A diverse network of pericoerulear neurons control arousal states

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Abstract: As the primary source of norepinephrine (NE) in the brain, the locus coeruleus (LC) regulates both arousal and stress responses[1,2]. However, how local neuromodulatory inputs contribute to LC function remains unresolved. Here we identify a network of transcriptionally and functionally diverse GABAergic neurons in the LC dendritic field that integrate distant inputs and modulate modes of LC firing to control arousal. We define peri-LC anatomy using viral tracing and combine single-cell RNA sequencing and spatial transcriptomics to molecularly define both LC and peri-LC cell types. We identify several cell types which underlie peri-LC functional diversity using a series of complementary approaches in behaving mice. Our findings indicate that LC and peri-LC neurons comprise transcriptionally and functionally heterogenous neuronal populations, alongside anatomically segregated features which coordinate specific influences on

behavioral arousal and avoidance states. Defining the molecular, cellular and functional diversity in the LC provides a road map for understanding the neurobiological basis of arousal alongside hyperarousal-related neuropsychiatric phenotypes.

Main:

The locus coeruleus (LC) is the primary source of norepinephrine (NE) across the central nervous system, and is widely recognized as a critical modulator of arousal states[1,2]. The LC has opposing roles in pain, avoidance and reinforcement learning, exploration, and feeding, which are produced by distinct LC subpopulations with diverse morphology, topographic location, and electrophysiological properties[3–10]. Additionally, the behavioral effect of LC activity depends heavily on its tonic and phasic activity patterns[11,12]. However, it remains unclear how LC ensembles and their activity dynamics are controlled. Long-range neuromodulatory inputs to the region potently modulate the LC, but many do not target LC neurons directly[13–17]. LC dendrites extend medially to form a pericoerulear zone (the peri-LC)[18] that receives inputs from the medial prefrontal cortex (mPFC), dorsomedial and paraventricular hypothalamus, central amygdala, medial preoptic area, and dorsal raphe[13–15]. An uncharacterized population of GABAergic neurons in the peri-LC has been shown to modulate physiological arousal by inhibiting the LC[13,19,20]. We hypothesized that the peri-LC^{GABA} network plays critical and important roles in coordinating LC ensembles and can influence behavioral arousal by integrating distant inputs to modulate tonic and phasic modes of LC firing, thus providing finely tuned control of the LC via GABAergic and local peptidergic interactions.

Here, we report that peri-LC neurons directly inhibit the LC and reveal that peri-LC^{GABA} neurons increase their activity in behaving mice in response to salient stimuli, during exploration, and after feeding. Using a combination of optogenetic and chemogenetic manipulations, we found

that peri-LC^{GABA} circuits potently decrease arousal, while inhibiting peri-LC^{GABA} circuits increases anxiety-like behaviors. Complementary single-nuclei and spatial RNA transcriptomics uncovered the diverse properties of both peri-LC and LC cell types, and these results informed experiments that found differential firing patterns in neuropeptide-containing peri-LC subpopulations during behavior. Together, our results reveal specialized roles for this group of locally projecting, inhibitory peri-LC neurons in tuning LC-NE activity to regulate arousal and avoidance behaviors.

Peri-LC^{GABA} neurons integrate distant inputs and directly control LC activity

We first examined the anatomical properties of peri-LC^{GABA} neurons in the pericoerulear zone, the area medial to the LC and spanning the length of the LC dendritic field, from approximately 5.3 to 5.8 mm caudal to bregma[18]. Using Cre-inducible viral expression of enhanced yellow fluorescent protein tagged channelrhodopsin-2 (AAV5-DIO-ChR2-eYFP) and immunostaining of tyrosine hydroxylase in cleared tissue of vesicular GABA transporter (vGAT)-Cre mice, we found that peri-LC^{GABA} neurons form a dense web on the ventromedial face of the LC, where they intertwine with LC^{NE} dendrites (**Fig. 1A, Supplemental Video 1**). Notably, these GABAergic projections are almost exclusively local; after examining the whole brains of mice, only a single long-range projection was found outside of the LC region, within the lateral interpeduncular nucleus. Additionally, using monosynaptic rabies tracing onto peri-LC^{GABA} starter cells, we found diverse inputs to peri-LC^{GABA} neurons from distant brain regions including the bed nucleus of the stria terminalis (BNST), the central amygdala (CeA), the lateral hypothalamus (LH) (**Extended Data Fig. 1A**), the medial cerebellar nucleus (Med), and numerous brainstem areas.

Next, we determined whether peri-LC^{GABA} neurons directly modulate LC activity using *ex vivo* patch-clamp electrophysiology. In acute brain slices from vGAT-Cre mice expressing DIO-

ChR2-eYFP in peri-LC^{GABA} neurons (**Fig. 1B**), we recorded from LC^{NE} neurons identified visually and confirmed by the presence of an evoked α_2-adrenergic receptor-mediated inhibitory current (**Extended Data Fig. 1B**), which is a fundamental characteristic of LC^{NE} neurons[21]. Photostimulation of peri-LC^{GABA} neurons and their terminals evoked fast IPSCs (latency 2.0±0.1ms) insensitive to TTX and 4-AP treatment, demonstrating that peri-LC^{GABA} neurons make monosynaptic inhibitory connections onto LC^{NE} neurons (**Fig. 1C-E**). Photostimulation inhibited ~78% of LC^{NE} neurons examined (**Extended Data Fig. 1C**) and was ineffective when ChR2 was expressed lateral to the LC. Moreover, applying DNQX did not affect optically evoked IPSC amplitude in LC^{NE} neurons, and picrotoxin reduced IPSC amplitude by more than half, indicating that the IPSCs are primarily $GABA_A$ receptor-mediated (**Fig. 1F**); thus, we hypothesize that glycinergic currents are responsible for the other portion. Interestingly, stimulating peri-LC^{GABA} neurons at high frequency also caused a pause in LC tonic firing, followed by a burst of activity upon ceasing photostimulation (**Fig. 1G**). Together, these results suggest that peri-LC^{GABA} neurons directly inhibit LC^{NE} activity via GABA release to regulate different modes of LC^{NE} firing. We also found that peri-LC^{GABA} neurons were tonically active at baseline, indicating a level of basal tonic inhibition onto LC^{NE} neurons (**Extended Data Fig. 1D-F, Extended Data Table 1**).

Peri-LC^{GABA} neurons respond to salient stimuli and feeding

To investigate whether peri-LC^{GABA} neurons process arousal-related stimuli, we used photometry to monitor neuronal (calcium) activity in peri-LC^{GABA} neurons. We injected AAV-DIO-GCaMP6s and implanted an optical fiber above the peri-LC of vGAT-Cre mice, then used a battery of diverse behavioral tests to determine how arousing stimuli with different modalities and valence affect peri-LC^{GABA} neuron activity (**Fig. 1H**). First, to determine whether peri-LC^{GABA} neurons respond to arousing odorant stimuli, we exposed mice to 30s of various odors in a chamber

with lateral flow. Exposure to the aversive odor of predator urine or 2MT, a derivative of predator urine[22] (**Fig. 1I, Extended Data Fig. 1G**), to appetitive 2-phenylethanol (2-PE; rose oil extract) or the urine of opposite-sex mice[23,24] (**Fig. 1J, Extended Data Fig. 1H**), and to peppermint odor as a novel stimulus (**Extended Data Fig. 1I**), all significantly increased the activity of peri-LC^{GABA} neurons. Air flow without a corresponding odor did not increase peri-LC^{GABA} activity (**Extended Data Fig. 1N**). Taken together, these findings demonstrate that the presentation of salient odors, irrespective of valence, increases peri-LC^{GABA} neuronal activity. As salient stimuli generate phasic bursting in LC^{NE} neurons[25], this suggests that peri-LC^{GABA} neurons may suppress certain LC^{NE} subpopulations to increase the relative influence of other specific LC^{NE} populations, or that peri-LC^{GABA} signaling may coordinate LC^{NE} phasic bursting (**Fig. 1G**).

Second, we determined how peri-LC^{GABA} neurons respond to threat using aversive conditioning to a 20s tone that coterminated with a 1s 0.5mA foot shock. While the foot shock caused a robust increase in peri-LC^{GABA} activity (**Fig. 1K**), the conditioned stimulus alone did not elicit an increase. We also measured peri-LC^{GABA} neuronal activity in less hyper-arousing contexts by using the elevated-zero maze (EZM) and a brightly illuminated open field. We found that peri-LC^{GABA} neurons increased activity when animals entered the anxiogenic open arm of the EZM or the center of the open field, suggesting that they are likely involved in online processing of threatening contextual and location-based information (**Fig. 1L and Extended Data Fig. 1J-K**). As bulk activation of peri-LC^{GABA} neurons generally suppresses LC^{NE} tonic activity, the increase in peri-LC^{GABA} activity we observed may reflect activation of a subset of peri-LC^{GABA} neurons. This effect may correspond to a selective suppression of LC^{NE} subpopulations, while other LC^{NE} populations undergo the increase in tonic activity associated with anxiety-like behavior[17].

Next, to determine whether peri-LC^{GABA} neurons respond to novel salient stimuli, as previously established for LC^{NE} neurons[26,27], we performed novel-object testing as described[28]. In

this experiment, peri-LC^{GABA} neurons increased activity during an animal's interaction with a novel object, but not with a familiar object (**Fig. 1M**), suggesting that the peri-LC^{GABA} neurons respond to novel information, and not to the animal's conditioned memory of the stimulus[29]. The increase in bulk peri-LC^{GABA} neuron activity was surprising, but likely represent the activation of a subset of neurons which selectively inactivate a subset of LC^{NE} neurons or coordinate LC^{NE} neurons for a phasic burst. A similar mechanism has been proposed for local GABAergic control of dopamine activity in the ventral tegmental area[30–32].

LC^{NE} neurons and a glutamatergic peri-LC neuronal population have been recently implicated in feeding[33]; thus we sought to determine whether feeding behavior impacts peri-LC^{GABA} activity. In food-deprived animals, peri-LC^{GABA} activity strongly decreased during chow consumption and increased upon the cessation of consumption (**Fig. 1N-O**). This pattern of activity was also observed when food-deprived animals attempted to eat a false food pellet, which caused a decrease in activity that increased after stopping (**Extended Data Fig. 1L**). When sated mice consumed a highly palatable food item, no change in peri-LC^{GABA} activity was observed, though these neurons increased activity when mice ceased an eating bout (**Extended Data Fig. 1M**). These data indicate that peri-LC^{GABA} neurons are recruited during food consumption behavior in a deprived state. Taken together, the results from these behavioral tests and recordings indicate that peri-LC^{GABA} neurons integrate both salient arousing stimuli as well as internal states.

Selective control of peri-LC^{GABA} neuron activity alters arousal states, feeding, and pain responsivity

Because peri-LC^{GABA} stimulation decreases LC activity (**Fig. 1G**), we determined whether arousal behavior, positively correlated with LC^{NE} activity[17,34], is reduced with peri-LC^{GABA} stimulation. We injected AAV5-EF1a-DIO-hChR2-(H134R)-eYFP and implanted optical fibers

bilaterally in the peri-LC of vGAT-Cre mice (**Fig. 2A**). As changes in pupil diameter positively correlate with changes in LC^{NE} activity and arousal level[20,35], we measured pupil diameter subsequent to peri-LC^{GABA} photostimulation in anesthetized mice (**Extended Data Fig. 2A**). Photostimulation at 20Hz for 10s decreased pupil diameter, consistent with strong LC^{NE} inhibition (**Fig. 2B**), which caused a decrease in locomotor activity in a real-time place preference assay, consistent with decreased behavioral arousal (**Fig. 2C** and **Extended Data Fig. 2B**). Similarly, peri-LC^{GABA} stimulation did not directly modify anxiogenic behaviors in the EZM, open field, and light-dark box, but did decrease mean velocity of animal locomotion (**Extended Data Fig. 2C-E**). Moreover, stimulating peri-LC^{GABA} neurons did not impair aversive memory acquisition when photostimulation was delivered during tone-shock pairing, nor did it impact novel object interaction or food consumption (**Extended Data Fig. 2F-H**). Peri-LC^{GABA} stimulation was not positively reinforcing when animals were allowed to nosepoke for photostimulation (**Extended Data Fig. 2I**). These findings suggest that peri-LC^{GABA} neurons can modulate sympathetic and behavioral arousal, yet the disparity between photometry and activation results suggest that a more discrete regulation underlies peri-LC^{GABA} function.

As peri-LC^{GABA} neurons tonically inhibit the LC at baseline (**Extended Data Table 1**), we hypothesized that inhibiting these neurons would release the LC^{NE} population from this restraint, in turn increasing anxiogenic and hyperarousal states[20,36]. We injected AAV5-EF1a-DIO-hM4D(Gi), an inhibitory DREADD, into the peri-LC of vGAT-Cre mice (**Fig. 2D**) and measured the behavioral impact of inhibiting peri-LC^{GABA} neurons with 1 mg/kg CNO. This dose of CNO was used because administering 5 mg/kg CNO to DREADD-expressing mice caused behavioral arrest (**Extended Data Fig. 2J-K**), as previously observed when LC^{NE} neurons are hyperactivated[37]. Indeed, selective inhibition of peri-LC^{GABA} increased anxiogenic behavior in the

open field (**Fig. 2E** and **Extended Data Fig. 2L**), light-dark box (**Fig. 2F**), and EZM tests (**Fig. 2G** and **Extended Data Fig. 2M**).

A recent study showed that LC activity decreases during food consumption and that LC^{NE} activation attenuates feeding[38]; consistent with this finding, we found that DREADD-mediated inhibition of peri-LC^{GABA} neurons significantly decreased food consumption (**Fig. 2H**). The LC has also been reported to encode differential effects on pain processing based on projection target, wherein LC projections to the spinal cord reduce pain responses and projections to the forebrain exacerbate pain responses[8]. We therefore tested whether inhibiting peri-LC^{GABA} neurons could alter nocifensive behavioral responses. DREADD-expressing animals injected with CNO (1 mg/kg), exposed to a noxious thermal stimulus in the hot plate (**Fig. 2I**) and tail-flick (**Fig. 2J**) assays, had an increased latency for a nocifensive response relative to non-expressing controls, consistent with an induced analgesic effect.

Together, these results indicate that peri-LC^{GABA} neurons exert a tonic influence on LC^{NE}-mediated behaviors and bidirectionally modulate arousal level, affective responses, and nocifensive behavior. These results strongly suggest that the peri-LC^{GABA} system is a polymorphic structure with complex and heterogeneous functional roles during behavior.

LC and peri-LC neurons are composed of diverse molecular subpopulations

Having observed diverse and sometimes counter-intuitive LC and peri-LC activity responses in different behavioral paradigms, we therefore hypothesized that molecular heterogeneity of LC and peri-LC neuronal subpopulations may underlie these functional variations. To better understand the cellular diversity within the LC and peri-LC, we used single-nuclei RNA sequencing (snRNAseq) in brain sections containing both the LC and peri-LC from adult wild-type male or female mice and processed them by droplet-based nuclear mRNA

partitioning and sequencing (**Fig. 3A**, see methods for details)[39]. After removing low-quality cells and doublets, we identified 30,838 total cells and performed graph-based weighted nearest neighbor clustering analysis. We classified 15,050 cells (48.8%) as neurons, based on their expression of the canonical neuronal markers *Stmn2* and *Thy1* (**Fig. 3B-C; Extended Data Fig. 3A-G**)[39]. We excluded VGLUT1 neurons in our samples because they were of low-quality (**Extended Data Fig. 3E-F**), were detected in only a single sample, and *Slc17a7* (VGLUT1) was not expressed in the medial peri-LC zone in fluorescent *in situ* hybridization (FISH) (**Extended Data Fig. 3K**).

Interestingly, LC and peri-LC neurons formed clusters based on the glutamate, GABA, and the monoamine neurotransmitters they release (**Fig. 3D; Extended Data Fig. 3H**). The LC^{NE} cluster was highly enriched in genes involved in NE synthesis and transport, including *Dbh*, *Th*, *Ddc*, *Slc10a4*, *Slc6a2* (NET), and *Slc18a2* (VMAT2), but low in *Slc6a3* (DAT), the gene involved in dopamine transport, suggesting that these are predominantly LC^{NE} neurons and not dopamine neurons[40]. GABAergic neurons, on the other hand, were separated into 7 clusters that displayed high expression of *Slc32a1* (VGAT), *Gad1*, and *Gad2*, with one cluster co-expressing high levels of *Slc17a6* (VGLUT2). Five additional classes of VGLUT2 neurons were identified, but no cluster highly expressed *Slc17a7* (VGLUT1) or *Slc17a8* (VGLUT3). The last cluster of neurons expressed *Slc10a4*, *Slc18a3*, *Bche*, *Chat*, *Prima1*, and *Slc5a7*, which are typically characteristic of cholinergic (ACh) neurons[41].

Furthermore, we identified LC and peri-LC neurons which expressed many neuropeptides and their cognate G-protein coupled receptors (GPCRs), likely important for modulatory tuning of LC functional diversity (**Extended Data Fig. 3I-J**). LC^{NE} neurons were highly enriched in *Calca* (calcitonin/CGRP), *Cartpt*, and *Gal* (galanin), as well as *Calcr* (calcitonin receptor). VGLUT2 neurons expressed high levels of *Adcyap1*, which encodes PACAP, with subpopulations also

expressing *Cck* or *Bdnf*. A glutamatergic cluster (GLUT-3) and the Ach cluster were enriched in *Crh*; the cognate receptor *Crhr1* was primarily expressed on non-noradrenergic neurons. CRH input to the LC region caused by stress has previously been linked to increases in LC activity and subsequent anxiety-like behavior; however, monosynaptic connections onto LC^{NE} neurons were not able to be isolated[17]. Thus, these results suggest that CRH inputs may influence LC^{NE} activity and subsequent stress response via interactions with the peri-LC^{GABA} population.

The LC has long been associated with opioid neuropeptide and receptor signaling in stress, pain, and analgesia[42,43]. *Pdyn* (preprodynorphin) expression was low, except within one glutamatergic population (GLUT-4), but *Oprk1* (kappa opioid receptor) was primarily expressed in non-noradrenergic, glutamatergic neurons, consistent with previous studies showing that dynorphin signaling likely acts on presynaptic terminals in the LC region[44,45]. *Oprm1* (μ opioid receptor) expression was highest in LC^{NE} neurons, consistent with previous studies[46,47]; expression was also common across many non-noradrenergic groups. *Hcrtr1* and *Hcrtr2*, the two orexin receptors, were expressed on different cell populations, implicating differential roles in regulating arousal, motivation, and opioid abuse-related behaviors[48,49].

Additional analyses were performed to characterize the transcriptional diversity in physiology, structure, and disease-associated genes within the LC and peri-LC. We found differential expression in neuropeptide signaling, neurotransmitter reuptake, endocannabinoid signaling, hormone receptors, LC developmental markers[50–52], and genes involved in cell morphology and adhesion (**Extended Data Fig. 4A-C**). Differential expression of gap junction proteins (**Extended Data Fig. 4B**) may allow LC ensembles to form by electrotonic coupling and may explain sub-millisecond LC synchrony[53]. Differences in the expression of genes associated with psychiatric diseases, such as *Maoa*, *Ptger3*, and *Peg10*[54] (**Extended Data Fig. 4D**), and with dementia, such as genes involved in amyloid and tau interactions (**Supplementary Table S2**) were

also found in our sample. We also report heterogenous expression of ion channels, genes associated with neurotransmitter loading and release, and GPCRs and genes associated with GPCR regulation (**Supplementary Table S2**).

Because our photometry, and optogenetic and chemogenetic manipulation data imply that heterogeneity may exist within LC^{NE} and peri-LC^{GABA} neurons, we sought to identify subpopulations by performing conservative reclustering (resolution=0.8) only in neurons expressing *Dbh* or *Th* (**Fig. 3E-F; Extended Data Fig. 3L**); or expressing *Slc32a1*, *Gad1*, or *Gad2* (**Fig. 3E,G; Extended Data Fig. 3M**). Given other recent reports describing sexual dimorphism in LC structure and function[54–56], we also identified sexually dimorphic genes in all cells, the LC^{NE} subset, and the peri-LC^{GABA} subset (**Supplementary Table S1**). Subpopulations of LC^{NE} neurons defined by co-expression of glutamatergic markers and GABAergic markers may indicate a role in lateral inhibition or excitation within the LC^{NE} cells. Striking differences in expression are found in the gap junction proteins (**Extended Data Fig. 5C**), which may underlie some of the previously reported electrotonic coupling between LC^{NE} ensembles[57]. LC^{NE} neurons also differ in their receptors for neurotransmitters and neuromodulators, including α_2-adrenergic receptors (**Fig. 3G; Supplementary Table S2**) and in ion channel expression (**Supplementary Table S2**), which may facilitate the heterogeneous, intrinsic, electrophysiological differences in LC neurons[6,58], and responses to synaptic inputs. We also found that peri-LC^{GABA} subpopulations exhibit key differences (**Fig. 3F**), including differential expressions of *Penk*, *Pnoc*, and *Tac1* neuropeptides. Interestingly, GABA-3 neurons co-express *Pnoc* and *Slc6a5* (glycine transporter) (**Extended Data Fig. 6B**), suggesting that peri-LC^{Pnoc} neurons may be glycinergic. Taken together, the single-nuclei transcriptional profiles reveal distinct and molecularly heterogenous subpopulations of LC and peri-LC neurons.

LC and peri-LC RNA transcripts are spatially distributed in anatomically discrete locations

We next sought to corroborate our snRNAseq by determining the spatial distribution of RNA transcripts using Pixel-seq, a spatial barcoding approach in which a gel covered by high-density DNA polymerase colonies (polonies) allows capture of tissue mRNA followed by sequencing of RNA transcripts along with spatially-identified barcodes[59]. Matching the barcodes to their position in the gel allows for the segmentation of highly spatially resolved transcripts into individual cells. Tissue containing the LC and peri-LC was dissected from 10-μm sections, its RNA hybridized to the barcoded polony gel, and the cDNA then amplified and sequenced (**Extended Data Fig. 7A**). In total, ~4.6 million unique molecular identifiers (UMIs) at an estimated read saturation of 0.93 were obtained for the ~8mm^2 sections (**Extended Data Fig. 7B-C**). ~84% of the mapped transcripts were from protein-coding genes (**Extended Data Fig. 7D**). The LC region had high density of mRNAs (**Fig. 3J**), indicating that LC^{NE} neurons may be highly transcriptionally active relative to surrounding cells. The spatially resolved transcripts from three sections were aggregated into 8,181 pseudo-cells; then, leveraging on cell type-enriched markers found in our snRNAseq experiments, we clustered cells into neurons and non-neuronal subtypes (**Extended Data Fig. 7E**). Among the neurons, we further identified subpopulations, which when spatially mapped, showed a dense core of noradrenergic neurons surrounded by GABAergic neurons (**Fig. 3J**), consistent with our anatomical studies in **Fig 1A**. Noradrenergic markers (*Dbh*, *Slc18a2*, *Slc6a2*, and *Gal*) were densely expressed (**Extended Data Fig. 7F**), in contrast to the various neuropeptides identified by snRNAseq (*Adcyap1*, *Calca*, *Npy*, *Nts*, *Penk*, *Pnoc*, *Sst*, and *Tac1*), which were sparsely distributed throughout the peri-LC (**Extended Data Fig. 7G**).

To better appreciate the functional significance of genetically distinct peri-LC clusters in behavior, we selected three peptidergic subpopulations to investigate further: pENK, Pnoc, and Tac1. This was based on their high gene expression in the peri-LC and the high gene expression

of their cognate receptors within the LC^{NE} clusters (**Fig. 3H; Extended Data Fig. 3N-O**). We validated the peri-LC expression of these peptide mRNAs using FISH (**Fig. 3K-M**), and then examined the responses of these subpopulations during behavioral studies using photometry.

Peri-LC neuropeptidergic subpopulations are differentially activated by salient stimuli

We hypothesized that the peptidergic peri-LC subpopulations could have differential roles in the peri-LC response to salient stimuli, as several prior reports have suggested that different neuropeptide GPCRs can regulate LC^{NE} function[17,60–64] in various ways. Based on isolation of highly expressed peptide genes in the peri-LC and cognate receptor genes in the LC (**Fig. 3H**), we investigated putative peptidergic neurons expressing pENK, Pnoc, and Tac1, which showed limited overlap in expression within the peri-LC (**Fig. 3I; Extended Data Fig. 3P**). We injected AAV-DIO-GCaMP6s and implanted an optical fiber in the peri-LC of vGAT-Cre, pENK-Cre, Pnoc-Cre, and Tac1-Cre mice to determine how different stimuli impact the activity of these peri-LC subpopulations (**Fig. 4A-B**, **Extended Data Fig. 8A**).

Exposure to a predator odor significantly increased the activity of peri-LC GABAergic, pENK, and Pnoc populations, but not of Tac1 neurons (**Fig. 4C**). The same three populations also increased activity when presented with the odor of opposite-sex mice (**Extended Data Fig. 8B**), demonstrating that the valence of the stimulus did not affect the activity of these populations. Curiously, exposure to 2MT (**Extended Data Fig. 8C**), 2-PE (**Fig. 4D**), and peppermint odor (**Extended Data Fig. 8D**) caused a significant increase in activity in the peri-LC GABAergic, pENK, and Tac1 populations, but not in Pnoc neurons. Control air flow did not affect the activity of any peri-LC population (**Extended Data Fig. 8E**) These experiments demonstrate that peri-LC subpopulations exhibit diverse physiological responses to a variety of salient odorant stimuli. We also found that peri-LC^{GABA} and peri-LC^{pENK} neurons increased activity when an animal interacted

with a novel object but did not change significantly when exposed to a familiar object (**Extended Data Fig. 8F**). By contrast, peri-LC^{Tac1} neurons increased activity during novel object interactions, but decreased activity during familiar object interactions (**Extended Data Fig. 8F**). Together, these results indicate that peri-LC^{GABA}, peri-LC^{Tac1}, and peri-LC^{pENK} populations likely have distinct roles in integrating novel stimuli.

We also examined the calcium activity of peri-LC subpopulations during food consumption. On one hand, the GABAergic, pENK, and Pnoc populations decreased activity during chow consumption in food-deprived animals, and increased activity after stopping consumption (**Fig. 4H**). Tac1 neurons, on the other hand, did not significantly change activity during or immediately after consumption. Additionally, no population changed activity during the consumption of highly palatable food when sated, with only the peri-LC^{GABA} neurons increasing in activity after an eating bout (**Extended Data Fig. 14J**), indicating that an unidentified subpopulation of peri-LC^{GABA} neurons likely contributes to hedonic feeding.

Finally, we determined whether activities of the peri-LC neurons differentially respond to hyperarousing stimuli, including threat and anxiogenic contexts using fear conditioning (**Fig 4E**), EZM, and open field tests. In aversive conditioning, a pre-testing showed no inherent response to the tone (**Extended Data Fig. 14G**) in any of the four peri-LC populations, but the footshock caused a sharp increase in activity in all cell populations. However, after this initial increase, the peri-LC GABAergic and pENK neurons remained elevated in activity, whereas Pnoc neurons decreased activity to below pre-shock baseline (**Fig. 4E**). Interestingly, the next day, none of these peri-LC populations responded to the tone onset; however, peri-LC^{GABA} neurons showed a small but significant decrease in activity at the end of tone presentation, while Tac1 neurons sharply increased activity at tone offset (**Extended Data Fig. 14H**). Another noxious stimulus, a foot pinch, also elicited increased activity in all four populations (**Fig. 4F**). In experiments involving

anxiogenic contexts, we found that peri-LC^{GABA} neurons increased activity when animals entered the open arm of an EZM or the center of an open field while pENK and Pnoc populations increased activity only in the center of the open field, but not upon entering the open arm of an EZM (**Fig. 4G** and **Extended Data Fig. 14I**). Peri-LC^{Tac1} neurons, by contrast, did not change activity in the center of open field, but decreased activity in the open arm of an EZM. Combined, these experiments show that peri-LC^{pENK} and peri-LC^{Pnoc} populations are recruited during the danger of exploration in an anxiogenic environment, while Tac1 neurons may decrease activity during exploratory threat. Taken together, these results identify a molecular and functionally heterogeneous peri-LC structure which processes salient stimuli in a host of internal metabolic, cognitive, and arousal states.

Discussion

Here we defined a heterogenous peri-LC^{GABA} neuronal population whose inhibitory inputs shape LC firing. We identified the molecular diversity and spatial organization of the LC and peri-LC, and generally characterized peri-LC activity during behavioral state transitions. We report that increasing the activity of peri-LC^{GABA} neurons can diminish LC tonic activity, but triggers rebound spiking (**Fig. 1G**), revealing that peri-LC^{GABA} neurons likely perform complex modulation of LC firing, rather than simply inhibiting LC phasic bursting, as described before[65]. Additionally, given the predominant local projections of peri-LC^{GABA} neurons (**Fig 1A**), and that most, if not all, LC^{NE} neurons were subject to modulation by peri-LC^{GABA} neurons, peri-LC^{GABA} likely controls LC-NE mediated arousal states via direcxt interactions. Consistent with this idea, we found that activating peri-LC^{GABA} neurons decreased arousal and exploratory behavior (**Fig. 1G**, **2B-C**), whereas inhibiting these neurons increased anxiety-like behavior (**Fig. 2D-G**), consistent with disinhibition of LC activity[66]. We defined the peri-LC^{GABA} in cognitive areas that LC^{NE} system is known to

control, including arousing, aversive and appetitive stimulus processing (**Fig. 1I-J, Extended Data Fig. 1G-I**), sexual behavior (**Extended Data Fig. 1A**), feeding (**Extended Data Fig. 1L**), nocifensive response (**Fig. 1K, 4K**) and novel object processing (**Fig. 1M**). Thus, peri-LC^{GABA} neurons likely act both as an integrator and a filter of information sent to the LC.

Transcriptomics revealed significant molecular and spatial diversity in peri-LC neurons that translates to functional diversity. In particular, peri-LC^{GABA} neurons expressing a wide repertoire of neuropeptides (**Fig. 3F**) surround the LC^{NE} dendritic field as seen in our Pixel-seq data (**Extended Data Fig. 7G**). As differing groups of LC neurons also express different sets of cognate receptors (**Fig. 3G**, **Extended Data Fig. 5A**), the combinations of neuropeptide-receptor interactions between the peri-LC and LC likely give rise to polymodal LC ensembles that can flexibly change in response to different internal states and behavioral needs. For instance, given that peri-LC^{GABA} activity decreased during food consumption (**Fig. 1N**), we hypothesized that inhibiting these neurons would increase feeding, but chemogenetic inhibition of these neurons instead decreased feeding (**Fig. 2H**). likely by disinhibiting LC^{NE} projections to the lateral hypothalamus involved in feeding[38]. This seemingly paradoxical finding is likely because chemogenetic inhibition affected more neurons than involved in natural feeding behavior, engaging vastly different LC^{NE} ensembles and projections (compare [67] vs. [38]). We also found that inhibiting peri-LC^{GABA} neurons promoted analgesia (**Fig. 2I-J**), likely by preferentially disinhibiting LC^{NE} projections to the dorsal horn of the spinal cord, which act to inhibit noxious afferents[68], rather than disinhibiting LC^{NE} projections to the BLA[69] and PFC[8], which contribute to the negative affect in pain response. Furthermore, although novel and salient stimuli were generally associated with increased peri-LC activity (**Fig. 4B,E**), Tac1 neurons decreased activity during exploration in contrast to peri-LC^{pENK}, peri-LC^{Pnoc}, and peri-LC^{GABA} population as a whole

(**Fig. 4G, Extended Data Fig. 8I**). These examples highlight that the peri-LC acts as a functionally diverse, heterogeneous population.

Our findings identify diverse peri-LC^{GABA} circuitry at molecular, cellular, and systems levels, opening many avenues in follow up studies on these newly identified populations in a host of ethologically relevant homeostatic behaviors. We isolate unique peri-LC neuronal groups which act to integrate ascending and descending LC-NE control via both direct fast neurotransmission and neuropeptide signaling. The expression of several genetically defined subpopulations with differing functional properties represents a subset of putative critical integrators of arousal states mediated via the LC-NE system. Sub-populations of peri-LC^{GABA} neurons thereby form distinct modular components of the LC-NE system, impacting noradrenergic tone, physiological arousal and behavior. The molecular and functional diversity of the peri-LC we identified here presents unique opportunities for altering NE function in disease-related behavioral states.

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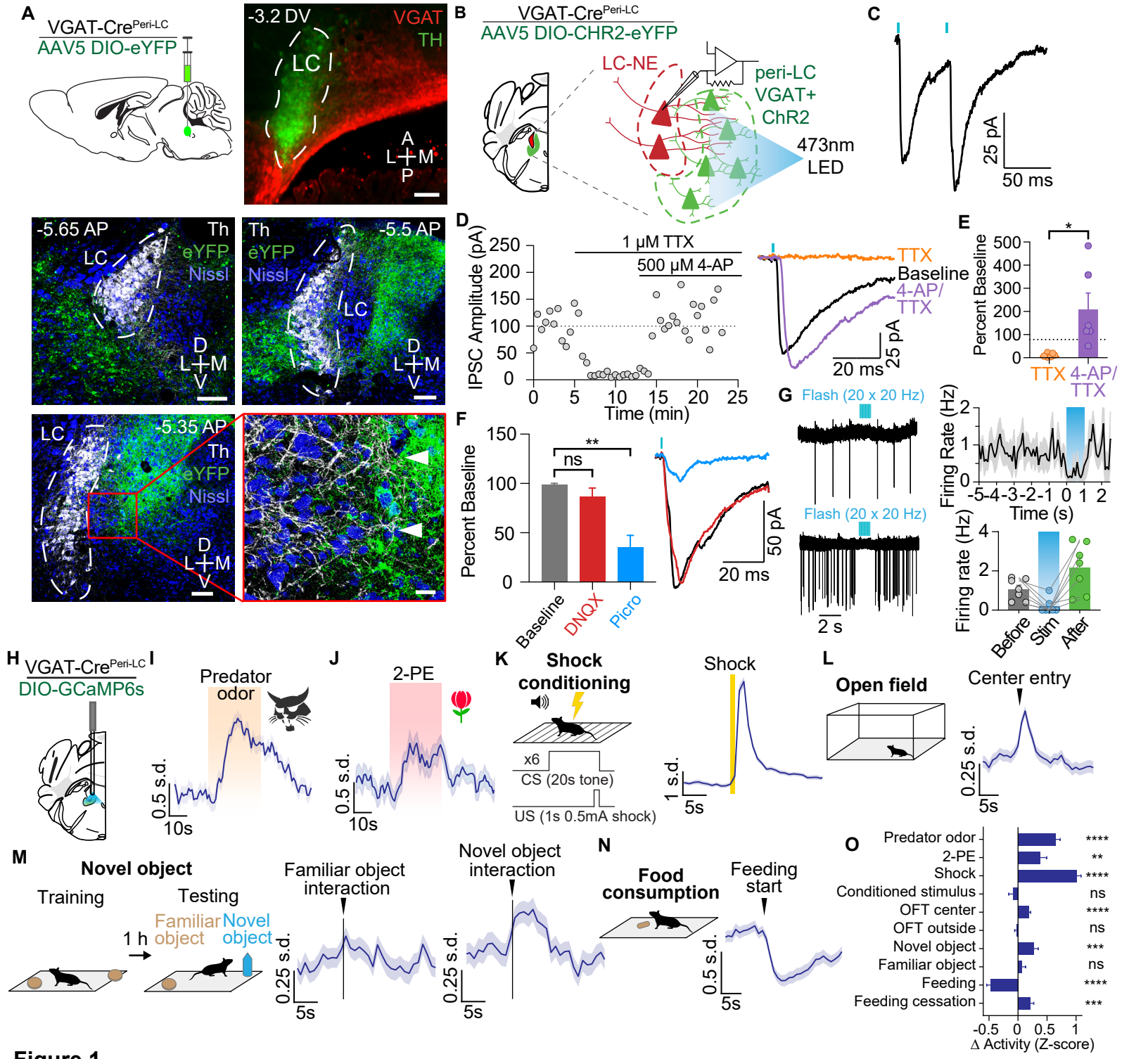
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AAV5 DIO-eYFP
-3.2 DV
VGAT
TH
LC
A
L M
P
-5.65 AP
Th
eYFP
Nissl
LC
D
L M
V
-5.5 AP
-5.35 AP
B
VGAT-Cre Peri-LC
AAV5 DIO-CHR2-eYFP
LC-NE
peri-LC
VGAT+
ChR2
473nm
LED
C
25 pA
50 ms
D
1 μM TTX
500 μM 4-AP
IPSC Amplitude (pA)
Time (min)
TTX
Baseline
4-AP/
TTX
20 ms
25 pA
E
Percent Baseline
TTX
4-AP/
TTX
F
Percent Baseline
ns
**
Baseline
DNQX
Picro
50 pA
20 ms
G
Flash (20 x 20 Hz)
2 s
Firing Rate (Hz)
Time (s)
Firing rate (Hz)
Before
Stim
After
H
VGAT-Cre Peri-LC
DIO-GCaMP6s
I
Predator
odor
0.5 s.d.
10s
J
2-PE
0.5 s.d.
10s
K
Shock
conditioning
x6
CS (20s tone)
US (1s 0.5mA shock)
Shock
1 s.d.
5s
L
Open field
Center entry
0.25 s.d.
5s
M
Novel object
Training
Testing
1 h
Familiar
object
Novel
object
Familiar object
interaction
0.25 s.d.
5s
Novel object
interaction
0.25 s.d.
5s
N
Food
consumption
Feeding
start
0.5 s.d.
5s
O
Predator odor ****
2-PE **
Shock ****
Conditioned stimulus ns
OFT center ****
OFT outside ns
Novel object ***
Familiar object ns
Feeding ****
Feeding cessation ***
Δ Activity (Z-score)

Figure 1

Fig. 1. *In vitro* and *in vivo* identification of the pericoerulear GABAergic neural network.

(A) Schematic of viral injection in vGAT-Cre mice is shown on top left. Light-sheet image of cleared mouse brain immunostained for TH (green) and eYFP-expressing GABAergic cells (red) is shown on top right. Confocal images depicting peri-LC^{GABA} soma with eYFP (green), Th (white), and Nissl (blue) (bottom) are shown on the bottom. Scale bars are 100 μm, except for inset (20 μm). AP and DV distances in mm from bregma.

(B) Schematic of whole-cell patch clamp electrophysiology recordings of optically-evoked inhibitory postsynaptic currents (IPSCs).

(C) Representative optically evoked IPSC is shown (53.8 ± 7.3 pA, n=18) with a paired pulse ratio of 1.1 at a 50 ms interpulse interval (n=16).

(D) (Left) Representative recording shows rescue of tetrodotoxin (TTX, 1 μM)-sensitive light-evoked IPSCs by 4-aminopyridine (4-AP, 500μM). (Right) Average IPSCs during 5 min baseline (black), 3-8 min post TTX (orange), and 5-10 min post 4-AP (purple).

(E) IPSC amplitudes in the presence of TTX or TTX + 4-AP normalized to baseline (n=6).

(F) (Left) Average amplitudes of optically evoked IPSCs normalized to baseline (n=5) during the wash on of DNQX (10 μM) followed by picrotoxin (100 μM). (Right) Representative traces showing PSCs during 5 min of baseline (black), 5-10 min post DNQX (red), and 5-10 min post picrotoxin (green).

(G) Representative trace of a single sweep (top) and an overlay of 10 sweeps (bottom) recorded from an LC-NE neuron in cell attached mode. Blue hash marks indicate the duration of light stimulation (1ms pulses at 20 Hz for 1 s). (Right top) Time course of action potential firing rate for an average of 6 cell-attached recordings with a pulse train for 250 ms (20 Hz, 1 ms pulses). (Right bottom) Firing rate before, during, and after photostimulation.

(H) Schematic of fiber photometry recordings in peri-LC^{GABA} neurons.

(I) Peri-LC^{GABA} neuronal activity increases during 30s exposure to bobcat urine odor.

(J) Peri-LC^{GABA} neuronal activity increases during 30s exposure to 2-phenylethanol (2-PE) odor.

(K) Peri-LC^{GABA} neuronal activity increases after a 1s 0.5mA shock preceded by a coterminating 20s tone.

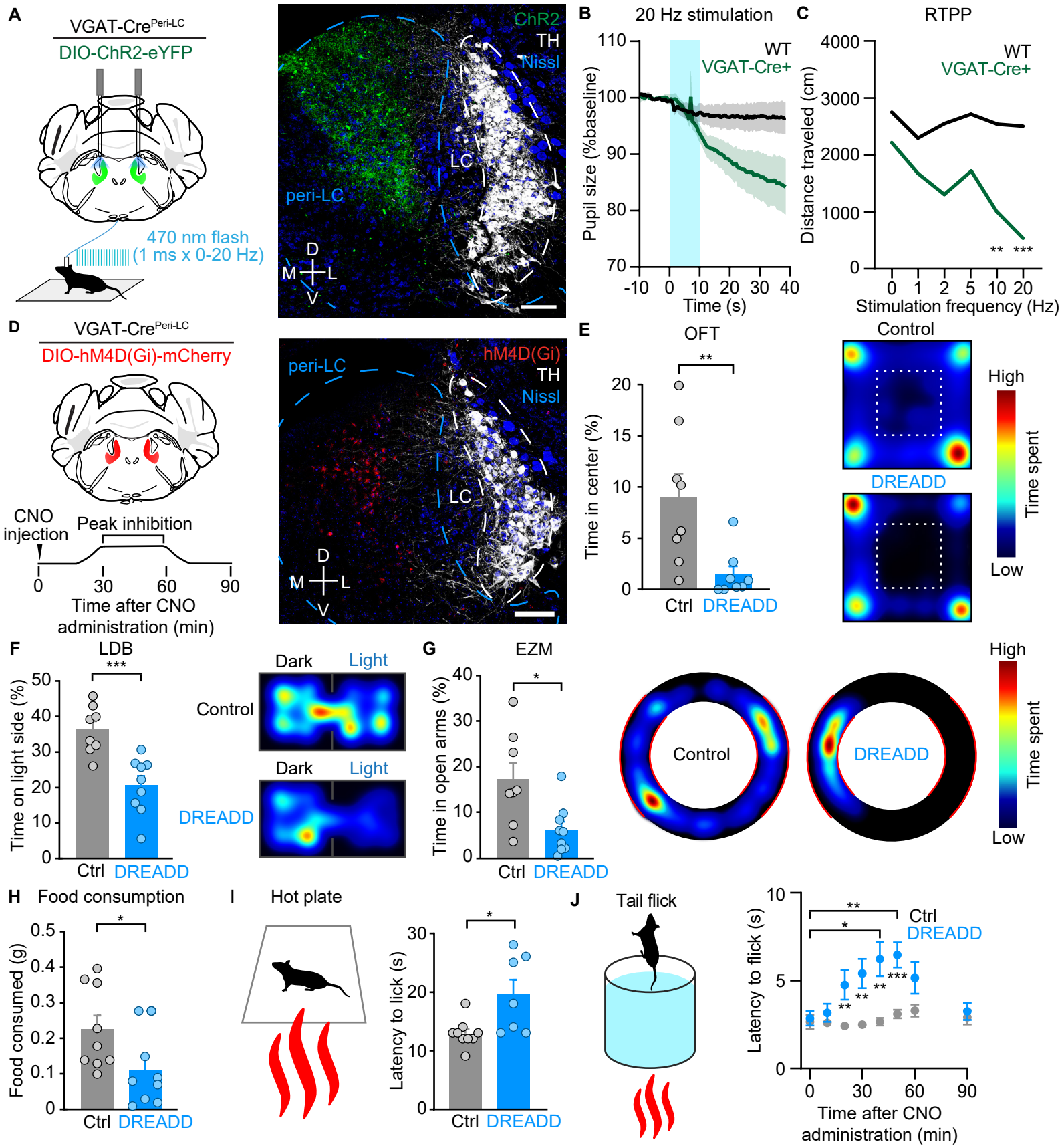
(L) Peri-LC^{GABA} neuronal activity increases upon center entry in an open field test.

(M) Schematic (left) of novel object assay; peri-LC^{GABA} neuronal activity increases when animal interacts with a novel object (right), but not a familiar (left) object.

(N) Peri-LC^{GABA} neuronal activity decreases during food consumption in food-deprived animal.

(O) Summary of peri-LC^{GABA} fiber photometry experiments. Bars represent change in activity 10s before and after event or intervention (30s before and after for odor exposure experiments).

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 using paired two-tailed t-tests. ns, not significant. Error bars indicate SEM.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ChR2
TH
Nissl
LC
peri-LC
D
M
L
V
B
20 Hz stimulation
WT
VGAT-Cre+
Pupil size (%baseline)
Time (s)
C
RTPP
Distance traveled (cm)
Stimulation frequency (Hz)
D
VGAT-Cre Peri-LC
DIO-hM4D(Gi)-mCherry
CNO injection
Peak inhibition
Time after CNO administration (min)
hM4D(Gi)
E
OFT
Time in center (%)
Ctrl
DREADD
Control
High
Low
Time spent
F
LDB
Time on light side (%)
Dark
Light
G
EZM
Time in open arms (%)
H
Food consumption
Food consumed (g)
I
Hot plate
Latency to lick (s)
J
Tail flick
Latency to flick (s)

Figure 2

Fig. 2. Manipulation of pericoerulear GABAergic neurons alters diverse behavioral states.

(A) (Left) Schematic of viral injection and optogenetic simulation of peri-LC^{GABA} neurons. (Right) Image shows ChR2-eYFP (green), tyrosine hydroxylase (TH, white), and Nissl (blue) expressed in the LC and peri-LC region. Scale bar is 100 µm.

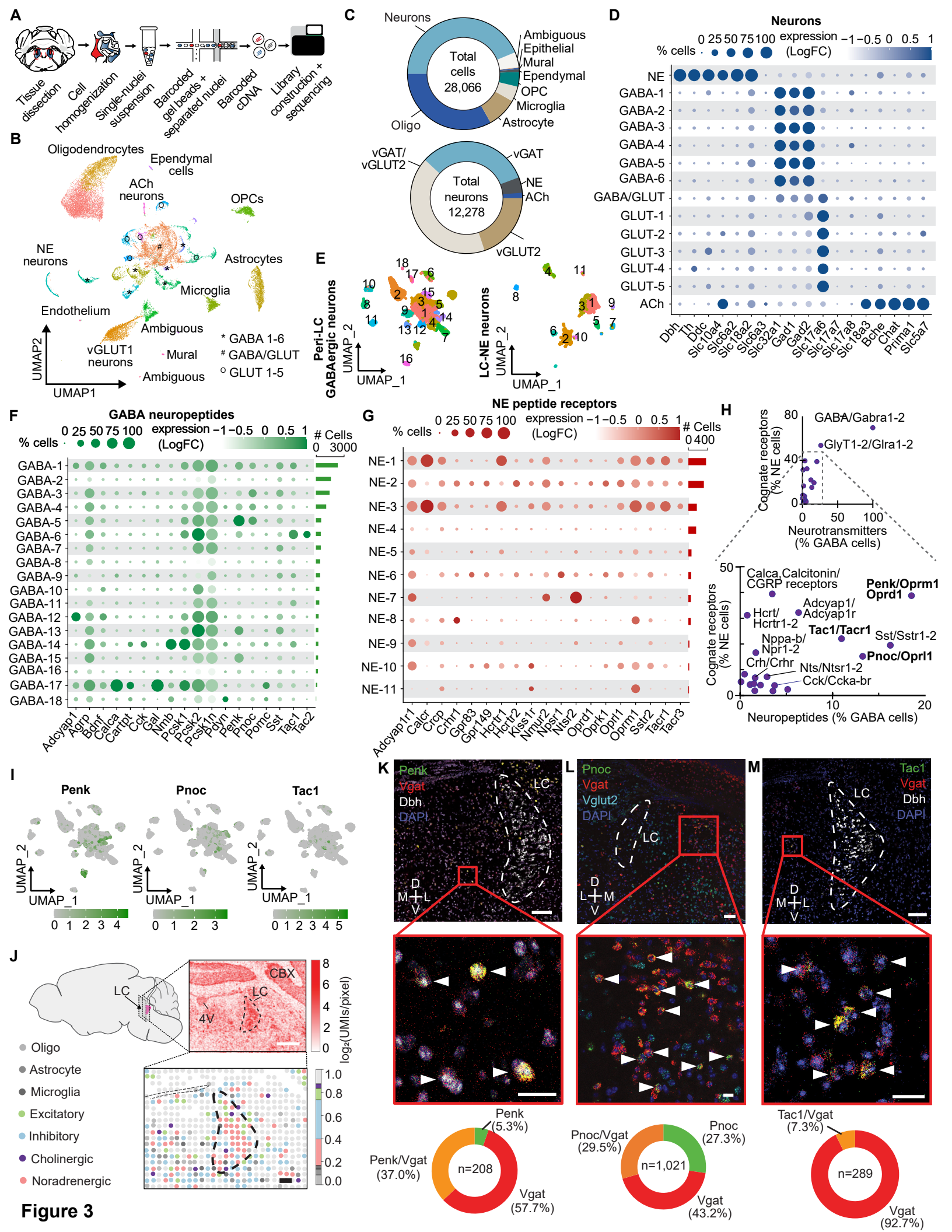
(B) Graph shows pupil size in response to 10s of 20Hz stimulation (2mW, 5ms pulse width) in wildtype control or vGAT-Cre mice starting at 10s.

(C) Graph shows distance traveled in real-time place preference assay with varying photostimulation frequencies 0-20 Hz (2mW, 5ms pulse width).

(D) (Left) Schematic of viral injection and chemogenetic inhibition of peri-LC^{GABA} neurons. (Right) Image shows hM4D(Gi)-mCherry (red), TH (white), and Nissl (blue) in the LC and peri-LC region. Scale bar is 100 µm.

(E-J) Graphs compare chemogenetic inhibition (1 mg/kg CNO) of peri-LC^{GABA} neurons in vGAT-Cre mice versus non-expressing wild-type controls in **(E)** time spent in the center of an open field, **(F)** time spent on the light side of a light-dark box test (LDB), **(G)** time spent in the open arms of an elevated-zero maze (EZM), **(H)** food consumed in a 20 min period, **(I)** latency to paw lick on a 55°C hot plate, and **(J)** latency to tail flick in 54°C water. Corresponding location heat maps are shown for OFT, LDB, and EZM.

*p<0.05, **p<0.01, ***p<0.001 by unpaired two-tailed *t*-test (E-I) or two-way ANOVA (C and J). Error bars indicate SEM.


A
Tissue dissection
Cell homogenization
Single-nuclei suspension
Barcoded gel beads + separated nuclei
Barcoded cDNA
Library construction + sequencing
B
Oligodendrocytes
Ependymal cells
ACh neurons
OPCs
NE neurons
Astrocytes
Microglia
Endothelium
Ambiguous
vGLUT1 neurons
Mural
Ambiguous
* GABA 1-6
# GABA/GLUT
o GLUT 1-5
UMAP2
UMAP1
C
Neurons
Total cells 28,066
Ambiguous
Epithelial
Mural
Ependymal
OPC
Microglia
Astrocyte
Oligo
vGAT/ vGLUT2
vGAT
NE
ACh
Total neurons 12,278
vGLUT2
D
Neurons
% cells
expression (LogFC)
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GABA-1
GABA-2
GABA-3
GABA-4
GABA-5
GABA-6
GABA/GLUT
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Gad1
Gad2
Slc17a6
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Slc17a8
Slc18a3
Bche
Chat
Prima1
Slc5a7
E
Peri-LC GABAergic neurons
LC-NE neurons
UMAP_2
UMAP_1
F
GABA neuropeptides
% cells
expression (LogFC)
Cells
GABA-1
GABA-2
GABA-3
GABA-4
GABA-5
GABA-6
GABA-7
GABA-8
GABA-9
GABA-10
GABA-11
GABA-12
GABA-13
GABA-14
GABA-15
GABA-16
GABA-17
GABA-18
Adcyap1
Agrp
Bdnf
Calca
Cartpt
Cck
Gal
Nmb
Pcsk1
Pcsk2
Pcsk1n
Pdyn
Penk
Pnoc
Pomc
Sst
Tac1
Tac2
G
NE peptide receptors
% cells
expression (LogFC)
Cells
NE-1
NE-2
NE-3
NE-4
NE-5
NE-6
NE-7
NE-8
NE-9
NE-10
NE-11
Adcyap1r1
Calcr
Crcp
Crhr1
Gpr83
Gpr149
Hcrtr1
Hcrtr2
Kiss1r
Nmur2
Npsr1
Ntsr2
Oprd1
Oprk1
Oprl1
Oprm1
Sstr2
Tacr1
Tacr3
H
Cognate receptors (% NE cells)
GABA-A/Gabra1-2
GlyT1-2/Glra1-2
Neurotransmitters (% GABA cells)
Calca,Calcitonin/ CGRP receptors
Penk/Oprm1 Oprd1
Adcyap1/ Adcyap1r
Hcrt/ Hcrtr1-2
Tac1/Tacr1
Sst/Sstr1-2
Nppa-b/ Npr1-2
Pnoc/Oprl1
Crh/Crhr
Nts/Ntsr1-2
Cck/Ccka-br
Neuropeptides (% GABA cells)
I
Penk
Pnoc
Tac1
UMAP_2
UMAP_1
J
LC
CBX
4V
log2(UMIs/pixel)
Oligo
Astrocyte
Microglia
Excitatory
Inhibitory
Cholinergic
Noradrenergic
K
Penk
Vgat
Dbh
DAPI
LC
L
Pnoc
Vgat
Vglut2
DAPI
LC
M
Tac1
Vgat
Dbh
DAPI
LC
Penk (5.3%)
Penk/Vgat (37.0%)
n=208
Vgat (57.7%)
Pnoc/Vgat (29.5%)
Pnoc (27.3%)
n=1,021
Vgat (43.2%)
Tac1/Vgat (7.3%)
n=289
Vgat (92.7%)

Figure 3

Fig. 3. Isolating the locus coeruleus and pericoerulear transcriptome.

(A) Schematic shows the steps in the single-nuclei RNA sequencing (snRNAseq) experiments.

(B) UMAP visualization based on transcriptional profile is shown for n=30,838 cells pooled from 3 different samples.

(C) Distribution of cell types (left, n=28,066) and neuronal classes (right, n=12,278) in snRNAseq sample after vGLUT1 neurons were removed.

(D) Dot plots of n=12,278 neurons are shown, with neuronal clusters on the y-axis and gene transcripts on the x-axis. Size of circles corresponds to percent of cells in the cluster expressing a specific transcript, while color intensity corresponds to the relative expression level of the transcript. Plots correspond to transcripts related to neurotransmission.

(E) UMAP visualizations of reclustered neurons using *Slc32a1* (vGAT), *Gad1*, or *Gad2* expression for the peri-LC inhibitory neurons (left, n=11,182); and *Dbh* or *Th* expression for the LC-NE neurons (right, n=1,324).

(F) Dot plots of the subset of neurons that express *Slc32a1* (vGAT), *Gad1*, or *Gad2* (n=11,182) are shown, with the GABAergic subpopulations on the y-axis and neuropeptide genes on the x-axis. Circle size corresponds to percent of cells in the cluster expressing a specific transcript, while color intensity corresponds to its relative expression. Cell number in each cluster is graphed to the right.

(G) Dot plots of the subset of neurons that express *Dbh* or *Th* (n=1,324) are shown, with LC-NE subpopulations on the y-axis and neuropeptide receptor genes on the x-axis. Circle size corresponds to percent of cells in the cluster expressing the specific transcript, while color intensity corresponds to its relative expression. Cell number in each cluster is graphed to the right.

(H) Graphs show the distribution of neurotransmitter/neuropeptide gene expression in GABAergic neurons on the x-axis and the gene expression of their cognate receptors in LC-NE neurons on the y-axis.

(I) Feature plots show the peri-LC inhibitory neuron clusters expressing Penk (left), Pnoc (middle), and Tac1 (right).

(J) Schematic for spatial transcriptomics shows a fresh tissue section containing the LC (pink) and neighboring regions. 4V, 4th ventricle; CTX, cerebellum. An example of the resulting spatial UMI density plot is shown for a fresh, coronal mouse brain section (10 μm thickness) centered in the LC region. Scale bar is 500 μm. Image on the bottom shows the single-nuclei RNA sequencing-guided annotation of binned (50 μm x 50 μm) pseudo-cells.

(K-M) Representative images for *in situ* hybridization of vGAT (*Slc32a1*) (**K-M**), *Dbh* (**K,M**), and vGLUT2 (*Slc17a6*) (**L**) with (**K**) *Penk*, (**L**) *Pnoc*, **(M)** *Tac1* mRNA. Scale bars are 100 μm (top) and 20 μm (bottom). The respective quantification of the relevant *in situ* cell populations are shown in the bottom row.

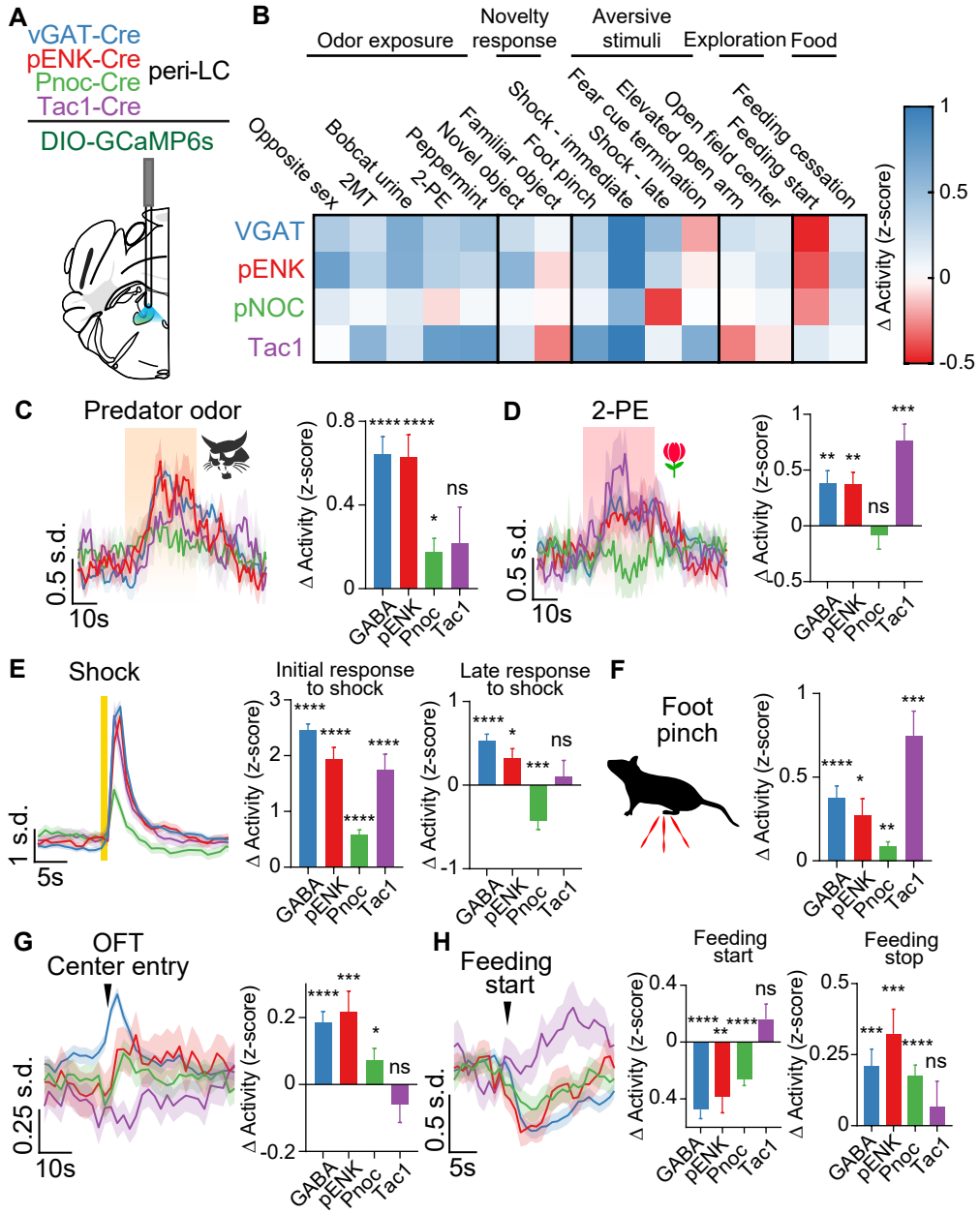
A
vGAT-Cre
pENK-Cre
Pnoc-Cre
Tac1-Cre
peri-LC
DIO-GCaMP6s
B
Odor exposure
Novelty response
Aversive stimuli
Exploration
Food
Opposite sex
Bobcat urine
2MT
2-PE
Peppermint
Novel object
Familiar object
Foot pinch
Shock - immediate
Shock - late
Fear cue termination
Elevated open arm
Open field center
Feeding start
Feeding cessation
VGAT
pENK
pNOC
Tac1
Δ Activity (z-score)
C
Predator odor
0.5 s.d.
10s
D
2-PE
E
Shock
1 s.d.
5s
Initial response to shock
Late response to shock
F
Foot pinch
G
OFT
Center entry
0.25 s.d.
H
Feeding start
Feeding stop
GABA
pENK
Pnoc
Tac1

Figure 4

Fig. 4. Functional heterogeneity of peri-LC neuronal subpopulations.

(A) Schematic depicts fiber photometry recordings from peri-LC neurons expressing Cre-dependent GCaMP6s in vGAT-Cre, pENK-Cre, Pnoc-Cre, or Tac1-Cre mice.

(B) Heat map summary of peri-LC fiber photometry experiments.

(C-H) Photometry traces and their quantification for the peri-LC neuronal subpopulations (vGAT, pENK, Pnoc, Tac1) are shown for responses to 30s exposure to bobcat urine odor **(C)**, to 30s exposure to 2-phenylethanol (2-PE) odor **(D)**, to early (left, 0-5 s after shock) and late (right, 5-20 s after shock) responses to a 1s 0.5mA footshock **(E)**, to a foot pinch **(F),** to entering the center of an open field **(G)**, and to the start and end of a food consumption bout **(H)**. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$ by paired two-tailed *t*-test. Error bars indicate SEM.

Methods

Animals

Adult wild-type C57BL/6J (Jackson Laboratory #000664), vGAT-IRES-Cre (*Slc32a1*$^{tm2(cre)Lowl}$/J; Jackson Laboratory #028862)[70], pENK-IRES-Cre (*B6;129S-Penk*$^{tm2(cre)Hze}$/J; Jackson Laboratory #025112), Tac1-IRES-Cre (B6;129S-Tac1$^{tm1.1(cre)Hze}$/J; Jackson Laboratory # 021877) and prepronociceptin (*Pnoc*)-IRES-Cre (*Pnoc*$^{tm1.1(cre)Mrbr}$/J; Jackson Laboratory # 034278) [71] mice were group housed, given access to food and water *ad libitum*, and maintained on a 12:12 hr reverse light:dark cycle (lights off at 9:00 AM and on at 9:00 PM). All animals were kept in an isolated and sound-attenuated holding facility within the lab and adjacent to behavior rooms one week prior to surgery, post-surgery, and throughout the duration of the behavioral assays to minimize stress. Males and female mice were used in all experiments. All procedures were approved by the Animal Care and Use Committee of Washington University and the University of Washington and conformed to US National Institutes of Health guidelines.

Stereotaxic Surgeries

After the animals were acclimated to the holding facility for at least seven days, the mice were anesthetized in an induction chamber (2% isoflurane) and placed into a stereotaxic frame (Kopf Instruments, Model 1900) where they were maintained at 1-2% isoflurane. Mice were then injected using a Nanoject (Drummond Scientific #3000204 and #3000207) with a glass tip of 7-20 µm diameter. Mice were injected in the peri-LC (AP -5.40 mm, ML +0.75 mm, DV -3.70 mm relative to bregma) with three bursts of 9.2 nL each (3 minutes apart; 27.6 nL total) at a rate of 23 nL/s. Injections were unilateral for tracing and fiber photometry experiments; bilateral for optogenetic or chemogenetic experiments. The needle was slowly removed from the brain 10 min after cessation of injection to allow for diffusion. For fiber photometry and optogenetic experiments, mice were also implanted with a 400-µm fiber optic (Doric Inc., MFC_400/430-0.48_MF2.5_FLT) or custom-made optical fibers of ≥70% optical efficiency in the same surgery. Mice received carprofen and recovered for at least 6 weeks prior to behavioral testing, permitting optimal expression of the virus. For optogenetic inhibition, fiber optics were placed bilaterally. Fiber optic implants were secured using Metabond (Parkell #S380).

Viruses

AAV5-EF1a-DIO-hChR2-(H134R)-eYFP	WashU HOPE Center Viral Core
AAV-DJ-EF1a-DIO-GCaMP6s	Stanford Gene Vector and Viral Core
AAV5-CAG-FLEX-RG	UNC Vector Core
AAV8-EF1a-FLEX-TVA-mCherry	UNC Vector Core
EnvA-G-Deleted Rabies-eGFP	Salk

Immunohistochemistry

Immunohistochemistry was performed as previously described[72]. In brief, mice were anesthetized with sodium pentobarbital and transcardially perfused with 4% paraformaldehyde (PFA), post-fixed overnight in 4% PFA, and cryo-protected in 30% sucrose for at least 24 hours. Brains were then sectioned (30 µm) and placed in 0.1M PB until immunohistochemistry. Free-floating sections were washed in 0.1M PBS for 3 x 10 minutes intervals. Sections were then placed in blocking buffer (0.5% Triton X-100 and 5% natural goat serum in 0.1 M PBS) for 1 hr at room temperature. Sections were incubated with primary antibody (TH – Aves TYH 1:2000; mCherry – Iowa DSHB-mCherry-3A11 1:1000) at 4°C overnight. After 3 x 10 minute 0.1M PBS, sections were incubated in Alexa Fluor secondary antibodies (1:1000) and NeuroTrace (1:400, 435/455

blue fluorescent Nissl stain, Invitrogen #N21479) for 1 hour, followed by 3 x 10 minute 0.1M PBS then 3 x 10 minute 0.1M PB washes. Sections were then mounted and coverslipped with Vectashield Vibrance HardSet mounting medium (Vector Laboratories, CAT #H-1700) and imaged on Olympus Fluoview 3000 confocal microscope. Animals that did not show targeted expression were excluded.

Fluorescent *in situ* Hybridization (FISH)

Animals were anesthetized and rapidly decapitated. Brains were quickly removed and fresh frozen in dry ice, then stored at -80°C. Sections were cut at 20µm, mounted on slides, and stored at -80°C. Sections were fixed in 4% PFA for 15 min, dehydrated in serial ethanol concentrations (50%, 70%, and 100%) and processed with RNAscope (Advanced Cell Diagnostics, cat. No. 320293). Sections were hybridized with the probes listed below. Sections were then counterstained with DAPI and coverslips were applied. Confocal images were obtained on an Olympus FV3000RS microscope.

RNAScope Probes

DBH	Advanced Cell Diagnostics	*Cat#464621*
vGLUT1 (*Slc17a7*)	Advanced Cell Diagnostics	*Cat#416631*
vGAT (*Slc32a1*)	Advanced Cell Diagnostics	*Cat#319191*
vGLUT2 (*Slc17a6*)	Advanced Cell Diagnostics	*Cat#319171*
pENK	Advanced Cell Diagnostics	*Cat#318761*
Tac1	Advanced Cell Diagnostics	*Cat#410351*
Pnoc	Advanced Cell Diagnostics	*Cat#437881*

Slice electrophysiology

For electrophysiology experiments, slices of the LC were prepared from injected mice at least 21 days after stereotaxic surgery. Anesthetized mice underwent cardiac perfusion with ice cold cutting solution (in mM: 75 NaCl, 2.5 KCl, 6 $MgCl_2$, 0.1 $CaCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 2.5 D-glucose, 50 sucrose; bubbled with 95% O_2 and 5% CO_2). The brain was then submerged in cutting solution and placed on a VT1200 vibratome (Leica), where 240 µm horizontal LC slices were collected. Slices were transferred to a 32°C holding chamber containing oxygenated artificial cerebrospinal fluid (ACSF; in mM: 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 1.2 NaH_2PO_4, 21.4 $NaHCO_3$, and 11.1 D-glucose; bubbled with 95% O_2 and 5% CO_2), where they recovered for at least 1 hour.

Whole cell and cell attached recordings were made using an Axopatch 200B Amplifier (Molecular Devices). Data were acquired with Axograph X (Axograph Scientific) at 5 kHz and filtered to 2 kHz. Cells were visualized with a BX51WI microscope (Olympus). LC-NE cells were identified by morphology, location (between the edge of the 4th ventricle and motor neurons of the mesencephalic tract), and the presence of an electrically evoked current (α2-mediated IPSC)[21]. Channelrhodopsin2 expressing peri-LC cells were identified by the presence of a steady state current during a 150 ms light pulse and were medial to LC-NE cells. In the recording chamber, slices were continuously perfused at 1.5 - 2 mL per minute with ACSF warmed to 30 +/- 2°C. Pipettes (1.5 - 2.0 MΩ) were pulled from borosilicate glass (World Precision Instruments). For whole cell voltage clamp recordings, cells were held at -60 mV and the internal pipette contained: 135 mM KCl, 0.1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES, 0.038 mM EGTA, 1 mg/ml ATP, 0.1 mg/ml GTP, 1.5 mg/ml phosphocreatine (pH 7.4, 275 mOsm). Currents were evoked with wide-field stimulation using a 1 ms pulse from an LED (470 nm, ~0.5-1.0 mW/mm^2) through a 40x water immersion objective. Paired pulse ratios were measured using a 50 ms interpulse

interval. Glutamatergic and GABAergic synaptic components were blocked using DNQX and picrotoxin, respectively. TTX and 4-aminopyridine were used to isolate monosynaptic ChR2-evoked currents. Current clamp recordings were collected (using the same internal solution) to measure action potentials and resting membrane potential of peri-LC cells. For cell attached recordings, the intracellular pipette solution contained: 126 mM NaCl, 2.5 mM KCl, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, and 1.2 mM NaH_2PO_4. Firing rate was measured in 500 ms bins.

Brain clearing and imaging LC and peri-LC

VGAT-Cre mice were injected with 300 nl of AAV5-EF1α-DIO-ChR2-(H134R)-eYFP ($1x10^{13}$ vg/ml) at -5.40 AP, +0.75 ML, -4.0 to -3.7 DV from bregma with a lateral-facing beveled syringe. After 5 weeks, mice were euthanized, perfused with phosphate buffered saline and paraformaldehyde, and the brains were collected. Tissue clearing was modified from iDISCO+ protocol[73]. Briefly, brains were dehydrated with methanol, delipidated with dichloromethane, and bleached with hydrogen peroxide solution. After rehydration and blocking, brains were incubated with 1:1000 chicken anti-GFP antibody (Aves GFP-1020) and 1:1000 mouse anti-tyrosine hydroxylase antibody (BioLegend 818001), with boosters over 5 days for a final concentration of 1:200. After washing, secondary antibodies were added: 1:500 goat anti-chicken IgG Alexa Fluor 647 (Invitrogen A21449) and 1:500 goat anti-mouse Alexa Fluor 488 (Life Technologies A11001), with boosters over 5 days for a final concentration of 1:100. After washing and dehydration, brain clearing and refraction index matching were performed with dibenzyl ether. Brains were imaged with a light sheet microscope (UltraMicroscope II, LaVision Biotec), and imaging data were analyzed using ImageJ and arivis Vision4D (3.0.0).

Single-nuclei RNA sequencing

Adult mice were euthanized with 0.01 ml/kg of phenytoin/pentobarbitol solution, and perfused transcardially with cold NMDG-aCSF (96 mM NMDG, 2.5 mM KCl, 1.35 mM NaH_2PO_4, 30 mM $NaHCO_3$, 20 mM HEPES, 25 mM glucose, 2 mM thiourea, 5 mM Na+ ascorbate, 3 mM Na+ pyruvate, 2 mM N-acetyl-cysteine, 0.5 mM $CaCl_2$, 10 mM $MgSO_4$, 0.6 mM glutathione-ethyl-ester; pH 7.35-7.40, 300-305 mOsm, oxygenated with 95% O_2 and 5% CO_2) with the following inhibitors: 500 nM TTX, 10 μM APV, 10 μM DNQX, 5 μM actinomycin, 22.7 μM anisomycin. Extracted brains were sliced using a vibratome (Leica, VT1200), the locus coeruleus and its neighboring regions dissected out, and the tissue flash frozen on dry ice and stored at -80°C. Later, coerulear sections were lysed, homogenized with glass pestle, filtered via a 40 μm strainer, centrifuged initially to remove cellular debris, and again in differing density solvents (50% and 30% OptiPrep) in which the pelleted nuclei were collected. Nuclei were resuspended in PBS as needed for >400 nuclei/μl.

The cDNA libraries were constructed using the Chromium Next GEM Single Cell 3' Reagents Kits v3.1 Dual Index, 10x Genomics. Briefly, ~10,000 nuclei were load into the chip, single nuclear mRNAs were captured by barcoded beads, and the transcripts were reverse transcribed. The resulting cDNAs were then PCR amplified, fragmented, ligated with adaptors, and added with sample index. cDNA libraries were sequenced on Illumina Hiseq 2x150bp and NovaSeq PE150, and sequence alignment to mouse genome done with 10x Genomics Cell Ranger Count v6.1.2 pipeline.

Integration of data sets, clustering, and differential gene expression analysis were performed using Seurat v4.0[74] and R. Nuclei of potentially low quality (total UMI<700 or total UMI>15,000, or percentage of mitochondrial genes >20%) were removed from downstream analysis. Suspected doublet cells were computationally removed using DoubletDecon 1.02[75]. Cells were clustered by weighted-nearest neighbor analysis of normalized gene expression and these

clusters were validated using known markers for cell types in the brain[74]. Differentially-expressed genes between clusters were identified using a non-parametric Wilcoxon rank sum test[76]. Gene lists were acquired and modified from ontology lists at the Mouse Genome Database (MGD), Jackson Laboratory, Bar Harbor, Maine (informatics.jax.org) (December 2021)[77].

PIXEL-seq

Pixel-seq was performed as previously described[59]. Crosslinked polyacrylamide gels were cast on a coverslip and 368-bp DNA templates were synthesized on the gel. Each template contained 24-bp random spatial indices, which served as spatial barcodes, a 20-bp poly(T) probe, which captures RNAs, and two Taq1 restriction sites, which are digested to expose the poly(T) probes for RNA capture. Polonies were amplified to reduce gaps between the polony templates, and polony sequencing using sequencing-by-synthesis was used to build a spatial index of barcode coordinates. A 10μm-thick section of fresh-frozen brain containing the LC was dissected and placed on the gel in a flat layer. Next, *in situ* reverse transcriptase reaction was performed, followed by library construction. cDNA libraries were sequenced on NovaSeq PE150. Finally, spatial barcodes were used to match captured transcripts to their location within the sample.

Fiber photometry

Fiber photometry recordings were performed as previously described[71]. Briefly, an optic fiber was attached to the implanted fiber by a ferrule sleeve, then GCaMP6s was stimulated by two LEDs, a 531-Hz sinusoidal light (Thorlabs M470F3), bandpass filtered at 470 ± 20nm, and a 211-Hz sinusoidal light (Thorlabs M405FP1), bandpass filtered at 405 ± 10nm. (Filter cube: Doric FMC4; LED driver: DC4104). The 470 nm signal evokes Ca^{2+}-dependent emission, while the 405 nm signal evokes Ca^{2+}-independent isosbestic control emission. Prior to recording, a 180s period of GCaMP6s excitation with both light channels was used to remove the majority of baseline drift. Laser intensity at the optic fiber tip was adjusted to ~50 μW before each day of recording. GCaMP6s fluorescent signal was isolated by bandpass filtering (525 ± 25nm), transduced by a femtowatt silicon photoreceiver (Newport 2151), and recorded by a real-time processor (TDT RZ5P). The envelopes of 531 Hz and 211 Hz signals were extracted in real time by the TDT program Synapse at a sampling rate of 1017.25 Hz.

Fiber recordings were analyzed using custom MATLAB scripts available on Github. Baseline drift due to slow photobleaching artifacts was corrected by fitting a double exponential curve to the raw trace, then the photometry trace was z-scored relative to the mean and standard deviation of the signal. The mean z-score during the ten seconds preceding and following an event were compared using paired t tests.

Behavior

Odor delivery

Odor delivery was performed in a polyethylene chamber approximately 12 x 12 x 24 cm, out of which air was continuously vacuumed at a rate of 2L/min. To minimize odor release into the room, the chamber was placed in a fume hood and the vacuumed air was passed through a carbon filter system. Mice were habituated to the room and chamber three times before odor was delivered and were habituated to the room for 30 min before testing. Odor was delivered for 30s periods.

Food consumption

Feeding assays were performed in a square plexiglass arena (27cm × 27cm). Mice were habituated to the room and chamber at least 3 times before testing and the room 30 min before

trials. For food-deprived conditions, mice were food deprived for 24 h before trial. For highly-palatable food, mice were habituated to the food item one week before trial to eliminate food neophobia. Food items were placed in the corner of the arena and mice were introduced to the area for 30 min. To record the amount of food consumed, food was measured before and after the assay; the bottom of the chamber was scraped after each trial to collect all crumbs resulting from chewing without consumption. When multiple stimulation or inhibition conditions were tested, they were performed in a counterbalanced fashion across mice. For DREADD experiments, clozapine N-oxide (CNO) (1 mg/kg, Enzo Life Sciences, Cat#BML-NS105) was administered 30 minutes prior to testing.

Threat Conditioning

Pavlovian threat conditioning was performed in Med Associates Fear Conditioning Chambers (NIR-022MD). This equipment consisted of a conductive grid floor inside a 29.53cm L x 23.5cm W x 20.96cm H chamber inside of a soundproof box lit by an infrared light. Stimuli occurred at random timing, at least 110-140s apart. On day 1, mice were exposed to six 20s tones without an associated shock. On day 2, mice were conditioned to six 20s tones co-terminating with 1-s 0.5mA shocks. On Day 3, mice were presented with six 20s tones in the absence of shock.

False food

False food testing was performed as previously described[78]. Mice were food deprived for 24 hours before testing, and were habituated to the room for 30 minutes before testing. Mice were then placed in a dimly-lit (25 lux) plexiglass arena (27 cm x 27 cm) with a foam plug shaped like a piece of chow placed in the middle. Videos were scored for attempts to eat the false food.

Novel object

Novel object testing was performed as described previously[28]. Briefly, mice were habituated to the room for 30 minutes before testing. Mice were then placed in a dimly-lit (25 lux) plexiglass arena (27 cm x 27 cm) with two identical objects (test tubes or petri dishes; counterbalanced) fixed at the corners. Mice were given ten minutes to explore the objects, then returned to the homecage for one hour, then were placed in the arena with one familiar object and one novel object. Objects were thoroughly cleaned with ethanol and allowed to dry between trials. Videos were scored for interactions with each object.

Elevated Zero Maze (EZM)

EZM testing was performed as previously described[72]. The EZM (Harvard Apparatus) was made of grey plastic, 200 cm in circumference, comprised of four 50 cm sections (two opened and two closed). The maze was elevated 50 cm above the floor and had a path width of 4 cm with a 0.5 cm lip on each open section. Mice were connected to fiber optic cables, positioned head-first into a closed arm, and allowed to roam freely for 7 min. Open arm time was the primary measure of anxiety-like behavior. For optogenetic experiments, animals received 4 Hz (5 ms pulse width) constant photostimulation (2mW light power). For DREADD experiments, CNO was administered 30 minutes prior to testing.

Open Field Test (OFT)

OFT was performed as described previously[72] in a plexiglass arena (50 cm x 50 cm). Center zone was defined as the middle 50% of the arena size. Mice were connected to fiber optic cables, positioned head first into a closed arm, and allowed to roam freely for 20 min. For optogenetic experiments, animals received 4 Hz (5 ms pulse width) constant photostimulation (2 mW light

power). For DREADD experiments, CNO was administered 30 minutes prior to testing. Center time was the primary measure of anxiety-like behavior.

Real-Time Place Preference (RTPP)

Mice were placed into a custom-made unbiased, balanced two-compartment conditioning apparatus (52.5cm x 25.5cm x 25.5cm) as previously described[72] and allowed to freely roam the entire apparatus for 20 min. Entry into one compartment triggered constant photostimulation (0-20 Hz; 2 mW light power) while the animal remained in the light-paired chamber. Entry into the other chamber ended the photostimulation. The side paired with photostimulation was counterbalanced across mice and between days. Time spent in each chamber and total distance traveled was measured using Ethovision 10 (Noldus).

Light-dark box

Light-dark box was performed in a RTPP chamber where one side was brightly lit (1000 lux) and the other side was dimly lit (5 lux). Animals were allowed to freely move throughout the chamber for 20 minutes. Time spent in the light side was the primary measure of anxiety-like behavior. For optogenetic experiments, animals received 4 Hz (5 ms pulse width) constant photostimulation (2mW light power). For DREADD experiments, CNO was administered 30 min prior to testing.

Foot pinch

Animals were lightly anesthetized at 1.5% isoflurane and placed in a stereotax. Their pupil size was monitored under infrared lighting[35]. Mice were pinched on their contralateral paw for 10s.

Tail flick

The tail-flick assay was performed as previously described[79]. 2-4 cm of the mouse's tail was immersed into 54°C water and the time between immersion and withdrawal was recorded, up to a maximum of 10s in order to prevent tissue damage. Mice were injected with 1mg/kg CNO after the initial test (time 0) and tested every 10 min for one hour, then again at 90 min.

Hot plate

The hot-plate assay was performed as previously described[80]. Mice were placed on a 55°C hot plate and the time for a nocifensive response (paw withdrawal or licking, stamping, leaning, or jumping) was recorded, up to a maximum of 30s in order to prevent tissue damage. Mice were injected with 1mg/kg CNO 30 min before the test.

Statistical analyses

All summary data are expressed as mean ± SEM. Statistical significance was taken as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, as determined by two-tailed Student's t-test (paired and unpaired) or two-way repeated measure analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. Statistical analyses were performed in GraphPad Prism 9. For sequencing experiments, analyses were performed as described in the corresponding methods section.

Data availability: All data are available in the manuscript or the supplementary materials. RNA sequencing data is deposited in in the NCBI Gene Expression Omnibus (GEO) database with

accession number GSE201998. The full behavioral dataset supporting the current study are available from the corresponding author upon reasonable request.

Code availability: Custom MATLAB analysis code was created to appropriately organize, process, and combine photometry recording data with associated behavioral data. Custom R scripts were written to analyze single nuclei RNA sequencing datasets. Code supporting the current study are available from the corresponding author upon reasonable request.

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Acknowledgments: We thank Dylan Blumenthal, Michelle Chung, Megan Votoupal, Taylor Hobbs, and Carina Pizzano for animal colony maintenance; Christine Stander and Azra Suko for laboratory management and support; the Bruchas laboratory and UW NAPE Center for discussions; Marcus Basiri and the Stuber Lab for assistance with single-cell sequencing; Jane Chen and Richard Palmiter for mouse lines and assistance with single-cell sequencing. We thank Chris Stander, K. Deisseroth, the Washington University HOPE Vector Core, and the University of North Carolina (UNC) Vector Core for viral constructs, prep and packaging.

Funding: This study was supported by the National Institute of Mental Health (ATL, F31-MH122033; RCS, F31-MH117931; CPF & KB, R21-MH123085; MRB, R01-MH112355), the National Institute on Drug Abuse (LL, K99-DA054446; SAG, R00-DA045662, UG3-DA053802, & R01-DA054317; GDS, R37-DA032750 & R01-DA038168; CPF, R01-DA035821; LG & MRB, R61- DA051489; MRB, P30-DA048736), the National Cancer Institute (LG, UG3-CA268096), the National Institute of General Medical Sciences (ATL, T32-GM008151), the Foundation for Anesthesia Education and Research (LL), and the NARSAD Young Investigator Award 27082 (SAG). The content is solely the responsibility of the authors and does not necessarily represent the views of the National Institutes of Health nor any other funding institution.

Author contributions:

ATL, LL and MRB conceptualized and designed the entire study. ATL performed stereotaxic surgeries. ATL and TB performed behavioral experiments. ATL, LL, TB, EML, and AR performed histology. ATL, LL, XF, and LS performed Pixel-seq experiments. KB performed electrophysiological experiments. ATL and TB performed fiber photometry experiments. ATL, LL, and RCS performed single-nuclei RNA sequencing experiments and analysis. ATL, LL, and KB performed statistical analysis. LL and ADM performed tissue clearing and imaging. ATL, LL, XF, KB, and MRB made the figures. ATL, LL, and MRB wrote the manuscript with collective

input from all authors. SAG supervised tissue clearing and imaging. GS supervised single-nuclei sequencing. CPF supervised electrophysiological experiments. LG supervised Pixel-seq experiments. MRB supervised the entire study.

Competing interests: A provisional patent related to Pixel-seq has been filed by the University of Washington.

Supplementary Information:

Supplementary Table S1 – Sexually Dimorphic Genes.xlsx

Sexually dimorphic genes in all neurons, NE neurons, and GABA neurons are shown in order of decreasing significance.

Supplementary Table S2 – All Genes of Interest.xlsx

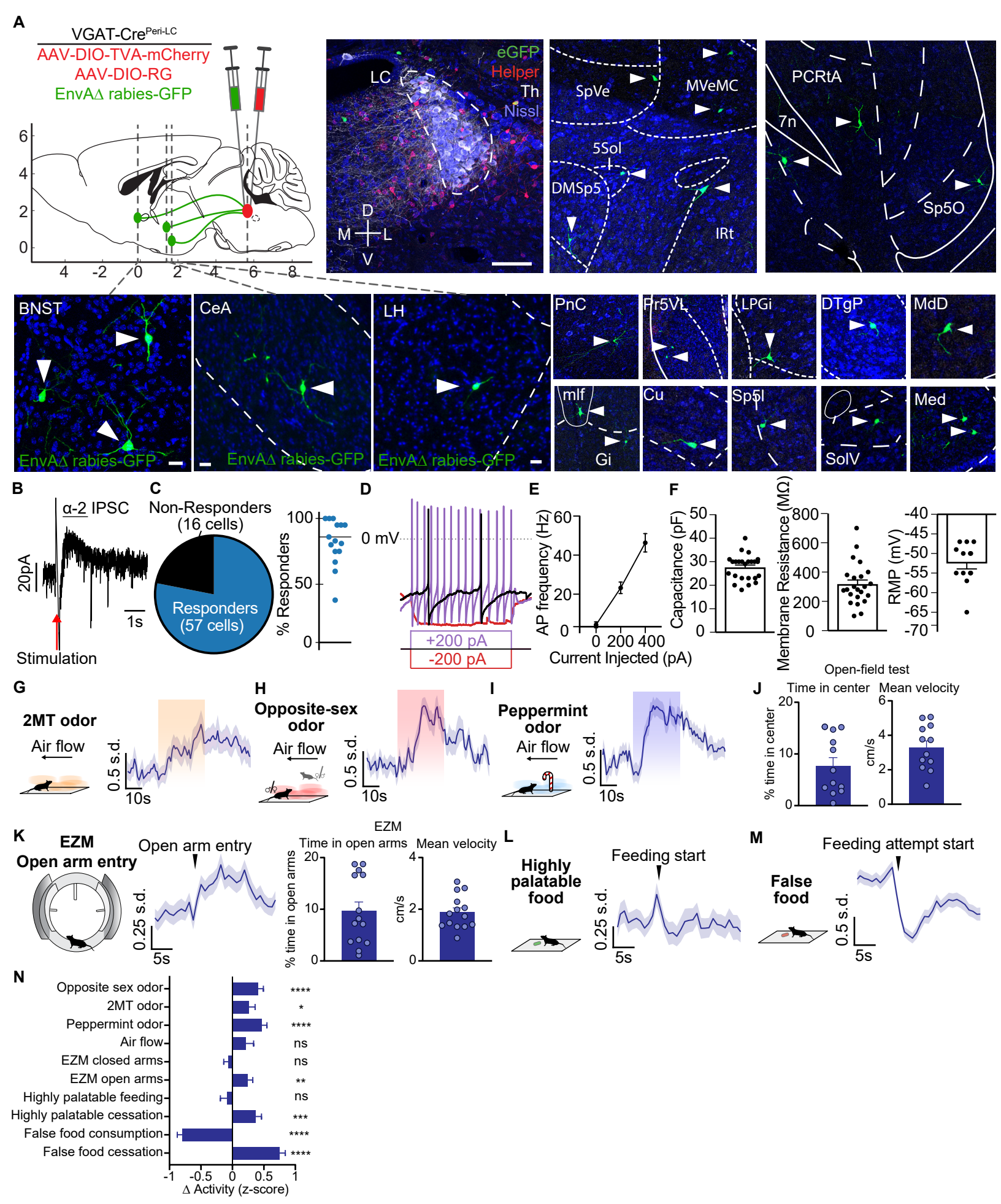
Relative expression of genes in clusters of cells in all neurons, NE neurons, and GABA neurons.

Supplementary Materials – Pathology, Neurotransmission, and GPCR-related genes

Dot plots of relative expression of genes involved in neuropathology, ion channels, GPCRs, and neurotransmission in clusters of cells in all neurons, NE neurons, and GABA neurons.

Supplementary Video 1

The video shows a representative 3D rendering in a cleared brain tissue of anti-tyrosine hydroxylase-stained LC neurons (green) and the neighboring anti-EYFP stained peri-LC GABAergic neurons (red) expressing ChR2-eYFP in a cre-dependent manner in a vGAT-cre mouse.


A
VGAT-Cre Peri-LC
AAV-DIO-TVA-mCherry
AAV-DIO-RG
EnvAΔ rabies-GFP
LC
eGFP
Helper
Th
Nissl
D
M
L
V
SpVe
MVeMC
5Sol
DMSp5
IRt
PCRtA
7n
Sp5O
BNST
CeA
LH
PnC
Pr5VL
LPGi
DTgP
MdD
mlf
Gi
Cu
Sp5I
SolV
Med
EnvAΔ rabies-GFP
B
α-2 IPSC
20pA
1s
Stimulation
C
Non-Responders
(16 cells)
Responders
(57 cells)
% Responders
D
0 mV
+200 pA
-200 pA
E
AP frequency (Hz)
Current Injected (pA)
F
Capacitance (pF)
Membrane Resistance (MΩ)
RMP (mV)
Open-field test
G
2MT odor
Air flow
0.5 s.d.
10s
H
Opposite-sex odor
Air flow
0.5 s.d.
10s
I
Peppermint odor
Air flow
0.5 s.d.
10s
J
Time in center
Mean velocity
% time in center
cm/s
K
EZM
Open arm entry
Open arm entry
0.25 s.d.
5s
EZM
Time in open arms
Mean velocity
% time in open arms
cm/s
L
Highly palatable food
Feeding start
0.25 s.d.
5s
M
False food
Feeding attempt start
0.5 s.d.
5s
N
Opposite sex odor ****
2MT odor *
Peppermint odor ****
Air flow ns
EZM closed arms ns
EZM open arms **
Highly palatable feeding ns
Highly palatable cessation ***
False food consumption ****
False food cessation ****
Δ Activity (z-score)

Extended Data Fig. 1. *In vitro* and *in vivo* identification and characterization of pericoerulear GABAergic neurons.

(A) Viral schematic for rabies tracing of monosynaptic inputs to peri-LC^{GABA} neurons. Starter cells in peri-LC express TVA-mCherry and rabies-GFP (scale bar: 100 μm in LC; 20 μm in input regions).

(B) Electrically evoked α2-mediated outward current recorded from LC^{NE} neuron.

(C) 78% of LC^{NE} neurons displayed measurable fast IPSCs, though the success rate appeared dependent on injection specificity as 6 of 14 injection sites had 100% response rate (each data point represents one hemisphere, line shown is median; injections that were completely off target and/or had 0% response rate were excluded).

(D) Properties of peri-LC neurons. ChR2-expressing peri-LC neurons were identified by the presence of eYFP expression and a steady state response to a 150 ms light pulse (not shown). Representative trace of a current clamp recording with steps of 200 pA current injection.

(E) Peri-LC^{GABA} neuron firing rate upon current injection (mean values ± SEM).

(F) Peri-LC^{GABA} neuron capacitance, membrane resistance, and resting membrane potential (RMP) (mean values ± SEM).

(G) Peri-LC^{GABA} neurons increase activity during 30s exposure to 2MT.

(H) Peri-LC^{GABA} neurons increase activity during 30s exposure to odor of opposite-sex mice.

(I) Peri-LC^{GABA} neurons increase activity during 30s exposure to peppermint oil odor.

(J) Summary of exploration in open-field test.

(K) Peri-LC^{GABA} neuronal activity increases upon open arm entry in an elevated zero maze.

(L) Peri-LC^{GABA} neurons are not affected by the consumption of highly-palatable food.

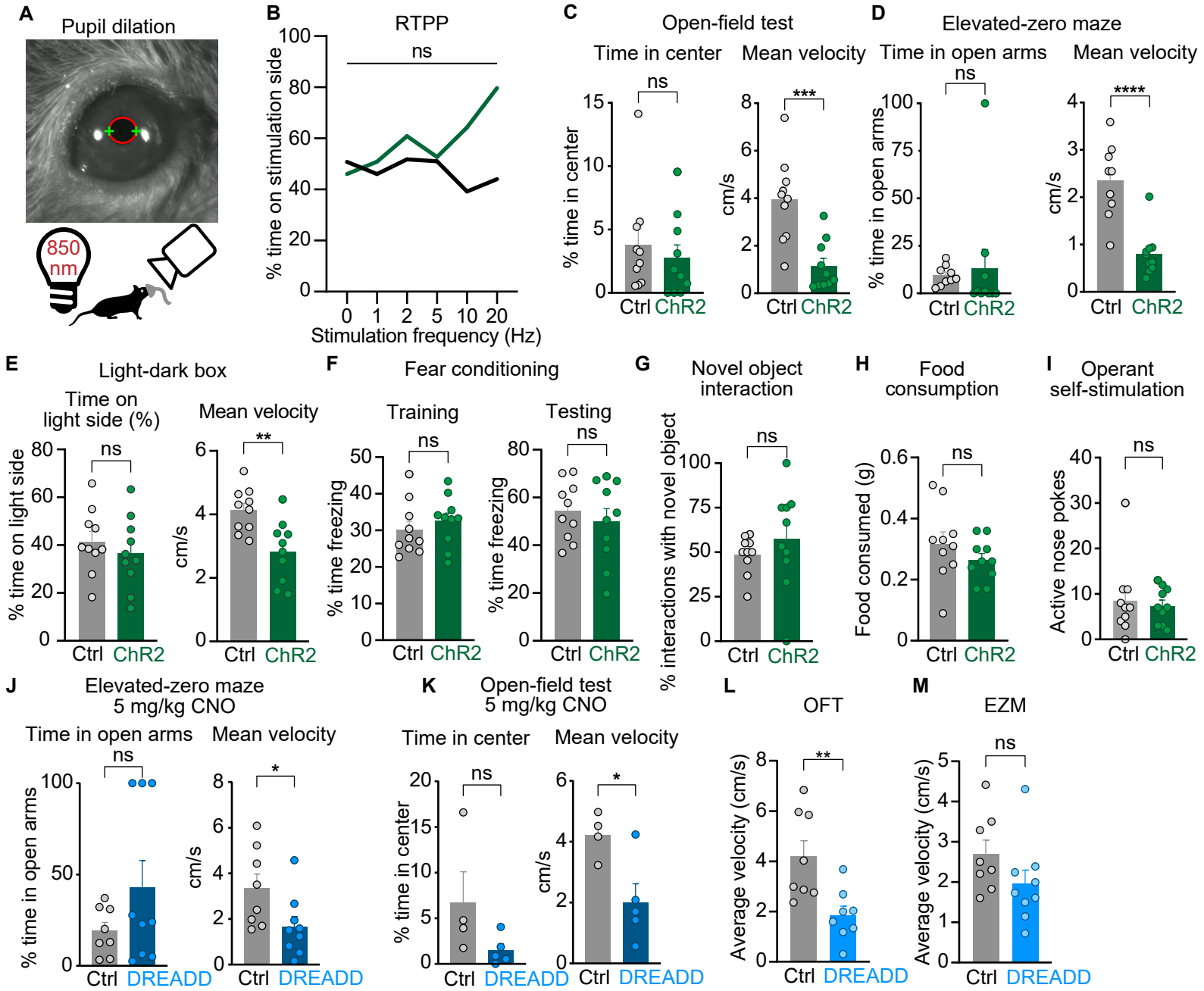
(M) Peri-LC^{GABA} neurons decrease activity during attempts to eat a false food pellet.

(N) Summary of fiber photometry experiments. Bars represent change in activity 10s before and after event (30s before and after for odor exposure experiments).

*$p < 0.05$, ** $p<0.01$, *** $p<0.001$, ****$p<0.0001$ by paired two-tailed *t*-test. Error bars indicate SEM.

Extended Data Table 1.
Electrophysiological properties of recorded LC and peri-LC GABAergic neurons.

LC-NE neurons			
	IPSC amplitude	53.8 ± 7.3 pA	n/N = 18/9
	Paired pulse ratio (50 ms IPI)	1.13 ± 0.11	n/N = 16/9
	Rise time	3.15 ± 0.47 ms	n/N = 18/9
	Width	16.1 ± 1.7 ms	n/N = 18/9
	Decay constant	17.2 ± 1.2 ms	n/N = 18/9
Peri-LC GABA neurons			
	Capacitance	27.5 ± 1.1 pF	n/N = 24/12
	Membrane resistance	316.3 ± 29.8 mΩ	n/N = 23/12
	Resting membrane potential	-53.5 ± 1.4 mV	n/N = 13/7
	Basal firing rate	3.5 ± 1.0 Hz	n/N = 6/5
	Firing rate +200 pA	11.6 ± 1.5 Hz	n/N = 11/7
	Firing rate +400 pA	23.2 ± 2.3 Hz	n/N = 11/7


A
Pupil dilation
850 nm
B
RTPP
ns
% time on stimulation side
Stimulation frequency (Hz)
C
Open-field test
Time in center
Mean velocity
% time in center
cm/s
D
Elevated-zero maze
Time in open arms
Mean velocity
% time in open arms
E
Light-dark box
Time on light side (%)
Mean velocity
% time on light side
F
Fear conditioning
Training
Testing
% time freezing
G
Novel object interaction
% interactions with novel object
H
Food consumption
Food consumed (g)
I
Operant self-stimulation
Active nose pokes
J
Elevated-zero maze 5 mg/kg CNO
Time in open arms
Mean velocity
K
Open-field test 5 mg/kg CNO
Time in center
Mean velocity
L
OFT
Average velocity (cm/s)
M
EZM
Ctrl
ChR2
DREADD

Extended Data Fig. 2. Selective manipulation of pericoerulear GABA neurons modulates arousal.

(A) Schematic and image of pupil dilation experiment.

(B) Photostimulation did not cause a real-time place preference.

(C-E) Photostimulation (2 mW, 4 Hz, 5 ms pulse width) affected movement, but not anxiety-like behavior, in open-field test (C), elevated-zero maze (D), and light-dark box (E).

(F) Photostimulation (2 mW, 4 Hz, 5 ms pulse width during 20s presentation of cue during fear conditioning) did not affect acquisition of fear response.

(G) Photostimulation (2 mW, 4 Hz, 5 ms pulse width) did not affect novel object interaction.

(H) Photostimulation (2 mW, 4 Hz, 5 ms pulse width) did not affect food consumption in food-deprived state.

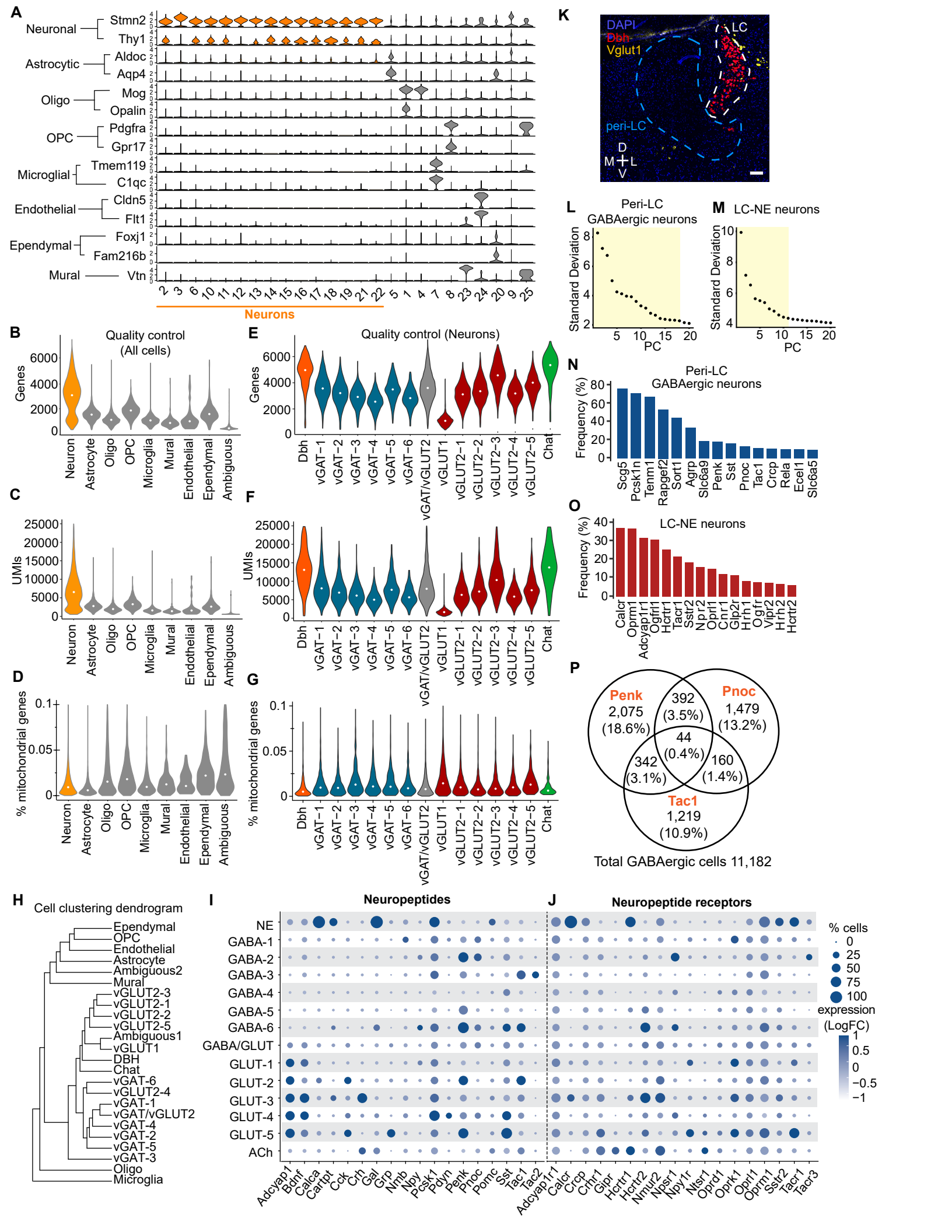
(I) Animals did not preferentially nose-poke to receive photostimulation (2 mW, 4 Hz, 5 ms pulse width).

(J, K) Chemogenetic inhibition (5 mg/kg CNO) of peri-LC^{GABA} neurons caused behavioral arrest in elevated-zero maze and open-field test.

(L) Chemogenetic inhibition (1 mg/kg CNO) of peri-LC^{GABA} neurons decreased movement in open-field test.

(M) Chemogenetic inhibition (1 mg/kg CNO) of peri-LC^{GABA} neurons did not affect movement in elevated-zero maze.

*$p < 0.05$, ** $p<0.01$, ***$p<.001$, ****$p<0.0001$ by unpaired two-tailed *t*-test. Error bars indicate SEM.


A
Neuronal
Stmn2
Thy1
Astrocytic
Aldoc
Aqp4
Oligo
Mog
Opalin
OPC
Pdgfra
Gpr17
Microglial
Tmem119
C1qc
Endothelial
Cldn5
Flt1
Ependymal
Foxj1
Fam216b
Mural
Vtn
Neurons
B
Quality control (All cells)
Genes
Neuron
Astrocyte
Oligo
OPC
Microglia
Mural
Endothelial
Ependymal
Ambiguous
C
UMIs
D
% mitochondrial genes
E
Quality control (Neurons)
Dbh
vGAT−1
vGAT−2
vGAT−3
vGAT−4
vGAT−5
vGAT−6
vGAT/vGLUT2
vGLUT1
vGLUT2−1
vGLUT2−2
vGLUT2−3
vGLUT2−4
vGLUT2−5
Chat
F
G
H
Cell clustering dendrogram
Ependymal
OPC
Endothelial
Astrocyte
Ambiguous2
Mural
vGLUT2-3
vGLUT2-1
vGLUT2-2
vGLUT2-5
Ambiguous1
vGLUT1
DBH
Chat
vGAT-6
vGLUT2-4
vGAT-1
vGAT/vGLUT2
vGAT-4
vGAT-2
vGAT-5
vGAT-3
Oligo
Microglia
I
Neuropeptides
NE
GABA-1
GABA-2
GABA-3
GABA-4
GABA-5
GABA-6
GABA/GLUT
GLUT-1
GLUT-2
GLUT-3
GLUT-4
GLUT-5
ACh
Adcyap1
Bdnf
Calca
Cartpt
Cck
Crh
Gal
Grp
Nmb
Npy
Pcsk1
Pdyn
Penk
Pnoc
Pomc
Sst
Tac1
Tac2
J
Neuropeptide receptors
Adcyap1r1
Calcr
Crcp
Crhr1
Gipr
Hcrtr1
Hcrtr2
Nmur2
Npsr1
Npy1r
Nlsr1
Oprd1
Oprk1
Oprl1
Oprm1
Sstr2
Tacr1
Tacr3
% cells
0
25
50
75
100
expression (LogFC)
1
0.5
0
−0.5
−1
K
DAPI
Dbh
Vglut1
LC
peri-LC
D
M
L
V
L
Peri-LC GABAergic neurons
Standard Deviation
PC
M
LC-NE neurons
N
Peri-LC GABAergic neurons
Frequency (%)
Scg5
Pcsk1n
Tenm1
Rapgef2
Sort1
Agrp
Slc6a9
Penk
Sst
Pnoc
Tac1
Crcp
Rela
Ecel1
Slc6a5
O
LC-NE neurons
Calcr
Oprm1
Adcyap1r1
Ogfrl1
Hcrtr1
Tac1
Sstr2
Npr2
Oprl1
Cnr1
Glp2r
Hrh1
Ogfr
Vipr2
Hrh2
Hcrtr2
P
Penk
2,075
(18.6%)
392
(3.5%)
Pnoc
1,479
(13.2%)
44
(0.4%)
342
(3.1%)
160
(1.4%)
Tac1
1,219
(10.9%)
Total GABAergic cells 11,182

Extended Data Fig. 3. Single-cell RNA-sequencing of locus coeruleus and pericoerulear region.

(A) Violin plots show expression of canonical marker genes in cells clustered by PCA based on transcriptional profile. The 15 neuronal clusters are shown in orange.

(B) Genes detected per cell type.

(C) Unique molecular identifiers (UMIs) per cell type.

(D) Percent mitochondrial genes per cell type.

(E) Genes detected per neuronal cell type.

(F) Unique molecular identifiers (UMIs) per neuronal cell type.

(G) Percent mitochondrial genes per neuronal cell type.

(H) Cluster tree shows relationships between clustered cell types.

(I,J) Dot plots of n=12,278 neurons are shown, with neuronal clusters on the y-axis and gene transcripts on the x-axis. Size of circles corresponds to percent of cells in the cluster expressing a specific transcript, while color intensity corresponds to the relative expression level of the transcript. Plots correspond to transcripts related neuropeptides (**I**), and neuropeptide receptors (**J**).

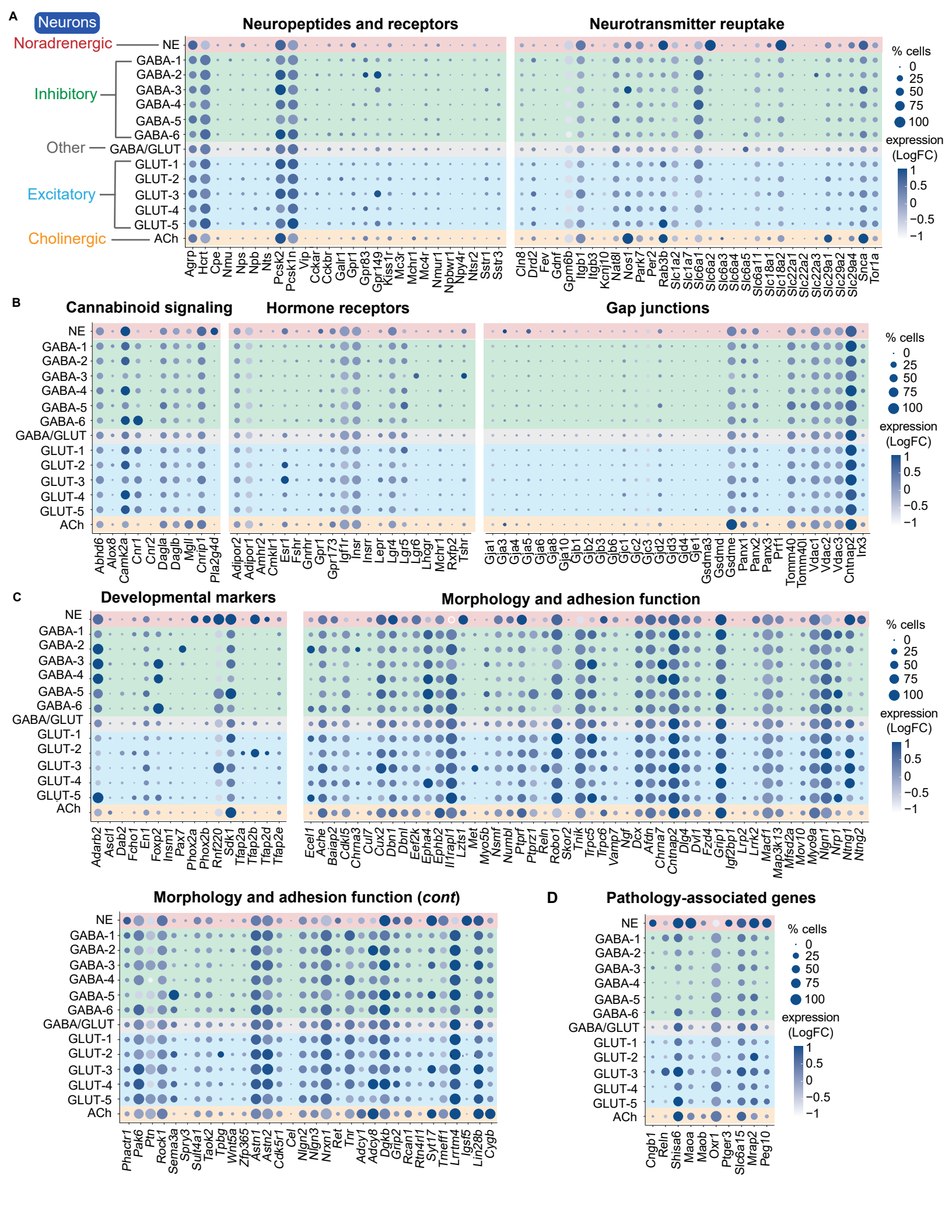
(K) Representative image for *in situ* hybridization of *Dbh* and vGLUT1 (*Slc17a7*) mRNA. Scale bar is 100 μm.

(L,M) Elbow plots show the number of principle components (PC) versus standard deviation for **(L)** peri-LC inhibitory neurons (Slc32a1, Gad1, Gad2) and **(M)** LC-NE neurons (Dbh, Th).

(N) Graph shows the frequency of neuropeptide-expressing peri-LC inhibitory neurons.

(O) Graph shows the frequency of neuropeptide receptor-expressing LC-NE neurons.

(P) Venn diagram shows the overlap of Penk-, Pnoc-, and Tac1-expressing neurons.


A
Neurons
Noradrenergic
Inhibitory
Other
Excitatory
Cholinergic
NE
GABA-1
GABA-2
GABA-3
GABA-4
GABA-5
GABA-6
GABA/GLUT
GLUT-1
GLUT-2
GLUT-3
GLUT-4
GLUT-5
ACh
Neuropeptides and receptors
Agrp Hcrt Cpe Nmu Nps Npb Nts Pcsk2 Pcsk1n Vip Cckar Cckbr Galr1 Gpr1 Gpr83 Gpr149 Kiss1r Mc3r Mchr1 Mc4r Nmur1 Npbwr1 Npy4r Ntsr2 Sstr1 Sstr3
Neurotransmitter reuptake
Cln8 Drd2 Fev Gdnf Gpm6b Itgb1 Itgb3 Kcnj10 Nat8l Nos1 Park7 Per2 Rab3b Slc1a2 Slc1a7 Slc6a1 Slc6a2 Slc6a3 Slc6a4 Slc6a5 Slc6a11 Slc18a1 Slc18a2 Slc22a1 Slc22a2 Slc22a3 Slc29a1 Slc29a2 Slc29a4 Snca Tor1a
% cells
0
25
50
75
100
expression (LogFC)
1
0.5
0
−0.5
−1
B
Cannabinoid signaling
Abhd6 Alox8 Camk2a Cnr1 Cnr2 Dagla Daglb Mgll Cnrip1 Pla2g4d
Hormone receptors
Adipor2 Adipor1 Amhr2 Cmklr1 Esr1 Fshr Gnrhr Gpr1 Gpr173 Igf1r Insr Insrr Lepr Lgr4 Lgr5 Lgr6 Lhcgr Mchr1 Rxfp2 Tshr
Gap junctions
Gja1 Gja3 Gja4 Gja5 Gja6 Gja8 Gja10 Gjb1 Gjb2 Gjb3 Gjb6 Gjc1 Gjc2 Gjc3 Gjd2 Gjd3 Gjd4 Gje1 Gsdma3 Gsdmd Gsdme Panx1 Panx2 Panx3 Prf1 Tomm40 Tomm40l Vdac1 Vdac2 Vdac3 Cntnap2 Irx3
C
Developmental markers
Adarb2 Ascl1 Dab2 Fcho1 En1 Foxp2 Insm1 Pax7 Phox2a Phox2b Rnf220 Sdk1 Tfap2a Tfap2b Tfap2d Tfap2e
Morphology and adhesion function
Ecel1 Ache Baiap2 Cdkl5 Chrna3 Cul7 Cux2 Dbn1 Dbnl Eef2k Epha4 Ephb2 Il1rapl1 Lzts1 Met Myo5b Nsmf Numbl Ptprf Ptprz1 Reln Robo1 Skor2 Tnik Trpc5 Trpc6 Vamp7 Ngf Dcx Afdn Chrna7 Cntnap2 Dlg4 Dvl1 Fzd4 Grip1 Igf2bp1 Lrp2 Lrrk2 Macf1 Map3k13 Mfsd2a Mov10 Myo9a Nlgn1 Nrp1 Ntng1 Ntng2
Morphology and adhesion function (cont)
Phactr1 Pak6 Ptn Rock1 Sema3a Spry3 Sulf4a1 Taok2 Tpbg Wnt5a Zfp365 Astn1 Astn2 Cdk5r1 Cel Nlgn2 Nlgn3 Nrxn1 Ret Tnr Adcy1 Adcy8 Dgkb Grip2 Rcan1 Rtn4rl1 Syt17 Tmeff1 Lrrtm4 Igsf5 Lin28b Cygb
D
Pathology-associated genes
Cngb1 Reln Shisa6 Maoa Maob Oxr1 Ptger3 Slc6a15 Mrap2 Peg10

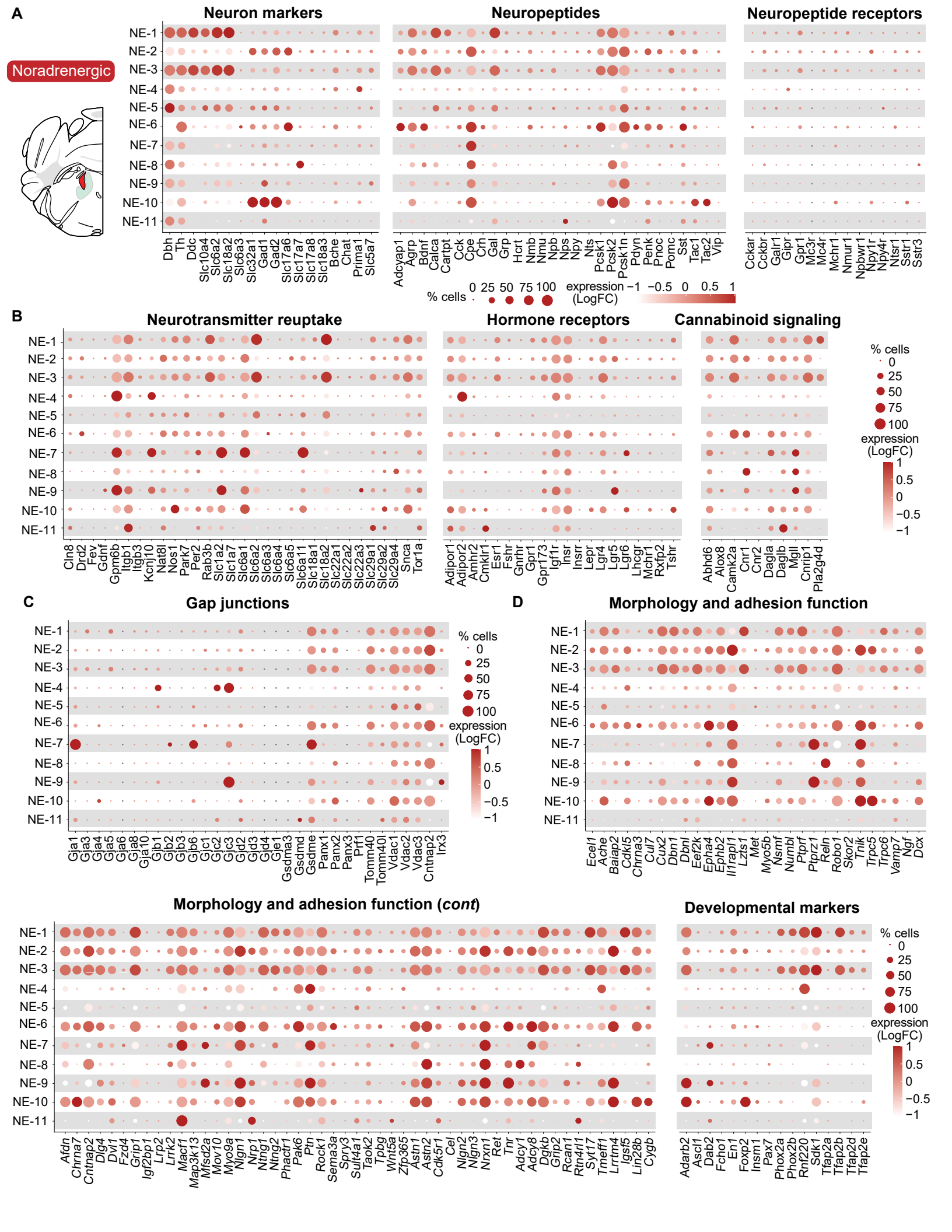
Extended Data Fig. 4. Neuromodulatory, signaling, structural, and disease-related genes in single-cell RNA sequencing of all peri-LC neurons (n=12,278).

(A) Dot plot of neuropeptide and receptor (left), and neurotransmitter reuptake (right) genes across all neuron clusters. Circle size corresponds to percent of cells in the cluster expressing the specific transcript, while color intensity corresponds to its relative expression.

(B) Dot plot of genes associated with cannabinoid signaling (left), hormone signaling (middle), and gap junctions (right) across all neuron clusters.

(C) Dot plot of genes associated with development (left), and morphology and adhesion functions (right) across all neuron clusters.

(D) Dot plot of genes associated with pathology across all neuron clusters.


A
Noradrenergic
Neuron markers
Neuropeptides
Neuropeptide receptors
NE-1
NE-2
NE-3
NE-4
NE-5
NE-6
NE-7
NE-8
NE-9
NE-10
NE-11
Dbh Th Ddc Slc10a4 Slc6a2 Slc18a2 Slc6a3 Slc32a1 Gad1 Gad2 Slc17a6 Slc17a7 Slc17a8 Slc18a3 Bche Chat Prima1 Slc5a7
Adcyap1 Agrp Bdnf Calca Cartpt Cck Cpe Crh Gal Grp Hcrt Nmb Nmu Npb Nps Npy Nts Pcsk1 Pcsk2 Pcsk1n Pdyn Penk Pnoc Pomc Sst Tac1 Tac2 Vip
Cckar Cckbr Galr1 Gipr Gpr1 Mc3r Mc4r Mchr1 Nmur1 Npbwr1 Npy1r Npy4r Ntsr1 Sstr1 Sstr3
% cells 0 25 50 75 100
expression (LogFC) −1 −0.5 0 0.5 1
B
Neurotransmitter reuptake
Hormone receptors
Cannabinoid signaling
Cln8 Drd2 Fev Gdnf Gpm6b Itgb1 Itgb3 Kcnj10 Nat8l Nos1 Park7 Per2 Rab3b Slc1a2 Slc1a7 Slc6a1 Slc6a2 Slc6a3 Slc6a4 Slc6a5 Slc6a11 Slc18a1 Slc18a2 Slc22a1 Slc22a2 Slc22a3 Slc29a1 Slc29a2 Slc29a4 Snca Tor1a
Adipor1 Adipor2 Amhr2 Cmklr1 Esr1 Fshr Gnrhr Gpr1 Gpr173 Igf1r Insr Insrr Lepr Lgr4 Lgr5 Lgr6 Lhcgr Mchr1 Rxfp2 Tshr
Abhd6 Alox8 Camk2a Cnr1 Cnr2 Dagla Daglb Mgll Cnrip1 Pla2g4d
% cells 0 25 50 75 100
expression (LogFC) 1 0.5 0 −0.5 −1
C
Gap junctions
Gja1 Gja3 Gja4 Gja5 Gja6 Gja8 Gja10 Gjb1 Gjb2 Gjb3 Gjb6 Gjc1 Gjc2 Gjc3 Gjd2 Gjd3 Gjd4 Gje1 Gsdma3 Gsdmd Gsdme Panx1 Panx2 Panx3 Prf1 Tomm40 Tomm40l Vdac1 Vdac2 Vdac3 Cntnap2 Irx3
D
Morphology and adhesion function
Ecel1 Ache Balap2 Cdkl5 Chrna3 Cul7 Cux2 Dbn1 Dbnl Eef2k Epha4 Ephb2 Il1rapl1 Lzts1 Met Myo5b Nsmf Numbl Ptprf Ptprz1 Reln Robo1 Skor2 Tnik Trpc5 Trpc6 Vamp7 Ngf Dcx
Morphology and adhesion function (cont)
Afdn Chrna7 Cntnap2 Dlg4 Dvl1 Fzd4 Grip1 Igf2bp1 Lrp2 Lrrk2 Macf1 Map3k13 Mfsd2a Mov10 Myo9a Nlgn1 Nrp1 Ntng1 Ntng2 Phactr1 Pak6 Ptn Rock1 Sema3a Spry3 Sult4a1 Taok2 Tpbg Wnt5a Zfp365 Astn1 Astn2 Cdk5r1 Cel Nlgn2 Nlgn3 Nrxn1 Ret Tnr Adcy1 Adcy8 Dgkb Grip2 Rcan1 Rtn4rl1 Syt17 Tmeff1 Lrrtm4 Igsf5 Lin28b Cygb
Developmental markers
Adarb2 Ascl1 Dab2 Fcho1 En1 Foxp2 Insm1 Pax7 Phox2a Phox2b Rnf220 Sdk1 Tfap2a Tfap2b Tfap2d Tfap2e

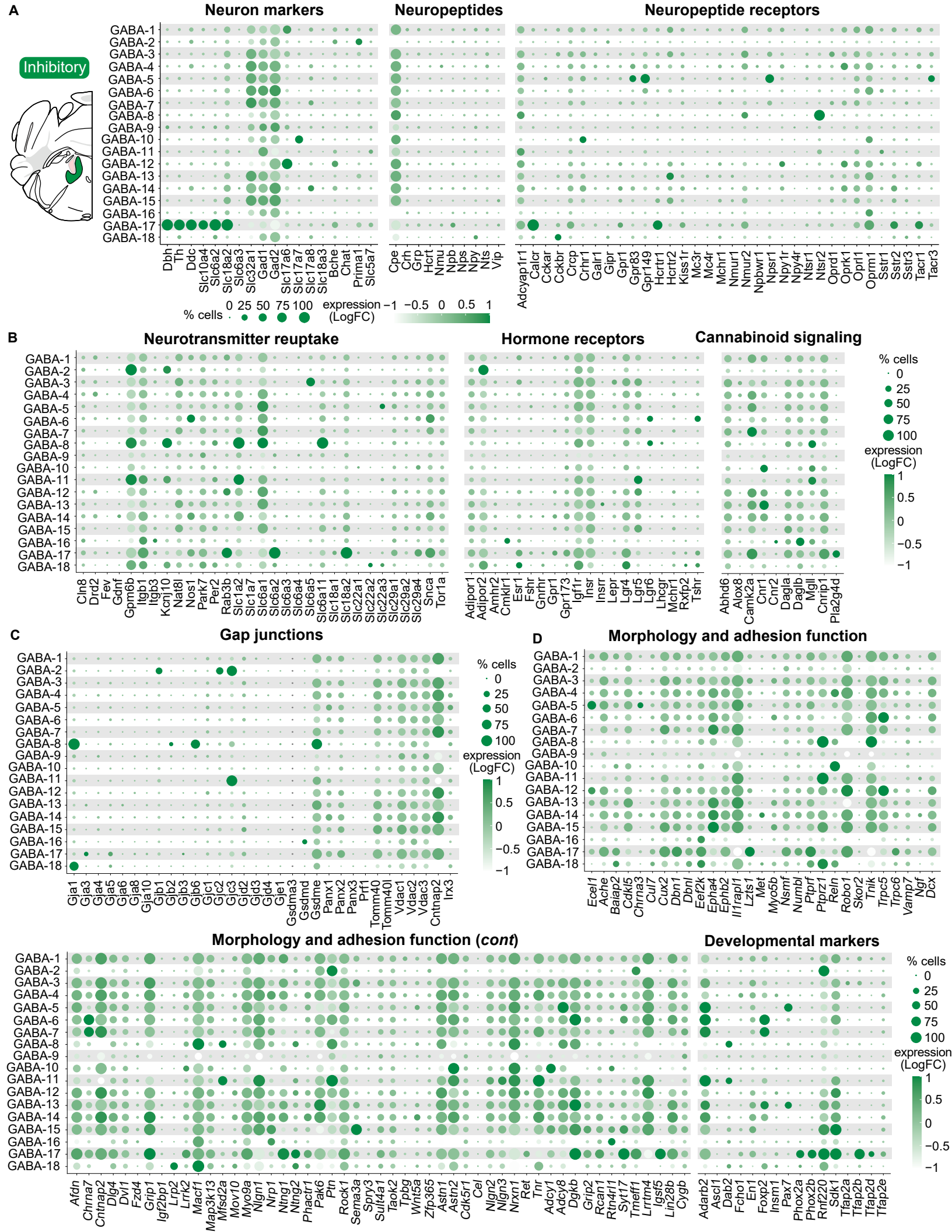
Extended Data Fig. 5. Neuromodulatory, signaling, and structural genes in single-cell RNA sequencing of LC^{NE} neurons (n=1,324).

(A) Cartoon of the sampled locus coeruleus noradrenergic neurons (left). Dot plot of neuronal subtype (left), neuropeptide (middle), and receptor (right) genes across LC^{NE} neuron clusters. Circle size corresponds to percent of cells in the cluster expressing the specific transcript, while color intensity corresponds to its relative expression.

(B) Dot plot of genes associated with neurotransmitter reuptake (left), hormone signaling (middle), and cannabinoid signaling (right) across LC^{NE} neuron clusters.

(C) Dot plot of genes associated with gap junctions across LC^{NE} neuron clusters.

(D) Dot plot of genes associated with morphology and adhesion functions and development across LC^{NE} neuron clusters.


A
Inhibitory
Neuron markers
Neuropeptides
Neuropeptide receptors
GABA-1
GABA-2
GABA-3
GABA-4
GABA-5
GABA-6
GABA-7
GABA-8
GABA-9
GABA-10
GABA-11
GABA-12
GABA-13
GABA-14
GABA-15
GABA-16
GABA-17
GABA-18
Dbh Th Ddc Slc10a4 Slc6a2 Slc18a2 Slc6a3 Slc32a1 Gad1 Gad2 Slc17a6 Slc17a7 Slc17a8 Slc18a3 Bche Chat Prima1 Slc5a7
Cpe Crh Grp Hcrt Nmu Npb Nps Npy Nts Vip
Adcyap1r1 Calcr Cckar Cckbr Crcp Crhr1 Galr1 Gipr Gpr1 Gpr83 Gpr149 Hcrtr1 Hcrtr2 Kiss1r Mc3r Mc4r Mchr1 Nmur1 Nmur2 Npbwr1 Npsr1 Npy1r Npy4r Ntsr1 Ntsr2 Oprd1 Oprk1 Oprl1 Oprm1 Sstr1 Sstr2 Sstr3 Tacr1 Tacr3
% cells
0 25 50 75 100
expression (LogFC)
−1 −0.5 0 0.5 1
B
Neurotransmitter reuptake
Hormone receptors
Cannabinoid signaling
Cln8 Drd2 Fev Gdnf Gpm6b Itgb1 Itgb3 Kcnj10 Nat8l Nos1 Park7 Per2 Rab3b Slc1a2 Slc1a7 Slc6a1 Slc6a2 Slc6a3 Slc6a4 Slc6a5 Slc6a11 Slc18a1 Slc18a2 Slc22a1 Slc22a2 Slc22a3 Slc29a1 Slc29a2 Slc29a4 Snca Tor1a
Adipor1 Adipor2 Amhr2 Cmklr1 Esr1 Fshr Gnrhr Gpr1 Gpr173 Igf1r Insr Insrr Lepr Lgr4 Lgr5 Lgr6 Lhcgr Mchr1 Rxfp2 Tshr
Abhd6 Alox8 Camk2a Cnr1 Cnr2 Dagla Daglb Mgll Cnrip1 Pla2g4d
% cells
0 25 50 75 100
expression (LogFC)
1 0.5 0 −0.5 −1
C
Gap junctions
Gja1 Gja3 Gja4 Gja5 Gja6 Gja8 Gja10 Gjb1 Gjb2 Gjb3 Gjb6 Gjc1 Gjc2 Gjc3 Gjd2 Gjd3 Gjd4 Gje1 Gsdma3 Gsdmd Gsdme Panx1 Panx2 Panx3 Prf1 Tomm40 Tomm40l Vdac1 Vdac2 Vdac3 Cntnap2 Irx3
% cells
0 25 50 75 100
expression (LogFC)
1 0.5 0 −0.5 −1
D
Morphology and adhesion function
Ecel1 Ache Balap2 Cdkl5 Chrna3 Cul7 Cux2 Dbn1 Dbnl Eef2k Epha4 Ephb2 Il1rapl1 Lzts1 Met Myo5b Nsmf Numbl Ptprf Ptprz1 Reln Robo1 Skor2 Tnik Trpc5 Trpc6 Vamp7 Ngf Dcx
Morphology and adhesion function (cont)
Developmental markers
Afdn Chrna7 Cntnap2 Dlg4 Dvl1 Fzd4 Grip1 Igf2bp1 Lrp2 Lrrk2 Macf1 Map3k13 Mfsd2a Mov10 Myo9a Nlgn1 Nrp1 Ntng1 Ntng2 Phactr1 Pak6 Ptn Rock1 Sema3a Spry3 Sult4a1 Taok2 Tpbg Wnt5a Zfp365 Astn1 Astn2 Cdk5r1 Cel Nlgn2 Nlgn3 Nrxn1 Ret Tnr Adcy1 Adcy8 Dgkb Grip2 Rcan1 Rtn4rl1 Syt17 Tmeff1 Lrrtm4 Igsf5 Lin28b Cygb
Adarb2 Ascl1 Dab2 Fcho1 En1 Foxp2 Insm1 Pax7 Phox2a Phox2b Rnf220 Sdk1 Tfap2a Tfap2b Tfap2d Tfap2e

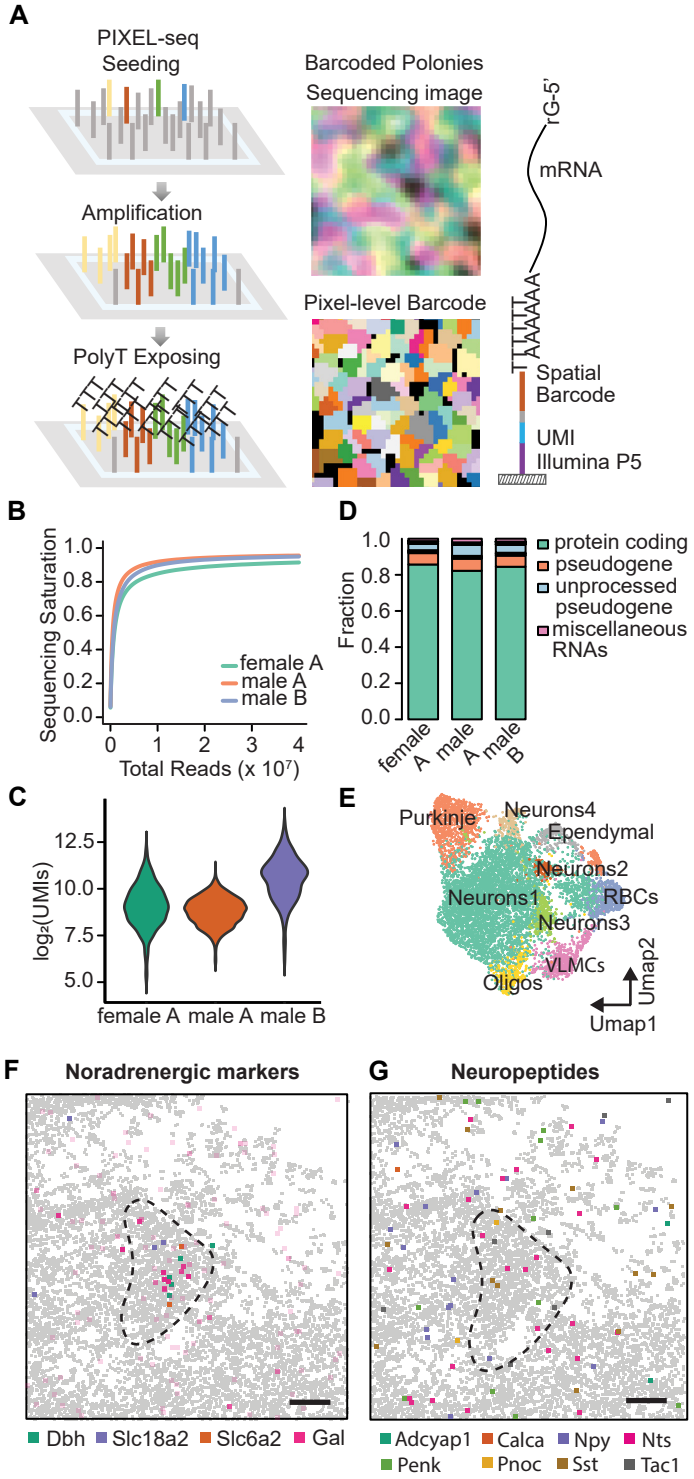
Extended Data Fig. 6. Neuromodulatory, signaling, and structural genes in single-cell RNA sequencing of peri-LC^{GABA} neurons (n=11,182).

(A) Cartoon of the sampled peri-LC inhibitory neurons (left). Dot plot of neuronal subtype (left), neuropeptide (middle), and receptor (right) genes across peri-LC^{GABA} neuron clusters. Circle size corresponds to percent of cells in the cluster expressing the specific transcript, while color intensity corresponds to its relative expression.

(B) Dot plot of genes associated with neurotransmitter reuptake (left), hormone signaling (middle), and cannabinoid signaling (right) across peri-LC^{GABA} neuron clusters.

(C) Dot plot of genes associated with gap junctions across peri-LC^{GABA} neuron clusters.

(D) Dot plot of genes associated with morphology and adhesion functions and development across peri-LC^{GABA} neuron clusters.


A
PIXEL-seq
Seeding
Amplification
PolyT Exposing
Barcoded Polonies
Sequencing image
Pixel-level Barcode
rG-5'
mRNA
TTTTTTAAAAAA
Spatial
Barcode
UMI
Illumina P5
B
Sequencing Saturation
Total Reads (x 10^7)
female A
male A
male B
C
log2(UMIs)
female A
male A
male B
D
Fraction
protein coding
pseudogene
unprocessed
pseudogene
miscellaneous
RNAs
female A
male A
male B
E
Purkinje
Neurons4
Ependymal
Neurons2
Neurons1
RBCs
Neurons3
VLMCs
Oligos
Umap2
Umap1
F
Noradrenergic markers
Dbh
Slc18a2
Slc6a2
Gal
G
Neuropeptides
Adcyap1
Calca
Npy
Nts
Penk
Pnoc
Sst
Tac1

Extended Data Fig. 7. PIXEL-seq quality.

(A) Schematic depicts the Pixel-seq process. Barcoded DNA templates were grafted onto polyacrylamide gel and the spatial coordinates of barcodes were determined by Illumina sequencing by synthesis method. Cartoon on the right shows the gene library after *in situ* reverse transcriptase reaction and library construction.

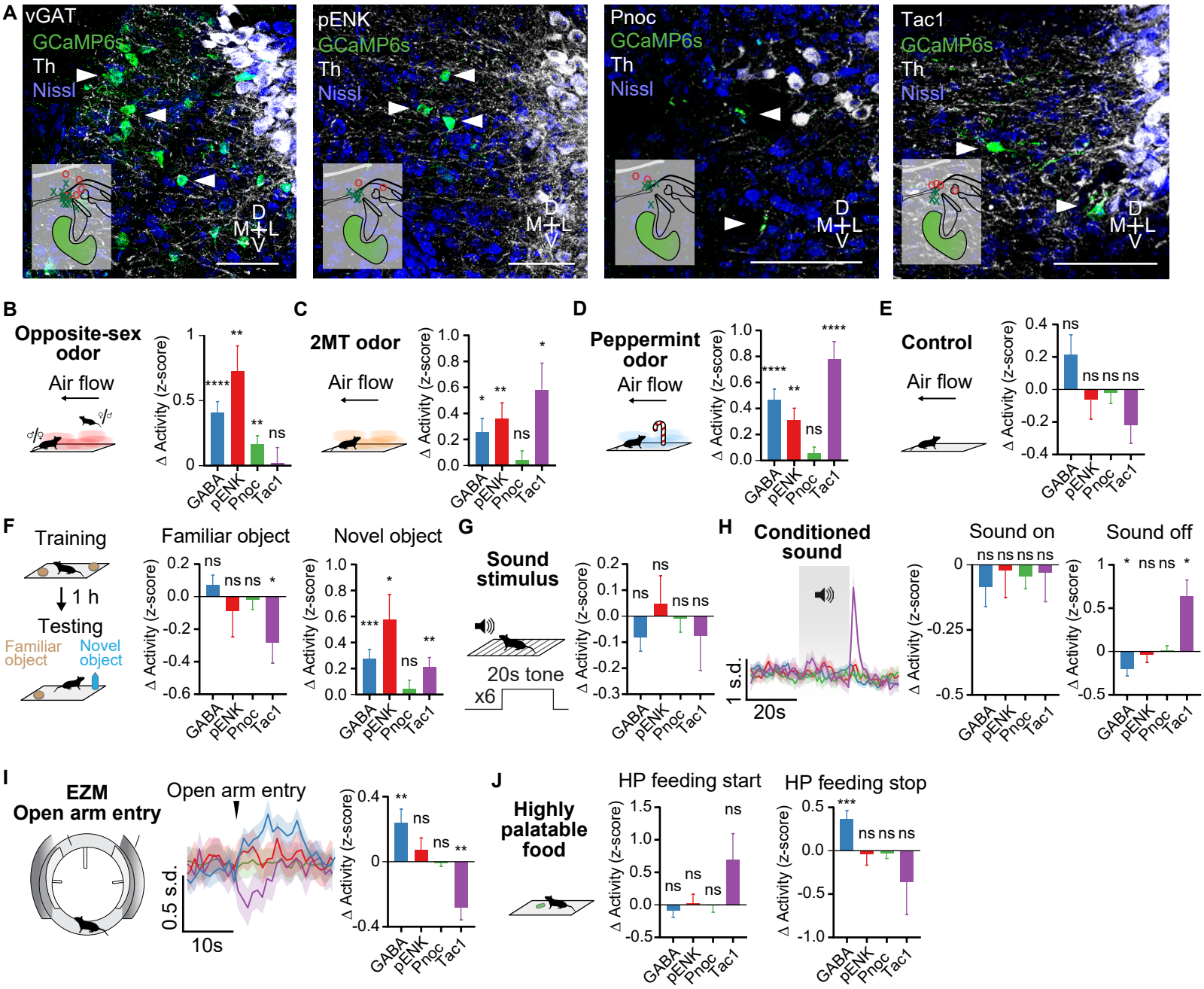
(B) Sequencing reads and saturation for the 3 brain slices (2 male and 1 female) containing the locus coeruleus used for PIXEL-seq.

(C) Unique molecular identifier (UMI) counts for the 3 brain slices used for PIXEL-seq.

(D) Proportion of the RNA type in the 3 brain slices used for PIXEL-seq.

(E) UMAP visualization is shown for n=8,181 cells obtained from Pixel-seq from 3 sections of 2 mice.

(F,G) Spatial distributions of noradrenergic neuronal markers **(F)**, and neuropeptides **(G)** are shown. Scale bars are 100 μm.


A
vGAT
GCaMP6s
Th
Nissl
pENK
Pnoc
Tac1
D
M+L
V
B
Opposite-sex odor
Air flow
C
2MT odor
D
Peppermint odor
E
Control
Δ Activity (z-score)
GABA
pENK
Pnoc
Tac1
F
Training
1 h
Testing
Familiar object
Novel object
G
Sound stimulus
20s tone
x6
H
Conditioned sound
1 s.d.
20s
Sound on
Sound off
I
EZM
Open arm entry
0.5 s.d.
10s
J
Highly palatable food
HP feeding start
HP feeding stop

Extended Data Fig. 8. Peri-LC neuronal subpopulations differentially respond to arousing and stress stimuli.

(A) Example images of GCaMP6s expression in peri-LC subpopulations. Insets show placements of fibers, with 'x' representing on-target fibers and expression and 'o' representing no or off-target expression. Scale bars is 50 μm.

(B) Peri-LC neurons are modulated by 30 s exposure to odor of opposite-sex mice.

(C) Peri-LC neurons are modulated by 30 s exposure to 2MT.

(D) Peri-LC neurons are modulated by 30 s exposure to peppermint oil odor.

(E) Peri-LC neurons are not affected by 30 s of blowing air through chamber.

(F) Peri-LC neurons are modulated by interactions with a novel object or a familiar object.

(G) Peri-LC neurons are unaffected by exposure to a tone that had not yet been paired with a shock.

(H) Peri-LC neurons do not respond to a conditioned tone, but GABA neurons decrease activity and Tac1 neurons increase activity after the tone stops.

(I) Peri-LC GABA neurons increase activity and Tac1 neurons decrease activity when an animal enters the open arm of an elevated zero maze.

(J) Peri-LC^{GABA} neurons increase activity after they stop eating highly palatable food; no other conditions are affected.

*$p < 0.05$, ** $p<0.01$, ***$p<.001$, ****$p<0.0001$ by paired two-tailed *t*-test. Error bars indicate SEM. Bars represent change in activity 10s before and after event (30s before and after for odor exposure experiments; 20s for sound stimulus).