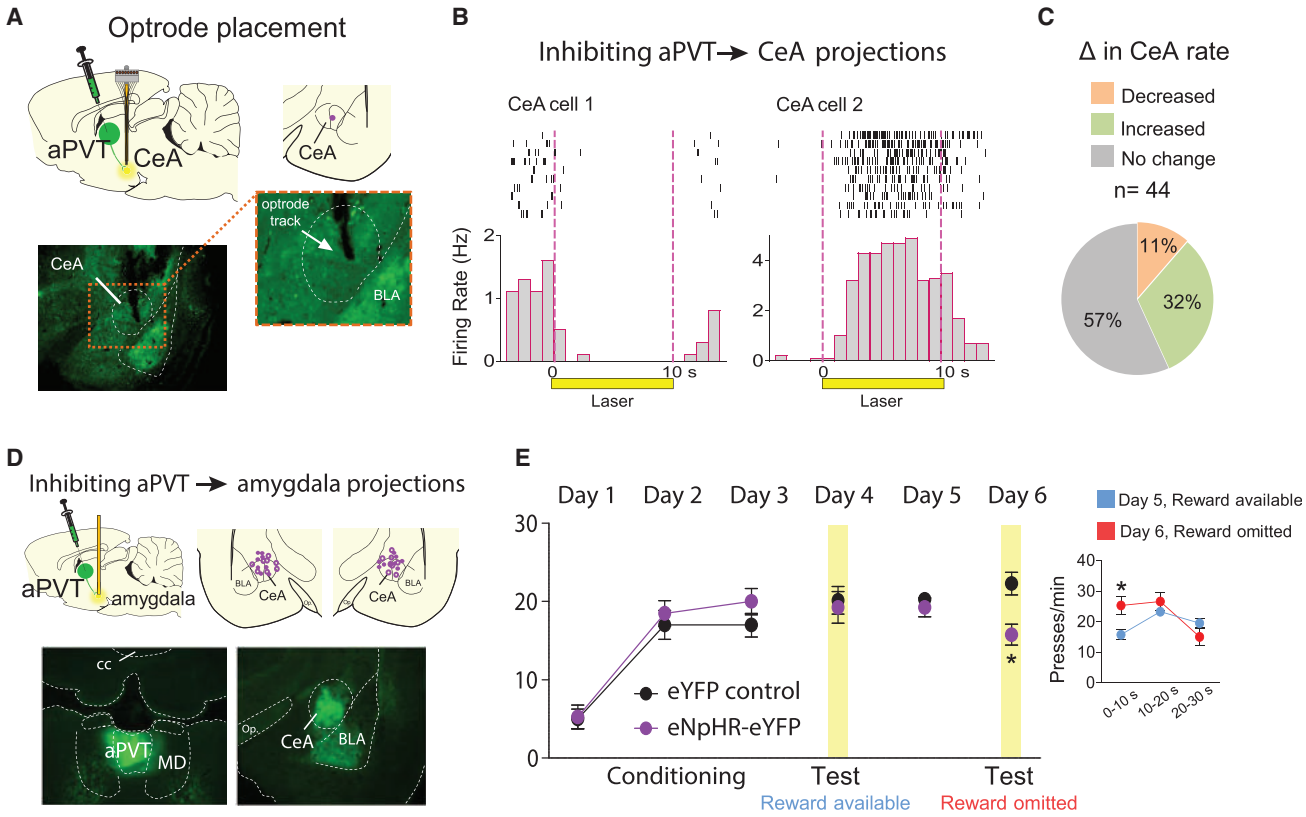

Figure 7. Photoinhibition of aPVT to Amygdala Projections Decreases Pressing When Reward Is Omitted

(A) Diagram of recording placement and representative micrograph showing optrode tracks in the central nucleus of the amygdala (CeA) following infusion of eNpHR-eYFP into the anterior PVT (aPVT).

(B) Raster plot and PSTH of representative CeA neurons responding to photoinhibition (yellow bar) of aPVT inputs in the amygdala showing either inhibition (left) or excitation (right).

(C) Pie chart summarizing changes in CeA firing rate with photoinhibition of aPVT terminals in the amygdala (11% decreased, 32% increased, 57% did not change, unpaired t test, all p values < 0.05 compared with laser-off period, n = 44 neurons from 1 rat).

(D) Diagram of optical fiber placement and representative micrograph showing eNpHR-eYFP expression in the anterior PVT (aPVT) and fiber location in the amygdala.

(E) Rats expressing eYFP (control, black dots, n = 14) or eNpHR-eYFP (purple dots, n = 8) in aPVT and implanted with optical fibers aimed at the CeA were trained in cued sucrose seeking (days 1–3). Photoinhibition of aPVT→ amygdala projections (yellow vertical bar) had no effect on sucrose seeking when reward was available (day 4, unpaired t test, p = 0.75, t = 0.32) but reduced sucrose seeking when reward was unexpectedly omitted (day 6, unpaired t test, p = 0.007, t = 2.98). Inset: eYFP-control rats pressed significantly more during the first 10 s of the reward-omitted session (day 6, red dots) compared to the previous day in which reward was available (day 5, blue dots, ANOVA repeated measures $F_{(2,52)} = 3.99$, p = 0.024; Duncan post hoc test, *p = 0.022). Data shown as mean ± SEM, first trial of each day. *p < 0.05. See also Figures S6–S8.

that PVT releases glutamate in the NAc (Ligorio et al., 2009), activity in aPVT→ NAc glutamatergic projections would therefore be responsible for reducing food seeking. This idea seems to be at odds with previous studies showing that glutamatergic afferents from the ventral hippocampus and BLA to the NAc increase reward seeking (Britt et al., 2012; Stuber et al., 2011). A plausible explanation for this divergence is the observation that PVT neurons have postsynaptic targets in the NAc that differ from those of ventral hippocampus and BLA. Whereas PVT efferents preferentially modulate NAc neurons expressing the inhibitory dopaminergic receptor D2 (Zhu et al., 2016), inputs from ventral hippocampus and BLA modulate NAc neurons expressing the excitatory dopaminergic receptor D1 (Floresco et al., 2001; Stuber et al., 2011). Moreover, NAc neurons expressing D1 versus D2 receptors display different electrophysiological properties (Cepeda et al., 2008) and exhibit opposing roles in reward-seeking behavior (Kravitz et al., 2012; Yawata et al., 2012).

Previous studies have suggested that the core and the shell subregions of the NAc play dissociable roles in guiding reward-seeking behavior. Whereas the core subregion has been involved in learning and action during goal-directed behavior, the shell subregion has been implicated in processing hedonic and motivated behavior (Burton et al., 2015; Castro and Berridge, 2014; Saddoris et al., 2015; West and Carelli, 2016). Such functional differences have been attributed to distinct input sources and output targets, with the core being mainly interconnected with regions involved in motor responses and the shell

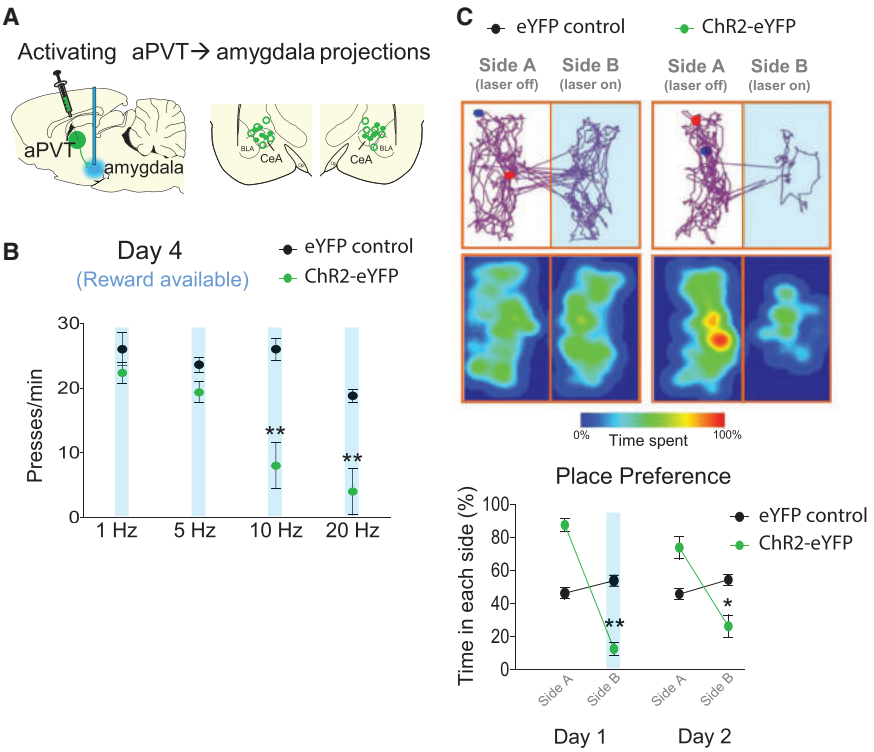

Figure 8. Photoactivation of aPVT to Amygdala Projections Inhibits Sucrose Seeking and Induces Place Aversion

(A) Diagram of optical fiber placement in the amygdala of rats expressing ChR2-eYFP in the aPVT.

(B) Rats expressing eYFP (control, black dots, n = 5) or ChR2-eYFP (green dots, n = 6) in aPVT and implanted with optical fibers aimed at the central nucleus of the amygdala (CeA) were trained in cued sucrose seeking (days 1–3, data not shown). Photoactivation of aPVT→ amygdala projections on day 4 (reward available) reduced cued sucrose seeking at frequencies of 10 Hz and 20 Hz (unpaired t test, **all p values < 0.01), but not at 1 Hz and 5 Hz (unpaired t test, all p values > 0.05).

(C) Top: representative real-time place preference tracks and heatmaps showing laser-evoked behavioral aversion (at 10 Hz) in ChR2-eYFP group (right, n = 6), but not in eYFP-control group (left, n = 5). Bottom: quantification of laser-evoked behavioral aversion. Photoactivation of PVT→ amygdala projections reduced the time spent on the side of the chamber paired with laser stimulation (day 1, unpaired t test, **p < 0.01, t = 5.21), an effect that persisted on the following day without photoactivation (day 2, unpaired t test, p = 0.011, t = 3.11). Data shown as mean ± SEM. *p < 0.05, **p < 0.01.

being mainly interconnected with regions implicated in incentive motivation (Brog et al., 1993; Groenewegen et al., 1999). This distinct pattern of innervation has been also described for PVT efferents to the NAc, which are denser to the shell than to the core (Li and Kirouac, 2008; Moga et al., 1995; Vertes and Hoover, 2008). In our optogenetic experiments, aPVT inputs preferentially innervated the shell portion of the NAc, and the optical fibers were aimed specifically at this subregion; however, we cannot exclude the possibility that the light also affected aPVT fibers located in the core portion of the NAc. Similarly, because viral injections into the aPVT may have also involved the adjacent paratenial nucleus, which also projects to the nucleus accumbens (Vertes and Hoover, 2008), we cannot exclude the possibility that part of the observed effects were due to modulation of paratenial nucleus fibers in the NAc.

Our observation that aPVT→ NAc projections modulate sucrose seeking during reward omission suggests that aPVT neurons are recruited during negative outcomes, as has been previously demonstrated for drug withdrawal (Zhu et al., 2016). Our tracer finding in aPVT showing that NAc-projecting neurons are denser than CeA-projecting neurons is consistent with previous neuroanatomical findings (Li and Kirouac, 2008) and may explain why pharmacological inactivation of aPVT had effects similar to photoinhibition of PVT→ NAc projections. In contrast to NAc, aPVT projections to the amygdala increase sucrose seeking during reward omission, as evidenced by photoinhibition of the PVT→ amygdala pathway. Prior studies showed that activation of CeA neurons increases cued food seeking (Holland and Hsu, 2014; Robinson et al., 2014), but most relevant to our findings is the observation that CeA activity increases during unexpected omission of reward (Calu et al., 2010; Lee et al., 2010). Similarly, a population of BLA neurons show increased activity during reward omission, a response that positively correlates with the maintenance of reward seeking during this frustrative condition (Tye et al., 2010). Our findings suggest that increased amygdalar activity during reward omission may be due to aPVT inputs.

A role of PVT in communicating aversive information to the CeA has been recently demonstrated in Pavlovian fear conditioning (Do-Monte et al., 2015b; Penzo et al., 2015), suggesting that the aversive states observed in conditioned fear and reward omission may recruit similar circuits. However, considering that different populations of CeA neurons are activated during fear conditioning and reward omission (Purgert et al., 2012), it is likely that different subsets of PVT neurons signal these two aversive experiences. Together with our tracer findings, it is possible that activity in dual-projecting neurons in aPVT would both increase fear responses (by activating CeA neurons) and reduce sucrose seeking (by activating NAc neurons). In contrast, activity in aPVT neurons projecting to the CeA, but not to the NAc, would mediate the increased sucrose seeking observed during reward omission. This could explain why bulk photoactivation of PVT→ amygdala projections in our experiments increased anxiety/aversion rather than promoting reward seeking.

Consistent with our optogenetic findings, we observed that aPVT neurons responded differently depending on reward availability. Inhibitory responses were observed more frequently when reward was available and were no longer observed when reward was omitted. Reward-modulated responses have been

previously described for midline thalamic neurons (Li et al., 2016). We also observed a set of aPVT neurons showing excitatory responses exclusively during reward omission. The targets of these two types of PVT neurons is not known, but based on our findings, we speculate that neurons with inhibitory responses project to NAc_{sh} (permitting pressing when reward is available), whereas neurons with excitatory responses project to CeA (increasing pressing when reward is omitted). Further studies using mice and combining cre-recombination system with optrode recordings are needed to identify the specific targets of these two populations of aPVT neurons.

aPVT receives hypothalamic peptidergic inputs implicated in the control of feeding (e.g., neuropeptide Y; Lee et al., 2015), arousal (e.g., orexin; Kirouac et al., 2005), and stress responses (e.g., corticotropin releasing factor; Hsu and Price, 2009). The aPVT also receives GABAergic projections from the anterior portion of the lateral hypothalamus (LHA; Stamatakis et al., 2016), and increasing GABAergic activity in the LHA promotes feeding (Jennings et al., 2015). It is possible, therefore, that GABAergic projections from the LHA inhibit aPVT neurons when reward is available, thereby promoting sucrose seeking. aPVT is also robustly innervated by efferents from the prefrontal cortex and ventral subiculum (Li and Kirouac, 2012), both implicated in decision making and goal-directed behavior (Ciocchi et al., 2015; Piantadosi et al., 2016). Accordingly, a recent study in mice have demonstrated that photoactivation of prefrontal cortex projections to PVT suppresses cue-induced sucrose seeking (Otis et al., 2017). This unique set of inputs places aPVT in an optimal position to integrate internal physiological states with emotionally salient information (Kirouac, 2015). Omission of expected reward activates the hypothalamic-pituitary adrenal axis, increasing corticosteroid levels (Coover et al., 1971; Mitchell and Flaherty, 1998; Romero et al., 1995). The high density of corticosteroid receptors in PVT (Ahima et al., 1991; Jaferi and Bhatnagar, 2006), together with our findings, suggests that PVT neurons may play a role in mediating the frustrative effects of reward omission (Amsel, 1958; Papini, 2003). Therefore, top-down modulation of aPVT may adjust ascending signals from the hypothalamus during reward omission.

In a natural environment, the availability of food sources is highly dynamic. In order to survive, animals must adjust their foraging behavior when food is no longer available. This adaptive strategy may serve to first invigorate food seeking in the face of reward loss and then re-direct such responses to other potential sources (Amsel, 1992; Papini, 2003). Although a great deal of information has been gathered about the behavioral and physiological consequences of reward omission (Burokas et al., 2012; Jensen and Fallon, 1973; Komorowski et al., 2012; Manzo et al., 2015; Stout et al., 2002), far less attention has been paid to the neural circuits involved. In humans, the sudden loss of previously established gains (e.g., loss of employment) has been implicated in the onset and maintenance of psychiatric disorders, such as anxiety, depression, and substance abuse (Huston et al., 2013; Papini et al., 2015). Thus, elucidating the neural circuits underlying unexpected reward loss and frustration may help to understand adaptive and motivated behaviors, as well as the pathophysiological mechanisms of psychiatric illnesses.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information includes eight figures and one movie and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2017.03.036.

AUTHOR CONTRIBUTIONS

F.H.D.-M., A.M.-T., and E.M.M.-C. performed behavioral, immunohistochemical, and optogenetic experiments. F.H.D.-M. and K.Q.-L. performed single-unit recording in anesthetized and behaving rats. F.H.D.-M., A.M.-T., K.Q.-L., E.M.M.-C., and G.J.Q. designed the study, interpreted results, and wrote the paper.

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