Excitatory SST neurons in the medial paralemniscal nucleus control repetitive self-grooming and encode reward

Graphical abstract

Highlights

- MPL\textsuperscript{SST} neurons promote self-grooming and show grooming-related neuronal activity
- MPL\textsuperscript{SST} neurons are glutamatergic and encode reward value
- The neuropeptide SST facilitates the rewarding impact of MPL\textsuperscript{SST} neurons
- Inhibition of MPL\textsuperscript{SST} neurons impairs post-stress anxiety regulation

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In brief

Sun et al. identify a CeA-MPL\textsuperscript{SST-VTA}\textsuperscript{DA} circuit controlling self-grooming and post-stress anxiety alleviation by activation of the reward system in mice. This study provides insights into how repetitive behavior-related neurons connect to and influence emotion regulation-related neurons, with significant implications for the neuropsychological mechanisms of body-focused repetitive behaviors (BFRBs).
Excitatory SST neurons in the medial paralemniscal nucleus control repetitive self-grooming and encode reward

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SUMMARY

The use of body-focused repetitive behaviors (BFRBs) is conceptualized as a means of coping with stress. However, the neurological mechanism by which repetitive behaviors affect anxiety regulation is unclear. Here, we identify that the excitatory somatostatin-positive neurons in the medial paralemniscal nucleus (MPLSST neurons) in mice promote self-grooming and encode reward. MPLSST neurons display prominent grooming-related neuronal activity. Loss of function of MPLSST neurons impairs both self-grooming and post-stress anxiety alleviation. Activation of MPLSST neurons is rewarding and sufficient to drive reinforcement by activating dopamine (DA) neurons in the ventral tegmental area (VTA) and eliciting dopamine release. The neuropeptide SST facilitates the rewarding impact of MPLSST neurons. MPLSST neuron-mediated self-grooming is triggered by the input from the central amygdala (CeA). Our study reveals a dual role of CeA-MPLSST-VTADA circuit in self-grooming and post-stress anxiety regulation and conceptualizes MPLSST neurons as an interface linking the stress and reward systems in mice.

INTRODUCTION

Body-focused repetitive behaviors (BFRBs) in humans refer to a group of recurrent nonfunctional behaviors directed toward the body (e.g., hair pulling and skin picking). Individuals report increased nervousness and an urge prior to engaging in BFRBs and subsequently report pleasure, relief, and great satisfaction during or after performing BFRBs (Bohne et al., 2002; Stein et al., 2008). BFRBs may serve for emotion regulation, enabling individuals to control negative emotions by means of reducing arousal and resisting anxiety upon stress exposure (Teng, 2002; Schreiber et al., 2011; Roberts et al., 2013). Although there are now psychological models addressing BFRBs, the neural circuitry-level mechanism by which these repetitive behaviors correlate with emotion regulation in the mammalian brain is still not clear.

Similar to BFRBs in humans, many animals engage in self-grooming upon stress exposure. Self-grooming is a type of evolutionarily conserved repetitive behavior involving a sequenced structure that comprises of repeated stereotyped movements sequentially from the nose to the face, to the head, and finally ending with body licking (Cromwell and Berridge, 1996; Berridge et al., 2005; Kalueff et al., 2007, 2016). Known self-grooming-related brain regions in rodents include the orbitofrontal cortex (OFC), the ventromedial striatum (VMS), the medial/central amygdala (MeA/CeA), the ventral lateral septum (LSv), the ventral striatal islands of Calleja (ICj), the caudal part of the spinal trigeminal nucleus (Sp5C), the periaqueductal gray (PAG), the lateral hypothalamus (LHA), and the cerebellum (Berridge, 1989; Berridge and Whishaw, 1992; Welch et al., 2007; Ahmari et al., 2013; Burguiere et al., 2013; Hong et al., 2014; Alé et al., 2015; Mangieri et al., 2018; Gao et al., 2019; Mu et al., 2020; Xie et al., 2022; Zhang et al., 2021). However, there is still a debate with respect to the emotional impact of self-grooming (Xu et al., 2019; Mu et al., 2020), and it remains unclear how repetitive behavior-related neurons connect to and influence emotion-regulation-related neurons in the mammalian brain.

The medial paralemniscal nucleus (MPL) in rodents is located in the rostral pons and bordered by the auditory relay nuclei of the lateral lemniscus laterally, the oral part of the pontine reticular formation medially, and the rubrospinal tract ventrally (Dobolyi et al., 2003; Varga et al., 2008; Dobolyi et al., 2010). The MPL contains parathyroid hormone 2 (PTH2, also referred to as...
Data are represented as mean ± SEM.

In this study, we discovered that the somatostatin (SST)-positive neurons in the MPL significantly promote self-grooming and display grooming-related neuronal activity in mice. MPLSST neurons encode reward value and the neuropeptide SST facilitates the rewarding impact of MPLSST neurons. By characterizing the relevant upstream and downstream connectivity, we functionally validated a CeA-MPLSST-VTADA circuit in the regulation of self-grooming and post-stress anxiety alleviation.

RESULTS

MPLSST neurons promote self-grooming and respond to stress

We started searching for the neurons innervating both the ventral tegmental area (VTA) and the LHA, two brain regions that encode reward (Hu, 2016; Stuber and Wise, 2016), by using intersection-subtraction (IS) reporter mice in which the neurons having both Cre and Flpo recombinases express GFP (He et al., 2016). Retrogradely transported adeno-associated virus (AAV)-expressing Cre recombinase and Flpo recombinase were delivered into the LHA and the VTA of the IS reporter mice, respectively. We identified four brain regions containing GFP-positive neurons innervating both the VTA and the LHA (Figure S1A). Among them, the MPL triggered our particular interest because we found that this brain region was not only responsible for self-grooming but also responsive to stress.

Insight into the MPL being functionally involved in self-grooming originated from the observation that self-grooming was markedly induced upon optogenetic stimulation in the LHA of SST-Cre; Ai32 mice, in which the SST-positive neurons were genetically tagged with channelrhodopsin-2 (ChR2) (Figures S1H–S1J). Because the LHA contains very few SST-positive neurons, we searched for the soma of these LHA-projecting SST-positive neurons responsible for self-grooming. By injecting the retrogradely transported AAV expressing Cre-dependent EGFP (retroAAV-DIO-EGFP) into the LHA of SST-Cre mice, we identified a group of EGFP-positive neurons in the MPL (Figures 1A and 1B). We confirmed that these LHA-projecting neurons in the MPL expressed SST mRNA by single-molecule fluorescent in situ hybridization (smFISH) (Figure 1C). The MPL contained a total number of approximately 1,300 SST-positive neurons (referred to as MPLSST neurons) (Figures S1K and S1L). To examine whether the LHA-projecting MPLSST neurons are responsible for the observed self-grooming behavior, we injected retrogradely transported AAV expressing Cre-dependent ChR2 (retroAAV-DIO-ChR2-mCherry) into the LHA of SST-Cre mice, resulting in the specific expression of ChR2-mCherry in the LHA-projecting MPLSST neurons (Figure S1M). The optic fiber was implanted directly above the MPL. We found that optogenetic activation of the LHA-projecting MPLSST neurons induced robust self-grooming behaviors (Figure S1N).

To corroborate whether activating the population of MPLSST neurons induces self-grooming, we injected AAV-DIO-ChR2-mCherry (or AAV-DIO-mCherry as the control) into the MPL of SST-Cre mice, resulting in the specific expression of ChR2-mCherry in the MPLSST neurons (Figure 1D). The optic fiber was implanted directly above the MPL. Optogenetic activation of the MPLSST neurons elicited robust self-grooming (Figures 1E–1G; Video S1). The self-grooming induced by MPLSST neuron activation was not specific to a particular sex: a similar intensity of self-grooming was observed in both male and female mice (Figure 1E). A higher frequency of photostimulation resulted in a longer grooming time and a shorter grooming onset latency (Figures 1G and 1H), with an approximate 4-s grooming onset latency upon 20-Hz photostimulation (Figure 1H).

In the control experiments, we validated that the blue light pulses evoked phase-locked action potentials in the ChR2-expressing MPLSST neurons (Figures S1O–S1Q). We also ruled out the possibility that the non-SST neurons in the MPL contributed to self-grooming. To activate the non-SST neurons in the MPL, we delivered AAV-hSyn-Fas-ChR2-mCherry (“Cre-off”) (Saunders et al., 2012), together with AAV-Ef1a-DIO-EGFP (“Cre-on”), into the MPL of SST-Cre mice (Figures S2A and S2B). Optogenetic activation of the non-SST neurons in the MPL failed to induce self-grooming and instead slightly inhibited it (Figure S2C), indicating the specificity of the SST neurons in the MPL in mediating the observed self-grooming behavior.
Figure 2. MPlSST neuronal activities are associated with self-grooming initiation and maintenance
(A) The fiber photometry recording of calcium signals from GCaMP 7s-expressing MPLSST neurons. Scale bars, 100 μm.
(B) An example trace of calcium signals from MPLSST neurons during a bout of self-grooming.
(C and E) Heatmaps illustrating the MPLSST neuron calcium signals aligned to each bout of spontaneous self-grooming (C) and body-restraint-induced self-grooming (E).

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Subsequently, we employed a chemogenetic approach to examine the effect of prolonged activation of MPLSST neurons. We targeted hM3D to the MPLSST neurons by bilaterally injecting AAV-DIO-hM3D-mCherry (or AAV-DIO-mCherry as the control) into the MPL of SST-Cre mice (Figure S2D). Clozapine N-oxide (CNO, 50 μM)-evoked activation of hM3D-expressing MPLSST neurons was validated (Figure S2D). During 1-2 h after the administration of CNO (1 mg/kg), we observed a significant increase in self-grooming time in the mice with hM3D expression in MPLSST neurons compared with the control mice (Figure S2E), suggesting that prolonged activation of MPLSST neurons leads to persistent self-grooming. The selective serotonin reuptake inhibitor, fluoxetine, has an inhibiting effect on grooming (Shmelkov et al., 2010). We found that the administration of fluoxetine for 7 consecutive days (5 mg/kg intraperitoneally [i.p.], q.d.) significantly inhibited the MPLSST neuron activation-induced self-grooming (Figures S2F and S2G), suggesting that the MPLSST-induced self-grooming is fluoxetine sensitive.

Insight into the MPL being responsive to stress originated from the observation that there was a significant increase of c-Fos-positive neurons in the MPL upon stress exposure (30-min body restraint) (Figures S1B and S1C). The c-Fos immunostaining in SST-Cre; LSL-H2B-GFP mice also showed that the MPLSST neurons responded to stress exposure (Figures S1D and S1E). Moreover, stress exposure significantly increased the percentage of c-Fos-positive cells in the subgroup of MPL neurons connecting to the VTA and the LHA (Figures S1F and S1G). These results suggest that the MPLSST neurons connecting to the LHA and VTA are responsive to stress.

**MPLSST neurons are glutamatergic excitatory neurons and corelease the neuropeptides SST and PTH2**

We next performed a spatial transcriptome analysis using the 10x Genomics Visium platform to profile potentially informative markers in the MPL from a coronal section (prenatal day 4.72 mm) of the post-natal day 60 (P60) male mice. After dimensionality reduction and clustering on the joint spatial gene expression profiles in a 2D t-distributed stochastic neighbor embedding (t-SNE) plot, we found that the gene expression signature of this brain section comprised 14 clusters (#0–13) of neurons (Figure 1I). The cluster #13 was characteristic of the MPL, based on the position and the expression of SST (Figure 1I). We identified the differentially expressed genes (DEGs) enriched in the MPL (cluster #13) compared with other regions (clusters #0–12). We found that PTH2 and SST were among the top-five-ranking DEGs enriched in the MPL (Figure 1J), both of which encode neuropeptides. The PTH2 and SST transcripts were mapped back to the tissue section and the t-SNE plot, indicating their enrichment in the MPL (Figures 1K and 1L). In addition to SST and PTH2, violin plots showed that the excitatory neuronal marker gene Vgat2 was also enriched in the MPL (cluster #13) (Figure S2H). The enrichment of Vgat2, along with a lack of obvious Vgat/GAD1/GAD2 expression (Figure S2H), clearly implies that MPLSST neurons are glutamatergic excitatory neurons. In contrast to most SST-positive neurons in the mice brain being inhibitory interneurons that release GABA (Ma et al., 2006; Urban-Ciecko and Barth, 2016), MPLSST neurons represent a special case in which these SST-positive neurons release the excitatory neurotransmitter glutamate.

By smfISH, we further confirmed the expression of these marker genes in the MPL. We found that 97.3% ± 0.9% of SST-positive neurons in the MPL expressed Vgat2, while these neurons had no signal for Vgat (Figures S2I and S2J). Intriguingly, we found that 56.5% ± 0.9% of SST-positive neurons in the MPL expressed PTH2, while 98.8% ± 0.7% of PTH2-positive neurons expressed SST (Figures S2K and S2M). These data validate that MPLSST neurons are excitatory neurons releasing the neurotransmitter glutamate, and more than half of them corelease the neuropeptides SST and PTH2 (Figure S2K).

**MPLSST neurons display grooming-related neuronal activity**

To examine how MPLSST neuronal activity contributes to self-grooming, we performed in vivo calcium recording from MPLSST neurons via fiber photometry. The AAV expressing Cre-dependent calcium indicator GCaMP7s (AAV-DIO-GCaMP7s) (Dana et al., 2019) was delivered into the MPL of SST-Cre mice, and the optic fiber was implanted directly above the MPL. We recorded the temporally demodulated 488-nm Ca\(^{2+}\)-dependent and 410-nm Ca\(^{2+}\)-independent fluorescence signals with fiber photometry from GCaMP7s-expressing MPLSST neurons in freely behaving mice (Figure 2A). A significant increase in calcium signals (as reflected in ΔF/F) was detected during spontaneous self-grooming, with the peak calcium increase reaching 23.1% ± 3.0% ΔF/F (Figures 2B–2D). The calcium dynamics from the MPLSST neurons increased immediately before each bout of grooming, remained elevated throughout the entire grooming period, and then slowly decreased to baseline after the termination of grooming (Figure 2D; Video S2).

Self-grooming behavior can be effectively induced by different stimuli under moderate to stressful conditions, e.g., by the water spray toward the face, the oil drop onto the face, or the body restraint treatment (Figures S3A–S3C). Correspondingly, we detected significantly increased calcium signals in MPLSST neurons immediately before and during self-grooming induced by water spray (Figures S3D and S3E), oil drop (Figures S3F and S3G), and body restraint (Figures S2E and S2F), with the peak calcium

(D and F) The average calcium signals from MPLSST neurons during spontaneous self-grooming (D, 28 trials from 7 mice) and body restraint-induced self-grooming (F, 23 trials from 3 mice).

(G and H) The kinetics of the MPLSST neuron calcium signals during spontaneous self-grooming (G, 9 trials from 1 mouse) and body restraint-induced self-grooming (H, 9 trials from 1 mouse).

(I) Example traces illustrating the relationship between self-grooming and the MPLSST neuron calcium signals.

(J) The self-grooming bout duration displays a positive correlation with the magnitude of the calcium signals (R = 0.76, 40 trials from 3 mice).

Data are represented as mean ± SEM. See also Figure S3.
signals reaching 22.2% ± 2.1%, 18.6% ± 1.9%, and 33.7% ± 3.3% ΔF/F, respectively (Figure S3H).

We further analyzed the temporal relationship between the onset of MPLSST calcium increase and the initiation of self-grooming by aligning the calcium signal with the initiation of each bout of self-grooming. The onset of calcium increase was defined as the time when the signal reached 15% of its peak amplitude (Xie et al., 2022). We found that the onset of calcium increase in MPLSST neurons preceded to the initiation of spontaneous, water-spray-induced, oil-drop-induced, and body-restraint-induced self-grooming by 254.6 ± 32.1, 232.0 ± 41.4, 298.4 ± 39.4, and 290.4 ± 23.52 ms, respectively (Figures 2G, 2H, and S3I–S3K). These data suggest that MPLSST neuronal activities contribute to the initiation of self-grooming. Moreover, by prolonged fiber photometry recording, we found that each bout of self-grooming could reliably induce a calcium increase in MPLSST neurons (Figure 2I). The magnitude of the calcium increase was positively correlated with the self-grooming bout duration: the longer the grooming bout, the larger the peak ΔF/F value (Figure 2J). These results suggest that MPLSST neurons are functionally involved in both the initiation and the maintenance of self-grooming.

The loss of function of MPLSST neurons impairs both stress-induced self-grooming and post-stress anxiety alleviation

Given that MPLSST neurons respond to stress, we next investigated whether MPLSST neurons are required for stress-induced self-grooming as well as post-stress anxiety regulation by chemogenetic inhibition of MPLSST neurons. We targeted hM4D to MPLSST neurons by bilaterally injecting AAV-DIO-hM4D-mCherry (or AAV-DIO-mCherry as the control) into the MPL of SST-Cre mice, resulting in the specific expression of hM4D-mCherry in MPLSST neurons (Figure 3A). We validated that CNO efficiently inhibited the action potentials in the hM4D-expressing neurons (Figure 3A). The mice were i.p. administered with CNO (3 mg/kg) 10 min before body restraint treatment. After a 30-min body restraint, the mice were subjected to a 20-min grooming test, followed by the anxiety-related behavioral assays including the open field (OF) test, the elevated plus maze (EPM) test, and the novelty suppressed feeding (NSF) test (Figure 3B). Compared with the mCherry-expressing control mice, the CNO/hM4D-mediated inhibition of MPLSST neurons significantly reduced the time spent in self-grooming after body restraint (Figure 3C), with a decrease in both grooming bout number and duration per bout (Figures 3D and 3E).

Subsequently, we found that chemogenetic inhibition of MPLSST neurons led to a significant decrease in the center time in the OF test (Figures 3F and 3G), a significant decrease in the open arm time in the EPM test (Figures 3I and 3J), and a significant increase in the latency to feed in the NSF test (Figures 3L and 3M). As for the control analyses, inhibition of MPLSST neurons did not change the locomotor activity in either the OF (Figure 3H) or the EPM test (Figure 3K) and had no effect on the home cage food consumption after the NSF test (Figure 3N). These results suggest that inhibition of MPLSST neurons impairs both stress-induced self-grooming and post-stress anxiety alleviation.

To further corroborate the dual role of MPLSST neurons in self-grooming and post-stress anxiety regulation, we ablated the MPLSST neurons based on the expression of a genetically encoded apoptosis mediator (caspase-3) (Yang et al., 2013). The AAV-DIO-caspase-3 (AAV-DIO-mCherry as the control) was bilaterally injected into the MPL of SST-Cre; LSL-H2B-GFP mice (Figure S4A), resulting in the ablation of 91.7% ± 1.3% of GFP-positive SST neurons inside the MPL and a slightly nonspecific ablation outside the MPL (Figure S4B). Ablation of MPLSST neurons significantly reduced the self-grooming time after body restraint (Figures S4C and S4D), with a decrease in both grooming bout number and duration per bout (Figures S4E and S4F). Ablation of MPLSST neurons also led to a significant decrease in the center time in the OF test after stress exposure (Figures S4G and S4H) but did not change the locomotor activity (Figure S4I). Taken together, these findings suggest that the loss of function of MPLSST neurons impairs both stress-induced self-grooming and post-stress anxiety alleviation.

MPLSST neuron-mediated self-grooming is triggered by the input from the CeA

To investigate the input circuitry to MPLSST neurons, we performed monosynaptic retrograde tracing using the pseudorabies virus (Wickersham et al., 2007). The AAVs expressing Cre-dependent avian-specific retroviral receptor (TVA) and rabies virus glycoprotein (RVG) were delivered into the MPL of SST-Cre mice, followed by RV-EnvA-ΔG-dsRed into the LHA, one of the MPLSST neurons’ projection fields (Figure 4A). TVA-expressing axons originating from MPLSST neurons were selectively competent for transduction by RV-EnvA-ΔG-dsRed. The neurons

Figure 3. Inhibition of MPLSST neurons impairs self-grooming and post-stress anxiety alleviation

(A) The viral injection and expression in the MPL. Scale bars, 75 μm. Right bottom: administration of CNO efficiently inhibited the action potentials of hM4D-expressing MPLSST neurons.

(B) A schematic diagram showing how to examine the impact of inhibiting MPLSST neurons on stress-induced self-grooming and anxiety-related behaviors. (C–E) Inhibiting MPLSST neurons significantly reduced the time (C, ***p < 0.001), bout number (D, **p < 0.01), and duration per bout (E, ***p < 0.001) of self-grooming following body restraint (mCherry: n = 12 mice; hM4D: n = 15 mice). (F and I) Example locomotor trajectories of mCherry- or hM4D-expressing mice in the OF test (F) and the EPM test (I). (G and J) Inhibiting MPLSST neurons significantly decreased the time spent in the center area of the OF test (G, mCherry: n = 11 mice; hM4D: n = 13 mice, *p < 0.05) and in the open arms of the EPM test (J, mCherry: n = 12 mice; hM4D: n = 12 mice, *p < 0.01). (H and K) Inhibiting MPLSST neurons did not change the locomotor activity in the OF test (H) and the EPM test (K). (L–N) Inhibiting MPLSST neurons significantly increased the latency to feed (M, *p < 0.05) but did not change the food consumption (N) in the NSF test (mCherry: n = 12 mice; hM4D: n = 12 mice).

Data are represented as mean ± SEM. See also Figure S4.
A

B

C

D

E

F

G

H

I

J

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expressing both dsRed and EGFP in the MPL represented the “starter cells” in the RV-based retrograde tracing (Figure 4A). We found that the LHA-projecting MPL^{SST} neurons receive inputs largely from six brain regions, including the CeA, the bed nucleus of the stria terminals (BNST), the ventral lateral PAG (vPAG), the paraventricular nucleus (PVN), the inferior colliculus (IC), and the auditory cortex (Au) (Figures 4B, high-magnification images, 4J, and SSA, low-magnification images).

We investigated the role of each upstream input to the MPL in the regulation of self-grooming. We targeted Chr2 to respective upstream input neurons in the CeA, BNST, vPAG, PVN, IC, and Au, by delivering retroAAV-hSyn-Cre to the MPL and AAV-DIO-Chr2-mCherry to each of these input regions in wild-type mice (Figures 4C–4H). Notably, we only observed a marked increase in self-grooming when the MPL-projecting CeA neurons were activated (Figure 4C). In contrast, activation of the MPL-projecting BNST or vPAG neurons inhibited self-grooming by eliciting other behaviors (e.g., circling or escaping) (Figures 4D and 4E), whereas activation of the MPL-projecting PVN, IC, or Au neurons had no effect on self-grooming (Figures 4F–4H). Different inputs may generate opposite effects due to the distinct transsynaptic property of a particular upstream-downstream connectivity. These results suggest that the input from the CeA to the MPL has a specific activating effect on the MPL^{SST} neuron-mediated self-grooming.

Next, we examined whether inhibition of MPL^{SST} neurons could block the self-grooming that was induced by activating the MPL-projecting CeA neurons. A combination of AAV-DIO-hM4D-mCherry (or AAV-DIO-mCherry as the control) and retro-AAV-hSyn-Flpo was injected into the CeA (Figure 4I). Compared with the control mice expressing mCherry, chemogenetic inhibition of MPL^{SST} neurons significantly impaired the self-grooming evoked by activating MPL-projecting CeA neurons (Figure 4I). Taken together, these results demonstrate that MPL^{SST} neuron-mediated self-grooming is specifically triggered by the input from the CeA.

**MPL^{SST} neurons send collateralized axon projections in a “one-to-many” configuration to coordinate self-grooming**

Recalling our findings that MPL^{SST} neurons send axon projects to the LHA and the VTA (Figure S1A), we wanted to determine whether MPL^{SST} neurons send additional axon collaterals to other downstream targets. In SST-Cre mice, the retrogradely transported AAV expressing Cre-dependent Flpo recombinase (retroAAV-DIO-Flpo) was injected into the LHA, and the AAV expressing Flpo-dependent mGFP (AAV-fDIO-mGFP) was injected into the MPL (Figure 5A). The expression of mGFP enabled visualization of the entire MPL^{SST} neurons including axon collaterals (Figure 5A). In addition to the VTA and LHA, we observed four additional trajectories of MPL^{SST} axons, including the dorsomedial hypothalamus (DMH), the vPAG, the periventricular area (PVA), and the medial preoptic area (MPO) (Figures S5B and S5C). To focus on the axon terminals, we employed the AAV expressing Cre-dependent synaptophysin-mCherry and validated this 6-region projection profile of MPL^{SST} neurons (Figure S5B).

To investigate the functional roles of MPL^{SST} neuron projections, we applied optogenetic stimulation to each of the axon projection fields of MPL^{SST} neurons, including the VTA, LHA/DMH, vPAG, and MPO. We examined the combined functional outputs of LHA and DMH projections because these two regions are positioned in close proximity. We found that activation of the axon terminals in the VTA, LHA/DMH, and vPAG elicited pronounced self-grooming (Figure 5D), but activation of the axon projections to the PVA and the MPO elicited a much milder extent of self-grooming (Figure 5D). These results suggest that multiple axon projections of MPL^{SST} neurons play a coordinating role in regulating self-grooming.

We performed loss-of-function experiments to examine the necessity of the axon projections to the VTA, LHA/DMH, and vPAG in self-grooming. We injected AAV-DIO-hM4D-mCherry (or AAV-DIO-mCherry as the control) into the MPL of SST-Cre mice. Through embedded guide cannulas, CNO (1 mM, 500 nL per side) was locally perfused into each of the axon projection fields in the VTA, LHA/DMH, and vPAG (Figures 5E–5G and S5C). Compared with the control mice expressing mCherry, local inhibition of MPL^{SST} neuron projections to the VTA, LHA/DMH, and vPAG all resulted in a significant decrease in stress-induced self-grooming (Figures 5E–5G), confirming that multiple axon projections of MPL^{SST} neurons are required for self-grooming.

The observation that MPL^{SST} neurons innervate multiple regions to coordinately regulate self-grooming prompted us to test whether MPL^{SST} neurons anatomically send collateralized axon projections in a “one-to-many” configuration. We performed pairwise retrograde tracing by injecting retroAAV-DIO-tdTomato and retroAAV-DIO-EGFP into different combinations of two axon projection fields of MPL^{SST} neurons in SST-Cre mice (Figures S5D–S5L). We found that 55.3% ± 1.7% of LHA-projecting MPL^{SST} neurons sent axon projections to the VTA, while 60.9% ± 6.5% of VTA-projecting MPL^{SST} neurons sent axon projections to the LHA (Figures S5D–S5F). Also, 67.8% ± 6.0% of LHA-projecting

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**Figure 4. The input from the CeA triggers MPL^{SST} neuron-mediated self-grooming**

(A) A schematic diagram showing retrograde monosynaptic tracing and the starter cells in the MPL. Scale bars, 30 μm.

(B) The six upstream inputs to MPL^{SST} neurons. Scale bars, 75 μm.

(C–H) Top: schematic diagrams showing the viral injection and optogenetic stimulation. Bottom left: the effects of activating MPL-projecting neurons in each upstream input region on self-grooming (CeA: n = 6 mice, ***p < 0.0001; BNST: n = 6 mice, *p < 0.05; vPAG: n = 7 mice, **p < 0.01; PVN: n = 6 mice; IC: n = 7 mice; Au: n = 7 mice). Bottom right: post hoc verification of the Chr2-mCherry expression and optic fiber placement. Scale bars, 75 μm.

(I) Inhibition of MPL^{SST} neurons significantly impaired self-grooming evoked by activating MPL-projecting CeA neurons (mCherry: n = 11 mice; hM4D: n = 15 mice, ***p < 0.0001).

(J) A schematic diagram showing the upstream inputs to MPL^{SST} neurons.

Data are represented as mean ± SEM.

See also Figure S5.
Downstream outputs of MPL\textsuperscript{SST} neurons

- Ventral tegmental area (VTA)
- Lateral hypothalamic area (LHA)/Dorsomedial hypothalamus (LHA/DMH)
- Ventrolateral periaqueductal gray (vIPAG)
- Paraventricular thalamic nucleus (PVA)
- Median preoptic nucleus (MPO)

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473 nm, 20 Hz, 20-ms pulse

VTA, LHA/DMH, vIPAG, PVA, MPO

Restraint-induced self-grooming

Grooming time/20 min

mCherry, hM4D

(legend on next page)
MPLSST neurons projected to the vlPAG, while 47.2% ± 0.2% of VTA-projecting MPLSST neurons projected to the LHA (Figures S5G–S5I). In addition, 77.1% ± 5.0% of VTA-projecting MPLSST neurons projected to the vlPAG, while 57.6% ± 4.1% of LHA-projecting MPLSST neurons projected to the VTA (Figures S5J–S5L). These data indicate that MPLSST neurons send collateralized axon projections in a “one-to-many” configuration.

To further understand how subpopulations of MPL neurons projecting to the VTA and LHA segregate, retroAAV-hSyn-Cre was injected into the LHA, and retroAAV-hSyn-flpO was injected into the VTA in the IS mice. The percentage of MPL neurons projecting to both the LHA and the VTA (GFP+) was 55.2% ± 7.3% among the total LHA-projecting MPL neurons (dTomato+ and GFP+) (Figure SS5M). In a similar way, we found that the percentage of MPL neurons projecting to both the LHA and the VTA (GFP+) was 52.3% ± 5.8% among the total VTA-projecting MPL neurons (dTomato+ and GFP+) (Figure SS5N). These data suggest that the subgroup of MPL neurons projecting to both the LHA and the VTA and the subgroup of MPL neurons projecting to solely the LHA (or solely the VTA) are approximately half-and-half.

MPLSST neurons encode reward value

Considering that MPLSST neurons innervate the brain regions related to reward (the VTA and the LHA), we wanted to know whether activating MPLSST neurons is rewarding. We targeted ChR2 to MPLSST neurons by injecting AAV-DIO-ChR2-mCherry (or AAV-DIO-mCherry as the control) into the MPL of SST-Cre mice and implanted the optic fiber above the MPL (Figure 6A). We first used a conditioned place preference (CPP) paradigm (Zhao et al., 2019) to test whether activating MPLSST neurons encodes reward value (Figure 6B). Compared with the control mice expressing mCherry, the mice expressing ChR2 in MPLSST neurons displayed a consistent positive preference for staying in the chamber coupled with optogenetic stimulation (Figure 6C). After 4 days of conditioning, these mice developed a profound preference for the chamber previously coupled with optogenetic stimulation (Figure 6D), indicating that MPLSST neurons encode a marked reward value.

We further investigated whether activating MPLSST neurons is rewarding and sufficient to drive positive reinforcement by the intracranial self-stimulation (ICSS) paradigm (Wise, 1996), whereby the mice learned to poke an active port to deliver optogenetic stimulation to themselves (Figure 6E). In the fixed ratio 1 (FR1) schedule of ICSS, each successful nose poking to the active port would trigger the delivery of photostimulation. We observed a profound preference toward the active port to administer self-stimulation in the mice expressing ChR2 in MPLSST neurons (Figure 6F). After 5 days of the FR1 schedule, the mice were tested with a progressive ratio 2 (PR2) reinforcement schedule in which each delivery of photostimulation required two additional nose pokes. Compared with the control mice expressing mCherry, the mice expressing ChR2 in MPLSST neurons displayed a significant increase in nose poking toward the active port in PR2 schedule (Figures 6G and 6H). Moreover, we observed a comparable rewarding effect upon activating the MPLSST neurons in female mice (Figures S6A–S6D), as that in male mice (Figures 6F–6H). These results demonstrate that activating MPLSST neurons is rewarding and sufficient to drive positive reinforcement.

Furthermore, we wanted to know whether MPLSST neuron activation in itself is sufficient to encode reward or whether self-grooming is a necessary component of MPL-induced reward. We made the mice wear a custom-made collar on the neck to prevent their self-grooming but not to influence their movement (Figure 6I). The mice were habituated to wearing the neck collar for 5 days and then tested by ICSS. We found that the ChR2-expressing mice with neck collars still developed a significant preference toward the active port to deliver self-stimulation in both the FR1 and PR2 schedules (Figures 6J and 6K), compared with the mCherry-expressing control mice with neck collars (Figure 6L). The ChR2-expressing mice with neck collars showed a comparable PR2 breakpoint number to those mice without neck collars (Figure 6M). These findings suggest that MPLSST neuron activation in itself is sufficient to encode reward, while blockage of self-grooming motor actions had little or no effect on MPLSST neuron activation-induced reward.

Moreover, we compared the effects of the outputs to the VTA or the LHA in MPL-induced reward. AAV-DIO-ChR2-mCherry was injected into the MPL of SST-Cre mice, and the optic fiber was implanted directly above the axon terminals in the VTA or in the LHA (Figures S6E and S6I). A much stronger rewarding effect was observed upon activating the output to the VTA, compared with activating the output to the LHA (Figures S6E–S6L). These findings suggest that the output to the VTA plays a more crucial role in MPLSST neuron activation-induced reward than the output to the LHA.

The neuropeptide SST facilitates the rewarding effect of MPLSST neurons

Recalling our findings that MPLSST neurons are glutamatergic and that more than half of these neurons corelease the neuropeptides SST and PTH2, we wanted to determine whether the

Figure 5. MPLSST neurons send axon collaterals in a “one-to-many” manner to coordinate self-grooming

(A) The viral injection and the expression of mGFP in MPLSST neurons. Scale bars, 100 μm.
(B) The six axon projection targets of MPLSST neurons. Scale bars, 150 μm.
(C) A schematic diagram showing the downstream outputs of MPLSST neurons.
(D) The effects of activating different MPLSST axon projections on self-grooming (VTA: n = 8 mice, ****p < 0.0001; LHA/DMH: n = 8 mice, ***p < 0.001; vlPAG: n = 9 mice, **p < 0.01; PVA: n = 9 mice, *p < 0.05; MPO: n = 8, *p < 0.05).
(E–G) Body restraint-induced self-grooming was significantly attenuated by local inhibition of MPLSST axon terminals in the VTA (E, mCherry: n = 7 mice; hM4D: n = 8 mice, *p < 0.01), in the LHA/DMH (F, mCherry: n = 7 mice; hM4D: n = 8 mice, *p < 0.01), and in the vlPAG (G, mCherry: n = 5 mice; hM4D: n = 7 mice, *p < 0.01).

Data are represented as mean ± SEM.

See also Figure S5.
release of SST or PTH2 may influence the rewarding impact of MPLSST neurons by using CRISPR-Cas9-mediated genome editing (Platt et al., 2014). In the MPL of SST-Cre; CAG-Cas9 mice, we bilaterally injected AAV carrying triple SST-targeting single-guide RNAs (sgRNAs) or triple PTH2-targeting sgRNAs to knock down the expression of endogenous SST or PTH2 in the MPL. In the control, we found that the knockdown of SST in the MPL significantly reduced both the FR1 poke numbers and PR2 breakpoint numbers (Figures 6O–6Q). In contrast, the knockdown of PTH2 in the MPL had little or no effect on the SST or PTH2 knockdown on the rewarding effect of MPLSST neuron activation using the ICSS paradigm. Compared with the control, we found that the knockdown of SST in the MPL significantly reduced both the FR1 poke numbers and PR2 breakpoint numbers (Figures 6O–6Q). In contrast, the knockdown of PTH2 in the MPL had little or no effect on the rewarding impact of MPLSST neuron activation (Figures 6O–6Q). These results clearly demonstrate that the neuropeptide SST facilitates the rewarding impact of MPLSST neurons.

**MPLSST neurons form monosynaptic excitatory synapses with VTA_{DA} neurons**

We next used both histological and electrophysiological approaches to examine whether MPLSST neurons form monosynaptic connections with VTA_{DA} neurons. We used AAV serotype 1 expressing recombinase Cre (Zingg et al., 2017) as an anterograde transsynaptic tracer to access the MPL-to-VTA connectivity. We injected AAV1-hSyn-Cre into the MPL and AAV-DIO-EGFP into the VTA in wild-type mice (Figure 7A). We observed many EGFP-expressing neurons in the VTA and these neurons were the ones receiving monosynaptic inputs from the MPL (Figure 7B). Tyrosine hydroxylase (TH) immunostaining showed that 20.2% ± 4.3% of TH-positive neurons expressed EGFP in the VTA (Figure 7B), indicating that a proportion of VTA_{DA} neurons received monosynaptic inputs from the MPL.

ChR2-assisted circuit mapping (CRACM) was subsequently performed to examine whether MPLSST neurons form monosynaptic excitatory synapses with VTA_{DA} neurons. In SST-Cre mice crossed with DAT-Flpo mice, we injected AAV-DIO-ChR2-mCherry into the MPL and the AAV expressing Flpo-dependent EYFP (AAV-DIO-EYFP) into the VTA (Figure 7C). We confirmed that the SST-Cre; DAT-Flpo mice could effectively label the dopamine (DA) neurons because 80.7% ± 1.8% of EYFP-positive neurons in the VTA expressed TH (Figure 7D). We performed whole-cell patch-clamp recording on the EYFP-positive neurons in the VTA. Upon a single pulse of 10-ms blue light stimulation, evoked excitatory postsynaptic currents (EPSCs) were recorded on 25 out of 49 neurons. We found that the blue light-evoked EPSCs could be completely blocked by the glutamate receptor blocker 6-cyano-7-nitroquinoline-2, 3-dione (CNQX, 40 μM) (Figure 7D), but not by the GABA_A receptor blocker picrotoxin (PTX, 100 μM) (Figure 7E). Administration of the sodium channel blocker tetrodotoxin (TTX, 2 μM) inhibited the evoked EPSCs, while the potassium channel blocker 4-aminopyridine (4-AP, 100 μM) reversed the TTX-inhibited EPSCs (Figure 7F). These results demonstrate that MPLSST neurons form monosynaptic excitatory synapses with VTA_{DA} neurons.

**Activation of MPLSST neurons and self-grooming behavior both trigger dopamine release**

Given that MPLSST neurons form excitatory synapses with VTA_{DA} neurons and considering that activating MPLSST neurons can generate reward and drive reinforcement, we next investigated whether activation of MPLSST neurons could trigger dopamine (DA) release in the nucleus accumbens (NAc), a major target field of the VTA_{DA} neurons. We employed a genetically encoded fluorescent DA sensor (DA2h) (Sun et al., 2020) that can detect changes in DA release in the NAc based on fiber photometry...
recording. The AAV expressing Cre-dependent ChrimsonR (AAV-DIO-ChrimsonR-ttdTomato) (Kiapetoeke et al., 2014) was injected into the MPL of SST-Cre mice, and the AAV expressing DA2h (AAV-hSyn-DA2h) or its mutant form DAmut (AAV-hSyn-DAmut as the control) was injected into the NAc (Figure 7G). The ChrimsonR-expressing MPLSST neurons were activated by the 640-nm laser light in the MPL, and the DA release were detected by the DA2h DA sensor in the NAc. We found that optogenetic activation of the MPLSST neurons (either 1 or 5 s) elicited a significant increase in DA signals in the NAc (Figures 7H, 7J, and 7K), reaching approximately 30% ΔF/F (Figure 7I). In the control mice expressing DAmut in the NAc, no change in DA signals was detected upon MPLSST neuron activation (Figures 7J and 7K). These findings demonstrate that activation of MPLSST neurons elicits a significant DA release in the NAc. We analyzed the temporal relationship between the onset of DA release in the NAc and the onset of MPLSST neuron activation. If we defined the onset of DA release as the time when the DA signal reached 15% of its peak amplitude, MPLSST neuron activation preceded the onset of DA release in the NAc by 101.0 ± 4.3 ms based on fiber photometry recording (Figures S7A and S7D).

Similar to the optogenetic activation of MPLSST neurons, both spontaneous and body restraint-induced self-grooming was associated with a marked increase in DA release in the NAc (Figures 7L–7N). The initiation of spontaneous self-grooming and restraint-induced self-grooming preceded the onset of DA release in the NAc by 253.8 ± 32.9 and 331.3 ± 52.6 ms, respectively (Figures S7B–S7D). Although acute stress is reported to have an activating effect on DA release (Holly and Miczek, 2016), we failed to detect a difference in the intensity of DA release between spontaneous and restraint-induced self-grooming (Figure 7O).

Given that grooming can be inhibited by high dose of DA D1 receptor (D1R) antagonist, SCH23390 (Starr and Starr, 1986), we wanted to know whether blockage of the DA D1R signaling impairs the self-grooming induced by activating the MPL-to-VTA projection. SCH23390 (1 mg/kg) was i.p. administered 15 min before optogenetic stimulation, and it significantly reduced the self-grooming induced by activating the MPL-to-VTA projection (Figures S7E and S7F). Moreover, we targeted hM4D to the VTA neurons bilaterally and ChR2 to the MPLSST neurons unilaterally in SST-Cre; DAT-Flpo mice (Figure S7G). CNO/hM4D-mediated inhibition of VTA neurons was validated (Figure S7G). We found that inhibition of VTA neurons led to a significant decrease in the MPLSST-neuron-activation-induced self-grooming (Figures S7H and S7I), and subsequently an increase in anxiety, as shown by the decreased center time in the OF test (Figure S7J). These results provide supporting evidence that blocking the VTA DA signaling impairs MPLSST-mediated self-grooming and anxiety regulation.

**DISCUSSION**

In this study, we revealed that MPLSST neurons respond to stress, promote self-grooming, and encode reward in mice. In contrast to most SST neurons being inhibitory interneurons, MPLSST neurons represent a special case in which these SST neurons are glutamatergic excitatory neurons. We functionally validated a CeA-MPLSST-VTA circuit controlling self-grooming and post-stress anxiety alleviation. Our study conceptualizes MPLSST neurons as an interface linking the stress and reward systems in the mammalian brain and helps shed light on the neurological mechanism in human BFRBs where relief and pleasure happens during and after repetitive behaviors.

Our data strongly support the role of MPLSST neurons in both the initiation and maintenance of self-grooming. Calcium recording via fiber photometry revealed that the onset of calcium increase in MPLSST neurons preceded the initiation of self-grooming behaviors, supporting the role of MPLSST neurons in self-grooming initiation. Other lines of evidence support the role of MPLSST neurons in self-grooming maintenance. For example, (1) MPLSST neuronal activities remained elevated throughout the entire grooming period and then slowly

Figure 7. Activation of MPLSST neurons and self-grooming behavior both trigger dopamine release in the NAc

(A) The viral injection for AAV1-mediated anterograde mono-transsynaptic tracing.

(B) Left: AAV1-hSyn-Cre mediated the expression of EGFP in the VTA and the TH immunostaining. Scale bars, 100 μm (low-mag) and 15 μm (high-mag). Right: the quantification of TH-expressing neurons that are connected with the MPL.

(C) A schematic diagram showing the measurement of synaptic transmission from MPLSST neurons to VTA neurons. Scale bars, 20 μm.

(D–F) The evoked EPSCs recorded in VTA neurons could be completely blocked by CNQX (D, n = 8 neurons from 6 mice, ****p < 0.0001), but not by PTX (E, n = 9 neurons from 6 mice). The evoked EPSCs could be inhibited by TTX (F, n = 7 neurons from 6 mice, **p < 0.01) and could be reversed by 4-AP (F, n = 7 neurons from 6 mice, *p < 0.01).

(G) A schematic diagram showing the fiber photometry recording of dopamine signals in the NAc with the dopamine sensor DA2h (DAmut) as a control in response to the optogenetic stimulation of the ChrimsonR-expressing MPLSST neurons.

(H) Heatmaps illustrating the DA2h fluorescence changes in the NAc in response to 1 (left) or 5 s (right) optogenetic stimulation of the ChrimsonR-expressing MPLSST neurons (11 trials from 1 mouse).

(I and K) The area under the curve (AUC) of the DA2h- and DAmut-fluorescence signals were calculated in response to 1 s (I, **p < 0.01) or 5 s (K, *p < 0.01) of optogenetic stimulation (1 s: 62 trials from 6 mice for DA2h and 60 trials from 6 mice for DAmut; 5 s: 55 trials from 6 mice for DA2h and 60 trials from 6 mice for DAmut).

(L) A schematic diagram showing the fiber photometry recording of the dopamine signals in the NAc during self-grooming.

(M and N) Heatmaps of the dopamine signals in the NAc during spontaneous self-grooming (M, 5 trials from 1 mouse) and restraint-induced self-grooming (N, 5 trials from 1 mouse).

(O) Both spontaneous self-grooming (29 trials from 6 mice) and restraint-induced self-grooming (23 trials from 4 mice) induced a comparable increase in dopamine release in the NAc. Data are represented as mean ± SEM.

See also Figures S6 and S7.
decreased to baseline after the termination of self-grooming. (2) The magnitude of the calcium signals was positively correlated with the self-grooming bout duration. (3) The loss of function of MPLSST neurons decreased total self-grooming time as well as the grooming duration per bout. These data provide supporting evidence that MPLSST neurons are involved in not only the initiation but also the maintenance of self-grooming.

We also noted that either hM4D-mediated inhibition or caspase-3-mediated ablation of MPLSST neurons failed to completely block self-grooming upon stress exposure, implicating additional mechanisms involved in stress-induced self-grooming. Previous studies offered us important insights into how self-grooming is regulated under normal circumstances and how it is affected in pathological conditions (Graybiel and Rauch, 2000; Aouizerate et al., 2004). Here, our study expands the knowledge about the neurobiological mechanisms in repetitive behaviors. Especially, we discover that MPLSST neurons function as an interface linking the stress and reward systems in the mice brain. With respect to the CeA-MPLSST-VTAD circuit, the input from the CeA specifically triggers the MPL-mediated self-grooming, while the output to the VTA occupies a central position in mediating the impact of MPL-mediated post-stress anxiety regulation.

Prior to our study, there was a debate on the emotional impact of self-grooming. A negative emotional impact was observed to be associated with self-grooming controlled by the PVN-LSv circuit (Xu et al., 2019), while a positive emotional impact was observed to be associated with self-grooming controlled by the LSv-Tu circuit (Mu et al., 2020). However, our study clearly supports a positive emotional impact associated with self-grooming controlled by MPLSST neuron activation. Notably, activation of MPLSST neurons in free behaving mice is rewarding. However, if the mice were prevented from self-grooming during the activation of MPLSST neurons, we instead observed an increased anxiety (Figure S7K). This suggests that preventing the mice from self-grooming upon stimulating MPLSST neurons would impair the rewarding effect of MPLSST neurons and lead to an opposite effect.

It is thought that self-grooming is a sensitive marker of stress levels in animals and cumulating evidence support the role of self-grooming in post-stress de-escalation (Kal枚eff et al., 2016; Song et al., 2016). Nevertheless, it should be cautious to limit the function of self-grooming within the confines of coping with stress. Self-grooming may have other functional or nonfunctional meanings for the animals because self-grooming is also very common under low-arousal situations in physiological conditions.

Here, our study provides new insights into the function and circuity of MPLSST neurons in controlling self-grooming and post-stress anxiety alleviation through activating the reward system. This expands our knowledge of how repetitive behavior-related neurons connect to and influence emotion regulation-related neurons in the mammalian brain, with an implication for the neurological mechanism in human BFRBs where relief and satisfaction is obtained along with repetitive behaviors.

**STAR METHODS**

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**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.neuron.2022.08.010.

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**AUTHOR CONTRIBUTIONS**

J.S., Y.Y., and X.W. conducted most of the experiments and data analyses. A.L., J.W., S.Y., B.L., Y.K., L.W., and K.Z. helped for immunostaining, smFISH, and viral tracing. T.Y., guided patch-clamp recording. J.H., J.S., and Y.Y. wrote the paper, and T.-L.X., Q.L., and S.Z. helped to edit.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Rabbit anti-c-Fos antibody | Abcam | Cat#ab222699; RRID:AB_2891049 |
| Rat anti-mCherry antibody | Thermo Fisher Scientific | Cat#M11217; RRID:AB_2536611 |
| Mouse anti-TH antibody | Millipore | Cat#MAB318; RRID:AB_2201528 |
| Goat anti-Rabbit IgG (H+L), Alexa Fluor 555 | Thermo Fisher Scientific | Cat#A21428; RRID:AB_2535849 |
| Goat anti-Mouse IgG (H+L), Alexa Fluor 488 | Thermo Fisher Scientific | Cat#A11001; RRID:AB_2534069 |
| Goat anti-Rabbit IgG (H+L), Alexa Fluor 633 | Thermo Fisher Scientific | Cat#A21070; RRID:AB_2535731 |
| Goat anti-Mouse IgG (H+L), Alexa Fluor 633 | Thermo Fisher Scientific | Cat#A21050; RRID:AB_2535718 |
| Goat anti-Rat IgG (H+L), Alexa Fluor 555 | Thermo Fisher Scientific | Cat#A21434; RRID:AB_2535855 |
| Bacterial and virus strains |        |            |
| AAV2/9-hEF1a-DIO-ChR2[H134R]-mCherry | Shanghai Taitool | Cat#S0170-9-L50 |
| AAV2/9-hSyn-DIO-GCaMP7s | Shanghai Taitool | Cat#S0590-9-H50 |
| RetroAAV2/2-hSyn-FLEX-tdTomato | Shanghai Taitool | Cat#S0255-2R-H50 |
| AAV2/9-hEF1a-DIO-EGFP | Shanghai Taitool | Cat#S0270-H50 |
| RetroAAV2/2-CAG-FLEX-Fipo | Shanghai Taitool | Cat#S0273-2R-H20 |
| AAV2/9-hEF1a-fDIO-mgFP | Shanghai Taitool | Cat#S0289-9-L50 |
| AAV2/9-CAG-DIO-taCasparase3 | Shanghai Taitool | Cat#S0236-9 |
| RetroAAV2/2-hSyn-Cre | Shanghai Taitool | Cat#S0278-2R-H20 |
| RetroAAV2/2-hSyn-Fipo | Shanghai Taitool | Cat#S0271 |
| AAV2/9-hEF1a-Fas DO-hChR2[H134R]-mCherry | Shanghai Taitool | Cat#S0333-9 |
| AAV2/9-hSyn-fDIO-hM4D(Gi)-mCherry | Shanghai Taitool | Cat#S0336-9 |
| AAV2/9-hSyn-FLEX-ChrimsonR-tdTomato | Shanghai Taitool | Cat#S0186-9 |
| AAV2/9-hU6-sgRNA_{1,3}(SST)-hEF1a-DIO-EGFP | Shanghai Taitool | Cat#WS0787 |
| AAV2/9-hU6-sgRNA_{1,3}(PTH2)-hEF1a-DIO-EGFP | Shanghai Taitool | Cat#WS0794 |
| AAV2/9-hU6-hEF1a-DIO-EGFP | Shanghai Taitool | Cat#S0883 |
| rAAV-nEF1a-IDIO-hChR2-EYFP | Brain VTA | Cat#PT-0080 |
| AAV2/9-Ef1a-DIO-His-EGFP-2A-TVA | Brain VTA | Cat#PT-0021 |
| AAV2/9-Ef1a-DIO-RVG | Brain VTA | Cat#PT-0023 |
| RV-EnvA-ΔG-dsRed | Brain VTA | Cat#R01002 |
| AAV2/9-Ef1a-DIO-hM4D(Gi)-mCherry | Brain VTA | Cat#PT-0043 |
| AAV2/9-Ef1a-DIO-mCherry | Brain VTA | Cat#PT-0013 |
| rAAV-nEF1a-IDIO-EYFP | Brain VTA | Cat#PT-0079 |
| RetroAAV2/2-Ef1a-DIO-ChR2[H134R]-mCherry | Brain VTA | Cat#PT-0002 |
| RetroAAV2/2-Ef1a-DIO-EGFP | Brain VTA | Cat#PT-0796 |
| AAV2/9-hSyn-DIO-Synaptophysin-mCherry | Brain VTA | Cat#PT-2755 |
| rAAV-Ef1a-DIO-hM3D(Gq)-mCherry | Brain VTA | Cat#PT-0042 |
| AAV9-hSyn-DA2h (DA4.3) | WZ Biosciences Inc | N/A |
| AAV9-hSyn-DAmut (2nd) | WZ Biosciences Inc | N/A |
| AAV1-hSyn-Cre-mCherry | ION gene editing core facility | N/A |
| Chemicals, peptides, and recombinant proteins |        |            |
| RNAscope® H2O2 and Protease Reagents | Advanced Cell Diagnostics | Cat#322381 |
| RNAscope® Multiplex Fluorescent Detection Reagents v2 | Advanced Cell Diagnostics | Cat#323110 |
| RNAscope® Wash Buffer Reagents | Advanced Cell Diagnostics | Cat#310091 |

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### REAGENT or RESOURCE SOURCE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| RNAscope® Multiplex TSA Buffer | Advanced Cell Diagnostics | Cat#322809 |
| Mm-SST-C1 | Advanced Cell Diagnostics | Cat#404631 |
| Mm-SST-C3 | Advanced Cell Diagnostics | Cat#404631-C3 |
| Mm-Pth2-C1 | Advanced Cell Diagnostics | Cat#1052361-C1 |
| Mm-Sltc17a6-C2 | Advanced Cell Diagnostics | Cat#319171-C2 |
| Mm-Sltc32a1-C2 | Advanced Cell Diagnostics | Cat#319191-C2 |
| EGFP-C3 | Advanced Cell Diagnostics | Cat#400281-C3 |
| Fluorescein Amplification Reagent (FITC) | Perkin Elmer | Cat#NEL741E001KT |
| Cyanine 3 Amplification Reagent (CY3) | Perkin Elmer | Cat#NEL744E001KT |
| Cyanine 5 Amplification Reagent (CY5) | Perkin Elmer | Cat#NEL745E001KT |
| DAPI | Cell Signaling Technology | Cat#4083S |
| Tetrodotoxin (TTX) | Tocris | Cat#1078 |
| CNQX | Tocris | Cat#1128 |
| 4-Aminopyridine (4-AP) | Sigma | Cat#275875 |
| Picrotoxin (PTX) | Tocris | Cat#C0832 |
| Clozapine N-oxide (CNO) | Abcam | Cat#ab120077 |
| Fluoxetine hydrochloride | Abcam | Cat#D054 |

### Deposited data

**Raw and analysed data**
This paper; Mendeley Data  
https://doi.org/10.17632/7grtvby7nz.1

**Spatial transcriptomic data**
This paper  
GEO: GSE194338  
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE194338

### Experimental models: Organisms/strains

**Mouse: C57BL/6J (B6/J)**
Slac Laboratory Animal  
N/A

**Mouse: SST-IRESCre**
Jackson Laboratory  
JAX:013044

**Mouse: Histone-GFP reporter**
Dr. Miao He at Fudan  
N/A

**Mouse: Intersection-Subtraction (IS) reporter**
Dr. Miao He at Fudan  
N/A

**Mouse: DAT-IRESCre**
Jackson Laboratory  
JAX:033673

**Mouse: Rosa26-Cas9 knockin**
Jackson Laboratory  
JAX:026179

**Mouse: Ai32**
Jackson Laboratory  
JAX:012569

### Oligonucleotides

**sgRNA targeting sequence: SST #1:**
CAGGCCAGCTTTTGCGTTCGG  
This paper  
N/A

**sgRNA targeting sequence: SST #2:**
TGACGGAGTCTGGGGTCCGA  
This paper  
N/A

**sgRNA targeting sequence: SST #3:**
GGGGCAAATCCTCGGGCTCCA  
This paper  
N/A

**sgRNA targeting sequence: PTH2 #1:**
CGCCAGGTGCAGCCCGCAGACA  
This paper  
N/A

**sgRNA targeting sequence: PTH2 #2:**
CGAGAACACAGCCGCTTCGGG  
This paper  
N/A

**sgRNA targeting sequence: PTH2 #3:**
TGTCAGGAGCCGCGAGAGAG  
This paper  
N/A

### Software and algorithms

**GraphPad Prism 8**
GraphPad  
https://www.graphpad.com/

**ImageJ**
NIH ImageJ  
https://imagej.nih.gov/ij/

**LAS X**
Leica  
https://www.leica-microsystems.com

**Inper Studio**
Inper Ltd.  
https://www.inper.com/

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**RESOURCE AVAILABILITY**

**Lead contact**
Further information and requests for resources and regents should be directed to and will be fulfilled by the lead contact, Dr. Ju Huang (juhuang@shsmu.edu.cn).

**Materials availability**
This study did not generate new unique reagents. Commercially available reagents are indicated in the key resources table.

**Data and code availability**
- Spatial transcriptomic data have been deposited at GEO: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE194338 and are publicly available as of the date of publication. The accession number is listed in the key resources table. Original images and experimental data have been deposited at Mendeley: https://doi.org/10.17632/7grtvby7nz.1 and publicly available as of the date of publication. The DOI is listed in the key resources table.
- The original code for spatial transcriptomic analyses has been deposited at Zenodo: https://doi.org/10.5281/zenodo.6960805. The DOI is listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Animals**
All experimental protocols were approved by the Institutional Animal Care and Use Committee at Shanghai Jiao Tong University School of Medicine (Protocol A-2019-060). All mice were housed at a controlled temperature (25°C) in a 12 h/12 h day/night cycle with-free access to rodent food and water. The following mouse lines were used in this study: wild-type C57BL/6J (SLAC laboratory Animal, Shanghai), SST-IRES-Cre mice (The Jackson Laboratory, Stock 013044), Histone-GFP (H2B-GFP) reporter mice (Miao He’s laboratory at Fudan University), Intersection-Subtraction (IS) reporter mice (Miao He’s laboratory at Fudan University), DAT-IRES-Flopo mice (The Jackson Laboratory, Stock 033673), and Rosa26-CAG-Cas9 mice (The Jackson Laboratory, Stock 026179), and Ai32 mice (The Jackson Laboratory, Stock 012569). Age- and gender-balanced littermate mice were randomly assigned to experimental groups. Adult male and female mice that were 3-4 months old were used in behavioral tests. We did not observe sex differences for the behavioral phenotype. All behavioral experiments were performed during the day cycle from 9 AM to 2 PM.

**METHOD DETAILS**

**Stereotaxic viral injection**
Stereotactic surgeries were performed on mice under anesthesia (100 mg/kg sodium pentobarbital, intraperitoneally (i.p.)). Body temperature was kept stable by using a temperature controller (RWD Life Science). Virus was injected at a volume of 200-300 nL/site at a flow rate of 25 nL/min using a quintessential stereotaxic injector controller (Stoelting Co.). After viral injection, the glass pipette was left in place for 10 min before withdrawal. The mice were injected with ketoprofen (5 mg/kg, i.p.) for postoperative analgesia after surgery. The animals were allowed at least 3 weeks in their home cage to recover from surgery and express virus before the behavioral tests were administered. In experiments for optogenetic stimulation and fiber photometry recording, an optic fiber (200 μm core, NA = 0.37) was planted in places as indicated. In experiments for chemogenetic inhibition, we implanted the cannulas bilaterally above axon projection fields as indicated. The fiber or cannula was secured to the skull using dental cement. We performed post hoc verification of the accuracy of viral infection and optic fiber or cannula implantation.
The corresponding coordinates of the target area were as follows (calculated from bregma): MPL (AP, -4.72 mm; ML, ±1.80 mm; DV, -3.70 mm), LHA/DMH (AP, -1.94 mm; ML, ±0.50 mm; DV, -5.25 mm), VTA (AP, -3.08 mm; ML, ±0.50 mm; DV, -4.50 mm), CeA (AP, -1.46 mm; ML, ±2.40 mm; DV, -4.50 mm), vlPAG (AP, -4.72 mm; ML, ±0.50 mm; DV, -2.50 mm), MPO (AP, 0.38 mm; ML, ±0.15 mm; DV, -5.45 mm), PVA (AP, -0.94 mm; ML, ±0.25 mm; DV, -2.90 mm), BNST (AP, 0.26 mm; ML, ±0.85 mm; DV, -4.10 mm), PVN (AP, -1.50 mm; ML, ±0.25 mm; DV, -4.90 mm), NAc (AP, 1.10 mm; ML, ±1.20 mm; DV, -4.30 mm). The virus and titers used in this study were as follows: AAV2/9-hEF1a-DIO-ChR2(H134R)-mCherry-WPRE-pA (3.40E+12 v.g./ml); AAV2/9-hSyn-DIO-GCaMP7s-WPRE-pA (2.5E+12 v.g./ml); RetroAAV2/2-hSyn-FLEX-tdTomato-WPRE-pA (1.44E+12 v.g./ml); AAV2/9-hEF1a-DIO-EGFP-WPRE-pA (1.78E+13 v.g./ml); RetroAAV2/2-CAG-FLEX-Flpo (3.18E+13 v.g./ml); AAV2/9-hEF1a-DIO-mGFP-WPRE-pA (2.00E+12 v.g./ml); AAV2/9-CAG-DIO-taCaspase3-TEVP-WPRE-pA (1.99E+13 v.g./ml); RetroAAV2/2-hSyn-Cre-WPRE-pA (2.55E+13 v.g./ml); RetroAAV2/2-hSyn-Fipo-WPRE-pA (2.32E+12 v.g./ml); AAV2/9-Ef1a-DIO-His-EGFP-2A-TVA-WPRE-pA (2.00E+12 v.g./ml); AAV2/9-Ef1a-DIO-EGFP-pA (2.5E+12 v.g./ml); RetroAAV2/2-Ef1a-DIO-ChR2(H134R)-mCherry-WPRE-pA (5.49E+12 v.g./ml); RetroAAV2/2-Ef1a-DIO-EGFP-WPRE-hGH-pA (5.85E+12 v.g./ml); AAV2/9-hSyn-DIO-Synaptophysin-mCherry-WPRE-hGH-pA (4.51E+12 v.g./ml); AAV9-hSyn-DA2h (5.99E+13 v.g./ml); AAV9-hSyn-DAmut (5.03E+13 v.g./ml); AAV2/9-hu6-sgRNA-3(SST)-hEF1a-DIO-EGFP-WPRE-pA (1.33E+13 v.g./ml); AAV2/9-hu6-sgRNA-3(PTh2)-hEF1a-DIO-EGFP-WPRE-pA (1.61E+13 v.g./ml); AAV2/9-hu6-hEF1a-DIO-EGFP-WPRE-pA (2.08E+13 v.g./ml); AAV1-hSyn-Cre (1.3E+13 v.g./ml); AAV2/9-hSyn-FLEX-ChrimsonR-tdTmato-WPRE-pA (3.76E+12 v.g./ml); AAV2/9-Ef1a-fDIO-hChr2(H134R)-EYFP (3.42E+12 v.g./ml); rAAV-Ef1a-DIO-hM3D(Gq)-mCherry-WPRE-pA (6.91E+12 v.g./ml); AAV2/9-hEF1a-fas-hChr2(H134R)-mCherry-WPRE-pA (1.23E+13).
Optogenetic activation

We injected AAV-DIO-ChR2-mCherry or AAV-DIO-mCherry into the MPL of SST-Cre mice and implanted the optic fiber above the MPL to activate the cell bodies of MPLSST neurons or above the respective downstream target regions, including the LHA/DMH, VTA, vIPAG, MPO, or PVA, to activate the axon terminals of MPLSST neurons. We injected retro-AAV-hSyn-Cre in the MPL of WT mice and AAV-DIO-ChR2-mCherry into respective upstream input regions, including the BNST, CeA, vIPAG, PVN, IC or Au to activate the MPLSST upstream input neurons. A 473-nm blue laser light with a 20-ms pulse duration at 20 Hz for 25 minutes (constant 1-minute on and 4-minute interval alternating for a total of 5 trials) was applied to activate the cell bodies or axon terminals of MPLSST neurons. In the experiments to measure dopamine release, we injected AAV-DIO-ChR2-mCherry into the MPL of SST-Cre mice. A 640-nm laser light was applied above the MPL to activate the cell bodies of ChrimsonR-expressing MPLSST neurons. In these experiments, we performed post hoc verification of the accuracy of viral injection sites and optic fiber placement. In the ChR2-assisted circuit mapping and whole-cell patch clamp recording experiments, we injected AAV-DIO-ChR2-mCherry into the MPL of SST-Cre or SST-Cre; DAT-Fplo mice. A 480-nm blue LED light with a single 10-ms pulse was applied to the acute slices for 8 sweeps with a 10-s intersweep interval to activate the axon terminals of MPLSST neurons.

Chemogenetic activation

For chemogenetic activation experiments, we injected AAV-DIO-hM3D-mCherry or AAV-DIO-mCherry bilaterally into the MPL of SST-Cre mice. We waited four weeks to allow animal recovery and viral expression. On the test day, the mice were first habituated in a test chamber (20 cm length, 20 cm width, 18 cm height) for 15 minutes. Then, the mice were intraperitoneally injected with CNO (1 mg/kg, Sigma), followed by the measurement of self-grooming behavior. The time spent in self-grooming was calculated during the period of 60-120 min after CNO injection.

Visium spatial transcriptomic profiling

The joint dataset consisted of a pool of 3651 individual spots, with each spot equivalent to a microdissection containing tens of cells, with an average of 5,233 genes and 17,575 unique transcripts per spot after filtering. The gene-spot matrix was generated by Space Ranger (versions 1.1.0) and analyzed with the Seurat package (versions 3.2.3) in R. Normalization was performed with the SCTransform function. We only considered ‘expressed’ genes to be those whose normalized expression values were more than 1.5. The expression values of the ‘nonexpressed’ genes were set to zero. Dimensionality reduction was performed by the RunPCA and RunTSNE functions. Clustering was performed by the FindNeighbors and FindClusters functions with the first 30 PCs at a resolution of 0.6. The clusters were visualized by the DimPlot and SpatialDimPlot functions. Heatmaps of spatial gene expression were generated with the SpatialFeaturePlot function. The tSNE plots of gene expression were generated with the FeaturePlot function. Differential expression analysis of the variable genes was performed by the FindAllMarkers function using the MAST test. We selected the significantly changed genes (adjusted p value < 0.05) and sorted them by the log-fold-change. The differentially expressed genes (DEGs) enriched in the MPL, compared to other regions, were passing the statistical threshold of the adjusted p value < 0.05 and log-fold-change > 1. The average expression level and percentage of expressed cells of the top three marker genes in each cluster were visualized by the DotPlot function. The violin plots of the marker gene expression level in each cluster were generated by the VlnPlot function. The average gene expression levels in cluster #13 compared with other clusters are shown by scatter plot. The highlighted marker genes of cluster #13 were selected with the criteria of log2FC > 1 and adjusted p value < 0.05.

Chemogenetic inhibition and neuronal ablation

For chemogenetic inhibition experiments, we injected AAV-DIO-hM4D-mCherry or AAV-DIO-mCherry virus bilaterally into the MPL of SST-Cre mice. To inhibit the axon terminals, the cannulas were implanted above the downstream target regions of MPLSST neurons. We waited four weeks to allow animal recovery and viral expression. Ten minutes before restraint treatment, we applied clozapine N-oxide (CNO, 3 mg/kg, Sigma) via i.p. injection to inhibit the cell bodies of MPLSST neurons or applied CNO (1 mM) into the cannulas with a microinjection pump (RWD Ltd.) at a rate of 0.5 µL/min and then waited an additional minute to allow the drug to be locally delivered into the respective axon terminal fields. In the Caspase-3-mediated neuronal ablation experiment, we injected AAV-DIO-taCaspase3 or AAV-DIO-mCherry bilaterally into the MPL of SST-Cre mice. After 4 weeks of recovery, we validated that the MPLSST neurons were effectively ablated.

Effect of fluoxetine on self-grooming

To test the effect of chronic treatment with fluoxetine hydrochloride (a 5-HT reuptake inhibitor) on MPL activation-induced self-grooming, we injected fluoxetine hydrochloride (5 mg/kg, Abcam) or saline intraperitoneally into the mice once a day for seven days. Twenty-four hours after the final injection, we measured chemogenetic-evoked self-grooming in saline-treated and fluoxetine-treated mice. The mice were first habituated in the test chamber (20 cm length, 20 cm width, 18 cm height) for 15 minutes, followed by intraperitoneal injection with CNO (1 mg/kg, Sigma). Self-grooming behavior was recorded and quantified by measuring the time spent in self-grooming during the time period of 60-120 min after CNO injection.
Monosynaptic retrograde tracing

For monosynaptic retrograde tracing to identify the upstream input of MPL\textsuperscript{SST} neurons, AAV-DIO-His-EGFP-2A-TVA and AAV-DIO-RVG were stereotactically injected into the MPL of SST-Cre mice. After three weeks of recovery and AAV expression, RV-EnvA-ΔGsRed was injected into the LHA in a biosafety level 2 environment. The mice were sacrificed one week after RV injection, and the brain tissue was fixed and sectioned. We obtained images of whole-brain sections using a Leica SP8 X confocal system.

Whole-cell patch clamp recording

We performed ChR2-assisted circuit mapping (CRACM) to examine whether the neurons in the VTA received excitatory synaptic input from MPL\textsuperscript{SST} neurons. We injected AAV-DIO-Chr2-mCherry into the MPL and AAV-IDIO-EYFP into the VTA in SST-Cre; DAT-Fipo mice. Four weeks after viral injection, we performed whole-cell patch clamp recording. Brain slices (250 μm) were prepared by vibratome (Leica, VT1200S) in ice-cold oxygenated high-Mg\textsuperscript{2+} and low-Ca\textsuperscript{2+} slicing solution containing 83 mM NaCl, 2.4 mM KCl, 0.5 mM CaCl\textsubscript{2}, 6.8 mM MgCl\textsubscript{2}, 24 mM NaHCO\textsubscript{3}, 1.61 mM NaH\textsubscript{2}PO\textsubscript{4}, 24 mM D-glucose, and 65 mM sucrose. Then, acute slices were recovered for 1 hour at 37 °C in artificial cerebrospinal fluid (ACSF). The ACSF contained 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH\textsubscript{2}PO\textsubscript{4}, 26 mM NaHCO\textsubscript{3}, 1.3 mM MgCl\textsubscript{2}, 2.5 mM CaCl\textsubscript{2}, and 10 mM D-glucose bubbled with 95% O\textsubscript{2}/5% CO\textsubscript{2}. During recording, the acute slices were transferred to a recording chamber that was perfused continuously with O\textsubscript{2}-saturated ACSF at a rate of 2 mL/min. The intracellular solution used for whole-cell patch clamp recording contained 147 mM K-glucosone, 3 mM KCl, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 4 mM ATP-Mg, 0.3 mM GTP-Na, 10 mM Na\textsubscript{2}HPO\textsubscript{4}, and 0.3 mM ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA). We performed recordings using a MultiClamp 700B amplifier digitized at 10 kHz by a Digidata 1550B D-A converter and Bessel-filtered at 2 kHz. The recording pipette resistance was 4-6 MΩ, and the series resistance was < 30 MΩ. The cells were excluded if the series resistance exceeded 30 MΩ. To deliver photostimulation, a pulse of 10-ms blue light from a light-emitting diode (LED) source was delivered through an Olympus 40x water-immersion lens. Drugs were applied at the following concentrations: PTX, 100 μM; CNQX, 40 μM; TTX, 2 μM; 4-AP, 100 μM; and CNO, 50 μM.

Fiber photometry recording of GCaMP7s signals

AAV-DIO-GCaMP7s virus was injected unilaterally at a volume of 300 nL into the MPL of SST-Cre mice. An optic fiber with a 200-μm outer diameter was implanted close to the viral injection site and fitted into a ceramic fiber holder on the head. Fiber photometry experiments were performed as previously described (Yang et al., 2021). We used a two-color fiber photometry system (Inper Ltd.) to record the in vivo calcium signal of MPL\textsuperscript{SST} neurons. The 488-nm excited GCaMP7s fluorescence signal and the 410-nm excited internal control signal were acquired at a 100-Hz sample rate. The LED light power was adjusted at the tip of the optic fiber to 30-50 mW to minimize bleaching. Self-grooming behavior was recorded by a video camera (Logitech). To record the calcium signal of MPL\textsuperscript{SST} neurons in freely moving mice, the mice were placed in a test chamber (20-cm length, 20-cm width, 18-cm height), and the calcium signal and control signal were recorded simultaneously. We recorded self-grooming-related calcium dynamics in MPL\textsuperscript{SST} neurons under different conditions: (a) physiological condition; (b) by 30-min body restraint; (c) by water spray (three times) on the face; and (d) by oil drop (50 μl) on the face. To measure the dynamics of fluorescence intensity before and after the onset of self-grooming, F/F\textsubscript{0} was represented as ∆F/∆t, where F\textsubscript{0} was the baseline fluorescence signal averaged over a 10-s window before self-grooming. ∆F/∆t values are presented as the mean with an S.E.M. envelope. To measure the peak ∆F/Δt around grooming onset (-1 s to 1.5 s) and the temporal relationship between calcium signal and self-grooming, GCaMP7s signals were aligned with the initiation of self-grooming, and the baseline fluorescence signals were averaged over a 0.5 s (-1 s to -0.5 s) window before self-grooming. The onset time of calcium signal increase was defined as the time when the peak reached 15% of its peak amplitude (Xie et al., 2022).

Fiber photometry recording of dopamine release

To record dopamine release in the NAc, we injected AAV-DIO-ChrimsonR-tdTomato into the MPL of SST-Cre mice. An AAV expressing dopamine sensor DA2h (AAV-hSyn-DA2h) or its mutant form DAmut as a control (AAV-hSyn-DAmut) was injected into the NAc. A 640-nm photostimulation (10 mW, 20 Hz, 20-ms pulse, 1-s or 5-s duration) was delivered to the MPL to activate the ChrimsonR-expressing MPL\textsuperscript{SST} neurons, and an optic fiber was implanted above the NAc to detect the fluorescent signal generated by DA2h at a 100-Hz sample rate with 488-nm excited light, allowing us to effectively measure the true fluorescence signal from the NAc without any potential contamination of the light signal coming from the laser stimulation at the MPL. To record dopamine release in the NAc correlated with restraint-induced self-grooming, the mice were first treated with a 30-min body restraint, followed by fiber photometry recording of dopamine signals in the NAc. Time 0 was defined as the trigger time of optogenetic stimulation (640-nm laser) or the behavioral onset of self-grooming. To measure the fluorescence dynamics of DA release before and after the onset of optogenetic stimulation or self-grooming, we calculated the area under the curve (AUC) between 0-1 s or 0-5 s relative to the optogenetic stimulation onset. To measure the temporal relationship between DA release and MPL activation (or self-grooming), DA2h signals (-1 s to 2 s) were aligned with the initiation of the optogenetic trigger time (or onset of self-grooming). The baseline fluorescence signals were averaged over a 1-s (-1 s to 0 s) window before the optogenetic trigger time (or onset of self-grooming). The onset time of DA release was defined as the time when the signal reached 15% of its peak amplitude.
Open field (OF) test
Mice were placed in one corner of an open field arena (40-cm length, 40-cm width, 30-cm height). A video-tracking system (EthoVision 3.0, Noldus) was used to measure the locomotor activity of the animal. The time spent in the center zone (20 cm x 20 cm) and the total distance and velocity traveled in the whole open field arena were measured over 20 minutes.

Elevated plus maze (EPM) test
The apparatus consisted of a central square (7-cm length, 7-cm width), two open arms without walls (30-cm length, 7-cm width) and two closed arms with walls (30-cm length, 7-cm width, 65-cm height). Mice were placed into the central area facing one closed arm and allowed to explore for 10 minutes. The time spent in the open arms and the total distance and velocity traveled in the whole apparatus were recorded by the video-tracking system (EthoVision 3.0, Noldus).

Novelty suppressed feeding (NSF) test
The mice were food-deprived for 24 hours. A single food pellet (regular chow) was placed on a piece of filter paper (10 cm in diameter) in the center of a new open field arena (40-cm length, 40-cm width, 30-cm height) whose floor was covered with bedding. The mouse was placed in one corner of the arena and allowed to explore for up to 10 minutes. The trial ended when the mouse initially bit and consumed the food pellet. After the NSF test, the mouse was immediately transferred into the home cage, where the mice were presented with a preweighed amount of chow for 5 minutes. The amount of consumed food was weighed at the end of the session as a control of feeding drive.

Conditioned place preference (CPP) test
A two-chamber apparatus was used with different visual (black vs. white sides) and textural cues (smooth transparent vs white matte floor) for the conditional place preference test. An overhead video camera was used to trace the position of the mice (Cineplex, Plexon). The mice were habituated in the apparatus for 30 minutes on the first day. On the following day, the mice were allowed to move freely in the two-chamber apparatus for 30 minutes for the measurement of initial place preference. On the 3\textsuperscript{rd} to 6\textsuperscript{th} days of conditioning, a 473-nm photostimulation (4 mW, 20 Hz, 10-ms pulse, constant 5 s ON with 5 s interval) was delivered to the mice in the less preferred chamber for 30 minutes per day. On the 7\textsuperscript{th} day, the mice were given free access to the entire two-chamber apparatus for 30 minutes without stimulation, and their positions were tracked. The change in the percentage of occupancy time on the light side (after conditioning- before conditioning) was calculated.

Intracranial self-stimulation (ICSS) test
The mice were placed in an operant conditioning chamber (25 cm x 18 cm x 20 cm, AniLab) that has two nose-poke ports (active and inactive) with infrared beam sensors. Nose-poking of the active port triggered the delivery of a 473-nm photostimulation (4 mW, 20 Hz, 20-ms pulse, 5-s duration), while nose-poking of the other port was inactive. Mice were first trained to associate a successful nose poke with photostimulation for a 1-hour session on an FR1 (fixed ratio 1) reinforcement schedule, in which each photostimulation required two additional nose pokes than the previous one, i.e., 1, 3, 5, 7, 2\textsuperscript{nd}, 3\textsuperscript{rd}, 4\textsuperscript{th}, 5\textsuperscript{th}, 6\textsuperscript{th} days before the ICSS test.

CRISPR/Cas9-mediated gene knockdown
CRISPR/Cas9-mediated gene knockdown methods were used to knockdown SST and PTH2 in MPL\textsuperscript{SST} neurons. The sgRNA target sequences were first selected by submitting the exon sequences of the SST and PTH2 genes to the CRISPR design tool (http://zlab.bio/guide-design-resources). Then, we performed additional Cas9 target selection from the top 6 candidates obtained from the CRISPR design tool. We transfectioned both the Cas9-, SST sgRNA-, and PTH2 sgRNA-expressing plasmids into Neuro2A cells. Three days after transfection, we harvested the cells for genomic DNA extraction followed by sequencing. Based on the analyses of CRISPR-mediated gene editing efficiency in Neuro2A cells, we selected the top 3 sgRNAs for both SST and PTH2. The three target sequences for the SST gene are as follows: CAGCCAGCTTGGTCTCCGA; TGACGGAGTCTGGGGTCCGA; and GGGCAAATCCTCGGGCTCCA. The three target sequences for the PTH2 gene are as follows: CGCCAGCTTGGTCTCCGA; TGACGGAGTCTGGGGTCCGA; and TGTCACAGCGCCGAGAG. The sgRNAs were then cloned into the construct according to the CRISPR-Cas9 mouse Toolbox. An AAV vector backbone without sgRNA (AAV-hU6-hEf1a-DIO-EGFP) was used as a control. AAV-hU6-sgRNA\textsubscript{1-3}(SST)-hEf1a-DIO-EGFP or AAV-hU6-sgRNA\textsubscript{1-3}(PTH2)-hEf1a-DIO-EGFP was injected into the MPL of SST-Cre mice crossed with Rosa26-CAG-Cas9 mice. After four weeks of recovery and AAV expression, mice were subjected to behavioral tests, including the stress-induced self-grooming test and the ICSS test. We performed post hoc smFISH experiments to validate the knockdown efficiency of endogenous SST and PTH2 in the MPL.
**Dopamine inhibition assay**

In SST-Cre; DAT-Flpo mice, we injected AAV-DIO-ChR2-mCherry unilaterally into the MPL and AAV-FDIO-hM4D-mCherry (AAV-FDIO-mCherry as the control) bilaterally into the VTA to inhibit VTA<sup>DA</sup> neurons. Grooming behavior was tested 4 weeks after viral injection. The mouse was i.p. injected with CNO (Sigma, 3 mg/kg), and 40 min later, a 473-nm blue laser light with a 20-ms pulse duration at 20 Hz (constant 1-minute on and 4-minute interval alternating for a total of 5 trials) was applied to activate the cell bodies of MPL<sup>SST</sup> neurons. Following the self-grooming test, the mice were subjected to a 10-min open field test. In the experiment with the use of dopamine D1R antagonist, SCH23390 (Sigma, 1 mg/kg) was i.p. administered 15 min before optogenetic stimulation. A 473-nm blue laser light with a 20-ms pulse duration at 20 Hz for 25 minutes (constant 1-minute on and 4-minute interval alternating for a total of 5 trials) was applied to the axon terminals of the MPL<sup>SST</sup> neurons in the VTA. Then we measured the effect of SCH23390 on the self-grooming induced by activating the MPL<sup>SST</sup> neuron projections to the VTA.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All data are presented as the mean ± SEM unless otherwise stated, and significance levels are indicated as *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. The data were analyzed using paired Student’s t-test in Figures 1E, 1G, 4C–4H, 5D, 7D–7F, S1J, S1N, S2E, S2G, S3A–S3C, and S7F. Unpaired Student’s t-tests were used in Figures 3C–3E, 3G, 3H, 3J, 3K, 3M, 3N, 4I, 5E–5G, 6D, 6H, 6L, 6M, 7J, 7K, S1C, S1E, S1G, S2E, S2G, S4D–S4F, S4H, S4I, S6C, S6D, S6H, S6L, S7F, and S7I–S7K. One-way analysis of variance (ANOVA) with Bonferroni’s multiple comparisons test is shown in Figures 1H, 6P, and 6Q. Linear regression and Pearson correlation were calculated by MATLAB for data analyses in Figure 2J. The Kolmogorov-Smirnov test was used to compare the cumulative poke numbers of the ICSS in Figures 6F, 6G, 6J, 6K, 6O, S6B, S6F, S6G, S6J, and S6K. All statistical analyses were performed using GraphPad PRISM 8 software or MATLAB_R2016b.