Connectivity characterization of the mouse basolateral amygdalar complex

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The basolateral amygdalar complex (BLA) is implicated in behaviors ranging from fear acquisition to addiction. Optogenetic methods have enabled the association of circuit-specific functions to uniquely connected BLA cell types. Thus, a systematic and detailed connectivity profile of BLA projection neurons to inform granular, cell type-specific interrogations is warranted. Here, we apply machine-learning based computational and informatics analysis techniques to the results of circuit-tracing experiments to create a foundational, comprehensive BLA connectivity map. The analyses identify three distinct domains within the anterior BLA (BLAa) that house target-specific projection neurons with distinguishable morphological features. We identify brain-wide targets of projection neurons in the three BLAa domains, as well as in the posterior BLA, ventral BLA, posterior basomedial, and lateral amygdalar nuclei. Inputs to each nucleus also are identified via retrograde tracing. The data suggests that connectionally unique, domain-specific BLAa neurons are associated with distinct behavior networks.

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The basolateral amygdalar complex (BLA) contains the lateral (LA), anterior and posterior basolateral (BLAa and BLAp), anterior and posterior basomedial (BMAa and BMAp), as well as the ventral (BLAv) amygdalar nuclei. Although recognized mostly for its role in fear conditioning and extinction1,2 the BLA is implicated in several behavior-related states and disorders including anxiety3, autism4, and addiction5. Optogenetic technology has enabled specialized investigations of circuit-specific functional assignments. It is therefore timely to advance understanding of connectivity-specific BLA neurons that will inform higher resolution cell type-specific interrogations.

BLA cell populations with unique connectional-functional phenotypes have been identified. For example, a population of BLA projection neurons that target the prelimbic cortical area (PL) are primarily activated during high fear conditions, while neurons that innervate the infralimbic cortical area (ILA) respond predominantly during fear extinction6. These functionally distinct cell groups defined by their projection targets are designated as “fear” and “extinction” neurons, respectively. Similarly, activation of pathways from BLA to the lateral part of the central amygdalar nucleus (BLA→CEA) and to the anterodorsal part of the bed nucleus of stria terminalis (BLA→BSTad) result in anxiolytic-like behaviors3,7, while BLA projections to ventral hippocampus (BLA→vHPC) have been implicated in anxiogenesis8. There is the supposition that these functionally specific, uniquely connected neurons are primarily intermingled within BLA6,9.

Studies also have demonstrated clear segregation of projection defined BLA cells. Anterior BLA (BLAa) neurons that project to PL10 and nucleus accumbens11 occupy medial aspects, and those that target the CEA localize in lateral aspects11, while ventral hippocampus projecting BLA neurons are reported in more caudal regions11,12. Topographic input to distinct BLAa regions also have been reported13,14, suggesting the existence of distinct BLAa domains with unique connections. So where are these target-specific BLA projection neurons located and where would investigators begin to probe cell type function? One approach is to acquire a systematic and detailed overview of BLA connectivity. Although BLA connectivity has been described previously15, it has been systematically acquired whole-brain input/output connectivity profile for BLA neurons has not been created for any species.

In this work, we apply circuit-level pathway tracing methods combined with computational techniques to provide a comprehensive connectivity atlas of the mouse BLA that includes three newly delineated BLAa domains. Online resources for viewing raw, reconstructed, and analyzed data are provided.

Results

Pathway tracing, computational analysis, and visualizations.

Connectivity data was collected at USC as part of the Mouse Connectome Project (MCP), currently at UCLA. Anterograde (PHAL, BDA, AAV) and retrograde (CTb, FG, AAV retro Cre) pathway tracers were placed in BLAa, BLAp, BLAv, BMAp, and LA (cases can be viewed at https://mouseconnectomeproject.github.io/amygdalar/iconnectome). Tracing data from injections targeting medial prefrontal cortex (MPF) and hippocampus (HPF) clearly delineated three BLAa domains: medial (BLA.am), lateral (BLA.al), and caudal (BLA.ac). Additional injections in cortex, thalamus, and striatum validated these domain distinctions (Fig. 1a–c; Supplementary Fig. 1a–c).

To determine BLAa domain boundaries, representative cases with distinct labeling in each domain were selected and all consecutive sections across the BLA were analyzed (Supplementary Fig. 2). Sections underwent image processing through our in-house software Connection Lens, where each section was matched and warped to its corresponding atlas level of the Allen Reference Atlas (ARA) and the labeling was segmented (Fig. 2a). Labels were manually mapped and aggregated atop the ARA, and approximate boundaries were delineated for BLA.am, BLA.al, and BLA.ac (Fig. 2b; Supplementary Fig. 3a, b). The same data was used as a training set for a machine learning algorithm to compute BLAa domain boundaries16–18. The automated boundary demarcations highly corroborated those of the manual delineations, returning an average agreement of 92% (Fig. 2b; Supplementary Fig. 3a). Utilizing these boundary approximations, anterograde and retrograde tracer injections were made to involve primarily BLA.am, BLA.al, or BLA.ac (Supplementary Fig. 4a) as well as BLAp, BLAv, LA (ventromedial region), or BMAp (Supplementary Fig. 4c).

To attain brain-wide connectivity patterns of each BLA nucleus, all data was processed through Connection Lens (Fig. 2a). Data was annotated at a 175 × 175 pixel grid resolution given the complexity of topographic projections from BLA to ROIs like the nucleus accumbens or olfactory tubercle (Fig. 1f, g). Subsequent Louvain community detection analysis assigned each injection site to a community based on labeling patterns of the individual grid cells. Community assignments were color-coded by injection site and visualized for 32 ARA sections to create anterograde and retrograde connectivity maps (Fig. 2a), which are available through a web application (https://mouseconnectomeproject.github.io/amygdalar/) (Supplementary Fig. 1d). Community analysis was isolated across separate categories of injections to identify regions of unique input and output. Data from BLA.am, BLA.al, and BLA.ac were aggregated, analyzed, and visualized, while separate analyses were conducted to compare BLAp to BLA.al and BLAv, BMAp. LA in addition, data for all BLA nuclei were combined and subjected to community detection analysis with resulting outputs visualized in a matrix (Fig. 3i, j; Fig. 4f, g).

Reported connections were validated using at least one of the following methods. Injections for all BLA nuclei were replicated and consistency across label patterns was manually assessed (Supplementary Fig. 4b). Retrograde tracers were injected into regions of anterograde terminal labeling to validate anterograde connections, but also to reveal the location of target-specific projection neurons (Fig. 2c). Anterograde tracers were injected into the location of back-labeled projection cells to validate retrograde BLA injection data (Supplementary Fig 4d, e). Quantitative comparisons of projection labels from BLAa domains were performed to supplement and validate the qualitative analysis (Supplementary Fig. 5b–m). Hierarchical clustering of anterograde projection data showed that repeated injections in BLAa domains produced highly consistent domain-specific label patterns, substantiating the validity and reproducibility of injection cases (Supplementary Fig. 6a). A total of 245 injections (44 in BLA) were used to generate nucleus-unique connectivity diagrams and a global wiring diagram of the entire BLA (https://mouseconnectomeproject.github.io/amygdalar/wiringdiagram) (Supplementary Fig. 6b).

Parcellation of BLAa connectivity-defined cell types projecting to medial prefrontal cortex (MPF) and hippocampal formation (HPF). BLAa shares extensive bidirectional connectivity with the ILA and PL in MPF14,19,20. These connections were validated and it was demonstrated that input to each MPF area originates from two regionally distinct BLAa neuron populations. An anterograde and retrograde tracer co-injection (BDA/FG) in PL labels overlapping fibers and projection cells in medial parts of BLAa (BLA.am), suggesting strong BLA.am→PL and PL→BLA.am connections (Fig. 3a). A co-injection (PHAL/CTb) in ILA shows axon terminals in BLA.am with retrogradely labeled...
projection neurons located mostly in lateral BLAa (BLA.al) (BLA.al → ILA) (Fig. 3a), suggesting a BLA.al → ILA → BLA.am circuit.

A PHAL/CTb co-injection in BLA.am confirms the presence of this circuit and reveals the detailed regional and laminar specificities of BLA.am-MPF connections. BLA.am neurons densely innervate PL layer II, but also layers V and VI [BLA.am → PL(II,V,VI)] (Fig. 3b; Supplementary Fig. 7a). ILA remains relatively void of inputs, corroborating sparse BLA.am → ILA connectivity. Further, BLA.am projecting PL neurons distribute primarily in layers II/III, and in layer III of ILA, confirming and refining the ILA(III) → BLA.
am→PL(II) pathway. Finally, PHAL and FG injections in BLA.al validate the unidirectional BLA.am→ILA connection and reveal laminar details [BLA.al→ILA(II-VI)] (Fig. 3c).

Neurons in the caudal BLAa (BLA.ac) share unique connections with MPF that differ from its BLA.of counterparts. Stronger BLA.ac projections to MPF are to PL, primarily layer II (Fig. 3c), but also layers III-VI [BLA.ac→PL(II-VI)], which lightly project back to BLA.ac (Fig. 3d, e). Most PL projections to BLA.ac are to BLA.am (PL→BLA.am), while stronger ILA projections are to BLA.ac [ILA(II/III)→BLA.ac] (Fig. 3d). BLA.ac neurons also target ILA [BLA.ac→ILA(II-VI)] (Fig. 3e). Multiple injections of anterograde tracers in BLA.am, BLA.al, and BLA.ac in a single animal highlight their distinct input to MPF structures (Fig. 3f). These unique BLA.ac-MPF connections are summarized in Fig. 3g, h.

BLA.ac neurons are the only BLAa cells to target HPF, and their connections with CA3 and parasubiculum (PAR) are primarily exclusive among all BLA nuclei. Retrogade tracer injections in CA3 and PAR selectively label BLA.ac projection neurons [BLA.ac→CA3; BLA.ac→PAR] (Fig. 4a, b). In addition, BLA.ac neurons target the pyramidal layers of CA1 (BLA.ac→CA1_sp) and ventral subiculum (BLA.ac→SUBv) (Fig. 4d, e). Besides CA3, all these HPF regions send inputs back to BLA.ac (CA1_sp/SUBv/PAR→BLA.ac) (Fig. 4e). See Fig. 4f for summarized connections of BLA and HPF and Fig. 4f, g for BLA-hippocampal connectivity matrices.

Global connections of the BLA.am, BLA.al, and BLA.ac. An overview of BLA.am, BLA.al, and BLA.ac projection neuron targets demonstrates their discrete brain-wide connectivity patterns (Fig. 1c; Supplementary Fig. 5a; Supplementary Fig. 8).

BLAa connects with other MPF areas. Projection cells in BLA.al provide input to deeper layers of dorsal peduncular area (DP) [BLA.al→DP(II,III)], without receiving much reciprocal input (Supplementary Fig. 7d–f). BLA.am projection neurons strongly target the anterior cingulate area (ACA), and in more caudal sections, the adjacent secondary motor area (MOS) suggested to correspond to frontal eye fields (MOs-feF)21 (Fig. 5a, d). These projections are directed toward both the dorsal (BLA.am→A-CAd) and ventral (BLA.am→ACA) ACA divisions across its rostral-caudal axis. Only rostral A-CAd contains neurons that project back to BLA.am (Supplementary Fig. 7b).

BLA.am neurons specifically target superficial layers of ventrolateral orbital area (ORBvl), while those in BLA.al target deeper layers of the lateral ORB (ORBl) [BLA.am→ORBvl(II/III); BLA.al→ORBvl(V/VI)] (Fig. 5c; Supplementary Fig. 7i). Input from medial ORB (ORBm) to BLA.am and BLA.ac are notable [ORBm(II/III)→BLA.am/BLA.ac] with some reciprocity [BLA.am/BLA.ac→ORBm(II-VI)] (Supplementary Fig. 7j).

Notable and topographic projections from agranular insular (AI) cortical areas to BLA are evident. The BLA.al is the primary recipient of input from dorsal AI (Ald) [Ald→BLA.al] (Fig. 5i; Supplementary Fig. 7k), while the BLA.am is the primary recipient of inputs from Alv (Alv→BLA.am) (Fig. 5j). Further, BLA.al projection neurons send axons to the gustatory cortical area (GU; BLA.al→GU) and neurons in GU(II-III) project back to BLA.al [GU(II-III)→BLA.al] (Fig. 5k).

The BLA is directly connected with only a few sensory cortical areas. BLA.am neurons target MOs-feF (Fig. 5a, d) and send sparse terminations to deep layers of secondary visual cortical areas like anteromedial (VISam) and anterolateral (VISal) areas.
BLA.al projects to more rostral parts of MOs, primary motor (MOp), and somatosensory (SSp) regions presumed to be associated with upper limb and orofacial information processing (Fig. 5m).

Projections from BLAa neurons to perirhinal (PERI), ectorhinal (ECT), and temporal association (TEa) areas are sparse. Observable projections from BLA.ac to caudal regions of ECT are noted, although strongest input to ECT arise from LA neurons (Supplementary Fig. 9a). Input from PERI, ECT, and TEa to BLAa are greater and topographically arranged. Neurons in more superficial layers of rostral TEa, PERI, and ECT project primarily to BLA.al [TEa/PERI/ECT(I-III)→BLA.al] (Supplementary Fig. 9b, c), while those in deeper layers target BLA.am [PERI/ECT(V-VI)→BLA.am] (Supplementary Fig. 9d). Strongest input to BLAa from TEa is provided by its caudal region to BLA.am (caudal TEa→BLA.am) (Supplementary Fig. 9e). See Fig. 3i, j for BLA-isocortical connectivity matrices.

Connections between claustrum (CLA) and BLAa are sparse. BLAa domains are the only nuclei with weak projections to the subcortical structure (Supplementary Fig. 9i).

BLAa domains uniquely connect with the entorhinal cortex. Projections from BLAa to lateral entorhinal cortex (ENTl) are weak (Supplementary Fig. 10a), but strong topographic
projections from ENT neurons terminate in BLAa. Cells in rostral (generally dorsal) ENT regions target BLA.al (rostral/dorsal ENT→BLA.al) (Supplementary Fig. 10b). More caudal intermediate ENT regions target BLA.am (caudal/intermediate ENT→BLA.am), while most ventrally located ENT neurons target BLA.ac (caudal/ventral ENT→BLA.ac) (Supplementary Fig. 10c). Connections between BLAa and medial ENT (ENTm) are sparse and mostly through BLA.ac (BLA.ac→medial ENTm; caudal ENTm→BLA.ac) (Supplementary Fig. 10f).

BLAa is connected with regions that process olfactory information. BLA.am and BLA.al cells project to anterior olfactory nucleus posteroverentral part (AONpv) (BLA.am/BLA.al→AONpv) (Supplementary Fig. 11a) and more strongly to the nucleus of the lateral olfactory tract (NLOT) (BLA.am/BLA.al→NLOT) (Supplementary Fig. 11d). Layer III NLOT cells project back to BLA.am and BLA.al [NLOT(III)→BLA.am/BLA.al] (Supplementary Fig. 11c). BLAa domains also have unique connections with dorsal taenia tecta, (TTd), piriform (PIR; Supplementary Fig. 11e, f), and postpiriform transition area (TR; Supplementary Fig. 12g, h).

Projections from BLA neurons to thalamus are light; however, all three domains are innervated by the midline thalamic nuclei. Generally, projection neurons in rostral midline nuclei (ARA 57, 61) target BLA.ac, while mid-caudal midline thalamic nuclei (ARA 69, 73, 75) target BLA.am and BLA.al (Fig. 6a). This is most evident with parataenial (PT) and paraventricular (PVT) projections (Fig. 6a, b). The PVT has rostral (rPVT) and caudal (cPVT) subdivisions based on anatomical22 and behavioral23,24 distinctions. Neurons in rPVT preferentially BLA.ac (rPVT→BLA.ac) (Fig. 6b, c), while those in cPVT primarily project to BLA.am and BLA.al (cPVT→BLA.am/BLA.al) (Fig. 6b; Supplementary Fig. 13a). Additional discriminating thalamic inputs are observed with PT neurons that primarily target BLA.ac (PT→BLA.ac) (Fig. 6b, d) and with intermediodorsal (IMD), parafascicular (PF, medial part (mPF)), and VMp neurons that innervate BLA.al (IMD/mPF/VMp→BLA.al) (Fig. 5i; Fig. 6b; Supplementary Fig. 13a). BLA.am/BLA.al also receive input from dorsal anteromedial (Amd), reuniens (RE), rhomboid (RH), and central medial (CM) thalamic nuclei (RE/RH/CM→BLA.am/BLA.al) (Fig. 6b; Supplementary Fig. 13b). Projections from lateral posterior thalamic nucleus (LP) are unique to BLA.am (LP→BLA.am) (Fig. 5e).

BLA connects with motor systems. Domain specific BLAa neurons target different regions of the coudoputamen (CP), BLA.al neurons target ventromedial (CP.vm) and ventrolateral (CP.vl) regions of intermediate CP (ARA 53), which are also targeted by AI, PIR, VISC, GU, and somatosensory and somatomotor regions related to mouth areas25 (BLA.al→CP.vl/vm) (Fig. 5h). BLA.al neurons also target ventral parts of caudal CP (CPcv), a region heavily innervated by GU and VISC25 (BLA.al→CPcv) (Fig. 5h). In contrast, BLA.am neurons target dorsomedial regions of intermediate CP (CPdm) (Fig. 5a; Supplementary Fig. 12b, c), where input from visual cortical areas, ACA, ENTm, and retrosplenial area (RSP) converge25 (BLA.am→CPdm) (Supplementary Fig. 12d, e). At caudal levels, BLA.am and BLA.ac neurons innervate dorsomedial domains (CPdm) (Fig. 5a, f), where visual information also converges25 (BLA.am/BLA.ac→CPdm) (Fig. 5f). Retrograde injections in dorsal and ventral CP validate these connections and illustrates the segregation of CP projecting BLA.am and BLA.al neuron populations (Fig. 1a; Supplementary Fig. 1c).

Within the nucleus accumbens (ACB) and olfactory tubercle (OT), clear topographic projections originate from BLA.am and BLA.ac neurons. BLA.am neurons preferentially target medial aspects of ACB and OT (BLA.ac→ACB medial/OT medial), while BLA.am neurons target their lateral aspects (BLA.am→ACB lateral/OT lateral) (Fig. 1f, g). BLA.al neurons sparsely target OT lateral and, more strongly, ACB lateral (BLA.al→OT lateral/ACB lateral) (Fig. 1f, g).

Neurons in BLA.al target additional areas like the substantia innominata (SI) (BLA.al→SI) (Supplementary Fig. 12i, k), which provide weak input to BLAa (SI→BLA.am/BLA.al) (Supplementary Fig. 12j, k). Only BLA.ac neurons target bed nuclei of stria terminalis (BST) specifically to its oval (BSTov) and rhomboid (BSTrh) nuclei (BLA.ac→BSTov/rh) (Fig. 7b).

Summarized brain-wide connections of projection neurons in BLA.am (Fig. 5b; Fig. 8e; Supplementary Fig. 14f), BLA.al (Fig. 5g; Fig. 8f; Supplementary Fig. 14g), and BLA.ac (Fig. 4c; Fig. 8g; Supplementary Fig. 14h) are provided. Although tracing experiments were conducted in male mice, BLAa domain-specific output was examined also in female mice, which displayed similar brain-wide patterns to those reported for males (Supplementary Fig. 15).

Functional pathways of projection-defined BLAa neurons. Optogenetically-assisted circuit mapping ex vivo studies were performed to demonstrate functional pathways through connectionally-defined BLAa subtypes. The BLA affects motor behavior through its outputs to CEA26. However, the CP is one of the main projection targets of BLAa neurons, which suggests that BLAa potentially integrates cortical information and affects motor output through CP. Our data suggests PL→BLA.am→CPdm and AId→BLA.al→CPcv connections, which were examined. An ILA→BLA.ac→CA3 pathway also was investigated.
An AAV-hSyn-ChR2-YFP (ChR2) injection was made into PL, while retrograde red microbeads (RR) were injected into CPc.dm to deliver ChR2 to PL axonal terminals in BLA.am and retrogradely label CP projecting BLA.am neurons (Fig. 8i). In slice preparations, whole-cell voltage-clamp recordings were made from CP projecting BLA.am neurons, while PL axons in BLA.am were optically stimulated. A 5-ms pulse of blue light elicited an excitatory current in the recorded neuron clamped at −70 mV in the presence of TTX (1 µM) and 4-AP (1 mM), which eliminate polysynaptic responses27,28 (Fig. 8r). Only evoked EPSPs were observed, suggesting excitatory PL to BLA.am projections, which monosynaptically innervate CP projecting BLA.am neurons (Fig. 8k).

To demonstrate an Ald→BLA.al→CPc.v connection, ChR2 was injected into Ald to label projection terminals in BLA.al, while an RR injection in CPc.v retrogradely labeled BLA.al.
Fig. 3 BLAa connections with medial prefrontal cortex. a Double co-injections of BDA/FG in PL(I/II) and PHAL/CTb in ILA(I/III) show the medial (BLA.am) and lateral (BLA.al) distinction of BLAa. PHAL and BDA fibers and FG are present in BLA.am, while CTb cells are in BLA.al suggesting PL→BLA.am, ILA→BLA.am, and BLA.al→ILA connections. These connections were validated with a BLA.am PHAL/CTb injection, which shows strong fiber labeling in PL, especially layer II/III [BLA.am→PL(I/II)] and CTb labeling in PL(II) [PL(II)→BLA.am] and ILA(III) [ILA(III)→BLA.am]. The LA FG injection delineates the ILA. c The BLA.al→ILA connection was validated with a BLA.al PHAL injection, which showed strong fiber labels in ILA and DP. FG injection in BLA.al confirms the absence of an ILA→BLA.al connection. * denotes lack of FG ILA labeling, d BLA.ac shows unique connections with MPF. A PHAL/CTb injection in PL shows CTb labeled BLA.ac projection cells, but sparse PHAL fiber labels in BLA.ac, suggesting a BLA.ac→PL connection. PHAL fibers from PL localize mostly in rostral BLA.am. A PHAL/CTb injection in ILA(V) shows strong fiber projections in BLA.ac suggesting a strong ILA→BLA.ac connection. Only a few CTb cells are present in BLA.ac suggesting a weak BLA.ac→ILA connection. e A BLA.ac PHAL/CTb injection validates these connections, showing strong projections to PL, especially layer II/III [BLA.ac→PL(I/II)] and CTb labeled cells in layers II-V of ILA (ILA→BLA.ac). f Top panel: anterograde labeling from tracer injections made primarily in BLA.am (PHAL) and BLA.al (AAV RFP) shows their topographic projections to PL/ACA and ILA/DP, respectively. Bottom panel: anterograde labeling from tracer injections made primarily in BLA.ac (PHAL) and BLA.al (AAV GFP) shows their distinct connections with PL, ILA, ACA, OT, and CP. Note the stronger projections from BLA.am to dorsal PL/ACA compared to projections from BLA.ac to more ventral parts of PL. This distinction can be seen in the anterograde map in (g). g BLAa domains share unique input/output connections with MPF, especially layers II/III, which is summarized in this schematic. Note (1) the reciprocal connections between the BLA.am and ACA and PL (dorsal), (2) BLA.al projections mostly to ILA, and (3) the unique BLA.ac connections with strong projections to PL (ventral), but strong input from ILA. h Schematic summarizing connections of all BLA nuclei with MPF areas. i Community detection confined to BLA projections to isocortical areas was run and visualized in a matrix. The matrix was reordered such that injection sites with their strongest projections are arranged along the diagonal. Grouped injection sites and their connections are boxed in different colors. The weighting of each connection is indicated by a color gradient from black (very strong) to white (very weak). Matrix analysis was ROI based, not grid-based. BLA.am and BLA.ac were grouped with projections to PL(I–III), ORBm, and ACAM among the strongest. The BLA.al and BLAP were grouped with strongest projections to ILA, GI, and PL(V). The BLAV shows strongest projections to AI, GI, and PERI, the BMAP to ILA, ORBm, PERI, and the LA to ECT, TEa, and ILA. j Community detection confined to isocortical projections to BLA was run and visualized in a matrix, which shows BLA.ac and BMAP grouped with strongest input from ILA(I/II/III). The BLA.al, BLA.am, BLAy, LA, and BLAp were individually grouped. Note the strong inputs to BLAv from agranular insular areas (AI) and to LA from auditory cortices (AUD). See Table 1 for full list of abbreviations.

BLAa neuron morphology. To assess whether connectionally-different BLAa domains share unique input/output connections with MPF areas. i Community detection confined to BLA projections to isocortical areas was run and visualized in a matrix. The matrix was reordered such that injection sites with their strongest projections are arranged along the diagonal. Grouped injection sites and their connections are boxed in different colors. The weighting of each connection is indicated by a color gradient from black (very strong) to white (very weak). Matrix analysis was ROI based, not grid-based. BLA.am and BLA.ac were grouped with projections to PL(I–III), ORBm, and ACAM among the strongest. The BLA.al and BLAP were grouped with strongest projections to ILA, GI, and PL(V). The BLAV shows strongest projections to AI, GI, and PERI, the BMAP to ILA, ORBm, PERI, and the LA to ECT, TEa, and ILA. j Community detection confined to isocortical projections to BLA was run and visualized in a matrix, which shows BLA.ac and BMAP grouped with strongest input from ILA(I/II/III). The BLA.al, BLA.am, BLAy, LA, and BLAp were individually grouped. Note the strong inputs to BLAv from agranular insular areas (AI) and to LA from auditory cortices (AUD). See Table 1 for full list of abbreviations.
provided by rostral PERI, ECT, TEa (rostral PERI/ECT/TEa→BLAp) (Supplementary Fig. 9h).

Strongest projections to hippocampal structures are to CA1v_sp, but mostly to SUBv (BLAp→CA1v/SUBv) (Fig. 4d). Input from these neurons back to BLAp are observable (CA1v/SUBv→BLAp) (Fig. 7n). Although projections to ENTI from BLAp neurons are sparse, input from ENTI to BLAp is evident (ENTI→BLAp) (Supplementary Fig. 10g).

Regarding olfactory areas, BLAp neurons target TTd, to a lesser extent its ventral counterpart, TTv (BLAp→TTd/TTv) (Supplementary Fig. 11g), and the medial AON (BLAp→AONm) (Supplementary Fig. 11b). Input from olfactory areas to BLAp is provided by PIR (PIR→BLAp) (Supplementary Fig. 11e, f) and dorsal endopiriform nucleus (EPd→BLAp) (Supplementary Fig. 11h). BLAp neurons project strongly to TR (BLAp→TR), which project back to BLAp (TR→BLAp) (Supplementary Fig. 12g, h).

Unlike BLAa, BLAp does not receive thalamic input. Some cPVT neurons provide sparse input to BLAp (cPVT→BLAp) (Supplementary Fig. 13a). Also unlike BLAa, BLAp neurons target medial subdivisions of the mediodorsal thalamic nucleus (MDm) (BLAp→MDm) (Supplementary Fig. 4e; Supplementary
**Fig. 4 BLA.ac connections with hippocampus.** a BLA.ac connections with hippocampal regions. A CTb (green) CA3 tracer injection selectively labels BLA projection neurons (BLA.ac→CA3). Note the absence of labeled cells in BLA.am. A PHAL (pink) and CTb (pink) injection in PAR show the BLA.ac→PAR and PAR→BLA.ac connections. b These connections were validated with a PHAL/CTb BLA.ac injection that shows strong PHAL fiber labels in CA3 and PAR. c Summarized brain-wide connections of BLA.ac projection neurons. For full abbreviation list see Table 1. d Top panels show projections from BLA.ac, BLA.al, BLA.c, BLA.p, BMAp, BMaV, and LA to hippocampal regions. The BLA.ac, BLA.p, and BMAp show strongest projections, with BLA.ac projecting to sp layers of CA1 and SUBv (BLA.ac→CA1_sp/SUBv), BLA.p to CA1v and SUBv (BLA.p→CA1v_sp/SUBv), and BMaV to sp and m layers of CA1v and SUBv (BMaV→CA1v_m/SUBv). Bottom panels show validation of these connections with FG and CTb injections marked 1-3 on top panels. Injections 1 and 2 show FG and CTb injections in intermediate (CA1i and ventral (CA1v) CA1, respectively. Both injections back-label projection neurons in BLA.ac, while only the injection in CA1v labels BLA neurons. Injection 3 is a CTb injection in SUBv, which labels BLA.c, BLA.p, and BMAp neurons. e Top panels show BLAa projections to rostral CA1 and more caudal CA1 and SUB. Boxed regions are numbered and magnified to the right. Bottom panels show projections from CA1 and SUB back to BLAa. Note exclusive BLA.ac connections with the hippocampus, particularly its caudal regions (Fig. 7n shows PHAL validation of these connections). f Community detection confined to BLA projections to hippocampal areas was run and visualized in a matrix. The matrix was reordered such that grouped injection sites and their strongest projections are arranged along the diagonal and boxed in different colors. The weighting of each connection is indicated by a color gradient from black (very strong) to white (very weak). Note (1) projections from BLA.ac to PAR and CA3, (2) from BLA.p to CA1_sp and SUBv_sp, (3) from BLA.p to ENTl (layers II and V), and (4) from BMaV to SUBv_sr. * indicates strong connections that were not validated. g Community detection confined to hippocampal inputs to BLA was run and visualized in a matrix. Note (1) BLA.ac and BMaV are grouped with strong inputs from CA1 and SUBv and (2) BLA.al and BLA.am grouped with strong input from ENTl. Matrix analysis was ROI based, not grid based. h Schematic summarizing connections of all BLA nuclei with hippocampal areas. See Table 1 for full list of abbreviations.

Most prominent olfactory connections for BLA.v is through PIR (PIR→BLA.v) (Supplementary Fig. 11e, f). Neurons in Epd (Epd→BLA.v) (Supplementary Fig. 11h) project to BLA (Epd→BLA) (Supplementary Fig. 12h), anterior (COAa) and posterior medial (COAp) cortical amygdala areas (COAa/COAp→BLA.v) (COAa/COAp→BLA.v) (Supplementary Fig. 11b). Summarized brain-wide connections of BLA.v projection neurons are provided (Fig. 7o).

**Global connections of LA.** LA projection neurons provide input to ILA [ILA→ILA(I-VI)] and neurons in ILA project back to LA [ILA(II/III)→ILA] (Supplementary Fig. 1e, f). LA also provides input to ORBm (ILA→ORBm), which is reciprocated [ORBm(II/III)→ILA]; LA→ORBm (Supplementary Fig. 7i). Inputs to AI are sparse, although Ald and Aiv neurons target LA (Ald/Aiv→LA) (Fig. 5i, j). Strongest cortical projections from LA neurons are to ECT and TEa (LA→ECT/TEa) (Supplementary Fig. 9f), which in turn target LA (ECT/TEa→LA) (Supplementary Fig. 9g). LA also receives strong input from primary (AUDp) and ventral (AUDv) auditory areas (AUDp/AUDv→LA) (Fig. 8c).

Weak hippocampal connections were detected for LA neurons (ENTI→LA; Supplementary Fig. 10i) (LA→CA1v/SUBv; Fig. 8b) and (LA→PAR; Fig. 4a).

LA does not project strongly to olfactory regions although neurons in olfactory areas provide input to LA [PIR/EPd/NLOT(III)→ILA] (Supplementary Fig. 11c, e, h). The cortical olfactory area, piriform-amygdala area (PAA), also targets LA (PAA→LA), while posterior lateral COA (COAp) receives some input (LA→COAp) (Supplementary Fig. 14c).

Thalamic structures that provide input to LA include cPVT, PT, and LP (caudal PVT/PT/LP→LA) (Fig. 4d; Supplementary Fig. 13d-f). Strongest inputs originate from dorsal (MGd) and ventral (MGv) medial geniculate neurons (MG) and SPP (subfascicular nucleus, parvocellular part) and PPR (peripeduncular nucleus) nuclei (MGd/MGv/SPP/PP→LA) (Supplementary Fig. 13g, h).

Connections from LA to motor systems include a specific region in ACB medial (LA→ACB medial) (Supplementary Fig. 12a), BSTov (LA→BSTov) (Fig. 7d), and CP (LA→CP medial) (Supplementary Fig. 12b). Finally, although LA neurons provide weak input to hypothalamic VMH (LA→VMH) (Fig. 7m), VMH neurons send strong projections back to LA (VMH→LA) (Fig. 8d). Summarized brain-wide connections of LA projection neurons are provided in Fig. 8a.
| Acronym | Full structure name |
|---------|---------------------|
| AAA     | Anterior amygdalar area |
| ac     | Anterior commissure |
| ACAd    | Anterior cingulate cortical area, dorsal part |
| cACAd   | Anterior cingulate cortical area, dorsal part, caudal region |
| rACAd   | Anterior cingulate cortical area, rostral region |
| ACAv    | Anterior cingulate cortical area, ventral part |
| cACAv   | Anterior cingulate cortical area, ventral part, caudal region |
| rACAv   | Anterior cingulate cortical area, ventral part, rostral region |
| ACB     | Nucleus accumbens |
| AD      | Anterodorsal nucleus of the thalamus |
| ADp     | Anterodorsal preoptic nucleus |
| ADN     | Anterior hypothalamic nucleus |
| AI      | Agranular insular cortical area |
| Ald     | Agranular insular cortical area, dorsal part |
| Alp     | Agranular insular cortical area, posterior part |
| Alv     | Agranular insular cortical area, ventral part |
| AM      | Anteromedial nucleus of the thalamus |
| AMd     | Anteromedial nucleus of the thalamus, dorsal part |
| AMv     | Anteromedial nucleus of the thalamus, ventral part |
| AON     | Anterior olfactory nucleus |
| AONm    | Anterior olfactory nucleus, medial part |
| AONpv   | Anterior olfactory nucleus, posteroverentral part |
| ARH     | Arcuate hypothalamic nucleus |
| AUD     | Auditory cortical area |
| AUDd    | Dorsal auditory cortical area |
| AUDp    | Primary auditory cortical area |
| AUDv    | Ventral auditory cortical area |
| AV      | Anteroventral nucleus of the thalamus |
| AVN     | Anteroventral preoptic nucleus |
| AVPV    | Anteroventral periventricular nucleus |
| bic     | Brachium of the inferior colliculus |
| BLA     | Basolateral amygdalar complex |
| BLAa    | Basolateral amygdalar nucleus, anterior part |
| BLAac   | Basolateral amygdalar nucleus, anterior part, caudal domain |
| BLAal   | Basolateral amygdalar nucleus, anterior part, lateral domain |
| BLAam   | Basolateral amygdalar nucleus, anterior part, medial domain |
| BLAp    | Basolateral amygdalar nucleus, posterior part |
| BLAv    | Basolateral amygdalar nucleus, ventral part |
| BMA     | Basomedial amygdalar nucleus |
| BMAa    | Basomedial amygdalar nucleus, anterior part |
| BMAp    | Basomedial amygdalar nucleus, posterior part |
| BST     | Bed nucleus of the stria terminalis |
| BSTal   | Bed nucleus of the stria terminalis, anterolateral nucleus |
| BSTam   | Bed nucleus of the stria terminalis, anteromedial nucleus |
| BSTdm   | Bed nucleus of the stria terminalis, dorsomedial nucleus |
| BSTif   | Bed nucleus of the stria terminalis, interfascicular nucleus |
| BSTmg   | Bed nucleus of the stria terminalis, magnocellular nucleus |
| BSTpr   | Bed nucleus of the stria terminalis, principal nucleus |
| BSTtr   | Bed nucleus of the stria terminalis, transverse nucleus |
| BSTv    | Bed nucleus of the stria terminalis, ventral nucleus |
| CA1     | Hippocampal field CA1 |
| CA1d    | CA1 dorsal |
| CA1slm  | CA1 stratum lacunosum-moleculare |
| CA1so   | CA1 stratum oriens |
| CA1sp   | CA1 pyramidal layer |
| CA1sr   | CA1 stratum radiatum |
| CA1v    | CA1 ventral |
| CA2     | Hippocampal field CA2 |
| CA2so   | CA2 stratum oriens |
| CA3     | Hippocampal field CA3 |
| CA3so   | CA3 stratum oriens |
| CA3sp   | CA3 pyramidal layer |
| CEA     | Central amygdalar nucleus |
| CLA     | Claustrum |
| CM      | Central medial nucleus of the thalamus |
| COA     | Cortical amygdalar area |

Table 1 (continued)

| Acronym | Full structure name |
|---------|---------------------|
| COAa    | Cortical amygdalar area, anterior part |
| COAp    | Cortical amygdalar area, posterior part, lateral domain |
| COApml  | Cortical amygdalar area, posterior part, medial domain |
| CPC     | Caudoputamen |
| CPCv    | Caudoputamen, caudal part |
| CPCvdm  | Caudoputamen, caudal part, dorsomedial domain |
| CPCvtr  | Caudoputamen, caudal part, ventral domain |
| CPI     | Caudoputamen, intermediate part |
| CPIvm   | Caudoputamen, intermediate part, ventromedial domain |
| DMH     | Dorsomedial nucleus of the hypothalamus |
| DP      | Dorsal peduncular area |
| ECT     | Ectorhinal cortical area |
| cECT    | Ectorhinal cortical area, caudal region |
| rECT    | Ectorhinal cortical area, rostral region |
| ENH     | Entorhinal cortical area |
| cENH    | Entorhinal cortical area, lateral part |
| rENH    | Entorhinal cortical area, rostral part |
| ENTm    | Entorhinal cortical area, medial part |
| EP      | Endopiriform nucleus |
| EPd     | Endopiriform nucleus, dorsal part |
| Epv     | Endopiriform nucleus, ventral part |
| fr      | Fasciculus retroflexus |
| MS      | Fundus of the striatum |
| Gu      | Gustatory cortical area |
| GAd     | Interanterodorsal nucleus of the thalamus |
| ILA     | Infralimbic cortical area |
| IMD     | Intermediodorsal nucleus of the thalamus |
| int     | Internal capsule |
| LA      | Lateral amygdalar area |
| LD      | Laterodorsal nucleus of the thalamus |
| LHA     | Lateral hypothalamic area |
| LP      | Lateral posterior nucleus of the thalamus |
| LPO     | Lateral preoptic nucleus |
| LS      | Lateral septal nucleus |
| LSc     | Lateral septal nucleus, caudal part |
| Lsr     | Lateral septal nucleus, rostral part |
| Lsv     | Lateral septal nucleus, ventral part |
| MB      | Midbrain |
| MD      | Mediodorsal nucleus of the thalamus |
| MDm     | Mediodorsal nucleus of the thalamus, medial part |
| MEA     | Medial amygdalar nucleus |
| MEAd    | Medial amygdalar nucleus, anterodorsal part |
| MEAv    | Medial amygdalar nucleus, anteroventral part |
| MEApd   | Medial amygdalar nucleus, posterodorsal part |
| MEApv   | Medial amygdalar nucleus, posteroverentral part |
| MEPO    | Median preoptic nucleus |
| MG      | Medial geniculate complex |
| MGd     | Medial geniculate complex, dorsal part |
| MGm     | Medial geniculate complex, medial part |
| MGv     | Medial geniculate complex, ventral part |
| MM      | Medial mammillary nucleus |
| MOp     | Primary motor cortical area |
| MOp m   | Primary motor cortical area, mouth region |
| MOPl    | Primary motor cortical area, upper limb region |
| rmOPl   | Primary motor cortical area, rostral region |
| MOs     | Secondary motor cortical area |
| MOs mf  | Secondary motor cortical area, frontal eye field region |
| MOs ul  | Secondary motor cortical area, upper limb region |
| rMOs    | Secondary motor cortical area, rostral region |
| MPN     | Medial preoptic nucleus |
| MPO     | Medial preoptic area |
| MRN     | Midbrain reticular nucleus |
Table 1 (continued)

| Acronym | Full structure name |
|---------|---------------------|
| MS      | Medial septal nuclei |
| mtf     | Mammmillothalamic tract |
| NDB     | Nucleus of the diagonal band |
| NLOT    | Nucleus of the lateral olfactory tract |
| ORB     | Orbital cortical area |
| ORBl    | Orbital cortical area, lateral part |
| ORBm    | Orbital cortical area, medial part |
| ORBvl   | Orbital cortical area, ventrolateral part |
| OT      | Olfactory tubercle |
| PA      | Posterior amygdalar nucleus |
| PAA     | Piriform amygdalar area |
| PAR     | Parasubiculum |
| PERI    | Perirhinal cortical area |
| cPERI   | Perirhinal cortical area, caudal region |
| rPERI   | Perirhinal cortical area, rostral region |
| PF      | Parafascicular nucleus of the thalamus |
| PH      | Posterior hypothalamic nucleus |
| PIR     | Piriform cortical area |
| PL      | Prelimbic cortical area |
| PMv     | Ventral premammillary nucleus |
| POST    | Postsubiculum |
| PP      | Peripeduncular nucleus |
| PRE     | Presubiculum |
| PT      | Parataenial nucleus of the thalamus |
| PTlp    | Posterior parietal association area |
| PVp     | Periventricular hypothalamic nucleus, posterior part |
| PVT     | Paraventricular nucleus of the thalamus |
| cPVT    | Paraventricular nucleus of the thalamus, caudal region |
| rPVT    | Paraventricular nucleus of the thalamus, rostral region |
| RCH     | Retrochiasmatic area |
| RE      | Reuniens nucleus of the thalamus |
| RH      | Rhomboid nucleus of the thalamus |
| RR      | Retrorubral area |
| RSP     | Retrosplenial cortical area |
| RSPagl  | Retrosplenial cortical area, agranular part |
| RSPd    | Retrosplenial cortical area, dorsal part |
| RSPv    | Retrosplenial cortical area, ventral part |
| RT      | Reticular nucleus of the thalamus |
| SC      | Superior colliculus |
| SI      | Substantia innominata |
| sm      | Stria medullaris |
| SN      | Substantia nigra |
| SNc     | Substantia nigra, compact part |
| SNr     | Substantia nigra reticulate part |
| SPFp    | Subfascicular nucleus, parcellarvular part |
| SSP     | Primary somatosensory cortical area |
| SSPm    | Primary somatosensory cortical area, mouth region |
| SSS     | Supplementary somatosensory cortical area |
| SUB     | Subiculum |
| SUBd    | Subiculum, dorsal part |
| SUBv    | Subiculum, ventral part |
| SUBv_m  | Subiculum, ventral part, molecular layer |
| SUBv_sp | Subiculum, ventral part, pyramidal layer |
| SUBv_sr | Subiculum, ventral part, stratum radiatum |
| TEa     | Temporal association cortical area |
| cTEa    | Temporal association cortical area, caudal region |
| rTEa    | Temporal association cortical area, rostral region |
| TM      | Tuberomammillary nucleus |
| TMd     | Tuberomammillary nucleus, dorsal part |
| TMv     | Tuberomammillary nucleus, ventral part |
| TR      | Postpiriform transition area |
| TT      | Taenia tecta |
| TTd     | Taenia tecta, dorsal part |
| TTv     | Taenia tecta, ventral part |
| TU      | Tuberal nucleus |
| VIS     | Visual cortical area |

Table 1 (continued)

| Acronym | Full structure name |
|---------|---------------------|
| VISal   | Anterolateral visual cortical area |
| VISam   | Anteromedial visual cortical area |
| VISL    | Lateral visual cortical area |
| VISp    | Primary visual cortical area |
| VISC    | Visceral cortical area |
| VLPO    | Ventrolateral preoptic nucleus |
| VMH     | Ventromedial hypothalamic nucleus |
| VMHd    | Ventromedial hypothalamic nucleus, dorsal part |
| VP      | Ventral pallidium |
| VPMpc   | Ventral posteromedial nucleus of the thalamus, parvocellular part |

Global networks of BMAp. BMAp neurons target ILA [BMAp→ILA(II-VI)] and in turn, receive input from the MPF area [ILA(V)→BMAp] (Supplementary Fig. 1f, f). BMAp neurons also provide input to ORBm (BMAp→ORBm) (Supplementary Fig. 7j) and receive input from Alp cells (Alp→BMAp) (Fig. 5j).

Besides BLAv, BMAp neurons are the only BLA projection neurons to provide strong input to ENTI [BMAp→ENTI(V/VI)] (Supplementary Fig. 10h). Like all other BLA nuclei, BMAp receives input from ENTI (ENTI→BMAp) (Supplementary Fig. 10h). Similar to BLA.ac and BLAp, BMAp neurons project to stratum radiatum of ventral CA1 (CA1v_sr) and to SUBv, except BMAp neurons target specifically the molecular (SUBv_m) and stratum radiatum (SUBv_sr) layers of SUBv, a distinguishable feature of BMAp injections (BMAp→CA1v_sr/SUBv_msr) (Fig. 4d). Neurons in CA1v_sp and especially SUBv project back to BMAp (CA1v_sp/SUBv→BMAp) (Fig. 7n).

For olfactory processing, the BMAp receives strong input from ventral PIR (PIRv→BMAp) (Supplementary Fig. 11e). Of all nuclei, the BMAp is most strongly connected with olfactory cortical areas and receives input from the PAA (PAA→BMAp) (Supplementary Fig. 14c), TR (TR→BMAp) (Supplementary Fig. 12h), and the anterior (COAa) (Supplementary Fig. 14b), posterior lateral (COAp) (Supplementary Fig. 14c), and posterior medial (COAp) (Supplementary Fig. 14d) cortical amygdalae areas (COAa/COAp/COApm→BMAp). BMAp neurons project back to each of these areas (BMAp→COAa/COAp/COApm/PAA/TRA) (Supplementary Fig. 14a, c, e; Supplementary Fig. 12g). Strong connections between EPd and BMAp are evident (BMAp→EPd; EPd→BMAp) (Supplementary Fig. 11h).

BMAp-thalamic connections were observed. BMAp neurons send weak projections to MDm (BMAp→MDm) (Supplementary Fig. 13c) and, like the BLA.ac, receive input from rPVT (rPVT→BMAp), and PT (PT→BMAp) (Fig. 6d; Supplementary Fig. 13e).

Within the ventral striatum, BMAp neurons target the ACB medial, where inputs from BLA.ac, BLAp, and LA also terminate (BMAp→ACB medial) (Supplementary Fig. 12a). BMAp neurons sparsely project to OT medial (BMAp→OT medial) (Fig. 1f). In addition, BMAp neurons share connections with SI (BMAp→SI; SI→BMAp) (Supplementary Fig. 12i–k) and BST, particularly with the anteromedial (BSTam) and principal (BSTpr) nuclei (BMAp→BSTam/BSTpr) (Fig. 7a, d). As such, while BLAp preferentially targets lateral anterior BST, BMAp neurons target medial anterior BST (Fig. 7a–d).

Finally, BMAp neurons send appreciable projections to the medial hypothalamic column, such as the medial preoptic area (MO), medial preoptic nucleus (MPN) (Fig. 7k) (BMAp→MO/MPN), dorsomedial hypothalamus (DMH)
**Discussion**

In this work, we provided a comprehensive, systematically collected, dataset on mouse BLA connectivity. Discrete BLAa projection neuron types were identified based on their connectivity.
Fig. 5 Unique connections of BLA.am and BLA.al neurons. a BLA.am projections to visual processing areas. Top panels: whole brain sections with labeled fibers in ACAd, ACAv, MOs-if, CP caudal dorsomedial, and deep layers of VlSaml and VlSal following a BLA.am PHAL injection. Bottom panels: magnified versions of PHAL labeled fibers in visual areas labeled. b Summarized brain-wide connections of BLA.am projection neurons. For full list of abbreviations see Table 1. e BLA.am connections with ORBvl. BLA.am neurons project to ORBvl(V/VI), while BLA.al neurons project to ORBvl(V/VI). Projections in ORBvl from BLA.ac case were not validated. A CTb ORBvl injection solely back-labels BLA.am neurons confirming a BLA.am→ORBvl connection. ORBvl neurons do not project back to BLA.am as shown by the PHAL ORBvl injection. Schematic adapted from20 demonstrates pattern of inputs to frontal cortex from visual information processing areas like VIS, ACAd, ACAv, and PTlp, which is similar to prefrontal input patterns from BLA.am. d Anterograde map shows BLA.am projections to ACAv and MOs-if validated with retrograde injections of CTb and FG (BLA.am→ACAv/MOs-if). e Left: retrograde map with back-labeled neurons in visual LP following a retrograde tracer injection into BLA.am. Right: PHAL injection in visual LP labels fibers in BLA.am, and also LA, confirming LP→BLA.am/LA projections. f Left: anterograde map of BLA.am and BLA.ac projection fibers to CP caudal dorsomedial (CPc.dm) at ARA 61 superimposed with CP caudal domains. Right: a screenshot of our cortico-striatal map at ARA 61 showing projections from visual areas like VlSp, VlSal, VlSal, ACA, RSP, and PTlp to CP caudal dorsomedial (http://www.mouseconnectome.org/CorticalMap/page/map/5). FG injection in CPc.dm back-labels BLA.am neurons confirming the BLA.am→CPc.dm connection. g Summarized brain-wide connections of BLA.al neurons. For full list of abbreviations see Table 1. h BLA.am projections to gustatory/visceral CP. Top left shows anterograde map of BLA.al projection fibers at ARA 53 superimposed with domains of CP intermediate (CPi). BLA.al neurons target CPi vl and CPi vm, which also receive input from AI, PIR, VISC, GU, and somatosensory and somatomotor regions associated with mouth movement. Retrograde tracers CTb 555 and CTb 647 in these CP domains back-label projection neurons in BLA.al. Bottom panels: CP caudal ventral (CPc.v), which receives input from GU and VISC is also targeted by BLA.al. A CTb injection in CPc.v back-labels BLA.al neurons to confirm this (BLA.al→CPc.v). i Top: AId neurons target BLA.al and LA as shown with an AId PHAL injection. Bottom: BLA.al retrograde injection back-labels AId neurons (AId→BLA.al). j AId shows weak projections to BLA.al and stronger projections to LA and BLA.v. AId neurons target mostly BLA.v and BMAP. k Retrograde map shows neurons in GU that project to BLA.al (GU→BLA.al). A FG injection in GU (V) reveals BLA.al projection neurons target GU (BLA.al→GU (V)). l Retrograde map shows back-labeled neurons in PF/VPMpc from BLA.al retrograde injection suggesting a PF/VPMpc→BLA.al connection. An anterograde AAV-RFP injection in the thalamic region labels BLA.al, validating the projection. m BLA.al projection neurons target somatomotor and somatosensory regions presumably associated with orofacial information processing including the MOs/MOp ul (upper limb) and SSp/ MOp m (mouth). Retrograde tracer injections in these regions clearly label BLA.al neurons. See Table 1 for full list of abbreviations.

characteristics. These distinguishable connections provide insight into the fundamental organizational principles of amygdalar circuits that regulate different behavioral output. Some potential hypotheses are presented below.

The BLA.am is in a network of structures associated with visual information processing and eye movement (Fig. 6e). Visual information can reach the BLA.am from caudal TEa, which receives abundant visual and auditory inputs32, from the visual LP thalamic nucleus, and from thalamic RE, which contains head direction cells.34 In turn, BLA.am projections to motor areas like CPc.dm can regulate behavioral output. The CPc.dm is implicated in visual information processing given that it integrates inputs from areas like VIS, ACA, and RSP. The BLA.am also sends input to deep layers of secondary visual areas (VlSaml, VlSal), the ACAv, MOs-if, and ORBvl. The ORBvl is in a cortical network that processes visual and spatial information. BLA connections to primary visual cortex are reported in primates35 although, to our knowledge, not in mice. The relevance of these connections are apparent in the context of primate studies showing BLA involvement and facial expressions36. They may also be relevant in rodents. Although BLA (BLA.a, BLA.p) unimodal neurons responsive only to visual cues are sparse, neurons responsive to a variety of sensory modalities, including visual, auditory, somatosensory, and gustatory, are dispersed throughout37. Further, significantly more multimodal neurons that respond to stimuli previously paired in a conditioning task are located in BLA (BLA.a, BLA.p). Our data suggests multimodal neurons involving visual information may be in BLA.am. Since re-evaluation of stimuli for outcome prediction is BLA dependent38, it is a reasonable assumption that BLA.am would be equipped with the ability to survey and communicate about stimuli of various modalities, including those that are visual in nature.

The BLA.al has connections that suggest its role in gustatory information processing (Fig. 6f). Distinct areas within the insular cortex (AId, AIV, ALP, and GU) are shown to code palatable and unpalatable tasters, with neurons responding to sweet tasters located more rostrally than those that respond to bitter39. Unique amygdalar projections from the “sweet” cortical area to BLA process the hedonic valence of the tastant. Our data showed that the strongest projections from rostral AId (location of sweet neurons) are to BLA.al. BLA cells targeting AId and GU also are primarily located in BLA.al. In addition, BLA.al receives input from rostral ECT/PERI, which share extensive connections with somatic sensorimotor mouth areas. BLA.al neurons, in turn, specifically target CP domains that receive convergent inputs from the AI, GU, and somatic and somatomotor mouth regions, suggesting a role for BLA.al in feedback mechanisms potentially involving these regions. In fact, we showed that AId fibers directly innervate BLA.al projection neurons that target CPc.v, providing a functional circuit for gustatory information processing and behavioral motor output. Thalamic input to BLA.al comes from the caudal PVT, the IMD, medial part of PF, and VPMpc. The medial part of the thalamic PF nucleus was recently shown to project to A143, the caudal PVT receives input from the AI42, and the VPMpc receives gustatory information from the medial parabrachial nucleus (PBN) within the gustatory taste pathway43. Taste neurons have been identified in BLA and following odor-taste associations, the number of BLA neurons that respond to both stimuli dramatically increases44. BLA.al could be a candidate region in which these conditioned responsive cells involving taste stimuli are located. BLAp and BLAv are additional possibilities.

Unlike BLA.am and BLA.al, BLA.ac neurons are connected with hippocampal regions like SUBv, CAIV, but most particularly with CA3 and PAR. Connections with these latter two regions are exclusive to BLA.ac neurons. Compared to BLA.am and BLA.al, which provide input to ACB lateral and OT lateral46, BLA.ac neurons provide input predominantly to ACB medial shell and OT medial47. Unique thalamic input to BLA.ac is provided by PT and rostral PVT45. These BLA.ac neuronal connections potentially suggest a role in context-induced reinstatement of drug seeking behavior (Fig. 6g). Reexposure to the environment in which drugs are self-administered facilitates relapse following abstinence. This contextually promoted vulnerability is modeled in laboratory animals using context-induced reinstatement. The BLA, medial shell ACB, SUBv, CAIV, CA3, PVT, and PL/JILA are contributors to the circuitry that mediates contextual reinstatement.
Fig. 6 BLAa-thalamic connections and BLAa functional diagrams. a Bar chart showing proportion of back-labeled thalamic neurons from representative retrograde tracer injections in BLA.am, BLA.al, and BLA.ac (n = 1 each). ROIs for grids with strongest labels from each injection is included. A grid can include multiple ROIs [e.g., (PT, PVT)]. Note the greater proportion of thalamic PVT labeling from BLA.ac injection at rostral levels (ARA 57, 61) versus the larger proportion of thalamic PVT label from BLA.am and BLA.al injections in caudal levels (ARA 73), which substantiates the rostral and caudal PVT distinction. Also, greatest thalamic input to BLA.am is from caudal PVT (caudal PVT → BLA.am), greatest input to BLA.al is from caudal PVT, PF, and IMD (caudal PVT/PF/IMD → BLA.al), and greatest input to BLA.ac is from PT and rostral PVT (PT/rostral PVT → BLA.ac). ** denotes connections that were not validated with anterograde tracing.
b Retrograde maps from ARA 57–75 showing back-labeled thalamic nuclei from injections in BLA.am, BLA.al, and BLA.ac that corroborate the bar chart in (a). c PHAL injection in rostral PVT validates the rostral PVT → BLA.ac connection. d PHAL injection in PT validates the PT → BLA.ac connection, but also shows strong PT projections to LA and BMAp (PT → BMAp/LA). e BLA.am connections with areas associated with visual processing. f BLA.al connections with areas associated with gustatory/orofacial information processing. g BLA.ac connections with areas involved in reinstatement of drug seeking behavior. h BLAv connections with visceral/gustatory information processing areas. See Table 1 for full list of abbreviations.
Anterograde maps showing BLAp and BMAp neuron projections to BST. BLAp neurons target lateral parts of BST, while those in BMAp target medial BST. BLA neurons show increased activity during extinction (Fig. 6h). There is a striking similarity between BLAp projection targets and those of the thalamic IM, which is grouped into the dorsal midline thalamic nuclei speculated to be involved in visceros-limbic functions. These shared targets include the AI, ventral medial parts of intermediate CP, ventral parts of caudal CP, and a distinct ENTI(V) region. The ventral intermediate CP is a region where inputs from AI, GU, VISc, and somatomotor mouth regions converge. The ventral caudal CP receives densest input from VISC, but also from GU, and from thalamic CM, a region known for its dense connections with GU and VISC. Other candidates for this hypothesis would include the BLAp and BMAp.

Specifically, interactions between BLA and ACB medial shell, PL/ILA, and PTv are implicated. Here we show that ILA fibers innervate BLA projection neurons that target CA3, providing a functional circuit among these regions. Importantly, similar to the ACB medial shell, OT medial, and not OT lateral, is important for reinforcing effects of psychostimulants. The potential contribution of BLA neurons to the functional circuit among these regions may be important for fear extinction. This emotional conditioning and its resistance to extinction mimic the persistent pathological fears that manifest in post-traumatic stress disorder.

Methods

Subjects. Data from 8-week old male (n = 183) and female (n = 8) C57BL/6 mice (Jackson Laboratories) were used to trace the 245 pathways reported in this work. Animals were housed in pairs at 21–22 °C, humidity (51%), and light controlled (12 hr light/12 hr dark cycle) room. The mice were allowed at least 1 week to adapt to their living conditions prior to stereotoxic surgeries for the delivery of tracers. Subjects had ad libitum access to tap water and mouse chow.
throughout the experiments. All experiments were conducted according to the regulatory standards set by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and by the institutional guidelines set by the Institutional Animal Care and Use Committee at USC.

**Surgical methods.** Anterograde and retrograde tracers were delivered to anatomically delineated regions across the brain to assess their connectivity patterns. Stereotaxic surgeries for tracer infusions were performed under isoflurane anesthesia (Hospira, Inc.). Mice were initially anesthetized in an induction chamber primed with isoflurane and were subsequently mounted to the stereotaxic apparatus where they were maintained under anesthetic state via a vaporizer (Datex-Ohmeda). The isoflurane was vaporized and mixed with oxygen (0.5 L/min) and nitrogen (1 L/min). The percent of isoflurane in the gas mixture was maintained between 2 and 2.5. Tracers were delivered iontophoretically using glass micro-pipettes whose outside tip diameters measured ~10–30 µm. A positive 5 µAmp, 7-second alternating injection current was delivered for 10 min (Stoelting Co.).

**Tracing strategies.** Anterograde tracers included *Phaseolus vulgaris* leucoagglutinin (PHAL; 2.5%; Vector Laboratories) and adeno-associated viruses encoding enhanced green fluorescent protein (AAV GFP; AAV1-hSyn-EGFP-WPRE; 2.3 × 10^13 GC/mL; Addgene #105539) or tdTomato (AAV RFP; AAV1-CAG-tTomato-WPRE; 2.0 × 10^13 GC/mL; Addgene #105554). Retrograde tracers included cholera toxin subunit b conjugates 647, 555, and 488 (CTb; AlexaFluor conjugates, 0.25%; Invitrogen), Fluorogold (FG; 1%; Fluorochrome, LLC), and AAVretro-hSyn-Cre-WPRE (AAV retro Cre; 1.6 × 10^13 GC/ml; Addgene #105553). Anterograde and retrograde tracers were injected either in combination (e.g., co-injection of PHAL with CTb 647) or individually in a triple anterograde (PHAL, AAV-GFP, AAV-RFP) or a quadruple retrograde (CTb 647, CTb 555, CTb 488, or FG, AAV retro Cre) injection design.

For morphological information from BLAa domain specific projection neurons, G-deleted rabies virus injections (RVΔG-4tdTomato and RVΔG-4eGFP) were made into downstream targets of the domains. Cloning of pRVΔG-4tdTomato (Addgene #52500) and pRVΔG-4eGFP (Addgene #52487) has been described.
Fig. 8 LA connections, flatmaps of BLAa anterograde tracing, and BLAa functional recordings. a Summarized brain-wide connections of LA (ventromedial) neurons. For full list of abbreviations see Table 1. b Anterograde maps in top panels show weak projections from LA neurons to hippocampal regions S2Vb and CA1v. Ellipse denotes location of CTb injection, which validates these sparse connections. c Retrograde map shows labeled cells in auditory cortical areas (AUD) following a LA retrograde tracer injection. Ellipse denotes location of PHAL injection in the primary auditory cortical area (AUDp), which shows strong anterograde label in LA validating AUD→LA connections. d LA receives strong input from ventromedial hypothalamic nucleus (VMH), as shown by the retrograde map. An AAV-RFP injection in VMH validates the VMH→LA connection showing strong label in LA, but also in BMAd (VMH→BMAd). The outputs of BLA.alm (e), BLA.al (f), and BLA.ac (g) domains are represented at the macroscale level (gray matter region resolution) on a participant’s flattened brain map (25). The strength values of detected connections were binned into tertiles, and these are represented qualitatively as strongly (maroon), moderate (red), weak (pink), and none (gray). h Longitudinal half of the entire CN5 flatmap showing orientation and major brain divisions. The key to the color codes for connection strength is also shown. i-q Functional characterization of synaptic innervation of projection-defined BLAa neurons. i, o Schematic drawings of experimental design and proposed circuitry. AAV-hSyn-ChR2-YFP (ChR2) is injected into PL (i), AID (o), or ILA (p) to label projection axons in BLA.alm, BLA.al, and BLA.ac, respectively. Red retrobeads are injected into CP caudal dorsomedial (CPdm), CP caudal ventral (CPcv), or CA3 to back-label projection neurons in BLA.alm, BLA.al, or BLA.ac, respectively. Recordings are made from BLAa neurons labeled with red retrobeads while ChR2 labeled axons in BLA are stimulated. j-m p LED pulse (5 ms)-evoked averaged response traces of an example neuron recorded at −70 mV and +10 mV. Recordings were made in the presence of TTX and 4-AP to block polysynaptic inputs so that only monosynaptic excitatory responses are elicited. Average (± standard deviation) peak amplitude and latency of light pulse evoked EPSCs in red retrobead labeled BLA.alm (k, T/12 recorded neurons), BLA.al (n, 7/8 recorded neurons), and BLA.ac (q, T/12 recorded neurons) neuron populations. Source data are provided as a Source Data file. r Left: excitatory and inhibitory responses (superimposed traces) evoked by blue light stimulation (5 ms). Right, responses from the same recorded cell in the presence of TTX and 4AP to show blockade of IPSPs.

Production of B19G-enveloped rabies virus was done as described previously but using helper plasmids pCAG-B19N (Addgene #59924), pCAG-B19P (Addgene #59923), pCAG-IR (Addgene #59922), and pCAG-T7-pol (Addgene #59926) for the rescue step (29). The infectious units/ml for RV were adjusted to the correct ROI location. Further, oversaturation at the injection site prevents the detection of labeled fibers and boutons at the injection sites (BLAa) and their surrounding areas (LA, CEa). Therefore, with this injection strategy, it is difficult to assess intra-amygdalar connections especially within the BLA complex and the CEa. Due to their proximity to the injection sites, these areas appear as dense label in the threshold output subsequently affecting annotation and analysis. To obviate this, these areas of potentially false dense label at or surrounding the injection sites (specifically the BLA complex and CEa) were filtered out at the thresholding stage for all BLAa cases.

Intra-amygdalar connections were excluded from the analyses to reduce the challenge to accurately distinguish labels from background in nuclei located close to injection sites. Generally, only connections that were validated are reported. For certain computer-generated visualizations (e.g., bar charts, matrices), non-validated connections were reported. Such cases are clearly indicated on the visualizations. In addition, sometimes tracers produce very weak labeling in ROIs, which render interpretation of the data difficult. For example, tracers will label only a few (~2–3) axon terminals or cells within an ROI. Such connections are not reported.

Reproducibility of data. For most figures, labels from representative cases are presented. However, injections in all BLA nuclei were injected. The following are sample sizes for anterograde tracer injections: BLA.alm (n = 8), BLA.al (n = 6), BLA.ac (n = 7), BLAvc (n = 2), BLAp (n = 2), BMAd (n = 2), and LA (n = 2). For retrograde tracer injections, repeated sets of injections were made for each BLA nucleus. The consistency of labeling across repeated cases is clear from manual analysis of the data (Supplementary Fig. 4b). The label similarity also is evidenced in the output of the 2D hierarchical clustering analysis (described below), which groups injection sites based on the commonality of their projection targets. As can be seen in Supplementary Fig. 6a, BLA.alm injection groups together while BLA.alm injections form an individual group. This is in contrast to the different anterograde tracers used across the experiments (PHAL or AAV). As additional confirmation of reproducibility, BLA.alm, BLA.ac, and BLA.ac anterograde injections were replicated in female mice. Supplementary Fig. 15a–c shows the similarity in brain-wide label patterns from the same BLAa domain in males and females.

Tracer label validation. Importantly, injections reported for the BLAa domains are not entirely confined. For instance, one BLA.alm AAV-RFP injection encroached into the AAm domain (Supplementary Fig. 4a). Consequently, although the BLA.alm AAV-RFP injection largely represents BLAa projections, it also shows output more specific to BLA.alm and BLAp. Similarly, the BLA.al FG injection spread into the LA and BLA.alm showing labeling patterns of mostly BLA.al, but also of BLA.alm and LA. Tracer spread is expected given the small size of the domains and their close proximity to one another. In fact, infusion spread across ROIs is a valid and pervasive concern for all neuroanatomical studies. Several measures were taken to mitigate this. First, injection sites that were mostly confined...
to a single domain were selected for analysis so that the label would primarily represent connections of the target domain both in terms of presence and intensity of label. This strategy, combined with the grouping of the three domains for community detection analysis, which assigned grids to injection sites with the greatest pixel intensity value, helped to visualize labeling originating most likely from the target domain. Second, each of the injections was repeated in at least two cases for verification purposes. Third, only tracing data that was validated is reported. Anterograde labels were validated with retrograde tracers, while retrograde labels were validated with anterograde tracers. In the aforementioned BLA.al FG injection, back-labeled cells were observed in rostral ACAd. However, an AAV injection in the ACAd primarily labeled the BLA.am, suggesting that the ACAd labels were from tracer spread into the BLA.am (Supplementary Fig. 4d). In the case of the BLA.al AAV-RFP injection, the MDm and PL were labeled. However, retrograde injections placed in these two regions showed that the MDm label most likely originated from BLAp, while the PL label most likely resulted from tracer spread into the BLA.am (Supplementary Fig. 4e). Further, the PL label from the BLA.al was most evident in the analysis in which this domain was paired with the BLAp. However, once the BLA.al was grouped with the BLA.am and BLA.ac for
community detection analysis, projections to the PL got assigned to injection sites in the BLA.am or BLA.ac since injections in these two domains resulted in far denser labels in the PL than the BLA.al injection (Supplementary Fig. 4e).

**BLA injection site analysis.** BLA injection sites were rescanned under lower exposure parameters to acquire a more accurate assessment of their size and location. Sections containing the injections for each case were re-registered to ARA levels that best corresponded to the target BLA complex structures. For injections that spanned more than a single section, each section was registered and analyzed. An injection site annotation algorithm (detailed below) was run on the sections to identify injection location. The annotation was run atop a custom ARA atlas that contained the manually delineated boundaries of the BLAa domains (Supplementary Fig. 4a, c).

**Injection site annotation.** Correct annotation of the spatial location of injection site is critical in interpreting neuroanatomical data. In both anterograde and retrograde tracing experiments, the injection sites are typically surrounded by irregularly shaped, very high intensity background pixels. These high intensity background regions yield little useful connectivity information and tend to skew the overall connectivity quantification results as well as interfering with injection site annotation. To quantify the injection sites robustly and consistently, we employ a combination of multi-scale wavelet decomposition, non-linear adaptive intensity adjustment and maximally stable external region (MSER) detection.

Wavelet decomposition encodes both frequency and spatial information of the input data by successive application of high and low pass filters. Given a 2D image $f(x, y)$, its discrete wavelet decomposition of level $L$ is as following:

$$
\hat{f}(x, y) = \sum_{i,j} C_{ij}^{L} \cdot \phi_{ij}(x, y) + \sum_{l=1}^{2(L-1)} \sum_{i,j} C_{ij}^{l} \cdot \psi_{ij}^{l}(x, y)
$$

Where $C_{ij}^{L}$ is the level $l$ coefficients for the low pass band, and $C_{ij}^{1}, C_{ij}^{2}, C_{ij}^{3}$ are the scale level coefficients for horizontal, vertical and diagonal detail bands ($\Phi$ and $\Psi$ are the scaling and wavelet functions). For a brain section image $f_{\text{sec}}(x, y)$ containing an injection site, we decompose the image into $L$ levels and remove the details from levels 1, 2 and 3. At levels 4 and 5, diagonal detail coefficients with large magnitudes are amplified, while horizontal and vertical coefficients with small magnitudes are dampened. Level 5 low pass band coefficients are also dampened and further smoothered with a gaussian kernel. The reverse wavelet transformation of the modified coefficients $C_{ij}^{L}, C_{ij}^{1}, C_{ij}^{2}$ yields a reconstruction of the image $f_{\text{sec}}(x, y)$ with much of the background intensity in the injection site removed:

$$
\hat{f}_{\text{sec}}(x, y) = \sum_{i,j} C_{ij}^{L} \cdot \phi_{ij}(x, y) + \sum_{l=1}^{2(L-1)} \sum_{i,j} C_{ij}^{l} \cdot \psi_{ij}^{l}(x, y)
$$

The contrast of the reconstructed image $f_{\text{sec}}(x, y)$ is enhanced using the local adaptive mapping described in. For a normalized image $f(x, y) \in [0,1]$, an initial intensity mapping $T$ is defined as

$$
T(f(x, y), p) = \sin^{-1} \left(\frac{p}{2} f(x, y) \right), p > 0
$$

Using the first order Taylor expansion approximation of $\sin(a)$, the mapping is rewritten as

$$
T(f(x, y), c) = \frac{n^2}{4} (f(x, y))^2, c = 2p
$$

The mapping argument $c$ is defined as $c = c_1 \cdot \frac{f(x, y)}{(l_1 - f(x, y)/l_2)} + c_2$, where $f_1(x, y) = f(x, y) - g(x, y)$ denotes the convolution between $f(x, y)$ and a gaussian kernel $g(x, y)$, while $c_1, c_2$ are user specified values. Locally adaptive contrast enhancement is achieved as following:

$$
E(x, y) = f(x, y) T( f(x, y), c_1 ) + \frac{f(x, y)}{\Delta c} \left( f(x, y) - f_1(x, y) \right)
$$

The contrast enhancement further suppresses background pixels at the injection site. Finally, the injection site is extracted as a blob by MSER from E$(f(x, y))$, the result of applying wavelet filtering and adaptive contrast enhancement to the injection site image.

**BLAa boundary demarcation via machine learning.** Despite sharing many common input and output pathways, the medial (BLA.am), lateral (BLA.al), and caudal (BLA.ac) parts of BLAa each has discerning connections with different regions of the brain. These domain-specific connections were used to compute the boundaries between BLAa domains.

We first examined a collection of coronal sections containing BLAa to identify a subset of sections $S$ where division specific connections can be observed. Each section $s \in S$ was then associated with a division label $y = \text{medial, lateral, caudal}$, and registered to the Allen Reference Atlas (ARA). Due to the large z dimension sampling gap of 50 µm between adjacent ARA levels, the BLAa division boundary is computed individually for each ARA level. For any given level $l$, let $S_l$ be the subset of sections in $S$ that best matches with ARA at level $l$. Tracer signal foreground pixels were segmented for each section in $S_l$ to obtain a set of coordinates $D_l = \{(x_1^{l1}, y_1^{l1}), (x_2^{l1}, y_2^{l1})\}$. From (x1, x2) pairs inherit the section division label $y$. We used $D_l = \{(x_1, x_2)\}$ and its corresponding division labels $y$ to train an ensemble of RBF kernel support vector machines (SVM). The ensemble then determines BLAa subdivisions by assigning a division label to all spatial locations within BLAa.

A SVM classifier solves the following optimization problem:

$$
\min_{w \in \mathbb{R}^2} \frac{1}{2} w^T W w + C \sum_{i=1}^{n} \xi_i
$$

s.t. $y_i (w^T \phi(x_i) + b) \geq 1 - \xi_i$

$$
\xi_i \geq 0
$$

The classifier has two parameters: the soft margin parameter $C$ and the kernel (radial basis function) $\gamma$ parameter $\gamma$. A total of 64 classifiers were trained with $C$ and $\gamma$ and take values on a grid of powers of 2. For a classifier SVMl, a model accuracy $a_l$ is computed as the average accuracy from 10 threefold stratified cross-validation training and testing. Given any point $x = (x_1, x_2)$ within BLAa at level $l$, denote the division prediction by SVM, as $y(x)$. The BLAa division of $x$ at level $l$ is determined by a weighted averaging ensemble:

$$
E(x) = \arg\max_{y \in \mathbb{R}} \sum_{l} \cdot I(y(x) = y)
$$

Dense classification across BLAa with $E$ produces the optimal BLAa divisions based on division specific anatomical pathways.
Statistical analysis of BLAa projections. Quantitative comparisons of projection labels from BLAa domains were performed to supplement and validate the qualitative observations. Repeated anterograde tracer injections were made in each of BLAa domain (BLAa.m = 8; BLAa.al = 6; BLAa.ac = 7). Analysis was limited to 12 ARA levels (versus 32 levels) and to select ROIs (ACA, PL, ILA, DP, TTD, MOs, CA1, CA3, PAR, Subd, Subv). All the data was processed through Connection Lens (Fig. 2a) and the annotated data was subjected to statistical analyses. Two-sided paired Wilcoxon signed tests were performed, and the parameters that survived FDR correction for multiple testing with p values < 0.05 are reported and visualized in whisker plots (Supplementary Fig. S5–m). The quantitative results agreed with the qualitative analyses. Significant differences in projection densities were found in PL2 (BLAa.m vs BLAa.l, W = 47, p = 0.001, [0.006, 0.003]; BLAa.m vs BLAa.c, W = 4, p = 0.003, [−0.06, −0.001]; BLAa.l vs BLAa.c, W = 0, p = 0.001, [−0.008, −0.002]), ILA 37/39 (BLAa.m vs BLAa.l, W = 7, p = 0.02, [−0.003, −0.0005]; BLAa.m vs BLAa.c, W = 4, p = 0.003, [−0.002, −0.0005]), ILA 41/45 (BLAa.m vs BLAa.c, W = 0, p = 0.0003, [−0.006, −0.0015]; BLAa.l vs BLAa.c, W = 0, p = 0.0004, [−0.0006, −0.0005]). CA3 (BLAa.m vs BLAa.l, W = 1, p = 0.0025, [−0.0002, −0.0001]; BLAa.m vs BLAa.c, W = 0, p = 0.001, [−0.001, −0.0004]; BLAa.l vs BLAa.c, W = 0, p = 0.001, [−0.001, −0.0003]), ACAd (BLAa.m vs BLAa.l, W = 46, p = 0.002, [0.0003, 0.002]; BLAa.m vs BLAa.c, W = 44, p = 0.007, [0.0006, 0.003]; BLAa.m vs BLAa.c, W = 55, p = 0.0006, [0.001, 0.004]; BLAa.l vs BLAa.c, W = 37, p = 0.002, [0.0003, 0.001]), DP (BLAa.m vs BLAa.l, W = 0, p = 0.0006, [−0.001, −0.003]; BLAa.m vs BLAa.c, W = 42, p = 0.001, [0.0004, 0.001]), TD (BLAa.m vs BLAa.c, W = 5, p = 0.012, [−0.006, −0.0001]; BLAa.l vs BLAa.c, W = 42, p = 0.001, [0.0001, 0.0099]), CA1 (BLAa.m vs BLAa.c, W = 8, p = 0.04, [−0.004, −4.4e−6]; BLAa.m vs BLAa.c, W = 0, p = 0.0003, [−0.0002, −0.0001]), LGA.p (BLAa.m vs BLAa.c, W = 0, p = 0.0003, [−0.007, −0.003]; BLAa.m vs BLAa.c, W = 0, p = 0.001, [−0.007, −0.002]), and Subd (BLAa.m vs BLAa.c, W = 4, p = 0.003, [−0.0003, −4.7e−5]; BLAa.l vs BLAa.c, W = 6, p = 0.03, [−4.2e−4, −3.6e−5]).

Generation of 2D hierarchical clustering of anterograde projection data. To assess injection site reproducibility, repeated anterograde injections in BLAa.m (n = 8), BLAa.l (n = 6), and BLAa.c (n = 7) were used. For each case, the number of segmented anterograde projection pixels to each target region were compiled for each section. The pixel counts for regions spanning multiple ARA levels were summed for each case to form a projection vector with dimension equal to number of injection pixels and domain vectors that did not contribute to the consensus algorithm were discarded. The cosine distance metric was used during linkage. Row labels show the injection site and tracer used for each case. Column labels show name of the target region. Only regions receiving rich projections are shown in the plot for legibility. In our own analysis we included all target regions and obtained an identical row clustering outcome. We used scikit-learn for SVM implementation, but those close to the BLA.a or BLA.ac border were excluded. In addition, domain-specific neurons in deeper parts of the tissue (Z axis) farthest away from the edges of the sectioned tissue were selected for reconstruction. To assist in the accurate selection of the projection intensity threshold for each injection case, we also traced projections of 25 µm were created that aided in the identification of the BLAa domain boundaries.

3D tissue processing. To assess whether projection neurons located in BLAa.m, BLAa.l, and BLAa.c were morphologically distinct, representative neurons in each of the domains were labeled via a RVAG injection in the caudal dorsomedial caudoputamen (for BLAa.m), caudal ventral caudoputamen (for BLAa.l), and in the medial accumbens (for BLAa.c) (Fig. 1d). One week was allowed for tracer transport following injections, after which the animals were perfused. A 3D tissue processing workflow was followed for implementation of the SHIELD clearing protocol. Mice were perfused with ice-cold saline and SHIELD perfusion solution. The brains were extracted and incubated in the SHIELD perfusion solution at 4 °C for 48 h. The SHIELD perfusion solution was replaced with the SHIELD OFF solution and tissues were incubated at 4 °C for 24 h. The SHIELD OFF solution was replaced with the SHIELD ON solution and the tissues were incubated at 37 °C for 24 h. The whole brain was cut into 250 µm sections (for BLAa.m and BLAa.l) or 400 (for BLAa.c) µm sections and were cleared in the SDS buffer at 37 °C for 72 h. The sections were then washed three times with KPBS and incubated in KPBS at 4 °C for 24 h.

3D imaging protocol. Sections were mounted and coverslipped onto 25 × 75 × 1 mm glass slides with an index matching solution 100% (Easyindex, LifeCanvas Technologies, #EI-Z1001). Sections were imaged with a high speed spinning disk confocal microscope (Andor Dragonfly 202 Imaging System, Andor Oxford Instruments Company, CR-DFLY 202-2540). 10x magnification (NA 0.40, Olympus, UPLXAP010X) was used to acquire an overview after which 30x magnification (NA 1.05, Olympus, UPLSAPO30xIR) was used to image through the BLA ipsilateral to the injection site at ± 1 µm z steps. The BLA contralateral to the injection site was also imaged for the BLAa case.

3D reconstructions, visualization, and analysis of neuron morphology. Manual reconstruction of the neurons was performed using Aivia (version 8.5, DRVision) (Fig. 9d), and geometric processing of neuron models was performed using the Quantitative Imaging Toolkit (QIT) (also available at http://caibane.io/qitwiki). Although Cpc.d.m projecting BLAa neurons primarily are located in BLAa.m and BLAa.c as shown in the Cpc.d.m label mostly BLAa.m, but also some BLAa.l and BLAa.c neurons. To ensure the traced neurons in fact solely originated from their respective domains, for all cases, labeled cells visually grouped in the center of each domain (X-Y axes) were traced. Neurons located within the BLAa.m were identified for inclusion in the analysis, but those close to the BLAa.l or BLAa.c border were excluded. Similarly, neurons within BLAa.l and BLAa.c were identified for inclusion, but those close to the border of the BLAap were omitted. In addition, domain-specific neurons in deeper parts of the tissue (Z axis) farthest away from the sectioned tissue were selected for reconstruction. To assist in the accurate selection of the projection intensity threshold for each injection case, we also traced projections of 25 µm were created that aided in the identification of the BLAa domain boundaries. In total, 8 neurons were traced for the BLAa.m, 9 for the BLAa.l, and 6 for the BLAa.c.

Reconstructions of individual neurons poses a challenge due to dense labeling in the BLAa.m and BLAa.l regions (Fig. 1d). All labeled neurons in the BLAa.m contralateral to the injection site were also traced to show the details of dendritic morphology that could be captured and reconstructed with RVAG injections (n = 3; Fig. 9c) (Supplementary Movies 4–5). To mitigate the issue of dense labeling, but also to account for differences in slice thickness (250 µm for BLAa.m and BLAa.l and 400 µm for BLAa.c), we restricted our morphological analysis to those that were sufficiently close to the soma. We accomplished this by applying the NeuronTransform module in QIT to trim the contiguous portion of neurons that measured farther than 300 nm away from the center of the soma using the Euclidean distance (Supplementary Fig. 1g). With this distance, we were confident in our ability to accurately trace dendrites, regardless of neuron density. Due to the curvature of the dendrite, we applied a local regression filter to address the aliasing artifact and to regularize dendritic tortuosity. Specifically, the NeuronFilter module
in QIT was used to apply a locally weighted scatter-plot smoother (LOESS), which is a low bias approach that makes minimal assumptions (Supplementary Fig. 1h)72. The results were visualized in a matrix plot created using R (Fig. 9g, h). Similar to the PCA analysis, the data showed that groups of neurons in BLA_am, BLA_al, and BLA_ac all differed from one another, but the greatest differences were between BLA_am/BLA_al neurons compared to those in BLA_ac (Fig. 9b).

### Data availability

Raw data for amygdala cases used in the paper can be accessed through our Mouse Connectome Project website (https://mouseconnectomeproject.github.io/amygdalar/iconnectome). The color-coding visualized output of the community assignments for all injections are available (https://mouseconnectomeproject.github.io/amygdalar/), as well as the BLA global wiring diagram (https://mouseconnectomeproject.github.io/amygdalar/wiringdiagram). Reconstructions of BLA neuronal are cataloged in NeuroMorpho.org. SWF files and associated metadata for neuron reconstructions can be downloaded here: neuromorpho.org/datableFiles/dongSupplementary/Dong2020BLA.zip. Source data are provided with this paper.

### Code availability

An in-house software was used to process all image data presented in the manuscript. Connection Lens was specifically applied to register (warp), threshold (segment), and annotate the labels in all image data. This software has not been publicly released yet. However, custom code developed for data analysis is available at https://github.com/ibowman/BLA. For the Louvain algorithm implementation, the Brain Connectivity Toolbox (BCT) was employed, which is freely available at https://sites.google.com/site/bctnet/. The grid communities algorithm for matrix visualizations are available here at https://github.com/aestrivex/bctpy, https://sites.google.com/site/bctnet/. Geometric processing of neuron models was performed using the Quantitative Imaging Toolkit (QIT), available at http://caibeni.io/quitki. We also computed persistence diagrams using neuronTools (https://github.com/Nevermore520/NeuronTools) and then computed inter-neuron distances using the Wasserstein metric (https://bitbucket.org/grey_narn/geom_matching/src). We used scikit-learn for SVM implementation, and computed inter-neuron distances using the Wasserstein metric (https://bitbucket.org/grey_narn/geom_matching/src). We used scikit-learn for SVM implementation, and computed inter-neuron distances using the Wasserstein metric (https://bitbucket.org/grey_narn/geom_matching/src).

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Author contributions
H.H. planned and supervised the project, designed all the experiments, conducted manual analysis of data, wrote the manuscript, and prepared the figures and tables. H.-W.D. planned and supervised the project, and also edited the manuscript. I.B. led the informatics effort including execution of community detection analyses and generation of accompanying matrices. L. Garcia contributed to the generation of bar graphs and K. R.C. provided continuous support of Connection Lens software used for post-image processing and annotation of all data. A.J.T. aided with the wiring diagram. S.Y. provided support for all online platforms that host the data including creation of the web application that displays the anterograde and retrograde connectivity maps. D.L.J., M.F., D.L., S.A.U., and T.B. registered, thresholded, and annotated the connectivity data. M.Z. developed code for injection site annotation, for 2D hierarchical clustering of injections, and for the machine learning algorithm to automate detection of BLAa boundaries. J.S. created 3D videos of the BLAa neurons. B.P., L.I.Z., Z.-G.Z., and P.G. planned and executed optogenetic-assisted recording studies. N.K. organized and performed all SHIELD experiments. L.K. reconstructed neurons, ran quantitative analyses on morphometrics, generated the accompanying whisker plots, and aided with the associated text and figure panels. L.K. also analyzed connectivity data and generated the accompanying whisker plots. R.P.C. provided code to support 3D reconstruction of neurons and for attainment of morphometric data for analysis. L. Gou, L. Gao, C.C., S.A., and M. B. performed stereotaxic surgeries and subsequent histological processing and imaging of data. J.D.H. created the flatmaps for figures and contributed to manuscript edits. G.A.A. guided the analysis of morphometric data, the interpretations associated with their output, and contributed to the associated text. I.R.W. generated and provided rabies viruses used to label reconstructed neurons and contributed to the associated text and figure panels. M.S.F. contributed to interpretation of experiments related to emotional learning. N.N.F. provided input for experimental design and suggestions for text. M.S.B., N.L.B., and M.Y.S. offered constructive guidance for experiments and manuscript edits.

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The authors declare no competing interests.

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