Amygdala inputs to prefrontal cortex guide behavior amid conflicting cues of reward and punishment

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Orchestrating appropriate behavioral responses in the face of competing signals that predict either rewards or threats in the environment is crucial for survival. The basolateral nucleus of the amygdala (BLA) and prelimbic (PL) medial prefrontal cortex have been implicated in reward-seeking and fear-related responses, but how information flows between these reciprocally connected structures to coordinate behavior is unknown. We recorded neuronal activity from the BLA and PL while rats performed a task wherein competing shock- and sucrose-predictive cues were simultaneously presented. The correlated firing primarily displayed a BLA→PL directionality during the shock-associated cue. Furthermore, BLA neurons optogenetically identified as projecting to PL more accurately predicted behavioral responses during competition than unidentified BLA neurons. Finally photostimulation of the BLA→PL projection increased freezing, whereas both chemogenetic and optogenetic inhibition reduced freezing. Therefore, the BLA→PL circuit is critical in governing the selection of behavioral responses in the face of competing signals.

When animals engage in reward-seeking behaviors such as foraging or hunting, they often expose themselves to potential threats, and they must assess competing signals that may trigger conflicting motivational drives. The ability to appropriately weigh competing environmental cues and execute appropriate behavioral responses is paramount for survival and a key feature of mental health, yet little is known about the neural circuits that underpin this ability.

For decades, the amygdala has been identified as a focal point in emotional processing and is thought to be a hub for translating sensory information into motivated behaviors1,2. The BLA is important for the acquisition, encoding and retrieval of both positive and negative associations, and plasticity occurs in BLA neurons upon the encoding of cues that predict either positive or negative outcomes3–8. The BLA also shows prominent neuronal correlates of reward-seeking and fear-related responses in seminaturalistic tasks in which animals need to forage and retrieve food in the presence of imminent predator-like threats9,10.

An important target of the BLA thought to be crucial for the coordination of reward-seeking and fear-related behaviors is the medial prefrontal cortex (mPFC)11–13, which receives robust monosynaptic glutamatergic inputs from the BLA14,15 and sends a reciprocal connection in return16. Like the BLA, the mPFC has been widely implicated in the regulation of both reward-seeking17–18 and fear-related behavior19–21, and pharmacological inactivation of the mPFC produces deficits in the coordination of these behaviors22,23. Furthermore, the mPFC shows prominent neuronal responses that are highly correlated with the time course of behavioral manifestations of reward-seeking and fear-related behavior11,18. While some studies have examined the necessity of BLA activity for fear-related signaling in the mPFC24,25, little is known about how dynamic interactions between these structures may govern the coordination of reward-seeking and fear-related behavior upon presentation of competing signals. In this study, we focus on the PL subregion of the mPFC, though some experiments may also influence BLA projections to other subregions of mPFC.

In this study, we used electrophysiological recordings, optogenetically mediated photoidentification of BLA→PL neurons and supervised machine learning algorithms to decode behavior during competition, along with circuit-specific manipulations during a modified Pavlovian cue discrimination task in which conditioned stimuli predicting either sucrose or shock were presented separately on some trials and simultaneously in others. We address several questions. Is correlated firing between the BLA and PL dynamic upon presentations of cues associated with positive (rewards) or negative (punishments) outcomes? What is the directionality of information flow? Can we use neural activity and behavior during Pavlovian discrimination to accurately decode the behavior of an animal during the presentation of conflicting signals? And finally, is the BLA→PL projection necessary for and sufficient to promote fear-related behavior?

We also examined whether either brain region was particularly sensitive to the sucrose-predictive or the shock-predictive cue. We found that predominantly excitatory cross-correlations (CCs) between the BLA and PL developed a BLA→PL directionality during the shock-predictive but not the sucrose-predictive cue. On the basis of this finding, we hypothesized that this projection supplies information

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critical for driving fear responses. To further test this, we used channelrhodopsin-2 (ChR2) to activate BLA inputs to PL. This produced a selective enhancement of conditioned fear but not reward-seeking responses. Conversely, we used both optogenetic and chemogenetic approaches to inhibit BLA inputs to PL and observed suppression of freezing.

RESULTS

To investigate the neural dynamics that occur when an animal is challenged with competing environmental signals, we developed a variation of a Pavlovian discrimination task wherein one conditioned stimulus (CS) was paired with a sucrose reward (CS-Suc) whereas a second CS was paired with shock (CS-Shock). We used CSs of different sensory modalities (auditory and visual) to subsequently allow us to simultaneously present these stimuli during ‘competition’ trials without perceptual interference (Fig. 1a and Supplementary Fig. 1a).

Rats first learned a reward association in which a cue (for example, a light) was associated with the delivery of a palatable sucrose solution. Rats rapidly learned to explore the sucrose port during the cue presentation as compared to the inter-trial intervals (ITI; Supplementary Fig. 1c). Rats then learned to discriminate a second cue (for example, a tone) with an electrical shock, while still being exposed to the sucrose-predictive cue. Rats responded to the cue that predicted shock by freezing, which is a robust and consistent behavioral manifestation thought to be related to fear. By the third discrimination session, rats exhibited differential behavioral responses during the sucrose-predictive and shock-predictive cues. For simplicity, we used the terms reward and fear epochs to describe the time during which the cues associated with either sucrose or shock, respectively, were presented. Rats spent more time exploring the sucrose port than freezing during the reward epochs, whereas they spent more time freezing than exploring the sucrose port during the fear epochs (Fig. 1b,c and Supplementary Fig. 1d).

During subsequent competition sessions, the CS-Suc and CS-Shock associations were simultaneously presented to induce motivational conflict and competition of reward-seeking and fear-related behavior. Pairing of these associations produced a mean performance that was between the behaviors produced when the CS-Suc and CS-Shock were presented independently (Fig. 1d,e and Supplementary Fig. 1d). Throughout training, while the sucrose- and shock-predictive cues were of different sensory modalities, these cues were counterbalanced across animals, and differences in the cue modalities did not introduce significant differences in behavioral performance (Supplementary Fig. 1e–g).

BLA led more excitatory correlations with PL during the shock-predictive cue

To explore the potential contribution of interactions between the BLA and PL in governing behavioral selection in the face of conflicting cues signaling competing motivational drives, we first investigated the correlated activity across these two brain regions during distinct behavioral epochs during a discrimination session wherein the CS-Suc and CS-Shock were presented independently (Fig. 2). Recording sites are shown in Supplementary Figure 2a–d. Details on the detection of correlated activity are provided in the Online Methods (section “Cross-correlations”) and in Supplementary Figure 3a. Of 3,037 total possible pairs of BLA and PL neurons, we observed 639 pairs with positively correlated activity, which we termed “excitatory BLA/PL CCs” (Fig. 2a), and 107 pairs with negatively correlated activity, which we termed “inhibitory BLA/PL CCs” (Fig. 2b). Although excitatory CCs were more common during all task epochs (ITI, CS-Suc and CS-Shock; Fig. 2c,e), inhibitory CCs were also observed albeit less frequently and with more variability in the number of significantly correlated pairs across each epoch (Fig. 2d,f).

We wondered whether the directionality of information flow might be dynamic, depending on environmental cues predicting unconditioned stimuli of positive or negative valence. To begin exploring this question, we examined the proportion of pairs during each epoch wherein spiking of neurons in the BLA or PL preceded spiking of neurons in the other structure. We observed that a significantly greater proportion of BLA/PL neural pairs showing excitatory CCs were putatively led by neurons in the BLA during the CS-Shock, but not during either the CS-Suc or ITI epochs (Fig. 2g). Conversely, among inhibitory CCs, the BLA putatively led a greater proportion of cell pairs during the CS-Suc (Fig. 2h), though there was a smaller sample size among inhibitory CCs. Smaller bin widths showed more variable CCs, primarily due to sparse firing (Supplementary Fig. 4).

To control for stimulus generalization, we found that rats displayed neither reward-seeking nor fear-related behaviors during a habituation phase before any training or during the discrimination of a neutral cue (CS−) that was never paired with sucrose or shock (Supplementary Fig. 5a–c). Notably, significantly smaller proportions of BLA/PL cell pairs exhibited correlated activity during the CS− and during habituation (Supplementary Fig. 5d), suggesting that BLA/PL correlations developed over the course of training. We also confirmed that cue modality did not influence the BLA/PL lead and lag dynamics (Supplementary Fig. 6a,b). Furthermore, the lead and lag dynamics were consistent across distinct pairwise BLA/PL populations that exhibited correlated activity during different task events (Supplementary Fig. 6c,d). Of note, these dynamics were also strong in a BLA/PL population that exhibited excitatory correlations during all task events but that showed significant shifts in BLA and PL leading across task events (Supplementary Fig. 6c). Finally, these lead and lag dynamics were also maintained across multiple combinations of putative projection cells and interneurons (Supplementary Fig. 6e–i).

Populations of BLA cells encoding sucrose and shock cues exhibited similar levels of correlated activity with PL

Preferential encoding of the sucrose and shock associations by the BLA cells could potentially contribute to differential patterns of BLA/PL correlations. We examined this possibility by assessing CS-elicted changes in BLA activity and then quantifying correlations with PL for the reward- and fear-encoding BLA cells (Fig. 3a–d). Details on CS-evoked responses are provided in the Online Methods. While many BLA cells responded to either of the CSs or to both (Fig. 3a,b and Supplementary Fig. 7a,c,e), no significant differences were observed between the proportions of BLA cells that exhibited biased responses to either the CS-Suc or CS-Shock (Fig. 3c). Furthermore, the reward- and fear-biased populations showed similar levels of cross-correlated activity with PL (Fig. 3d). In addition, the patterns of lead and lag in the CCs were preserved across multiple combinations of BLA and PL cells that responded to either the reward- or fear-associated cues (Supplementary Fig. 7g–j). Therefore, the differential dynamics we observed in the BLA/PL CCs across reward and fear epochs could not solely be attributed to preferential encoding of the BLA cells of the reward and fear associations.

More PL cells encoded shock cues, and they exhibited stronger correlations with BLA than PL cells encoding sucrose cues

We next examined whether the PL cells that encoded the reward and fear associations exhibited distinct degrees of cross-correlated
activity with the BLA. Representative PL cells exhibiting significant responses to the reward- and fear-associated cues are shown in Supplementary Figure 7b. In contrast to the BLA, PL exhibited a larger proportion of cells that encoded the fear-associated cue (Fig. 3e,f and Supplementary Fig. 7d). Furthermore, a significantly greater overall population of PL cells exhibited biased responses to the fear-associated cue (Fig. 3g), and this population showed more CCs with the BLA than the population of PL cells that exhibited biased responses.

Figure 1 Behavioral tasks to examine the discrimination and competition of reward and fear memories. (a) During discrimination, discrete CS-Suc and CS-Shock cues predicted sucrose or shocks, respectively. Their sensory modalities (light versus tone) were counterbalanced across animals. Sucrose was removed from the port by vacuum (Vac) if animals did not collect it by the end of the CS. During competition, in addition to the individual CS-Suc and CS-Shock, animals were challenged by the co-presentation of these associations to induce conflicting motivational drives and competition between them. (b,c) We operationalize “reward” to refer to port entry and “fear” to refer to freezing. (b) Port entry responses per CS during the last discrimination session. Inset shows the average time that animals spent in the port per CS (paired t-test: t_{15} = 20.3, ***P < 0.001, n = 16 animals). (c) Freezing responses per CS during the last discrimination session. Inset shows the average time that animals spent freezing per CS (paired t-test: t_{15} = 20.6, ***P < 0.001, n = 16 animals). (d) Port entry responses during the last competition session. Inset shows the average time in the port per CS (repeated measures one-way ANOVA: F_{2,30} = 107.6, P < 0.001, n = 16 animals; Bonferroni post hoc tests: t_{15} > 6.85 and ***P < 0.001 for all comparisons). (e) Freezing responses during the last competition session. Inset shows the average time that animals spent freezing per CS (repeated measures one-way ANOVA: F_{2,30} = 89.0, P < 0.001, n = 16 animals; Bonferroni post hoc tests: t_{15} > 6.01 and ***P < 0.001 for all comparisons). Error bands in line plots and error bars in insets represent s.e.m.
to the reward-associated cue (Fig. 3h). This suggested that fear-biased cells in PL have greater functional connections with the BLA.

Taken together, these observations raised the possibility that fear-biased BLA→PL neurons may be driven by a common upstream site. These findings ultimately prompted us to perform optogenetically mediated photoidentification of BLA→PL neurons.

Most photoidentified BLA→PL neurons were excited by the shock-predictive cue

Given that the BLA, rather than PL, led more excitatory correlations during the CS-Shock, we pondered whether this might be related to monosynaptic input from the BLA to PL. To test this hypothesis, we used a dual virus approach as performed by Nieh et al., wherein a retrograde viral vector injected into PL (canine adenovirus, CAV2) resulted in expression of Cre recombinase, while an anterograde viral vector injected into the BLA allowed Cre-dependent expression of ChR2 fused to enhanced yellow fluorescent protein (eYFP; Fig. 4a). We first confirmed in an ex vivo preparation that this viral approach produced reliable ChR2 expression and selective photoresponses in BLA→PL neurons (Fig. 4b,c). We also determined in the ex vivo preparation the photoresponse latency threshold in these cells (Fig. 4d,e). For in vivo recordings, we implanted into the BLA an optrode (probe that combined an optical fiber with recording wires) to allow photoidentification of the BLA→PL cells shortly after the recording session during the behavioral task (Fig. 4f). Photoidentification parameters are provided in the Online Methods (section “In vivo photoidentification of the BLA→PL population”).

Among the BLA neurons recorded in vivo, 11 of 60 (18%) were identified as BLA→PL neurons, based on short-latency photoresponses...
BLA neurons biased toward encoding CS-Suc or CS-Shock exhibited similar proportions of correlated activity with PL, whereas a greater proportion of fear-biased PL cells exhibited correlated activity with BLA. (a,b) BLA populations based on the response to CS-Suc (R), CS-Shock (F) or both cues (R and F combinations). There were no significant differences in proportions across these populations (Bonferroni-corrected chi-square tests: \( \chi^2 < 9.50 \) and \( P > 0.056 \) for all comparisons). (c) Separation of reward- and fear-biased BLA cells, based on the peak response to each cue. Cells in the light gray zones exhibited larger responses to the reward-related cue and were deemed reward-biased. Cells in the dark gray zones exhibited larger responses to the fear-related cue and were deemed fear-biased. Inset shows the average proportion of reward- and fear-biased cells in the BLA per subject (12 subjects). (d) Reward- and fear-biased BLA cells showed similar proportions of cross-correlated activity with simultaneously recorded PL cells. These values were normalized to the total number of correlated neural pairs per subject per cue (repeated measures two-way ANOVA: cells, \( F_{1,22} = 0.01, P = 0.91 \); cue, \( F_{1,22} = 1.48, P = 0.24 \); interaction, \( F_{1,22} = 0.04, P = 0.85 \); n = 12 animals). (e,f) PL populations based on the response to the cues. The F+ population was significantly larger than most other populations (\( \chi^2 = 8.86 \) and \( P = 0.079 \) compared to R+F+; \( \chi^2 > 11.06 \) and \( *P < 0.024 \) compared to all other populations). (g) Separation of reward and fear biased PL cells, based on the peak response to each cue. A greater proportion of PL cells exhibited fear bias than reward bias (\( t_{11} = 3.03, *P = 0.011, n = 12 \) animals). (h) A greater proportion of fear-biased PL cells showed CCs with simultaneously recorded BLA cells (cells, \( F_{1,22} = 13.5, P = 0.0013 \); cue, \( F_{1,22} = 3.66, P = 0.069 \); interaction, \( F_{1,22} = 1.23, P = 0.28 \); CS-Suc, \( t_{11} = 3.32, **P = 0.0026 \); CS-Shock, \( t_{11} = 3.73, ***P < 0.001 \); n = 12 animals). Error bars represent s.e.m.
that were below the 12-ms threshold (Fig. 4g,h), which was determined in our visually guided ex vivo recordings (Fig. 4e). In addition, we observed a subpopulation of BLA cells (8 of 60, 13%) exhibiting inhibition in response to the photostimulation of BLA→PL neurons. We termed this subpopulation “network-inhibited cells,” though the precise number of synapses and distribution of the neurons in this network are not known.

We found that a greater proportion of the photoidentified BLA→PL neurons showed excitatory responses to the fear-associated

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**Figure 4** Most BLA→PL cells recorded showed selective excitation to the shock-predictive cue. An optogenetic approach was used to photoidentify these cells (“BLA→PL population”). (a–e) Assessment of photoresponse latencies in slices. (a) Ex vivo whole-cell patch-clamp recordings were performed after selectively expressing ChR2 in BLA→PL cells using a Cre-dependent viral system (n = 7 animals). Expressing cells (n = 6 cells) and nonexpressing neighbors (n = 24 cells) were recorded while stimulating with blue light (5-ms pulses at 1 Hz). (b) Representative traces from a ChR2+ BLA→PL cell and four nonexpressing neighbors. (c) Distribution of all cells sampled with whole-cell patch-clamp recording. (d) Representative traces show the latency of photoresponses at various light power densities (power range, 0.5–84 mW/mm²). Latencies were calculated from light onset to action potential peaks. (e) Distribution of photoresponse latencies for the BLA→PL cells. Dots represent individual cells and error bar represents s.e.m. (f–h) Photoidentification of BLA→PL cells in behaving animals. (f) Optrodes were chronically implanted in the BLA for neural recordings after selectively expressing ChR2 in BLA→PL cells. Optimal ChR2 expression and detection of photoresponses was achieved in a subset of animals (n = 2 of 6 animals, 33%). (g) BLA→PL cell displaying photoresponses in vivo (bin width, 20 ms). (h) Assessment of photoresponse latencies in vivo. Latencies were calculated from laser onset to the time at which cells exhibited a significant increase in firing frequency. Eleven of 60 cells (18%) were deemed BLA→PL cells, as they displayed photoresponse latencies shorter than 12 ms, which was the longest latency observed in slices. One cell displayed latencies greater than 12 ms (white-filled bin) and was excluded from further analyses. (i,j) Response profiles of photoidentified BLA populations, and peri-event heat maps reflecting z-scores for neurons referenced to the CS-Shock and CS-Suc. Error bands in line plots represent s.e.m. (i) BLA→PL population. A greater proportion of the BLA→PL cells sampled displayed selective excitatory responses to the fear-associated cue (F+, n = 6 of 11 cells, 55%). (j) An additional BLA population that exhibited significant inhibition during ChR2 stimulation. These cells thus did not terminate in PL, and they perhaps received inhibitory influence from the BLA→PL network. These cells were deemed network-inhibited cells (n = 8 of 60 cells, 13%). During the discrimination task, most of these cells exhibited either inhibitory responses to the fear cue (F–, 3 of 8, 38%) or excitatory responses to the reward cue (R+, 3 of 8, 38%).
The number of cells per population in BLA dots represent individual cells. On average, 85% of the competition trials showed inhibition. PC, principal component. Below, trial-by-trial behavioral output for a representative animal during each trial type. Schematic of the competition task, in which, in addition to CS-Suc and CS-Shock trials, animals were challenged by the co-presentation of these associations to induce behavioral competition. Below, trial-by-trial behavioral output for a representative animal during each trial type. The SVM model was trained using neural activity during the CS-Suc and CS-Shock trials. Data for the entire 20 s of CS presentation were used to classify neural activity. The model was then tested during competition trials to predict behavioral responses based on neural activity. For this example, this BLA→PL cell accurately predicted behavioral responses in 85% of the competition trials. PC, principal component. (e) Mean decoding accuracy for the distinct BLA populations. Superimposed dots represent individual cells (n values per population are reported in the bars). All BLA populations showed averaged decoding accuracies that were significantly higher than chance (Bonferroni-corrected paired t-test comparisons against scrambled data are represented by the asterisks above the number of cells per population: BLA→PL, t(3) = 3.31, *P = 0.013; network-inhibited, t(2) = 3.74, **P = 0.007; unidentified, t(24) = 2.29, *P = 0.028). Furthermore, the BLA→PL population but not the network-inhibited population showed significantly higher decoding accuracy than unidentified cells (one-way ANOVA: F(2,54) = 3.36, P = 0.042; Bonferroni post hoc tests: BLA→PL versus unidentified, t(2) = 2.74, *P = 0.017; network-inhibited versus unidentified, t(22) = 1.22, P = 0.23). (f) Mean decoding accuracy for the BLA populations, when their activity was paired with the activity of simultaneously recorded PL cells with which they showed either uncorrelated activity (Unc) or significantly correlated activity (Corr). Superimposed dots represent BLA/PL neural pairs (number of cell pairs per population are reported within the bars). All populations showed decoding accuracies that were significantly higher than chance (Bonferroni-corrected paired t-test comparisons against scrambled data are represented by the asterisks above the number of cells per population: BLA→PL Unc, t(26) = 2.91, **P = 0.006; BLA→PL Corr, t(26) = 6.75, ***P < 0.001; network-inhibited Unc, t(26) = 5.87, ***P < 0.001; network-inhibited Corr, t(24) = 10.5, ***P < 0.001; unidentified Unc, t(24) = 8.36, ***P < 0.001; unidentified Corr, t(24) = 4.24, ***P < 0.001). Furthermore, the BLA→PL cells showed significantly higher decoding accuracy when their activity was paired with correlated PL activity (one-way ANOVA: F(2,58) = 11.1, P < 0.001; Bonferroni post hoc tests: BLA→PL, Unc versus Corr, t(26) = 2.64, *P = 0.011; network-inhibited, Unc versus Corr, t(100) = 0.68, P = 0.50; unidentified, Unc versus Corr, t(100) = 1.18, P = 0.24). Error bars represent s.e.m.

Activity of BLA→PL neurons decoded behavior in moments of conflict more accurately than unidentified BLA neurons

On the basis of the above findings taken together, we hypothesized that the neural activity of photoidentified BLA→PL neurons during independent presentations of the fear- and reward-related cues would allow us to decode the behavior of animals challenged with competing signals, specifically the simultaneous presentation of CS-Suc and CS-Shock (Fig. 5). The selection of a behavioral output during competition trials varied between animals and even between trials within a single animal (Fig. 5b), as competition trials could produce behaviors related to either reward-seeking (port entry) or fear (freezing).

To test this, we used a support vector machine (SVM) algorithm to compare the decoding accuracy of individual neurons in the BLA. CS-Suc trials, which reliably induced port entry, and CS-Shock trials, which reliably induced freezing, were used as training data for the SVM (Fig. 5c; see “Machine learning to decode neuronal activity and predict behavior” in the Online Methods). The SVM algorithm was then tested on competition trials to determine the percentage of trials for which the activity of each individual BLA neuron accurately predicted behavioral selection (port entry or freezing; Fig. 5d). A representative BLA→PL neuron is shown that had 85% decoding accuracy (Fig. 5d).

Photoidentified BLA→PL neurons indeed showed a significantly higher mean decoding accuracy than unidentified BLA neurons (Fig. 5c).
Figure 6  Stimulation of BLA inputs to PL was sufficient to promote fear-related behavior and bias behavioral responses toward fear during competition. (a) Optogenetic strategy to stimulate (Stim) BLA inputs to PL. Illumination may have also reached other subregions of mPFC. The BLA was unilaterally transduced with either eYFP (n = 10 animals) or ChR2 (n = 8 animals), and an optical fiber was chronically implanted in dorsal PL to locally stimulate BLA inputs. (b) Schematic of the discrimination task, in which half of the trials were paired with 20-Hz blue light stimulation. The trial and laser sequences were pseudorandom. (c) Freezing behavior during CS-Shock trial, illustrated as the difference score in the percentage of time spent freezing in laser-on relative to laser-off. Stimulation of BLA inputs to PL significantly enhanced freezing responses (repeated measures two-way ANOVA; group, F1,16 = 11.4, P = 0.004; laser, F1,16 = 2.88, P = 0.11; interaction, F1,16 = 11.4, P = 0.004; eYFP versus ChR2 during laser-on: t8 = 4.78, ***P = 0.0002). (d) Port entry behavior during CS-Suc trials, illustrated as the difference score in the percentage of time spent in the sucrose port, relative to laser-off. No significant differences were detected for port entry responses (group, F1,16 = 0.95, P = 0.34; laser, F1,16 = 0.13, P = 0.72; interaction, F1,16 = 0.95, P = 0.34). (e) Pharmacology experiment to rule out a contribution from stimulation of fibers of passage or BLA terminals beyond PL. After unilateral transduction of the BLA with ChR2 (n = 8 animals), a cannula was chronically implanted above PL to allow the infusion of either ACSF or a combination of NBQX and AP5 ~10–15 min before inserting an optical fiber for optical stimulation and behavioral testing. (f) Experimental design for drug treatment and schematic of the competition task, in which half of the trials were paired with light stimulation. The trial and laser sequences were pseudorandom. (g) Freezing behavior during CS-Shock trials. Ruling out the possibility of stimulation of fibers of passage, the NBQX + AP5 treatment abolished the stimulation effect on freezing observed after the ACSF treatment (drug, F1,14 = 4.88, P = 0.044; laser, F1,14 = 7.64, P = 0.015; interaction, F1,14 = 4.88, P = 0.044; ACSF versus NBQX + AP5 during laser-on: t7 = 3.12, **P = 0.0076). (h) Port entry behavior during CS-Suc trials. No significant differences were detected (drug, F1,14 = 1.27, P = 0.28; laser, F1,14 = 4.58, P = 0.0504; interaction, F1,14 = 1.27, P = 0.28). (i) Freezing behavior during competition trials. Stimulation of BLA inputs to PL also enhanced freezing during competition trials under the ACSF treatment, and this effect was abolished by the NBQX + AP5 treatment (drug, F1,14 = 6.79, P = 0.02; laser, F1,14 = 1.89, P = 0.19; interaction, F1,14 = 6.79, P = 0.02; ACSF versus NBQX + AP5 during laser-on: t7 = 3.69, **P = 0.0024). (j) Port entry behavior during competition. There was a trend toward reduced port entry responses during competition (drug, F1,14 = 2.18, P = 0.16; laser, F1,14 = 1.83, P = 0.20; interaction, F1,14 = 2.18, P = 0.16; ACSF versus NBQX + AP5 during laser-on: t7 = 2.09, *P = 0.056). Error bars represent s.e.m.

Additionally, cross-regional neuronal pairs containing a photoidentified BLA→PL neuron had greater decoding accuracy if the PL neuron showed correlated firing with the PL-projecting BLA neuron (Fig. 5f). The significantly greater accuracy in predicting action selection during competition trials suggests that the BLA→PL projection encodes information that can guide behavior.

Photostimulation of BLA inputs to PL were sufficient to promote freezing
To test whether the BLA→mPFC projection was sufficient to promote fear-related behavior such as freezing, we photostimulated ChR2-expressing BLA terminals in the mPFC (Fig. 6a–d and Supplementary Fig. 8a). During the Pavlovian discrimination session, rats expressing ChR2 displayed significantly more freezing during laser-on than laser-off epochs relative to eYFP-expressing controls (Fig. 6c).

To control for the potential contribution of vesicle release from BLA terminals at targets other than PL, which may be induced by backpropagating action potentials or stimulation of axons of passage, in a separate experiment (Fig. 6e–j and Supplementary Fig. 8b,c) we used a standard pharmacological control for projection-specific optogenetic manipulation22. In ChR2-expressing animals, we either infused a glutamate receptor antagonist cocktail (NBQX plus AP5; see Online Methods) or artificial cerebrospinal fluid (ACSF) unilaterally into the PL, counterbalanced for order across two different sessions
Figure 7 The BLA→PL pathway is necessary for expression of the fear-associated memory, but not for reward-seeking behavior. (a) Optogenetic strategy to inhibit BLA inputs to PL. Illumination may have also affected BLA terminals in other subregions of mPFC. The BLA was bilaterally transsected to express either GFP (n = 6 animals) or the opsin ArchT (n = 6 animals), and optical fibers were chronically implanted just above BLA inputs locally. (b) Competition model, in which half of the trials were paired with constant yellow light to silence BLA inputs to mPFC. The trial and laser sequences were pseudorandomized. (c) Silencing of BLA inputs to mPFC significantly impaired freezing responses during CS-Shock trials (repeated measures two-way ANOVA: group, F_{1,10} = 5.64, P = 0.039; laser, F_{1,10} = 2.75, P = 0.14; interaction, F_{1,10} = 5.64, P = 0.039; GFP versus ArchT during laser-on: t_{10} = 3.36, **P = 0.0072). (d) No statistically significant differences were detected on port entry responses during CS-Suc trials (group, F_{1,10} = 2.53, P = 0.14; laser, F_{1,10} = 1.70, P = 0.22; interaction, F_{1,10} = 2.53, P = 0.14). (e) Significant group differences were detected for freezing during competition (group, F_{1,10} = 5.20, P = 0.046; laser, F_{1,10} = 4.37, P = 0.063; interaction, F_{1,10} = 5.20, P = 0.046; GFP versus ArchT during laser-on: t_{10} = 3.23, **P = 0.0091). (f) Significant group differences were also detected for port entries during competition (group, F_{1,10} = 10.5, P = 0.009; laser, F_{1,10} = 9.73, P = 0.011; interaction, F_{1,10} = 10.5, P = 0.009; GFP versus ArchT during laser-on: t_{10} = 4.58, ***P = 0.001). (g) Chemogenetic strategy to selectively silence BLA→PL cells. Using a Cre-dependent dual-virus method, BLA→PL cells were bilaterally transduced with either mCherry (n = 7 animals) or hM4D(Gi) (n = 7 animals). (h) Experimental design to treat animals with either vehicle (5% DMSO in 0.9% saline, i.p.; Veh) or CNO (10 mg/kg, i.p.) ~15–20 min before behavioral testing. (i) Silencing the BLA→PL cell population significantly impaired freezing responses during CS-Shock trials (group, F_{1,12} = 3.41, P = 0.09; drug, F_{2,24} = 7.96, P = 0.0022; interaction, F_{2,24} = 6.31, P = 0.006; mCherry versus hM4D(Gi) during CNO: f_{12} = 3.66, **P = 0.0033). (j) No significant differences were detected in port entry behavior during CS-Suc trials (group, F_{1,12} = 0.13, P = 0.72; drug, F_{2,24} = 0.69, P = 0.51; interaction, F_{2,24} = 0.57, P = 0.58). (k) Silencing the BLA→PL cell population impaired freezing responses during competition trials (group, F_{1,12} = 1.45, P = 0.25; drug, F_{2,24} = 0.09, P = 0.91; interaction, F_{2,24} = 2.67, P = 0.09; mCherry versus hM4D(Gi) during CNO: f_{12} = 2.56, *P = 0.025). (l) No significant differences were detected in port entry behavior during competition trials (group, F_{1,12} = 0.13, P = 0.72; drug, F_{2,24} = 0.60, P = 0.55; interaction, F_{2,24} = 0.17, P = 0.84). Error bars represent s.e.m.
wherein a subset of trials were paired with photostimulation. If off-site vesicle release contributed to the behavioral change in ChR2-expressing animals relative to eYFP animals, then we would expect those contributions to persist after the NBQX plus AP5 treatment. We found that infusion of NBQX plus AP5 abolished the light-induced increase in freezing observed in CS-Shock trials (Fig. 6g), as well as during competition trials (Fig. 6i), confirming that transmission from BLA terminals in the PL was sufficient to promote cue-induced freezing.

**Inhibition of the BLA→PL pathway suppressed cue-induced fear-related behavior**

Although our photostimulation experiments demonstrated that the BLA→PL projection is sufficient to augment freezing, fear-related behavior is likely governed by parallel, redundant circuits. We next tested the necessity of the BLA→mPFC pathway with optogenetic inhibition and the necessity of BLA→PL cells with chemogenetic inhibition during discrimination and competition (Fig. 7 and Supplementary Figs. 9 and 10).

For optogenetic inhibition, we bilaterally expressed the inhibitory opsin ArchT28,29 in BLA neurons and photoinhibited BLA terminals in the mPFC during the presentation of a subset of CS-Suc, CS-Shock and competition trials (Fig. 7a,b). We found that ArchT-expressing animals showed a significant reduction in freezing during the laser-on versus laser-off trials relative to GFP-expressing controls during CS-Shock trials (Fig. 7c) and competition trials (Fig. 7e).

Given the caveats associated with optogenetic inhibition, including the possibility of photoinhibition of adjacent mPFC subregions, effects of heating or sensory detection of the light stimulation, we also performed chemogenetic inhibition. We selectively expressed an inhibitory chemogenetic tool, hM4D(Gi) DREADDs (designer receptors exclusively activated by designer drugs)30, which are activated by clozapine-N-oxide (CNO), in BLA→PL neurons using the CAV2-Cre virus strategy (Fig. 7g). We then systemically injected either vehicle or CNO into animals before sessions wherein CS-Suc, CS-Shock and competition trials were presented in a pseudorandom order (Fig. 7h). As in our optogenetic inhibition experiment, we observed that selective inhibition of the BLA→PL neurons reduced freezing relative to mCherry-expressing controls during CS-Shock trials (Fig. 7i) and competition trials (Fig. 7k).

**DISCUSSION**

In this study, we developed a new model for studying the orchestration of competing mechanisms elicited by simultaneous presentation of cues associated with conflicting motivational drives. By presenting these stimuli in a trial structure, we enabled systematic observation of neural correlates (Fig. 1). Electrophysiological recordings revealed correlated neural activity in the BLA and PL that was predominantly excitatory during the entire task. However, the direction of information flow changed depending on the specific behavioral epoch: the BLA was more likely to lead excitatory correlations with PL during fear-related behavior (Fig. 2), and PL neurons encoding the fear-associated cue were more likely to be correlated with BLA activity (Fig. 3). This result was bolstered by our finding that most photodentified BLA→PL neurons recorded showed excitations in response to the fear-associated cue and more accurately decoded behavior in the face of competing signals (Figs. 4 and 5), showing that this effect was at least in part due to direct input from BLA to PL. Conversely, inhibitory CCs were more often led by the BLA upon presentation of the sucrose-predictive cue (Fig. 2h), and neurons inhibited by photodentified BLA→PL neurons (network-inhibited cells) showed a trend toward increased excitatory responding to the reward-associated cue or inhibition to the shock-predictive cue. Although the sample sizes for the inhibitory CCs and network-inhibited neurons were admittedly small, these data lead us to speculate that a competing reward-related network exists and is suppressed locally within the BLA2,31.

**Anatomical implications**

Several subtle effects from our experiments prompted us to speculate about the possible interactions between BLA and PL neurons. In our phototagging experiment (Fig. 4), we observed network-inhibited neurons in the BLA that were inhibited upon photostimulation of BLA→PL neurons. We speculate that BLA→PL neurons have collaterals that may synapse locally onto BLA interneurons that inhibit these network-inhibited cells, though it is possible that they collateralize to distal GABAergic neurons that have long-range projections back to the BLA. Though we are not aware of any direct evidence that the BLA contains GABAergic neurons that project to the PL, our experiments do not allow us to exclude this possibility.

Although in our optogenetic manipulations (Figs. 6 and 7) we aimed our optical fiber at the PL region of the mPFC, it is possible that we also targeted BLA terminals in surrounding areas. We took care to photoinhibit BLA terminals in mPFC only during the 20-s cue presentation, as we wanted to avoid paradoxical vesicle release associated with prolonged illumination of ArchT-expressing axon terminals32. Even so, we observed a minority of neurons in the PL (4%; Supplementary Fig. 9h) that showed an increase in activity upon photoinhibition of BLA terminals, which may result from network disinhibition. It may also be noteworthy that our optogenetic manipulations produced rather subtle behavioral changes (Figs. 6 and 7). The relatively small effect sizes may reflect either redundancy in the circuitry involved in this task or technical challenges related to using rats as opposed to mice (including greater volume of illumination required or lower relative expression levels achieved).

We found similar proportions of correlated BLA/PL cells during all task events, and a substantial proportion of cell pairs exhibited correlated activity during more than one event. This suggests that there are consistent anatomical relationships between the BLA and PL that are selectively modulated depending on the memory that is being retrieved. While we found evidence supporting bidirectional flow of information during reward-seeking epochs, the flow of information became biased toward the BLA→PL direction during fear epochs. This biased flow of information was not due to increased responsiveness to the shock-predictive cue compared to the sucrose-predictive cue in the BLA as a whole. More specific hypotheses of the underlying change in effective connectivity might include the possibility that BLA neurons that signal fear memory are more likely to connect functionally in a leading manner with PL. In addition, while BLA neurons that encode the same cue value may be more likely to facilitate each other than neurons that encode the opposite cue value, there is debate as to whether such populations are anatomically intermingled or segregated, and as to how they interact2,8,31,33. This suggests that statistical interrelationships are unlikely to remain completely independent across reward and fear retrieval. Despite this, we observed dynamic changes in the functional relationship between the BLA and PL that differentiated reward versus fear memory retrieval, with the BLA driving PL activity more strongly during the retrieval of fear than reward. A possible explanation for this could be that another structure mediates the changing relationship between the BLA and PL. One potential candidate is the ventral hippocampus, which is required for the expression of conditioned fear responses32 and preferentially modulates activity in both the BLA and PL during states of elevated fear and anxiety34,35.
Could another BLA→mPFC pathway preferentially guide reward-seeking behavior?

Other mPFC subregions, such as the infralimbic cortex (IL), have different functions than PL in the regulation of reward-seeking and fear responses. In the reward domain, while PL activity is required for the initial acquisition of goal-directed reward-seeking behavior, IL activity is required for habitual reward-seeking behavior in over-trained animals. Furthermore, IL shows stronger increases in activity than PL during reward-seeking tasks. In addition, IL activity has also been correlated with food enticement, contextually driven reward-seeking responses and Pavlovian-to-instrumental transfer of reward-seeking behavior. In the fear domain, while PL activity is crucial for fear expression, IL activity is crucial for fear extinction and the inhibition of fear responses later on. The ability of IL to inhibit fear could reliably allow reward-related signals to emerge without conflicting with fear signals, thus allowing reward-seeking behavior to occur. Future studies could focus on determining the function of the BLA→IL pathway during reward–fear discrimination and test the hypothesis that IL may be a reward-biased pathway between the BLA and the mPFC.

Potential implications for impact of emotion on cognition

While we found evidence for both ‘bottom-up’ (BLA→PL) and ‘top-down’ (PL→BLA) interactions, overall, BLA→PL regulation dominated over the reciprocal PL→BLA regulation during fear retrieval. This finding is consistent with other models that predict transfer of information from the BLA to PL during fear learning. Furthermore, recent studies that examined neural oscillations in the theta and gamma frequencies of local field potentials report synchronized activity between the BLA and PL. However, these oscillations do not clearly reflect preferential bottom-up BLA→PL regulation during fear retrieval. In contrast, top-down PL→BLA regulation has been reported to dominate during the presentation of ‘safety’ cues (for example, cues that do not predict electrical shocks) during anesthesia in untrained animals. Thus, it appears that bottom-up BLA→PL regulation dominates during high-fear states, whereas top-down PL→BLA regulation dominates during low-fear states. Nonetheless, we did not observe stronger top-down PL→BLA regulation during reward-seeking behavior, which is a low-fear state. Perhaps some bottom-up BLA→PL regulation might have occurred during reward-seeking that countered the opposite, top-down PL→BLA regulation during this low-fear, reward-seeking state.

After being trained using only neural activity during the individual fear- and reward-associated cues, machine learning algorithms were able to decode subject behavior using neural activity from competition trials (Fig. S). The ability to predict behavior in the face of conflicting cues suggests that limbic representations of singular motivational states are nested within the representation of these states in conflict. Indeed, the representation of emotional conflict in the BLA is not only associated with the cues that trigger positive or negative emotional states, but also the behavioral expression of those states. Despite variability across or even within animals, behavior on competition trials was decisive within individual trials, suggesting that emotional conflict is inherently unstable and quickly pivots toward simpler, singular motivational states. Our findings support the notion that the process of conflict resolution or state stabilization has, to some extent, already occurred in the information communicated by the BLA to the PL. In summary, the present study establishes a new model and identifies new vistas for exploration regarding the distal networks and microcircuity involved in the neural mechanisms guiding action selection in situations of conflict.
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ONLINE METHODS

Animals. All procedures were approved by the Committee on Animal Care of the Massachusetts Institute of Technology and the Animal Care and Use Review Office of the USAMRMC, in compliance with the PHS Policy on Humane Care and Use of Laboratory Animals (Public Law 99–158). Adult male Long-Evans rats weighing ~275–300 g (3 months old) were acquired from a commercial supplier (Taconic Biosciences) and were housed individually in Plexiglas home cages in a vivarium with controlled temperature, humidity and pressure. Rats were maintained on a regular 12-h light/dark cycle and all experiments were performed during the light phase. Water was available ad libitum. Standard rat chow was restricted to 20 g/d.

Surgery. Rats were anesthetized using isoflurane gas (~2.0%), and surgeries were performed using stereotaxic apparatuses (Kopf Instruments). Midline incisions were made down the scalp using surgical blades and craniotomies were opened using a dental drill. Coordinates to target the BLA were ~2.60 to ~2.80 mm anterior–posterior (AP), ±4.80 to ±5.00 mm medio–lateral (ML) and ~8.50 to ~8.80 mm dorsal–ventral (DV). Coordinates to target PL were ±3.00 to ±2.90 mm AP, ±0.50 to ±0.75 mm ML and ~3.75 to ~4.00 mm DV. All coordinates are relative to bregma. Implants were secured to the skull using stainless steel self-tapping screws (3.18 mm; Small Parts), adhesive cement (C&B Metabond, Parkell) and dental acrylic (Ortho-Jet, Lang Dental). Incisions were sutured and postoperative care and analgesia (5 mg/kg ketoprofen or 1.5 mg/kg meloxicam) were provided for 4 d. Rats were allowed to fully recover from surgery for 2 weeks.

Optogenetic manipulations. Viral vectors were infused during a surgical procedure that occurred at least 12–16 weeks before implanting optical fibers. All viral aliquots were obtained from the University of North Carolina Vector Core, unless otherwise specified. DNA sequences for viral constructs can be found online (http://www.optogenetics.org/). A 10-µL microsyringe with a 33-Ga needle (Nanofil, WPI) was used to deliver viral vectors into the targets at a rate of ~0.1 µL/min, using a microsyringe pump (UMP3/Micro4; WPI). Viral volumes of ~700–1,000 nL were infused per target. Needles were kept at the infusion site for an extra 5 min to allow viral diffusion. Needles were slowly withdrawn at an approximate rate of 1 mm/min.

For optogenetic stimulation, the BLA was unilaterally transduced with a serotype-5 adeno-associated viral vector (AAV5) encoding the blue-light-sensitive outward proton pump (Chlamydomonas reinhardtii channelrhodopsin-2) (ChR2), which was fused to enhanced yellow fluorescent protein (eYFP) and expressed selectively expressed in the BLA cells terminating in PL using a Cre-dependent dual-virus strategy in which the BLA was infected with a virus containing mCherry or hM4D(Gi) which silenced neuronal activity upon activation with a designer drug, mCherry or hM4D(Gi) were selectively expressed in the BLA cells terminating in PL using a Cre-dependent dual-virus strategy in which the BLA was infected with a virus containing mCherry or hM4D(Gi) in a double-floxed inverted open reading cassette (AAV5-HSyn-DIO-mCherry or AAV5-HSyn-DIO-hM4D(Gi)-mCherry), whereas PL was infected with a retrograde canine virus type-2 encoding Cre recombinase (CAX2-Cre (ref. 52); Institute of Molecular Genetics of Montpellier). Viral volumes of ~700–1,000 nL were infused per site at a rate of 0.1 µL/min. Viral expression was allowed for 12–16 weeks before behavioral testing. hM4D(Gi) was activated with the designer drug clozapine-N-oxide (CNO; Sigma-Aldrich), which was diluted in a solution of 5% DMSO and 0.9% saline. Systemic injections (i.p.) were performed ~15–20 min before behavioral testing at a dose of 10 mg/kg to inhibit neuronal activity. Behavioral testing was also performed after multiple vehicle injections (5% DMSO in 0.9% saline).

Behavioral tasks. Rats were trained in standard operant chambers (23 × 30 × 40 cm; Med Associates) located inside sound-deadening cubicles. Each chamber was equipped with a red house light, light cues, speakers for the delivery of tone or white noise cues, a syringe pump to deliver sucrose, a sucrose port that was equipped with an infrared beam for the detection of entries and exits, and a grid floor for the delivery of electrical shocks. A customized digital-relay circuit was added between the shock generator and grid floor to minimize electrical artifacts. Chambers were scrubbed with 70% isopropyl alcohol after testing each animal.

All training phases occurred in the context. The first phase of training consisted of the acquisition of a Pavlovian reward association in which rats learned to associate a conditioned stimulus with sucrose (that is, CS-Suc). To facilitate reward acquisition, rats were pre-exposed multiple times to sucrose in the home cage as well as in the training chambers. Reward conditioning consisted of the presentation of either a light cue or a sine wave tone cue (5 kHz, 80 dB) that lasted for 20 s and predicted the delivery of a 30% sucrose solution (120 µL/trial). Sucrose was delivered over 10 s during the cue presentation (5–15 s, relative to CS onset). Rats underwent three reward sessions (one per day), each consisting of a total of 25 trials delivered over ~35 min. The ITI was variable, with an average of 1 min. Sucrose was removed by vacuum immediately after cue offset if rats did not retrieve it during the CS.

The second phase of training consisted of the discrimination of conditioned stimuli that predicted sucrose reward (CS-Suc), aversive shocks (CS-Shock) or no outcome (CS−). The light and tone cues were counterbalanced across rats for the CS-Suc and CS-Shock associations. The white noise cue was always used for the CS−. The aversive shocks (0.40 mA) lasted for 0.5 s and co-terminated with the CS (19.5–20 s, relative to CS onset). CS-Suc, CS-Shock and CS− trials occurred in a pseudorandom manner. Rats underwent at least three discrimination sessions (one per day), each consisting of a total of 60 trials delivered over ~83 min. The ITI was variable, with an average of 1 min.

Pharmacology experiment. Optogenetic and pharmacological approaches were combined to rule out the possibility of stimulation of fibers of passage.
The third phase of training tested the direct competition of reward- and fear-related behaviors. In addition to individual CS-Suc and CS-Shock trials, this phase of training included competition trials, in which the CS-Suc and CS-Shock associations were co-presented to induce conflicting motivational drives and the potential for competition between reward- and fear-associated behaviors. CS-Suc, CS-Shock and competition trials occurred in a pseudorandom manner. A total of 60 trials were delivered over ~83 min during these competition sessions (variable ITI, with an average of 1 min).

**Reward and fear behaviors.** Entries into the sucrose port provided a readout of reward-related behavior. Timestamps for port entries and exits were sampled from beam breaks (Med-PC IV, Med Associates). These timestamps were used to quantify the amount of time that animals spent in the port. Freezing responses, which are defined as the lack of all movement except for respiration, provided a readout of fear-related behavior. Videos were sampled using infrared cameras at 30 fps, and freezing was quantified using an automated custom MatLab script that quantified frame-by-frame changes in total pixel intensity as approximations for animal motion. Frame-by-frame motion values were then converted into freezing scores using a binary method relative to a motion threshold (that is, motion levels above a certain threshold were classified as no freezing, whereas motion levels below a certain threshold were classified as freezing). The time that animals spent in the port was subtracted from the freezing quantification, as animals showed little motion while collecting sucrose.

**In vivo single-unit electrophysiology.** Extracellular single-unit recordings were performed using in-house-built multichannel electrodes. An electrode consisted of a 10 × 2 pin connector (Mill-Max Manufacturing Corp) that accommodated 16 microwires for single-unit recordings, an extra microwire for analog reference and a low-resistance 200-µm silver wire to provide ground (A-M Systems). A 22.9-µm HML-insulated nichrome microwire was used (Stabohlm 675, California Fine Wire). A 26-G stainless steel cannula was attached to one of the electrode pins to insert the microwire bundle. Microwires were secured to the connector pins using a silver print coating (GC Electronics). After testing for short circuits, all connections were secured using dental acrylic. Final cutting of the microwire tips was performed using serrated fine scissors (Science Tools). The microwire tips were gold-plated to reduce impedance and improve the signal-to-noise ratio. Gold plating was achieved by submerging the electrode tips in a solution containing (in mM) 125 potassium gluconate, 20 HEPES, 1 NaCl, 3 Mg-ATP, 8 biocytin and 2 Alexa Fluor 350 (pH 7.3; 287 mOsm). Recorded signals were amplified using a MultiClamp 700B amplifier (A-M Systems). Signals were digitized at 10 kHz using Digidata 1440 and recorded using the pClamp10 software (Molecular Devices).

After opening the cell membrane, neurons were confirmed to express ChR2 if they showed a constant inward current in voltage clamp in response to a 1-s constant blue light pulse, with a light power density of 84 mW/mm² (20 mW with a 40× objective) delivered via a 470-nm LED light source. From the 30 recorded neurons, 6 were confirmed to express ChR2. The remaining 24 neurons were confirmed to not express ChR2 nor to receive inputs from nearby ChR2 neurons, as they did not respond to the light stimulus. Cell bodies for the 24 neighboring cells were located at least 150 µm from the cell bodies of ChR2-expressing neurons.

Histology. Rats were euthanized with sodium pentobarbital (150 mg/kg) and microlesions were produced at the recording sites by passing an anodal electrical current (20 s at ~25–35 mA on at least four channels). Rats were surgically perfused with ice-cold phosphate buffer saline (PBS) and 4% paraformaldehyde (PFA, pH 7.3). Brains were collected and fixed in 4% PFA for 24 h and equilibrated in 30% sucrose for 48 h. Coronal sections were cut at 60 µm using a microtome (HM430, Thermo Fisher Scientific).

Brain sections containing the BLA and PL were incubated for 30 min in a DAPI-specific fluorescence probe (DAP1; 4′,6-diamidino-2-phenylindole; 1:50,000 dilution). After four washes in PBS (10 min each), sections were mounted on microscope slides using fluorescence-compatible PVD-DBAC medium. Confocal images were acquired with an Olympus FV1000 confocal laser-scanning microscope, using a 10×, 0.40 NA or 40×, 1.30 NA oil immersion objective.
Image stiches and serial z-stacks were assembled with commercial imaging software (Fluoview, Olympus). Expression of eYFP and DAPI was examined in sections containing various anterior–posterior coronal levels of the BLA and PL. Microlesions were examined on confocal images and reconstructed onto coronal drawings adapted from a rat brain atlas43.

Statistical analyses. All statistical analyses were based on two-tailed comparisons and were performed using GraphPad Prism (GraphPad Software, Inc.), unless otherwise specified. Although no statistical tests were used to predetermine sample sizes, our sample sizes are consistent with previous publications56–58. All data met the assumptions of every statistical tests used. The Kolmogorov–Smirnov normality test was used to determine whether data sets required parametric or non-parametric statistical tests.

Randomization and blinding. For behavioral training, the counterbalanced auditory and visual CSs were randomly assigned to animals. Pseudorandom trial sequences were generated to deliver CS-Suc, CS-Shock, CS−, competition, laser-off and laser-on trials. All animals received the same trial and laser sequences in any given experiment. Viral treatments for optogenetic and chemogenetic experiments were randomly assigned to animals. For the pharmacology experiment, the vehicle and drug treatments were randomly assigned during the initial test session, whereas they were counterbalanced during the second test session. Although blinding was not performed, behavioral testing was controlled by software and data analyses were performed using customized automated methods whenever possible. In addition, all experiments were designed with appropriate internal controls (for example, within-subject comparisons of laser-off versus laser-on trials, within-subject comparisons of CS-Suc versus CS-Shock trials, etc.).

Exclusion of animals, cells or data points. Several animals were excluded from this study due to either electrode misplacement (n = 2 animals), lack of viral expression (n = 5 animals), viral leakage (n = 2 animals) or breakage or misplacement of optical fibers (n = 3 animals). Several cells were also excluded from this study (BLA: n = 22 cells, PL: n = 23 cells) due to repetitions across channels. In addition, for cross-correlation analyses, we excluded cells that fired at low frequencies (<0.1 Hz), which typically produce unpopulated correlograms with sparse peaks and troughs. One data point was excluded from the decoding analysis as it was detected by the Grubbs’ test as a statistically significant outlier.

Behavioral data. Pearson’s correlation test was used on a subset of freezing data to determine whether our automated quantification method provided reliable values compared to hand-scoring (d.f. = 58; R = 0.989; P < 0.0001; n = 60 trials including CS-Suc, CS-Shock and competition; n = 3 animals). For the initial behavioral experiments, within-subject comparisons of port and freezing data across distinct CSs were performed using either paired t-test (in the case of two conditions) or one-way analysis of variance (ANOVA) with repeated-measures and Bonferroni post hoc tests (in the case of three or more conditions). For optogenetic and chemogenetic experiments, normalizations were performed to port entry and freezing data by calculating the difference between experimental conditions (% time values were used for the subtractions). For instance, the percentage of time that animals spent performing either of the behaviors during laser-off trials was subtracted from laser-on trials (that is, laser-on minus laser-off). Similarly, values obtained during the first test session in the chemogenetic experiment were subtracted from values obtained during subsequent sessions (for example, CNO minus Veh1). Statistical comparisons between groups were performed using two-way ANOVA with repeated measures and Bonferroni post hoc tests.

Quality of cluster sorting. Single units were considered for analysis if clusters met two sorting quality statistical parameters: (i) multivariate analysis of variance (MANOVA; probability threshold for significance was set to P < 0.01), which indicated that each cluster was positioned at a statistically different feature space location at any given feature space; and (ii) the non-parametric J3 statistic, which measured the ratio of between-cluster to within-cluster scatter. These cluster statistics were examined using Offline Sorter (Plexon Inc.). To avoid duplicates across channels, autocorrelograms and cross-correlograms of simultaneously recorded units were inspected using Neuroexplorer (NEX Technologies).

Putative principal cells and interneurons. Using a hierarchical clustering method, BLA and PL cells were separated into putative principal neurons versus interneurons based on spike width and firing frequency59,60. Three parameters were used: (i) duration of the depolarization phase at half amplitude, (ii) duration of the hyperpolarization phase at half amplitude and (iii) the average firing frequency during the entire recording session. Cross-correlations. Cross-correlations (CCs) were assessed to examine whether BLA and PL cells exhibited different patterns of functional interactions during distinct task epochs. Analyses for CCs were performed using a combination of tools in Neuroexplorer (NEX Technologies), Matlab (MathWorks) and R (R Core Team; https://www.R-project.org/). CCs were examined during various task epochs: (i) ITI, (ii) CS-Suc, (iii) CS-Shock, (iv) CS− and (v) competition. The ITI epochs were pseudorandomly generated such that they matched the number of CS-epochs in any given recording session and such that the ITI epochs were at least 5 s away from any CS epoch. To generate CCs, the BLA spikes were used as the reference events and the PL spikes were used as the target events. CCs were generated for a window of ±1.000 ms relative to the reference spikes, using bin widths of 25 ms (ref. 61).

Two correction methods were applied to control for apparent correlations that might be due to CS-elicited covariation or nonstationary firing rates: (i) a trial-shift predictor, in which spike trains from the reference and target neurons were compared during shifted trials (19 trial-shifts were applied per neural pair); and (ii) a spike-shuffle predictor, in which the spike trains of the reference neurons were repeatedly shuffled over time (100 random spike shuffles per trial were applied). The trial-shift predictor and spike-shuffle predictor correlograms were individually subtracted from the raw correlograms, and neural pairs were deemed significantly correlated if peaks or troughs reached statistical significance after application of both correction methods. The statistical significance of peaks and troughs was determined by z-score transformations of the corrected correlograms, relative to the average s.d. of the correlograms generated for each predictor. Significant peaks and troughs were evaluated within an experimental window of ±100 ms relative to the reference spikes, using a z-score criterion that was based on a two-tailed significance level of P < 0.01 and that was Bonferroni-corrected for multiple comparisons (that is, eight 25-ms bins within the ±100 ms window; actual P = 0.01/8 = 0.00125). The significance P-value of 0.00125 corresponded to z-score thresholds of z > 3.323 for excitation CCs or z < −3.23 for inhibitory CCs. CCs had to meet these significance thresholds for both predictor correlations (trial-shifting and spike-shuffling) to be considered for further analyses.

Since 25-ms bins were too broad to detect coincident firing, zero-lag ‘common-input’ correlograms62 were examined from 5-ms binned correlograms with the central bin centered at zero (that is, ±2.5 ms). If the 5-ms binned correlograms exhibited peaks or troughs centered at zero, correlations were considered to be due to common input and were excluded from analysis. The timing of peaks and troughs in the 25-ms-binned correlograms was examined to determine putative lead and lag. Excluding zero-lag cell pairs, correlations were considered to be led by BLA if peaks or troughs occurred after the BLA reference spikes (that is, within ±2.5 to +100 ms in the correlograms), whereas correlations were considered to be led by PL if peaks or troughs occurred before the BLA reference spikes (that is, within −100 to −2.5 ms in the correlograms). Proportions of significantly correlated cell pairs were compared across task epochs using Bonferroni-corrected chi-square tests. Results obtained with the 25-ms-binned correlograms were confirmed using 10-ms-binned correlograms63. However, the narrower bins in the setting of cells with low firing rates led to highly sparse correlograms with increased variability and the potential for false positive and false negative correlations. In addition, the narrower bins failed to detect many inhibitory correlations that were detected with the wider bins, due to increased variability and floor effects, especially in the low-firing-rate cell pairs.

CS-evoked responses. The response of individual cells to CSs was examined using a combination of the non-parametric Wilcoxon signed-rank test (as primary) and a z-score test (as secondary). Given the existence of cells in both the BLA and PL that typically show either transient or prolonged responses to CSs46–68, two signed-rank tests with 1,000 bootstraps and Bonferroni corrections were performed per CS. For transient responses, neural activity was binned in 25-ms intervals, and comparisons were made between a baseline window ranging from −1 to 0 s and an experimental window ranging from 0 to 300 ms, relative to CS onset. For prolonged responses, activity was binned in 50-ms intervals, and comparisons were made between a baseline window ranging from −100 to −2.5 s and an experimental window ranging from 0 to 1.5 s, relative to CS onset. The z-score test confirmed that the peak responses reached a certain significance threshold of either z > 2.58 for excitatory responses (corresponding to P < 0.01) or z < −1.96 for inhibitory responses (corresponding to P < 0.05). Cells that met both the Wilcoxon and z-score criteria were then considered CS-responsive cells. Proportions of CS-responsive populations were compared using chi-square tests with Bonferroni corrections for multiple comparisons.
Machine learning to decode neuronal activity and predict behavior. A machine-learning algorithm\textsuperscript{69,70} was used to determine whether behavioral responses during competition trials could be predicted based on how individual cells responded during CS-Suc and CS-Shock trials. Neural data for individual cells was extracted for the entire 20 s of each trial (20 trials for the CS-Suc, 20 trials for the CS-Shock and 20 trials for competition). These data were then preprocessed by binning neuronal activity within each individual trial into 50-ms bins. Spike density estimates for each trial were then generated by convolving the binned rasters with a Gaussian kernel (\textit{s.d.} of 200 ms). The dimensionality of each cell's data was further reduced using principal component analysis across all trial types\textsuperscript{71}, retaining the first four principal component scores for each trial. This procedure reduced the data from 400 data points per cell per trial (50-ms bins over 20 s) to only four data points per cell per trial. The same preprocessing steps were used for pairs of BLA and PL cells, except that the spike trains of each cell were concatenated before principal component analysis, and eight principal components were selected in total for each pair of cells. Each pair of cells was separately preprocessed using all possible combinations of simultaneously recorded cells.

The reduced, preprocessed data was then used to train a support vector machine (SVM) classifier\textsuperscript{72-73}. The SVM classifier was trained using linear kernels (\texttt{fitcsvm.m}, Matlab R2015b) to determine the optimal hyperplane that separated neural activity during the CS-Suc and CS-Shock trials. The identified separating hyperplane was then used to predict behavioral responses during competition trials by classifying neuronal activity as ‘more CS-Suc-like’ or ‘more CS-Shock-like’ on a trial-by-trial basis. Cross-validation was not necessary, as data from the competition trials were never used to train the classifier but only used to test it. The predicted classification was compared to the actual behavioral output of the animal to determine whether the prediction was correct or incorrect. The decoding accuracy for a given cell or cell pair was calculated as the percentage of competition trials in which the predicted behavioral response matched the actual response of the animal. The statistical significance of the calculated decoding accuracies were empirically determined using permutation tests, which compared the decoding accuracy for real training data to decoding accuracies obtained after scrambling the identity of the CS-Suc and CS-Shock trials 1,000 times, each time generating a permuted separating hyperplane that was used to classify the actual neural data from competition trials\textsuperscript{74}. The decoding accuracy obtained using the hyperplane derived from the actual CS-Suc and CS-Shock neural data was compared to the permuted distribution only once; therefore, there was no need to correct for multiple comparisons. For between-population comparisons of the decoding accuracies, we used one-way ANOVA with Bonferroni \textit{post hoc} tests. For within-population comparisons against scrambled data, we used Bonferroni-corrected paired \textit{t}-tests.

A \textbf{Supplementary Methods Checklist} is available.

\textbf{Data and code availability.} All relevant data and code supporting the findings of this study are available from the corresponding author upon reasonable request.