

Figure S1. Expression of genetic markers in the CeA, Related to Figure 1

(A-B) Expression of *Calcrl*, *Htr2a*, *Nts*, *Prkcd*, *Sst*, and *Tac2* in the CeA (A). Quantification of *Calcrl*, *Htr2a*, *Nts*, *Prkcd*, *Sst*, and *Tac2* smFISH expression in the CeC, CeL, anterior CeM (aCeM) and posterior CeM (pCeM) (A). Genes with no obvious expression in a CeA subdivision was not quantified for that subdivision. Boundaries for the CeC, CeL, aCeM, and pCeM depicted in cartoon (B). Representative histology of expression of gene markers in the CeA (B). Values represent mean \pm s.e.m of percentage of labeling out of total number of cells using the nuclear marker DAPI from n = 3 mice (A). Anterior-posterior (AP) distance from bregma (mm), scale bar, 250 µm. Astr, amygdala striatal transition zone.

(C) Proportion of CeL $Gad1^+$ neurons that are CeL $Prkcd^+$, CeL Sst^+ , and CeL $Crh^+Nts^+Tac2^+$ neurons and the proportion of CeM $Gad1^+$ neurons that are CeM Nts^+ , CeM Sst^+ , and CeM $Tac2^+$ neurons. Prkcd, Sst, and Tac2 label 96% of CeL $Gad1^+$ neurons, while 100% of CeL Prkcd, Sst, and Tac2 neurons are $Gad1^+$. Nts, Sst, and Tac2 label 94% of CeM $Gad1^+$ neurons, while 100% of CeM Nts, Sst, and Tac2 neurons are $Gad1^+$. Nts, Sst, and Tac2 label 94% of CeM $Gad1^+$ neurons, while 100% of CeM Nts, Sst, and Tac2 neurons are $Gad1^+$. Representative histology of Prkcd (red), Sst (red), Tac2 (red), and Gad1 (Green) in the CeL and Nts (red), Sst (red), Tac2 (red), and Gad1 (green) in the CeM (lower panel). Values represent mean \pm s.e.m from n = 3 mice (A). Scale bar, 125 µm (B).

(D) *Htr2a* colabelling with *Crh*, *Prkcd*, *Sst*, *Nts*, and *Tac2* in the CeL and/or CeM. White circles circles represent $Htr2a^+$ double labelled with corresponding genetic marker. Scale bar, 50 µm.



Figure S2. Genetic targeting of distinct CeA neurons, Related to Figure 2

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(A) Represented histology of viral targeting of distinct CeA neurons. The expression of ChR2 is shown across the anterior-posterior axis of the CeA using 100nL of a Cre-dependent ChR2 containing viral vector for targeting distinct CeA nuclei in Cre-expressing mice. Scale bar, 250 µm.

(B) Example expression of magnified micrograph of ChR2 (green) expression in the CeM of CeM Sst-ChR2 mice using confocal microscopy. White arrow, ChR2-expressing neurons. Scale bar, 50 µm.

(C) The values represent the mean \pm s.e.m of percentage of accurately targeted ChR2-expressing neurons amongst all ChR2-expressing neurons in adjacent or non-targeted sites. Cell counts are from 2 sections (anterior and posterior), sample sizes found in Figure 2.

(D) Cre⁻ animals underwent optogenetic stimulation experiments. Virus and fiber implant was targeted in Cre⁻ mice into the CeC (*Prkcd*-Cre⁻ mice) (n = 5) (top), CeL (*Sst*-Cre⁻ mice) (n = 6) (middle), and CeM (*Tac2*-Cre⁻ mice) (n = 5) (bottom) and underwent identical behavioral assay as Figure 2. With (ON) and without (OFF) photostimulation. Paired *t*-*test*, all comparisons between ON versus OFF resulted in no significant differences, P > .05.



Figure S3. Fos expression in genetically defined CeA neurons, Related to Figure 3

(A) Representative histology of *Fos* expression in response to several of the stimuli from quantified data in Figure 3. Experimental groups (odd rows) and control groups (even rows).

(B) Quantification of *Fos* expression in CeC *Calcrl*⁺ neurons in response to shock or no shock (B). Values are mean \pm s.e.m. from 2 sections per mouse from n = 3 mice. Significance for unpaired *t-test*, *P< 0.05.

- (C) Representative histology of Fos expression in CeC $Calcrl^+$ neurons in response to Shock or No Shock
- (C). White circles represent Caclrl⁺ neurons double labelled with Fos (C). Scale bar, 50 μ m.



Figure S4. CeM Drd1⁺ neurons are required for feeding and drinking, Related to Figure 4

(A-C) Feeding (A), drinking (B), and freezing (C) behavior in response to inhibition of CeM $Drd1^+$ neurons (n = 8). Significance for paired *t-test*, *P<0.05, ***P<0.001.

(D) Representative histology of eArch3.0 expression in the CeM of *Drd1*-cre mice. Scale bar, 250 µm.

(E-F) Raster plots of feeding (E) and drinking (F) bouts from inhibition of CeM $Drd1^+$ neurons (Cre⁺ mice 9-16) or controls (Cre⁻ mice 1-8). Time bins, 1 s.



Figure S5. Anatomical model of genetically defined CeA neurons, Related to Figure 5

(A) Quantification of retrogradely labelled PAG-projecting CTB^+ neurons with genetic markers in the CeA. Values represent mean \pm s.e.m. the percentage of CTB^+ neurons that the corresponding gene labels from n = 3 mice. No CTB^+ neurons were found in the CeC, thus CTB quantification was not performed for CeC *Prkcd*⁺ neurons.

(B) Representative histology of PAG CTB injection site. Scale bar, 1mm.

(C) Representative histology of CTB⁺ neurons in the CeA with *Prkcd*, *Crh*, *Nts*, *Sst* and *Tac2* expression. Magnified micrographs of the CeL, white circles represent double labelled cells (fourth column). Scale bar 200 μ m (column 1-3), 50 μ m (column 4).

(D) Anterograde fibers from genetically distinct CeA populations. Anterograde fibers in the PAG of several CeA ChR2-eYFP mice are shown. Scale bar, $250 \mu m$.

(E) Helper virus and rabies virus injected into the CeA of Cre⁻ mice, no visible immunofluorescence of helper (eGFP) or rabies virus (mCherry) was found in n = 3 mice.

(F) Targeting of rabies virus in distinct CeA cell populations. The values represent the mean \pm s.e.m of percentage of accurately targeted starter cells (mCherry⁺eGFP⁺) amongst all starter cells. Cell counts are from 2 sections (anterior and posterior) per mice from n = 3 mice.



Figure S6. Intrinsic electrophysiological properties and BLA connectivity of genetically defined CeA neurons, Related to Figure 6

(A-B) Resting membrane resistance of neurons in the CeC, CeL, and CeM (A) and genetically confirmed neurons in the CeC, CeL, and CeM (B). Red points represent neurons recorded from Ppp1r1b-cre mice green points represent neurons recorded from Rspo2-cre mice. Resting membrane resistance of CeC neurons is significantly lower than that of CeL and CeM neurons. Significance for one-way ANOVA with Bonferroni's multiple hypothesis correction, ****P<0.0001.

(C-D) Resting membrane potential of neurons in the CeC, CeL, and CeM (C) and genetically confirmed neurons in the CeC, CeL, and CeM (D). Red points represent neurons recorded from Ppp1r1b-cre mice

green points represent neurons recorded from Rspo2-cre mice. Resting membrane potential of CeC neurons is significantly lower than that of CeL and CeM neurons. Significance for one-way ANOVA with Bonferroni's multiple hypothesis correction, ****P<0.0001.

(E) qPCR traces for confirming genetic identity of CeA neurons. Single cell qPCR traces for *Prkcd* from CeC neurons. Single cell qPCR traces for *Nts*, *Sst*, and *Tac2* from CeM neurons. rfu, relative fluorescence units (units are scaled down 10^6).

(F-G) Latency of connection from BLA $Ppp1r1b^+$ neurons (F) and BLA $Rspo2^+$ neurons (G) to CeC, CeL, and CeM neurons.

(H-I) Latency of excitatory (*E*) and inhibitory (*I*) responses from BLA $Ppp1r1b^+$ (H) and BLA $Rspo2^+$ (I) in the CeC, CeL, and CeM.

(J-K) The type of connectivity from BLA to genetically defined populations of CeA neurons. The number and type of genetically confirmed neuron sorted by the type of response from BLA $Ppp1r1b^+$ (J) and BLA $Rspo2^+$ (K) neurons. Each circle represents a single genetically confirmed neuron.



Figure S7. Expression of striatal markers in the CeA, Related to Figure 7

(A-B) Expression of *Drd1*, *Drd2*, *Pdyn*, *Penk*, and *Tac1* in the CeA. Quantification of *Drd1*, *Drd2*, *Pdyn*, *Penk*, and *Tac1* smFISH expression in the CeC, CeL, anterior CeM (aCeM) and posterior CeM (pCeM)

(A). Genes with no obvious expression in a CeA subdivision was not quantified for that subdivision. Boundaries for the CeC, CeL, aCeM, and pCeM depicted in cartoon (B). Representative histology of expression of gene markers in the CeA (B). Values represent mean \pm s.e.m of percentage of labeling out of total number of cells using the nuclear marker DAPI from n = 3 mice (A). Anterior-posterior (AP) distance from bregma (mm), scale bar, 250 µm (B). Astr, amygdala striatal transition zone.

(C-D) Representative histology of double labelling of striatal markers in the CeA. Magnified micrographs of *Drd1* expression with *Drd2*, *Pdyn*, *Penk*, and *Tac1* (D). Circles represent double labelled neurons (D). Anterior-posterior (AP) distance from bregma (mm), scale bar, 250 μ m (C), 50 μ m (D).

(E) Proportion of CeL $Drd1^+$ neurons that are CeM Nts^+ , CeM Sst^+ , and CeM $Tac2^+$ neurons. Nts, Sst, and Tac2 label 95% of CeM $Drd1^+$ neurons, while 97% of CeM Nts, Sst, and Tac2 neurons are $Drd1^+$. Quantification from 2 sections per mouse from n = 3 mice.