The paraventricular thalamus controls a central amygdala fear circuit

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Appropriate responses to an imminent threat brace us for adversities. The ability to sense and predict threatening or stressful events is essential for such adaptive behaviour. In the mammalian brain, one putative stress sensor is the paraventricular nucleus of the thalamus (PVT), an area that is readily activated by both physical and psychological stressors1–5. However, the role of the PVT in the establishment of adaptive behavioural responses remains unclear. Here we show in mice that the PVT regulates fear processing in the lateral division of the central amygdala (CeL), a structure that orchestrates fear learning and expression6,7. Selective inactivation of CeL-projecting PVT neurons prevented fear conditioning, an effect that can be accounted for by an impairment in fear-conditioning-induced synaptic potentiation onto somatostatin-expressing (SOM+) neurons8,9. The pPVT–amygdala projections (Extended Data Fig. 2). Notably, pPVT neurons projecting to the CeL and BLA were largely non-overlapping (Extended Data Fig. 2).

To determine whether the pPVT–CeL pathway, the most prominent projection originating from the pPVT4,9, is involved in fear conditioning, we sought to selectively inhibit CeL-projecting neurons in the pPVT through a chemogenetic method11. We bilaterally injected the CeL with a retrograde canine adenovirus expressing Cre recombinase (CAV2-Cre)12, followed by injection into the PVT of AAV-DIO-hM4Di-mCherry, an adeno-associated virus harbouring a double-floxed inverted open reading frame (AAV-DIO) that expresses, in a Cre-dependent manner, an engineered G1-coupled receptor hM4Di tagged with a fluorescent protein mCherry (hM4Di–mCherry). This intersectional strategy effectively targeted CeL-projecting pPVT neurons (Fig. 1a, b), which can subsequently be suppressed by treating mice with clozapine-N-oxide (CNO) before conditioning or retrieval. Our results demonstrate that the PVT–CeL pathway constitutes a novel circuit essential for both the establishment of fear memory and the expression of fear responses, and uncover mechanisms linking stress detection in PVT with the emergence of adaptive behaviour.

To probe the sensitivity of the PVT to threatening events, we examined the expression of c-Fos, a marker of recent neuronal excitation, both following fear conditioning and after a fear memory retrieval test. Fear conditioning markedly increased the number of neurons expressing c-Fos in the posterior PVT (pPVT) (Extended Data Fig. 1), consistent with the finding that the pPVT receives direct inputs from the nociceptive parabrachial nucleus and the periaqueductal grey13,14. Notably, fear memory retrieval induced a similar increase in pPVT c-Fos expression (Extended Data Fig. 1). These results demonstrate that the pPVT is recruited by both the unconditioned stimulus and the threat-predicting conditioned stimulus, and raise the possibility that it might be instrumental in fear conditioning.

The pPVT strongly projects to the CeL,9,15, with weaker projections to other amygdala nuclei, such as the basolateral amygdala (BLA)10. To examine the distribution patterns of pPVT neurons innervating different amygdala subregions, we injected the CeL and BLA with the retrograde tracer cholera toxin subunit B conjugated to Alexa Fluor-488 (CTB-488) or Alexa Fluor-555 (CTB-555), respectively. This approach resulted in dense labelling throughout the ipsilateral pPVT, indicating prominent pPVT–amygdala projections (Extended Data Fig. 2). Notably, pPVT neurons in pPVT and the behavioural effect. CNO before conditioning, n = 15 mice; hM4Di with CNO before retrieval, n = 9 mice; hM4Di with CNO before conditioning, n = 13 mice; effect of treatments, F(2,68) = 5.14, P < 0.01; effect of treatment stimulus presentation, F(1,68) = 51.27, P < 0.001; interaction, F(2,68) = 7.42, P < 0.01; *P < 0.05; two-way analysis of variance (ANOVA) followed by Tukey’s test. The control group contains mice that were injected only with CAV2-Cre bilaterally in the CeL and were treated with CNO either before conditioning (n = 7 mice) or before retrieval (n = 8 mice). d, Correlation between viral infection efficiency in pPVT and the behavioural effect. CNO before conditioning, R2 = 0.59, P < 0.01; n = 9 mice; CNO before retrieval, R2 = 0.47, P < 0.05, n = 13 mice; linear regression lines are shown in grey. Data are presented as mean ± s.e.m.

Figure 1 | CeL-projecting pPVT neurons are essential for both learning and expression of conditioned fear. a, A schematic of the experimental approach. b, Representative images showing the expression of hM4Di–mCherry in CeL-projecting pPVT neurons. c, Quantification of freezing levels in memory retrieval test. Control, n = 15 mice; hM4Di with CNO before conditioning, n = 9 mice; hM4Di with CNO before retrieval, n = 13 mice; effect of treatments, F(2,68) = 5.14, P < 0.01; effect of condition stimulus presentation, F(1,68) = 51.27, P < 0.001; interaction, F(2,68) = 7.42, P < 0.01; *P < 0.05; two-way analysis of variance (ANOVA) followed by Tukey’s test. The control group contains mice that were injected only with CAV2-Cre bilaterally in the CeL and were treated with CNO either before conditioning (n = 7 mice) or before retrieval (n = 8 mice). d, Correlation between viral infection efficiency in pPVT and the behavioural effect. CNO before conditioning, R2 = 0.59, P < 0.01; n = 9 mice; CNO before retrieval, R2 = 0.47, P < 0.05, n = 13 mice; linear regression lines are shown in grey. Data are presented as mean ± s.e.m.

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(CNO), the agonist of hM4Di\(^{11}\). Notably, selective suppression of the CeL-projecting pPVT neurons during either conditioning or a 24-h memory retrieval test significantly impaired fear responses measured as freezing in the retrieval test (Fig. 1c and Extended Data Fig. 3). These behavioural effects significantly correlated with the number of hM4Di-expressing neurons in the pPVT (Fig. 1d), demonstrating the specificity and potency of our manipulation. Altogether, these results indicate that the pPVT is crucial for both the establishment and expression of fear memory.

We next determined the mechanisms by which the pPVT–CeL circuit contributes to fear regulation. Fear conditioning induces a potentiation of excitatory synapses onto SOM\(^+\) CeL neurons, a synaptic change that stores fear memory\(^5\). To investigate whether the pPVT is required for this plasticity, we labelled SOM\(^+\) CeL neurons with enhanced yellow fluorescent protein (eYFP) by injecting CeL with AAV-DIO-eYFP in Som-cre mice, in which the Cre recombinase is expressed under the endogenous Som promoter\(^6\). In addition, we injected the pPVT in the same mice with a mixture of AAV-GFP-Cre and AAV-DIO-hM4Di-mCherry (Fig. 2a–c). This strategy allowed us to inhibit pPVT neurons using the chemogenetic method during fear conditioning, and subsequently determine the effect on SOM\(^+\) (eYFP\(^+\)) CeL neurons (Fig. 2 and Extended Data Fig. 3).

As previously reported\(^4\), fear conditioning significantly enhanced excitatory synaptic transmission—measured as an increase in both the frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs)—onto SOM\(^+\) CeL neurons (Fig. 2d, e). This synaptic potentiation can be detected at both 3 h and 24 h following conditioning\(^7\). Notably, inhibition of pPVT neurons during fear conditioning did not affect this synaptic potentiation when examined 3 h after conditioning (Fig. 2d, e). In contrast, the same manipulation completely abolished synaptic potentiation measured 24 h after conditioning (Fig. 2d, e). These results indicate that the pPVT is required for the maintenance or consolidation, but not the initial induction, of CeL plasticity, and are consistent with findings in an accompanying study that long-term (>24 h), but not short-term (0.5 and 6 h), fear memories are susceptible to pPVT manipulations (see accompanying paper\(^3\)).

As mEPSCs do not allow us to distinguish between different synaptic pathways, we next examined whether pPVT inactivation impairs plasticity at lateral-amygdala–CeL synapses, a pathway that presumably conveys conditioned-stimulus information to the CeL. For this purpose we used the Som-cre;Rosa26-stagy\(^{12}\)-H2b–GFP (Som-cre;H2b-GFP) mice\(^14\) in which all SOM\(^+\) CeL neurons are readily identified on the basis of the nucleus-localized GFP signal (Extended Data Fig. 4a). We inhibited pPVT neurons during fear conditioning using the same method as described above (Fig. 2a, b). We then simultaneously recorded pairs of adjacent SOM\(^+\) (green fluorescent) and SOM\(^-\) (non-fluorescent) CeL neurons in acute brain slices, while mEPSCs were evoked by electrical stimulation in the lateral amygdala (Extended Data Fig. 4a). As previously reported\(^3\), in naive control mice AMPA receptor (AMPAR)-mediated mEPSCs were significantly larger in SOM\(^+\) than in SOM\(^-\) neurons (Extended Data Fig. 4b, c), indicating a clear distinction between these two cell types in their intrinsic functional connectivity. However, fear conditioning reversed this relationship such that AMPAR-mediated transmission was significantly stronger in SOM\(^+\) than in SOM\(^-\) neurons (Extended Data Fig. 4b, c).

Fear conditioning also induced a decrease in the paired-pulse ratio of mEPSCs (an indicator of increased presynaptic release probability) onto SOM\(^+\) CeL neurons (Extended Data Fig. 4d). This result, when considered together with data obtained from the paired recording (Extended Data Fig. 4a–c) and mEPSC recording (Fig. 2d, e) experiments (also see ref. 6), demonstrates that fear conditioning strengthens excitatory synaptic transmission onto SOM\(^+\) CeL neurons, and that an increase in presynaptic release probability is likely to be the major underlying mechanism. Notably, inhibition of the pPVT during conditioning largely blocked these synaptic changes in the lateral-amygdala–CeL pathway (Extended Data Fig. 4b–d). Altogether, these results indicate that the pPVT participates in fear memory formation by regulating the maintenance of fear-conditioning-induced plasticity at the lateral-amygdala–CeL synapses.

SOM\(^+\) and SOM\(^-\) neurons—the latter being predominantly protein kinase C-\(\delta\)-expressing (PKC-\(\delta\)^\(\delta\))—constitute two major CeL populations that are mutually inhibitory\(^6,11\). We reasoned that the pPVT might control CeL synaptic plasticity by regulating either one or both of these populations. To distinguish between these possibilities, we used a modified rabies virus system that can trace the monosynaptic inputs onto genetically identified neurons\(^16\) (Methods). This approach revealed that the pPVT projects to both populations (Fig. 3a–c), with the distinction that there were twice as many pPVT neurons innervating SOM\(^+\) neurons as those innervating PKC-\(\delta\) neurons (the connectivity indices, calculated as the ratio of the number of rabies-virus-labelled cells in the pPVT to that of starter cells in the CeL, for the two cell types are: SOM\(^+\), 0.99 ± 0.08, \(n = 3\) mice; PKC-\(\delta\), 0.49 ± 0.03, \(n = 3\) mice; \(P < 0.01\), t-test), suggesting that pPVT afferents in the CeL preferentially innervate SOM\(^+\) neurons.

To assess the functional connectivity between the pPVT and CeL, we used the Som-cre;Ai14 mice, in which SOM\(^+\) cells can be identified by their red fluorescence, and injected the pPVT with an AAV expressing channelrhodopsin-2 (AAV-ChR2-YFP) that allows photostimulation of axonal projections\(^17\) (Fig. 3d, e). Bright ChR2–YFP-labelled fibres were readily observed throughout the CeL in acute slices prepared from these mice, confirming a strong pPVT–CeL projection. To our surprise, brief light pulses, which reliably evoked excitatory synaptic transmission in BLA neurons (Extended Data Fig. 5a–c), failed to evoke any detectable fast synaptic transmission in all the recorded SOM\(^+\) or SOM\(^-\) CeL neurons (Fig. 3e, f). Notably, high-frequency photostimulation of pPVT afferents in the CeL induced a slow inward current exclusively in SOM\(^+\) neurons (Fig. 3e–g and Extended Data Fig. 5d–f). High-frequency photostimulation also generated a sustained increase in the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) onto SOM\(^+\) neurons (Extended Data Fig. 5g–i). Because SOM\(^+\) cells...
The pPVT (Fig. 3). These results suggest that BDNF might be a critical inhibitor of SOM+ cells in the CeL, these results suggest that pPVT inputs selectively facilitate the activation of SOM+ neurons, which in turn suppress SOM- neurons.

Given that stimulation of pPVT afferents in the CeL evoked slow inward currents, rather than canonical fast synaptic responses in SOM+ neurons, it is likely that the pPVT→CeLSOM transmission is mediated by a neuromodulator. One notable candidate for this is BDNF, a known modulator of synaptic function, the messenger RNA expression of which is highest in the pPVT within the dorsal thalamus. We found that the majority (∼70%; n = 5 mice) of CeL-projecting pPVT neurons did not express BDNF (Extended Data Fig. 6a, b). In contrast, only 29% (n = 2 mice) of BLA-projecting pPVT neurons expressed BDNF, a value that is probably an overestimation owing to contamination by CeL-projecting pPVT neurons caused by tracer diffusion into the CeL. (Extended Data Fig. 6c, d). In addition, CeL expression of the high-affinity BDNF receptor TrkB was largely restricted to SOM+ neurons (76% colocalization, n = 4 mice; Extended Data Fig. 7a), consistent with the preferential targeting of this particular cell type by inputs from the pPVT (Fig. 3). These results suggest that BDNF might be a critical factor mediating pPVT to CeL communication.

To examine the functional role of BDNF in the pPVT→CeLSOM interaction, we bred the Trkblox/lox,Som-Flp mice, which carry the Trkblox/lox conditional alleles and in which the Flp recombinase is expressed under the endogenous Som promoter. In these mice, TrkB can be deleted in the CeL by injection of the AAV-Cre–GFP, while SOM+ neurons can be tagged by injection with AAV-dIO-mCherry, an AAV harbouring a double-FRT-flanked inverted open reading frame (dIO) that expresses mCherry in an Flp-dependent manner, thereby allowing their identification in acute slices for electrophysiological recording (Extended Data Fig. 7b, c). Using this strategy, we found that the pPVT-driven slow excitatory inward currents were selectively abolished in SOM+ neurons in which TrkB was deleted (mCherry and GFP double-positive neurons; Extended Data Fig. 7c–f). Consistent with this result, bath application of the BDNF scavenger TrkB-Fc in acute slices abolished the pPVT-driven increase in inhibition onto SOM+ CeL neurons (Extended Data Fig. 7g, h), whereas exogenous application of BDNF mimicked this increase in inhibition (Extended Data Fig. 7i, j). These results indicate that BDNF/TrkB signalling is a mediator of pPVT→CeLSOM communication.

Next, to determine whether this BDNF/TrkB-mediated pPVT→CeLSOM communication is important for fear processing, we selectively deleted either Bdnf from the pPVT, or TrkB in SOM+ CeL neurons. To achieve the former, we employed a mouse line carrying the Bdnfluox/lox conditional alleles and injected the pPVT with AAV-GFP-Cre (Fig. 4a–c). To achieve the latter, we designed an AAV-dIO-Cre-GFP that expresses Cre–GFP (a Cre and GFP fusion protein) under the control of Flp (Extended Data Fig. 8). This virus, when injected into the CeL of the Trkblox/lox,Som-Flp mice, expresses Cre (and hence leads to TrkB deletion) specifically in SOM+ neurons. This intersectional recombinase-mediated areal- and cell-specific gene excision (IRASE) method can be generally applied for gene deletion with spatial and cell-type specificity.

Deletion of Bdnf in the pPVT depleted BDNF from the CeL (BDNF+ cells in CeL: GFP group, 156.36 ± 39.40 cells per mm², n = 8 mice; GFP–Cre group, 41.20 ± 12.00 cells per mm², n = 7 mice; P < 0.05, t-test) (Fig. 4a, b) and markedly impaired fear conditioning (Fig. 4c and Extended Data Fig. 3). In parallel, selective deletion of TrkB in SOM+ CeL neurons by IRASE similarly impaired fear conditioning (Fig. 4d–f and Extended Data Fig. 3). These results indicate that the pPVT is a major source of BDNF for the CeL, and that the BDNF/TrkB-mediated pPVT→CeLSOM interaction has an important role in fear processing.

Of note, Bdnf deletion in the pPVT or TrkB deletion in SOM+ CeL neurons not only impaired conditioned-stimuli-evoked freezing, a measurement of tone-associated memory, but also reduced pre-conditioned-stimuli freezing, which presumably represents contextual memory (Fig. 4c, e). Alternatively, or additionally, these behavioural effects could reflect a general reduction in fear responses, such as that caused by altered arousal or negative behavioural states.

To disentangle these issues, we first determined whether disruption of BDNF signalling affects the synaptic potentiation onto SOM+ CeL neurons that, as mentioned above, can serve as a fear memory trace. Deletion of TrkB in SOM+ CeL neurons by IRASE prevented the fear-conditioning-induced increase in frequency, but not amplitude, of mEPSCs (Extended Data Fig. 9a–d), suggesting that presynaptic potentiation, the major component of fear-conditioning-driven CeL plasticity, depends on BDNF signalling. We next examined the effects of enhancing BDNF signalling. Bath application of BDNF on slices was sufficient to induce long-term potentiation of excitatory synaptic transmission onto SOM+ neurons.
TrkB induces a slow excitatory current in SOM+ CeL neurons (Fig. 3d–g; Extended Data Fig. 7b–f) suggests that BDNF might increase the excitability of these neurons, thereby promoting fear expression. We found that bath application of BDNF on slices markedly increased the spike probability of SOM+ CeL neurons (Extended Data Fig. 10a–d), and notably, bilateral infusion of BDNF into the CeL of naive mice elicited robust freezing responses (Extended Data Fig. 10e, f and Supplementary Videos 1 and 2). These results, together with the above finding that the pPVT–CeL pathway is required for the expression of conditioned fear (Fig. 1), suggest that BDNF signalling in the pPVT–CeL pathway may facilitate the expression of fear by promoting SOM+ CeL neuron activation.

On the basis of our collective results, we propose that BDNF/TrkB-mediated pPVT–CeL communication promotes both synaptic plasticity and the excitability of SOM+ CeL neurons, thereby facilitating not only the formation of stable fear memories but also the expression of fear responses.

Recent studies suggest a role for the PVT in anxiety-related behaviours. In addition, altered BDNF signalling has been implicated in anxiety disorders. Our findings that the pPVT recruits BDNF/TrkB signalling to control CeL function define a functional framework for the pPVT that can subserve its role in the coordination of behavioural responses to stress.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions M.A.P. and B.L. designed the study. M.A.P. and V.R. conducted experiments. M.A.P. analysed data. J.T. assisted with the rabies viral tracing experiments. D.D.B. assisted with the BDNF infusion experiments. M.W. made the AAV-fDIO-Cre-GFP virus. M.D. made the CAV2-Cre virus. L.F.P. generated the Trkblox/lox mouse line. M.H. generated the Som-Flp mouse line. L.V.A., R.D.P. and Z.J.H. provided critical reagents and suggestions. M.A.P. and B.L. wrote the paper.

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METHODS

Mice. All procedures were approved by the Institutional Animal Care and Use Committees of Cold Spring Harbour Laboratory and conducted in accordance to the US National Institutes of Health guidelines. Mice were housed under a 12 light–dark cycle (9 a.m. to 9 p.m. light), with food and water available ad libitum. Som-cre, Prkcd-cre5, Som-Flp5, Bddylox28, Trkbllox28, A1f4, H2b-GFP4, and Rosa26-stoplox5,TA2 mice have all been described elsewhere. All mice were bred onto C57/Bl6J genetic background. Male and female mice 6–9 weeks of age were used for all experiments. All subjects were randomly allocated to the different experimental conditions used in this study.

Viral vectors. AAV-DIO-hM4Di-mCherry. AAV-DIO-eYFP, AAV-GFP-Cre, AAV-ChR2-YFP, and AAV-TRE-hGFP-TVA-G were produced by the University of North Carolina (UNC) Vector Core Facilities and have been described previously. For the generation of the AAV-DIO-Cre-TVA and AAV-DIO-mCherry strains, standard cloning procedures were used to subclone the Cre–GFP or mCherry cassettes into the backbone of a Flippase-dependent AAV-Ef1a-fDIO-YFP expression plasmid (gift from K. Deisseroth). Following DNA sequencing screening, the cassettes into the backbone of a Flippase-dependent AAV-Ef1a-fDIO-YFP expression plasmid was packaged into AAV serotype 8 virus from UNC Vector Core, deleted rabies-EnvA-SAD-21 (gift from K. Deisseroth). Following DNA sequencing screening, the cassettes into the backbone of a Flippase-dependent AAV-Ef1a-fDIO-YFP expression plasmid was packaged into AAV serotype 8 virus from UNC Vector Core. For chemogenetic manipulation of the pPVT, AAV-ChR2-expressing axons. The light source was a single-wavelength LED system (470 nm; http://www.coolled.com/) connected to the epifluorescence port of an Olympus BX51 microscope. Light pulses of 1 ms, triggered by a TTL signal from the Clampex software, were delivered at either 5 Hz or 30 Hz to drive synaptic responses. While most of the above electrophysiology experiments were carried out by V.R. who performed these experiments in a blinded manner. All electrophysiology experiments were carried out by M.A.P., plasticity results were replicated by Y.S. and T.B.P., whereas H.E. and S.S. contributed to the generation of the AAV vectors. Cell identification was based on their membrane potential (hGFP or tdTomato). Synaptic responses were evoked with a bipolar stimulating electrode placed in the BLA approximately 0.2 mm away from the recorded cell bodies in the CeL. Electrical stimulation was delivered every 10 s and synaptic responses were low-pass filtered at 1 KHz and recorded at holding potentials of ~ −70 mV (for AMPA-receptor-mediated responses), +40 mV (for NMDA-receptor-mediated responses), or 0 mV (for GABA-A-receptor-mediated responses). NMDA-receptor-mediated responses were elicited with a mean current of 1.5 ± 0.5 nA between 50 and 70 ms post-stimulation. Responses were averaged and plotted as a function of stimulation. Recordings were made in the ACSF. The internal solution for voltage-clamp experiments contained 115 mM NaCl, 2.5 mM KCl, 26.2 mM NaHCO3, 1 mM NaH2PO4, 20 mM glucose, 2 mM MgCl2 and 2 mM CaCl2 at 34 °C, pH 7.4, gassed with 95% O2 and 5% CO2. At least 40 min recovery time, slices were transferred to room temperature (20–24 °C) and were constantly perfused with ACSF.

Histology. Mice were deeply anaesthetized and transcardially perfused with PBS, followed by perfusion with 4% paraformaldehyde (PFA) in PBS. Brains were extracted and post-fixed in 4% PFA at 4 °C for 24 h for BDNF and TrkB immunohistochemistry and overnight for all other experiments. This was followed by cryoprotection in 30% PFS-buffered sucrose solution until brains were saturated (~ 36 h). Coronal brain sections (40 μm) were cut using a freezing microtome (SM 2010R, Leica). Brain sections were first washed in PBS (3 × 5 min) and then incubated in PBS (0.1% Triton X-100 in PBS) for 15 min at room temperature. Next, sections were blocked in 10% normal goat serum (NGS) in PBS for 30 min at room temperature, followed by incubation with primary antibodies overnight at 4 °C. Sections were then washed with PBS (5 × 15 min) and incubated with fluoro- rescent secondary antibodies at room temperature for 1 h. After washing with PBS (5 × 15 min), sections were mounted on glass slides with Fluoromount-G (Becton Dickinson). Images were taken using a LSM 780 laser-scanning confocal microscope (Carl Zeiss).

Antibodies. The primary antibodies used were: anti-c-Fos (1:2,000, rabbit, Santa Cruz, sc-52); anti-BDNF (1:100, rabbit, abcam, ab108383); and anti-TrkB (1:1,000, rabbit, Biosensis, R-149-100). Fluorophore-conjugated secondary antibodies were purchased from Invitrogen. Antibodies were diluted in PBS with 10% NGS and 0.1% Triton X-100.

Fear conditioning. Mice were initially handled and habituated to the conditioning cage, a mouse test cage (18 cm × 18 cm × 30 cm) with an electrifiable floor connected to a H13-15 shock generator (Coulbourn Instruments). The test cage was located inside a sound attenuated cabinet (H10-24A; Coulbourn Instruments). Before each conditioning session the test cage was wiped clean with 70% ethanol. During conditioning the cabinet was illuminated and the behaviour was captured with the second generation CCD-camera (Panasonic WV-BP334) at 3.7 Hz and stored on a personal computer. The FreezeFrame software (Coulbourn Instruments) was used to control the delivery of both tones and foot shocks. For habitation, five 4-KHz, 75-dB tones (conditioned stimulus), each of which was 30 s in duration, were delivered at variable intervals. During conditioning, mice received five presentations of the conditioned stimulus, each of which co-terminated with a 1–2, 1–ma foot shock (unconditioned stimulus). In the experiment to determine the effect of BDNF infusion on fear learning (Fig. 4g, h), weaker (2 s, 0.5 mA) foot shocks were used. The test for fear memory was performed 24 h following conditioning in a novel illuminated context, where mice were exposed to two presentations of unreinforced conditioned stimulus (120 s inter-stimulus interval). The novel context was a cage with a different shape (22 cm × 22 cm × 21 cm) and floor texture compared with the conditioning cage. Prior to each use the floor and walls of the cage were wiped clean with 0.5% acetic acid to make the scent distinct from that of the conditioning cage. Behavioural responses to the conditioned stimuli were recorded. Freezing behaviour was analysed with FreezeFrame (Coulbourn Instruments).

Electrophysiology. Mice used for electrophysiological experiments were anaesthetized with isoflurane, decapitated and their brains quickly removed and chilled in ice-cold dissection buffer (110 mM choline chloride, 25 mM NaHCO3, 1.25 mM NaH2PO4, 2.5 mM KCl, 5.0 mM CaCl2, 7.0 mM MgCl2, 25.0 mM glucose, 11.6 mM ascorbic acid and 3.1 mM pyruvic acid, gassed with 95% O2 and 5% CO2). Coronal slices (300 μm) containing the amygdala complex were cut in dissection buffer using a HM650 Vibrating-blade Microtome (Thermo Fisher Scientific), and were subsequently transferred to a storage chamber containing artificial cerebrospinal fluid (ACSF) (118 mM NaCl, 2.5 mM KCl, 26.2 mM NaHCO3, 1 mM NaH2PO4, 20 mM glucose, 2 mM MgCl2 and 2 mM CaCl2 at 34 °C, pH 7.4, gassed with 95% O2 and 5% CO2). After at least 40 min recovery time, slices were transferred to room temperature (20–24 °C) and were constantly perfused with ACSF.

For plasticity experiments, recordings were always performed on interleaved naive and fear-conditioned animals. Simultaneous whole-cell patch-clamp recordings from SOM+/SOM− neuronal pairs in the CeL were obtained with Multiclamp 700B amplifiers (Molecular Devices). Recordings were made under visual guidance using an Olympus BX51 microscope with transmitted light illumination, and SOM− cells were identified based on their fluorescence (hGFP or tdTomato). Synaptic responses were evoked with a bipolar stimulating electrode placed in the BLA approximately 0.2 mm away from the recorded cell bodies in the CeL. Electrical stimulation was delivered every 10 s and synaptic responses were low-pass filtered at 1 KHz and recorded at holding potentials of −70 mV (for AMPA-receptor-mediated responses), +40 mV (for NMDA-receptor-mediated responses), or 0 mV (for GABA-A-receptor-mediated responses). NMDA-receptor-mediated responses were elicited with a mean current of 1.5 ± 0.5 nA between 50 and 70 ms post-stimulation. Responses were averaged and plotted as a function of stimulation. Recordings were made in the ACSF. The internal solution for voltage-clamp experiments contained 115 mM NaCl, 25 mM KCl, 4 mM NaATP, 0.4 mM NaGTP, 10 mM sodium phos- hoacetate and 0.6 mM EGTA (pH 7.2). Evoked EPSCs were recorded with picro- toxin (100 μM) added to the ACSF. mEPSCs were recorded in the presence of tetrodotoxin (TTX; 1 μM) and picrotoxin (100 μM) and analysed using Mini Analysis software (Synaptosoft). To assess presynaptic function, a paired-pulse stimulation protocol (50 ms inter-stimulus interval) was used to evoke double-EPSCs, and the paired-pulse ratio was quantified as the ratio of the peak amplitude of the second EPSC to that of the first EPSC. While these electrophysiology experiments were carried out by M.A.P., plasticity results were replicated by V.R. who performed these experiments in a blinded manner.

To evoke pPV-driven synaptic transmission onto CeL neurons, the AAV-ChR2-YFP was injected into the pPV of Som-crcAi14 mice and allowed to express for 3 weeks. Acute brain slices were prepared and a blue light was used to stimulate ChR2-expressing axons. The light source was a single-wavelength LED system (λ = 470 nm; http://www.coolled.com/) connected to the epifluorescence port of the Olympus BX51 microscope. Light pulses of 1 ms, triggered by a TTL signal from the Clampex software, were delivered at either 5 Hz or 30 Hz to drive synaptic responses. For the BDNF assay, mBDNF scavenger TrkB–Fc (used at 1 μg ml−1) were purchased from R&D Systems.

Chemo genetic manipulations. For chemogenetic manipulation of the pPV, C57/Bl6J mice were bilaterally injected with the CAV2-Cre virus into the CeL and subsequently with the AAV-DIO-hM4Di-mCherry into the pPV. Three weeks
later mice were injected intraperitoneally with CNO (10 mg kg\(^{-1}\)) 40 min before either fear conditioning training or fear memory retrieval. For experiments that examine the effects of this manipulation on synaptic plasticity in the CeL, Som-cre and Som-cre::H2b-GFP mice were injected with a 40:60 mixture of AAV-Cre-GFP and AAV-DIO-hM4Di-mCherry into the pPVt (1 µl total volume). Som-cre mice were additionally injected bilaterally with 1 µl of AAV-DIO-YFP into the CeL.

**Cannula implantation and BDNF infusion.** Surgery. The surgical procedure was the same as described above. Stainless steel guide cannulae (26 gauge, 6.00 mm, Plastics One) were implanted bilaterally 0.5 mm above the CeL (−1.22 mm from bregma, 2.90 mm lateral from midline, and 4.25 mm vertical from the cortical surface) and were fixed to the skull with adhesive luting cement (C&B Metabond) and acrylic dental cement (Stoelting). A metal head bar was implanted posterior to the cannulae to facilitate restraining during infusion (see below). Following surgery, a dummy cannula was inserted into each guide cannula to seal off the opening. Mice were allowed to recover from surgery for a minimum of one week, during which they were handled and habituated to the infusion procedure on a daily basis. Infusion. Mice were briefly head-restrained, while the dummy cannulae were removed and an injection cannula (33 gauge, 6.50 mm, Plastics One) was inserted into each of the guide cannulae. The injection cannulae were designed to protrude 0.50 mm from the tip of the guide cannulae and thus penetrate into the CeL. A dose of 200 ng BDNF (or 0.9% sodium chloride as control) was slowly infused bilaterally into the CeL at a flow-rate of 0.5 µl per min to a total volume of 1 µl per infusion site. Following infusion, the injection cannulae were left in place for 1 min to allow the BDNF solution to diffuse from the cannula tips. The dummy cannulae were subsequently reinserted into the guide cannulae and mice were immediately tested for unconditioned freezing or returned to their home cage for 15 min before fear conditioning.

**Statistics and data presentation.** All statistical tests are indicated when used. The sample sizes used in this study were based on estimations by a power analysis (power = 0.9, α = 0.05). No mice or data points were excluded from analysis. All data are presented as mean ± s.e.m.

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Extended Data Figure 1 | PVT is activated following both fear conditioning and fear memory retrieval. a, A schematic of the experimental design. All mice were killed for the detection of c-Fos at 90 min after the last behavioural session. b, Representative images of c-Fos immunohistochemistry in the pPVT for the five groups indicated in a. c, Quantification of c-Fos expression as c-Fos⁺ cells per mm² (post-habituation 1 (mice that were subjected to one session of habituation), 131.17 ± 34.25, n = 4 mice; post-habituation 2 (mice that were subjected to two sessions of habituation), 180.68 ± 30.42, n = 5 mice; post-US (unconditioned stimulus; mice that were only exposed to five foot shocks), 443.3 ± 25.7, n = 3 mice; post-conditioning, 692.61 ± 46.68, n = 4 mice; post-retrieval, 565.51 ± 28.71, n = 3 mice; \( F_{(4,14)} = 19.3, P < 0.001 \); \( ***P < 0.01, ****P < 0.001 \); one-way analysis of variance (ANOVA) followed by Tukey’s test). Data are presented as mean ± s.e.m.
Extended Data Figure 2 | pPVT neurons projecting to the BLA and CeL are non-overlapping. a, A schematic of the approach to simultaneously label BLA- and CeL-projecting pPVT neurons. b, A representative image of the injection sites, where CTB-488 and CTB-555 were injected into the CeL and BLA, respectively. c, Representative images of pPVT cells labelled by CTB-488 (left) and CTB-555 (middle). These two populations were largely non-overlapping (right). Data was replicated in 4 mice.
Extended Data Figure 3 | Performance during conditioning. a–d, Freezing levels during conditioning are shown for mice used in Fig. 1c (a), Fig. 2 (b), Fig. 4c (c), and Fig. 4e (d). a, There was no significant difference in performance among groups (F(2,155) = 0.51, P > 0.05, two-way ANOVA). b, There was no significant difference in performance between saline-treated mice and CNO-treated mice (F(1,70) = 0.43, P > 0.05, two-way ANOVA). c, There was no significant difference in performance between the two groups (F(1,85) = 0.73, P > 0.05, two-way ANOVA). d, There was no significant difference in performance between the two groups (F(1,75) = 0.45, P > 0.05, two-way ANOVA). Data are presented as mean ± s.e.m.
Extended Data Figure 4 | The pPVT is required for fear-conditioning-induced synaptic plasticity in the lateral-amygdala–CeL\(^{SOM}\) pathway.

a, Top, a schematic of the whole-cell paired recording configuration. A pair of SOM\(^{+}\) and SOM\(^{-}\) CeL neurons were simultaneously recorded, and EPSCs were evoked by stimulation of the lateral amygdala. We used the Som-cre\(\Delta\)H2b-GFP mice, in which the SOM\(^{+}\) neurons were tagged with H2b–GFP and the pPVT neurons were infected with hM4Di as in Fig. 2a, b. Bottom, a representative image of a slice used for recording, in which a SOM\(^{+}\) (arrow) and an adjacent SOM\(^{-}\) (arrowhead) neuron were recorded. b, Sample EPSC traces obtained from the simultaneous paired recording experiment. Naive control mice (left) and fear-conditioned mice treated with either saline (middle) or CNO (right) were used. Saline or CNO was administered 40 min before, and recordings were performed 24 h after conditioning. Top and bottom traces represent EPSCs recorded at +40 mV and −70 mV holding potentials, respectively. c, Quantification of AMPA (left) and NMDA (right) currents (Control, \(n = 9\) pairs (2 mice); ‘Fear, saline’, \(n = 8\) pairs (3 mice); ‘Fear, CNO’, \(n = 14\) pairs (3 mice); *\(P < 0.05\), **\(P < 0.001\), n.s., non-significant; paired \(t\)-test). EPSC values are normalized to the average EPSC value of SOM\(^{-}\) cells for each group. d, Quantification of the paired-pulse ratio (PPR) (see Methods) of EPSCs measured at −70 mV (comparing control, ‘Fear, saline’, and ‘Fear, CNO’ groups for SOM\(^{-}\) neurons: *\(P < 0.05\), **\(P < 0.01\), n.s., non-significant; one-way ANOVA followed by Tukey’s test). Control mice for all experiments were injected with the same viral vectors as the experimental groups. Data are presented as mean ± s.e.m.
Extended Data Figure 5 | A different mode of communication in the pPVT–CeL pathway compared with the pPVT–BLA pathway. a–c, Optogenetic stimulation of pPVT afferents in the BLA drives fast synaptic transmission onto BLA neurons. a, b, Schematics of the experimental approach. c, Sample trace (average of 20) of the synaptic responses onto a BLA neuron following brief (1-s train of 5-Hz, 1-ms pulses) photostimulation of pPVT afferents expressing ChR2. Similar responses were observed in 5 out of 6 BLA neurons recorded. Data was obtained from the same mice as those in Fig. 3d–g.

d–f, Slow recovery of the pPVT-driven current in a SOM1 CeL neuron. d, e, Schematics of the experimental approach. f, Sample trace of the synaptic response onto a SOM1 neuron following prolonged (20-s train of 30-Hz, 1-ms pulses) photostimulation of pPVT afferents expressing ChR2, showing slow recovery after stimulus cessation.

g–i, Optogenetic stimulation of the pPVT–CeL pathway promotes intra-CeL inhibition. g, Representative traces of IPSCs onto SOM– (black) and SOM+ (red) CeL neurons. Blue bar indicates the 30 Hz photostimulation of pPVT afferents. h, Quantification of IPSC frequency, comparing pre- and post-photostimulation (SOM–, n = 14 neurons (6 mice), P < 0.001, t-test; SOM+, n = 11 neurons (6 mice), P > 0.05, t-test). i, Quantification of IPSC amplitude, comparing pre- and post-photostimulation (n.s., non-significant (P > 0.05), paired t-test). Data are presented as mean ± s.e.m.
Extended Data Figure 6 | CeL-projecting neurons in the pPVT express BDNF.  

a, A schematic of the experimental approach to retrogradely label CeL-projecting pPVT cells.  
b, Representative images of pPVT cells, which were labelled by CTB-488 (left) and an antibody recognizing BDNF (middle). CTB-labelled neurons largely overlapped with BDNF-positive somata (see overlay in right).  
c, d, BLA-projecting neurons and BDNF-expressing neurons in pPVT are largely non-overlapping.  
c, A schematic of the method used to label BLA-projecting neurons in the pPVT.  
d, Representative images of pPVT cells labelled by either CTB-555 (left) or the antibody recognizing BDNF (middle). These two populations were largely non-overlapping (see overlay in right).
Extended Data Figure 7 | BDNF/TrkB mediates pPVT–CeL communication. a, The TrkB receptor is selectively expressed by SOM⁺ CeL neurons. Top, representative images of the CeL in Som-cre;H2b-GFP mice, showing SOM⁺ neurons tagged with H2b–GFP (left) and TrkB expression recognized by an antibody (middle). Bottom, higher-magnification images of the boxed area in the top panel. TrkB-labelled cells largely overlap with SOM⁺ neurons (see overlay on right). b–f, TrkB mediates the pPVT–CeL transmission. b, A schematic of the experimental approach using the Trkblox/lox,Som-Flp mice to: (1) tag SOM⁺ CeL neurons with mCherry; (2) sparsely infect CeL neurons with GFP–Cre to delete Trkb; and (3) express ChR2 in the pPVT. c, Representative images resulting from the approach in b, showing CeL neurons expressing (from left to right) Cre–GFP, mCherry and TrkB. Neurons that expressed both mCherry and GFP–Cre represent SOM⁺ neurons in which Trkb was deleted (arrow; see overlay on right), whereas neurons that expressed mCherry, but not GFP–Cre, represent SOM⁺ neurons with intact Trkb (arrowhead; see overlay on right). d, A schematic of the whole-cell recording configuration. e, Sample traces of synaptic currents in mCherry-only (SOM⁺,Cre⁻; red) and mCherry/GFP–Cre double positive (SOM⁺,Cre⁺; yellow) neurons in response to prolonged high-frequency stimulation of pPVT afferents. f, Quantification of the synaptic responses (SOM⁺,Cre⁺, 8.06 ± 2.58 pA, n = 7 neurons (3 mice); SOM⁺,Cre⁻, 2.10 ± 0.76 pA, n = 7 neurons (3 mice); *P < 0.05, t-test). g–j, The pPVT input to the CeL promotes intra-CeL inhibition through BDNF/TrkB signalling. g, Representative traces of IPSCs recorded from SOM⁺ CeL neurons in response to the 30 Hz photostimulation (blue bars) of pPVT afferents. h, Quantification of the frequency of IPSCs recorded from SOM⁺ CeL neurons (comparing pre- and post-photostimulation: control, n = 14 neurons (6 mice; repetition of data from the SOM– cells in Extended Data Fig. 5h), P < 0.001, paired t-test; TrkB–Fc, n = 17 neurons (2 mice), P > 0.05, paired t-test). i, Representative traces showing the effect of BDNF bath application on spontaneous IPSCs recorded from CeL neurons. j, Quantification of the effect of BDNF on sIPSC frequency. Black line indicates the timing of BDNF application (n = 7; *P < 0.05 comparing baseline and BDNF application, paired t-test). Data are presented as mean ± s.e.m.
Extended Data Figure 8 | Characterization of the AAV-fDIO-CreGFP.

a. A schematic of the experimental approach to selectively target SOM⁺ CeL neurons in the Som-Flp mice with the AAV-fDIO-Cre-GFP. b. Representative images of CeL neurons expressing Cre–GFP (left), and PKC-δ⁺ CeL neurons (as a surrogate for SOM⁺ neurons) that were recognized by an antibody (middle). In the bottom panels are high magnification images of the boxed region in the top panel. These two cell populations were largely non-overlapping (see overlay on right), indicating that the AAV-fDIO-Cre-GFP selectively infects SOM⁺ neurons (data from one mouse). c. A schematic of the experimental approach to test the function of AAV-fDIO-Cre-GFP, whereby the CeL of Som-Flp mice was injected with a mixture of AAV-fDIO-Cre-GFP and AAV-DIO-hM4Di-mCherry. As the latter virus expresses mCherry in a Cre-dependent manner, observation of selective mCherry expression in GFP⁺ neurons would indicate that the AAV-fDIO-Cre-GFP is effective. d. Sample images of CeL neurons expressing Cre–GFP (left) and mCherry (middle). In the bottom panels are high magnification images of the boxed region in the top panel. Essentially, all mCherry⁺ neurons co-expressed GFP (see overlay on right), indicating selective expression of Cre by the GFP-labelled cells (data from one mouse).
Extended Data Figure 9 | BDNF/TrkB regulates synaptic plasticity onto SOM⁺ CeL neurons. a–d, Selective deletion of TrkB in SOM⁺ CeL neurons impairs fear-conditioning-induced synaptic plasticity. a, A schematic of the experimental approach to specifically delete TrkB in SOM⁺ CeL neurons. b, Representative traces of mEPSCs recorded from SOM⁺ CeL neurons in which TrkB was deleted, in naive control (top) and fear-conditioned (bottom) mice. c, d, Deletion of TrkB blocked the fear conditioning-induced increase in mEPSC frequency (c) (control, n = 19 neurons (3 mice); fear, n = 18 neurons (3 mice); P > 0.05, t-test), but not amplitude (d) (control, n = 19 neurons (3 mice); fear, n = 18 neurons (3 mice); P > 0.05, t-test). e–g, BDNF induces long-term potentiation at lateral-amygdala–CeLSOM synapses. e, A schematic of the whole-cell recording configuration. f, Top, sample EPSC traces recorded before (pre-BDNF) and after (post-BDNF) bath application of BDNF. Bottom, summary plot showing the effect of BDNF on EPSC peak amplitude, for which the first peak in the paired pulse was measured and normalized to the baseline (that is, the average pre-BDNF amplitude). BDNF significantly enhanced EPSC amplitude (pre-BDNF, 98 ± 1.74%; post-BDNF, 146 ± 17.6%; n = 6 neurons (3 mice), P < 0.05, paired t-test). g, BDNF application decreased the paired-pulse ratio (see Methods) of the EPSCs (pre-BDNF, 1.17 ± 0.18; post-BDNF, 0.80 ± 0.09; n = 6 neurons (3 mice), *P < 0.05, paired t-test). Data are presented as mean ± s.e.m.
Extended Data Figure 10 | Exogenous application of BDNF in the CeL increases the excitability of SOM1 neurons and elicits an unconditioned freezing response. a–d, BDNF increases the excitability of SOM1 CeL neurons. a, A schematic of the experimental approach, in which photostimulation was used to assess the excitability of SOM1 CeL neurons expressing ChR2. b, Sample traces of photostimulation-evoked spikes recorded in cell-attached mode, before (baseline; left) and after (right) bath application of BDNF (100 ng ml⁻¹). Light intensity was adjusted to evoke spikes with ~50% probability at baseline. c, A sample recording, in which the spike probability of a SOM1 CeL neuron was followed before, during and after BDNF application. d, Quantification of the effect of BDNF on spike probability (baseline, 0.50 ± 0.02; BDNF, 0.74 ± 0.08; n = 8 neurons (4 mice), *P < 0.05, paired t-test). e, f, Infusion of BDNF into the CeL elicits an unconditioned freezing response. e, Drawing of the cannula sites. Each dot denotes where the tip of the injection cannula was located in each mouse. f, Quantification of freezing levels following CeL infusion of saline and BDNF (saline, 2.93 ± 1.84%; BDNF, 32.22 ± 9.19%; n = 6 mice, *P < 0.05, paired t-test). Data are presented as mean ± s.e.m.