Basal forebrain projections to the lateral habenula modulate aggression reward

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Maladaptive aggressive behaviour is associated with a number of neuropsychiatric disorders1 and is thought to result partly from the inappropriate activation of brain reward systems in response to aggressive or violent social stimuli2. Nuclei within the ventromedial hypothalamus3–5, extended amygdala6 and limbic7 circuits are known to encode initiation of aggression; however, little is known about the neural mechanisms that directly modulate the motivational component of aggressive behaviour8. Here we established a mouse model to measure the valence of aggressive inter-male social interaction with a smaller subordinate intruder as reinforcement for the development of condition-place preference (CPP). Aggressors develop a CPP, whereas non-aggressors develop a conditioned place aversion to the intruder-paired context. Furthermore, we identify a functional GABAergic projection from the basal forebrain (BF) to the lateral habenula (lHb) that bi-directionally controls the valence of aggressive interactions. Circuit–specific silencing of GABAergic BF–lHb terminals of aggressors with halorhodopsin (NpHR3.0) increases lHb neuronal firing and abolishes CPP to the intruder-paired context. Activation of GABAergic BF–lHb terminals of non-aggressors with channelrhodopsin (ChR2) decreases lHb neuronal firing and promotes CPP to the intruder-paired context. Finally, we show that altering inhibitory transmission at BF–lHb terminals does not control the initiation of aggressive behaviour. These results demonstrate that the BF–lHb circuit has a critical role in regulating the valence of inter-male aggressive behaviour and provide novel mechanistic insight into the neural circuits modulating aggression reward processing.

To study individual differences in aggression, we adapted the sensory contact model of social defeat for CD-1 mice9–11, which exhibit a wide spectrum of aggressive behaviours. In this procedure (Fig. 1a), a sexually experienced adult male CD-1 mouse is presented with a series of novel 6–8-week-old subordinate male C57BL/6j intruder mice, who do not themselves exhibit any aggressive behaviours towards CD-1 mice (Extended Data Fig. 1a–i). This procedure identifies individual differences in antagonistic aggressive behaviours without producing lasting stress–related behavioural phenotypes (Extended Data Table 1). Ethological analysis revealed that approximately 70% (310/448) of mice exhibited aggressive behaviour (termed aggressors (AGGs)) during at least one session, while approximately 30% (138/448) failed to initiate aggressive behaviour (termed non-aggressors (NONs)) at any time (Fig. 1b).

After repeated intruder interactions, AGGs have elevated serum testosterone (Fig. 1c) and decreased corticosterone (Fig. 1d) levels relative to NONs, suggesting that NONs may be less dominant and experience forced intruder interactions as more stressful. Analysis of several common metrics for aggression revealed normalized distributions across AGGs that increased between screening sessions (Fig. 1e, f and Extended Data Fig. 2a–g). Importantly, the mean number of attack bouts (Extended Data Fig. 2f) and mean duration of attack bouts (Extended Data Fig. 2g) significantly correlate to mean attack latency. Therefore, attack latency provides a reliable index of aggression behaviours. Subsequently, we focused on AGGs that exhibited attack latencies within the most aggressive quartile of the sample distribution. These data confirm that outbred CD-1 mice exhibit a wide spectrum of aggressive behaviour and physiological responses to an intruder, leading us to hypothesize that there may be differences in the valence of intruder interactions among AGGs and NONs.

To assay the motivational state associated with intruder pairings, we developed an aggression-based CPP procedure. In this model, CD-1 mice are screened for aggression phenotype and then conditioned for CPP (Fig. 1g) by receiving novel C57BL/6j intruder-paired or intruder-unpaired sessions twice a day for three days. AGGs show a CPP for the intruder-paired context, while NONs show a conditioned place aversion (CPA) (Fig. 1h–j and Extended Data Fig. 3a–d). CPA in NONs does not appear to result from baseline differences in mood and anxiety or lack of interest in social targets (Extended Data Tables 1 and 2). However, we found that the valence of intruder interactions in AGGs and NONs is dependent upon intruder mice being freely moving and physically accessible during conditioning. Using a sensory CPP procedure in which the intruder mouse is placed in a protective cage within the intruder-paired context, both CPP and CPA are abolished (Fig. 1k–n and Extended Data Fig. 3e–h). These data demonstrate individual differences in the positive or negative valence of intruder interactions in AGGs versus NONs.

Clinical2,12 and preclinical8 studies have implicated BF structures, such as the nucleus accumbens (NAC), lateral septum and diagonal band nuclei (DBN), as potentially important brain regions controlling aggression-related behaviours. However, there has been limited functional evidence that the BF, or its projections, directly modulate the rewarding aspects of aggression. To define BF projections, we injected an adeno-associated virus (AAV) vector expressing enhanced yellow fluorescent protein (eYFP) under a neuronal-specific human synapsin (hSyn) promoter (AAV2-hSyn-eYFP) into the BF of CD-1 mice (Fig. 2a–c, top, and Extended Data Fig. 4a–c) targeted specifically to the more anterior septo-accumbal transition zone of the basal forebrain13 and observe a prominent axonal projection to the lHb (Fig. 2b, top). To characterize BF–lHb projections further, we injected the lHb (Fig. 2a, b, bottom) with a retrograde monosynaptic glycoprotein-dead rabies virus (G-deleted-rabies–eGFP)14. Within the anterior BF that overlaps with our anterograde viral infection, we observed retrograde labelling in the septum (~45%), DBN (~35%) and the medial NAc shell (~15%) (Fig. 2c, d, bottom). Within retrogradely labelled BF slices, we performed in situ hybridization for GAD67, a marker of inhibitory

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GABAergic neurons, and observed colocalization within the septum (~75%), DBN (~80%) and medial NAc shell (100%) (Fig. 2e).

To identify whether BF and lHb neurons are differentially activated by intruder interactions in AGGs and NONs, we examined c-Fos immunoreactivity 1 h after the final intruder screening. AGGs exhibit elevated c-Fos immunoreactivity in the septo-accumbal transition zone of the BF relative to NONs (Fig. 2f, g). Within the eYFP-positive BF terminal fields in the medial lHb, NONs exhibited increased c-Fos immunoreactive nuclei relative to AGGs (Fig. 2f, g). This finding was corroborated by slice electrophysiology, in which NONs exhibit an increase in lHb firing rates compared with AGGs 1 h after an intruder interaction that returns to baseline by 7 days after intruder interaction (Fig. 2h, i). Together, these data show that lHb neurons are differentially regulated by intruder interactions, possibly through inhibitory BF inputs.

To determine the functional contribution of BF–lHb projections, we conducted optogenetic circuit-specific terminal photostimulation in combination with slice electrophysiology with channelrhodopsin (AAV2-hSyn-Chr2(H134R)-eYFP) or halorhodopsin (AAV2-hSyn-NpHR3.0-eYFP), identifying photostimulation parameters that produce robust transient lHb activation or inhibition without rebound neuronal firing. Chr2BF–lHb terminal photostimulation with 40 Hz resulted in significantly decreased lHb firing rates (Fig. 2j, k), while NpHR3BF–lHb terminal photostimulation with 40 Hz resulted in significantly decreased lHb firing rates compared with AGGs 1 h after an intruder interaction in AGGs and CPA in NONs. lHb confirm inhibitory GABAergic circuitry and are both necessary and sufficient for the expression of CPA. Viral stimulation significantly affected the expression of CPP or CPA. Viral stimulation induced CPA (Fig. 3f–h), mimicking responses observed in control AGGs. Conversely, AGG::Chr2BF–lHb stimulation induced CPA (Fig. 3f–h), mimicking responses observed in control NONs. Neither NON::NpHR3BF–lHb or AGG::Chr2BF–lHb stimulation significantly affected the expression of CPP or CPA. Viral expression (Extended Data Fig. 6a–f) and locomotor activity (Extended Data Fig. 6g–j) were not different between conditions. These data confirm that BF–lHb circuitry modulates the rewarding component of aggressive behaviour and is both necessary and sufficient for the expression of CPP in AGGs and CPA in NONs.

To determine if these circuit-specific effects could be recapitulated by direct lHb cell body manipulation, we injected the lHb with AAV2-hSyn-Chr2-eYFP or AAV-hSyn-NpHR3.0-eYFP (Extended Data Fig. 7a–d) and directly stimulated lHb cell bodies using previously established optogenetic parameters for lHb [12]. NON::NpHR3.0BF stimulation to decrease lHb firing promoted CPP to the intruder-paired side (Extended Data Fig. 7e–g), whereas AGG::Chr2BF stimulation to GABA transporter (VGAT), but not vesicular glutamate transporter 1 (VGLUT1) (Extended Data Fig. 4g). To validate these findings within an intact system, we used multi-electrode recording of postsynaptic lHb firing rates in anesthetized mice in combination with terminal photostimulation (Extended Data Fig. 5a). Results show that activation (40 Hz Chr2BF–lHb), or inhibition (8 s on, 2 s off NpHR3BF–lHb) of presynaptic BF terminals in the lHb resulted in decreased or increased lHb postsynaptic neuronal firing, respectively (Extended Data Fig. 5b–d). These functional in vitro and in vivo recordings of Chr2BF–lHb and NpHR3BF–lHb confirm inhibitory GABAergic control over circuit activity and demonstrate reliable temporal control of lHb firing rates by optogenetic tools for in vivo behavioural analysis.

To investigate the functional consequences of BF–lHb neuronal firing on aggression reward, we paired photostimulation of Chr2BF–lHb and NpHR3BF–lHb in AGGs and NONs during the CPP test (Fig. 3a, b). NON::Chr2BF–lHb stimulation promoted CPP (Fig. 3c–e), mimicking responses observed in control AGGs. Conversely, AGG::NpHR3BF–lHb stimulation induced CPA (Fig. 3f–h), mimicking responses observed in control NONs. Neither NON::NpHR3BF–lHb or AGG::Chr2BF–lHb stimulation significantly affected the expression of CPP or CPA. Viral expression (Extended Data Fig. 6a–f) and locomotor activity (Extended Data Fig. 6g–j) were not different between conditions. These data confirm that BF–lHb circuitry modulates the rewarding component of aggressive behaviour and is both necessary and sufficient for the expression of CPP in AGGs and CPA in NONs.

Figure 1 | Individual differences in aggression-related reward behaviour. a, Aggression screening: experimental schematic. b, Percentage of mice exhibiting aggressive (AGG) versus non-aggressive (NON) behaviours. c, d, Serum testosterone (t16 = 2.23, *P < 0.05; two-tailed unpaired t-test, n = 9 per group) (c) and corticosterone (t16 = 3.231, ***P < 0.001; two-tailed unpaired t-test, n = 10–11 per group) (d). e, f, Mean latency to attack (e) (F2,138 = 49.37, two-way analysis of variance (ANOVA) P < 0.001; post-hoc test, ***P < 0.001; n = 138–310) and attack duration (f) (F2,138 = 22.35, two-way ANOVA P < 0.001; post-hoc test, ***P < 0.001; n = 138–310). g, Aggression CPP schematic.
Figure 2 | GABAergic BF–lHb circuit is differentially activated by intruder interactions. a, Schematic of anterograde and retrograde tracing strategies. b, Representative anterograde AAV2-hSyn-eYFP infections (top, terminals) or retrograde G-deleted-rabies-eGFP infections (bottom, infection site) in lHb. Scale bars: 500 μm; insets, 150 μm. c, Representative anterograde AAV2-hSyn-eYFP infections (top, infection site) or retrograde G-deleted-rabies-eGFP infections (bottom, cell bodies) in the BF. Scale bars: 400 μm; insets, 200 μm. d, Percentage retrograde-labelled eGFP+ neurons within subnuclei of the anterior BF (n = 3 mice, ~229 cells per mouse). Scale bars: 20 μm. e, Representative in situ hybridization colocalized GAD67 and eGFP in DBN (left) and quantification (right) within the BF (n = 3 mice, 14 cells per mouse). Scale bars: 20 μm. f, Representative images of AAV2-hSyn-eYFP infection and c-Fos immunoreactivity in medial NAc shell transition zone of the BF (top) and medial lHb terminals (bottom). Scale bars: 30 μm. g, Quantification of c-Fos immunoreactivity in the medial NAc shell–septum transition zone (t = 2.655, *P < 0.05; two-tailed unpaired t-test, n = 6–8 mice per group, 3 slices per mouse) and medial lHb (t = 5.678, ***P < 0.001; two-tailed unpaired t-test, n = 6–8 mice per group, 3 slices per mouse). h, Firing rate of lHb neurons in AGG and NON mice at 1 h or 7 days after intruder interaction (F1,35 = 10.56, two-way ANOVA P < 0.05; post-hoc test, **P < 0.01; n = 16–19 cells per group, 4–5 mice per group). i, Representative trace of lHb in vitro cell-attached firing rates. J, Representative trace of lHb in vitro cell-attached firing rates during ChR2BF–lHb photostimulation. k, Average firing rates of lHb neurons during ChR2BF–lHb (t = 3.679, **P < 0.01; two-tailed unpaired t-test, n = 6 cells). l, Representative trace of lHb in vitro cell-attached firing rates during NpHR3BF–lHb photostimulation. m, Average firing rates of lHb neurons during NpHR3BF–lHb (t = 11.68, ***P < 0.0001; two-tailed unpaired t-test, n = 10 cells) photostimulation. Data are represented as mean ± s.e.m. aLS, anterior lateral septum; DAPI, 4′,6-diamidino-2-phenylindole; mNACs, medial nucleus accumbens shell. Experiments were conducted once; n indicates biological replicates.

To determine if BF–lHb neuronal activity regulates the initiation or intensity of aggressive behaviour, we used ChR2BF–lHb and NpHR3BF–lHb (Fig. 4a) in AGGs and NONs during home-cage resident–intruder testing (Fig. 4b). Neither activation nor inhibition of BF–lHb terminals resulted in the initiation of aggressive behaviour (Fig. 4c, d), nor did it modulate social (Fig. 4e) and non-social (Fig. 4f) exploratory behaviours in NON mice. Similarly, AGG::ChR2BF–lHb stimulation failed to initiate immediate attack behaviour, as indexed by no change in attack latency (Fig. 4g). However, AGG::ChR2BF–lHb and AGG::NpHR3BF–lHb stimulation bi-directionally modulated the severity of the aggressive behaviour relative to each other, although a nonsignificant trend was observed when either were compared to AGG::GFPBF–lHb (Fig. 4h). As observed in NONs, AGG::ChR2BF–lHb and AGG::NpHR3BF–lHb photostimulation failed to alter either social (Fig. 4i) or non-social (Fig. 4j) exploratory behaviours. These data indicate that the BF–lHb circuit is important in modulating the intensity of aggressive behaviour; however, it is not a traditional attack initiation area.

On the basis of these data, we hypothesized that the BF–lHb circuit acts in other affective behavioural domains. We performed a behavioural battery to measure non-social generalized anxiety states and reward in naive CD-1 mice (Extended Data Fig. 8a). Both ChR2BF–lHb and NpHR3BF–lHb terminal photostimulation failed to modulate anxiety-like behaviours in the open field (Extended Data Fig. 8b, c) and elevated plus maze tasks (Extended Data Fig. 8d, e). However, ChR2BF–lHb stimulation potentiates the rewarding effects of cocaine by increasing the amount of time spent in the cocaine-paired chamber (Extended Data Fig. 8f). Therefore, while the BF–lHb circuit does not influence a generalized anxiety phenotype in the absence of social context or other stimuli, it does generalize to non-social rewarding stimuli such as cocaine.

Our results show individual differences in the rewarding properties of aggressive social interaction that are mediated by the BF–lHb circuit. When exposed to an intruder, AGGs exhibit increased activity of the BF and lHb in humans19, this is the first study to provide functional evidence that GABAergic BF projections produce inhibitory control of lHb neurons to regulate the valence of aggressive intruder-based interactions. Stimulation or inhibition of BF–lHb projections is both necessary and sufficient to alter the positive or negative valence of an intruder-paired context. Our findings advance the understanding of BF function in a behaviourally relevant animal model of aggression.
motivation and provide further understanding into the physiology and neural circuitry of aggression and reward-related behaviours.

While numerous functions have been ascribed to IHB neuronal activity, including anxiety21, addiction22 and depression23, there is a noticeable paucity of functional data addressing the role of IHB inputs, outside of those originating from the VTA region, within any of these behavioural domains. Indeed, anatomical tracing experiments have highlighted the complexity of IHB afferents24 and efferents25. With regard to the BF, the lateral septum, DBN and medial NAc shell, but not core, are known to send projections to the IHB26,27. Our study implies the septo-accumbal transition zone of BF as a critical source of GABAergic tone to the IHB within the context of motivated behaviour. However, on the basis of the fact that these BF GABAergic inputs to IHB exhibit high tonic activation in acute slice that can be rapidly inhibited by terminal inhibition with NpHR3 (Fig. 2i, m), it is unlikely that they are NAc medium spiny neurons. Finally, based on both in vitro and in vivo electrophysiological studies, as well as anatomical tracing, we note that there may be a small subset of cells in the BF that either release an excitatory neurotransmitter or act indirectly on the IHB via di-synaptic inputs. It will be interesting in the future to determine what role these neurons have in reward processing.

Our results may provide important information to clinical studies identifying novel targets of deep brain stimulation in the treatment of neuropsychiatric conditions that present with aggression co-morbidity such as substance abuse27 and depression28. Deep brain stimulation protocols within specific BF nuclei29 and the IHB30 have been successfully used to treat intractable major depressive disorder, which is associated with symptoms of increased aggression in men28. Overall, our findings demonstrate a previously unidentified functional role for the IHB and its inputs from the BF in mediating the rewarding component of aggression, and suggest that targeting shared underlying deficits in motivational circuitry may provide useful information for the development of novel therapeutic strategies for treating aggression-related neuropsychiatric disorders.

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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1. Anderson, D. J. Optogenetics, sex, and violence in the brain: implications for psychiatry. Biol. Psychiatry 71, 1081–1089 (2012).
2. Decety, J., Michalska, K. J., Aikutsu, Y. & Lahey, B. B. Atypical emotional responses in adolescents with aggressive conduct disorder: a functional MRI investigation. Biol. Psychol. 80, 203–211 (2009).
3. Yang, C. F. et al. Sexually dimorphic neurons in the ventromedial hypothalamus govern mating in both sexes and aggression in males. Cell 153, 896–909 (2013).
4. Wasman, M. & Flynn, J. P. Directed attack elicited from hypothalamus. Arch. Neural. 6, 220–227 (1962).
5. Lin, D. et al. Functional identification of an aggression locus in the mouse hypothalamus. Nature 470, 221–227 (2011).
6. Unger, E. K. et al. Medial amygdalar aromatase neurons regulate aggression in both sexes. Cell Reports 10, 453–462 (2015).
7. Yu, Q. et al. Optogenetic stimulation of DAergic VTA neurons increases aggression. Mol. Psychiatry 19, 635 (2014).
8. Takahashi, A. & Miczek, K. A. Neurogenetics of aggressive behavior: studies in rodents. Curr. Top. Behav. Neurosci. 17, 3–44 (2013).
9. Kudryavtseva, N. N., Bakshtanovskaya, I. V. & Koryakina, L. A. Social model of depression in mice of C57BL/6J strain. Pharmacol. Biochem. Behav. 38, 315–320 (1991).
10. Golden, S. A., Covington, H. E. III, Berton, O. & Russo, S. J. A standardized protocol for repeated social defeat stress in mice. Nat. Protocols 6, 1183–1191 (2011).
11. Miczek, K. A., DeBolt, J. F. & Thompson, M. L. Pharmacological, hormonal, and behavioral manipulations in analysis of aggressive behavior. Prog. Clin. Biol. Res. 167, 1–26 (1984).
12. Glenn, A. L. & Yang, Y. The potential role of the striatum in antisocial behavior and psychopathy. Biol. Psychiatry 72, 817–822 (2012).
13. Zahm, D. S., Parsley, K. P., Schwartz, Z. M. & Cheng, A. Y. On lateral septum-like characteristics of outputs from the accumbal hedonic “hotspot” of Peciña and Berndt with commentary on the transitional nature of basal forebrain “boundaries”. J. Comp. Neurol. 521, 50–68 (2013).
14. Callaway, E. M. & Luo, L. Monosynaptic circuit tracing with glycoprotein-deleted rabies viruses. J. Neurosci. 35, 8979–8985 (2015).
15. Lammel, S. et al. Input-specific control of reward and aversion in the ventral tegmental area. Nature 491, 212–217 (2012).
16. Herkenham, M. & Nauta, W. J. Afferent connections of the habenular nuclei in the rat. A horseradish peroxidase study, with a note on the fiber-of-passage tegmental area. Nature 242, 2426–2456 (1977).
17. Sutherland, R. J. The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex. Neurosci. Biobehav. Rev. 6, 1–13 (1982).
18. Lecca, S., Meyo, F. J. & Marmeli, M. The lateral habenula in addiction and depression: an anatomical, synaptic and behavioral overview. Eur. J. Neurosci. 39, 1170–1178 (2014).
19. Shelton, L. et al. Mapping pain activation and connectivity of the human habenula. J. Neurophysiol. 107, 2633–2648 (2012).
20. Hikosaka, O. The habenula: from stress evasion to value-based decision-making. Nature Rev. Neurosci. 11, 503–513 (2010).
21. Lee, E. H. & Huang, S. L. Role of lateral habenula in the regulation of exploratory behavior and its relationship to stress in rats. Behav. Brain Res. 204, 265–271 (2009).
22. Maroteaux, M. & Marmeli, M. Cocaine evokes projection-specific synaptic plasticity of lateral habenula neurons. J. Neurosci. 32, 12641–12646 (2012).
23. Li, B. et al. Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. Nature 470, 535–539 (2011).
24. Yetnikoff, L., Cheng, A. Y., Lavezzo, H. N., Parsley, K. F. & Zahm, D. S. Sources of input to the rostromedial tegmental area, ventral tegmental area, and lateral habenula compared: a study in rat. J. Comp. Neurol. 523, 2426–2456 (2015).
25. Quina, L. A. et al. Efferent pathways of the mouse lateral habenula. J. Comp. Neurol. 523, 32–60 (2015).
26. Felton, T. M., Linton, L., Rosenblatt, J. S. & Morell, J. I. First and second order maternal behavior related afferents of the lateral habenula. Neuroreport 10, 883–887 (1999).
27. Beck, A., Heinz, A. J. & Heinz, A. Translational clinical neuroscience perspectives on the cognitive and neurobiological mechanisms underlying alcohol-related aggression. Curr. Top. Behav. Neurosci. 17, 443–474 (2014).
28. Martin, L. A., Neighbors, H. W. & Griffith, D. M. The experience of symptoms of depression in men vs women: analysis of the National Comorbidity Survey Replication. JAMA Psychiatry 70, 1100–1106 (2013).
29. Bewernick, B. H., Kayser, S., Sturm, V. & Schlaepfer, T. E. Long-term effects of nucleus accumbens deep brain stimulation in treatment-resistant depression: evidence for sustained efficacy. Neuropsychopharmacology 37, 1975–1985 (2012).
30. Sartorius, A. & Henn, F. A. Deep brain stimulation of the lateral habenula in treatment resistant major depression. Med. Hypotheses 69, 1305–1308 (2007).

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.J.R. (scott.russo@mssm.edu).

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METHODS

Animals. Male CD-1 (ICR) mice (35–45 g, sexually experienced retired breeders; Charles River Laboratories (CRL)) were obtained at 4 months of age. All breeders were confirmed by CRL to have had equal access, experience and success as breeders. Male C57BL/6j mice (20–30 g; The Jackson Laboratory) were obtained at 7–8 weeks of age and used as novel intruders. All mice were allowed 1 week of acclimation to the housing facilities before the start of experiments. CD-1 mice were single housed, and C57BL/6j mice were group housed. All mice were maintained on a 12 h light/12 h dark cycle with ad libitum access to food and water. Procedures were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and the Icahn School of Medicine at Mount Sinai Institutional Animal Care and Use Committee.

Aggression screening and ethological analysis. Aggression screening was performed as previously described. After a minimum of 1 week habituation to home cages, experimental CD-1 mice were exposed to a novel C57BL/6j intruder for 5 min daily over 3 consecutive days. Each intruder presentation was performed in the home cage of the CD-1 mouse between 1–3 PM daily under white light conditions. During screening sessions the cage top along with feeding and water apparatus were replaced with a clear Plexiglass cover to allow unimpeded viewing and video recording of screening sessions. The duration and number of screening sessions were selected to prevent induction of stress- and anxiety-related behaviours in CD-1 mice (Extended Data Tables 1 and 2), which has been shown to occur during extended antagonist encounters. This allows for separation between aggression and stress-related states. All screening sessions were video recorded for later ethological analysis using a digital colour video camera. Ethological analysis of aggression behaviour was performed by two blinded observers, recording (1) latency to initial aggression, (2) the number of aggressive bouts, (3) the total duration of aggression, and (4) the mean duration of aggressive bouts. Operational definitions for these behaviours were defined as follows: initiation of aggression is defined by the first clear physical antagonist interaction initiated by the CD-1 mouse, not including grooming or pursuit behaviour. Aggressive bouts are defined by cycles of initiated aggression with continuous orientation by the CD-1 mouse towards the intruder, and only defined as completed when the CD-1 mouse has physically reoriented away from the intruder. This definition allows for slight breaks (less than 5 s) in continuous physical interaction within an aggressive bout, assuming the CD-1 mouse has remained oriented towards the intruder throughout. CD-1 mice were defined as AGG if they initiated aggression during any of the three screening sessions and NON were defined as those that showed no aggression during any screening sessions. All aggression screening was halted if an intruder showed any signs of injury in accordance with our previously published protocol.

Aggression CPP and behavioural analysis. The aggression CPP protocol, developed on the basis of a previously published cocaine CPP protocol, consisted of three phases: pre-test, acquisition, and test. Mice were acclimated to the testing facility for 1 h before testing. All phases were conducted under red light and sound-attenuated conditions. The CPP apparatus (Med Associates) consisted of two unique conditioning chambers with a neutral middle zone that allowed for unbiased entry into either conditioning chamber at the initiation of each trial. All CPP sessions were video recorded using Noldus Ethovision 3.0 (Noldus Information Technology). During the pre-test phase, mice were placed into the middle chamber of the conditioning apparatus and allowed to freely explore the full extent of the CPP apparatus for 20 min. There were no group differences in bias for either chamber, and conditioning groups were balanced in an unbiased fashion to account for start side preference. The acquisition phase consisted of two successive days with two conditioning trials each day for a total of four acquisition trials. Morning trials (between 8:00 AM and 10:00 AM) and afternoon trials (between 3:00 PM and 5:00 PM) consisted of CD-1 mice confined to one chamber for 20 min paired with an intraperitoneal injection of cocaine (10 mg kg⁻¹). Afternoon sessions were paired with saline injections. All groups were counterbalanced for conditioning chamber. On the test day, CD-1 mice were placed into the middle arena of the CPP apparatus and allowed to freely explore both chambers for 30 min. Analysis of duration spent within either context was used to identify a CPP or CPA to the cocaine-paired context. For optogenetic experiments, stimulation was performed during the full duration of the test phase. Total locomotor responses were also assessed to ensure equal exploratory behaviour between groups. Operative analysis of aggression CPP data was performed by assessing (1) normalized CPP (test phase duration spent in the intruder-paired chamber minus test phase duration spent in the intruder-unpaired chamber, accounting for test session behaviour only), and (3) group and individual durations in both pre-test and test sessions.

Sensory CPP. Sensory CPP was performed and analysed identically to the aggression CPP procedure, with the exception that the intruder C57BL/6j was placed within a physical barrier to provide only sensory contact with the resident CD-1 mice.

Cocaine CPP. A previously published cocaine CPP protocol was used, which consisted of three phases: pre-test, acquisition, and test. Mice were acclimated to the testing facility for 1 h before testing. All phases were conducted under red light and sound-attenuated conditions. The CPP apparatus (Med Associates) consisted of two unique conditioning chambers with a neutral middle zone that allowed for unbiased entry into either conditioning chamber at the initiation of each trial. All CPP sessions were video recorded using Noldus Ethovision 3.0 (Noldus Information Technology). During the pre-test phase, mice were placed into the middle chamber of the conditioning apparatus and allowed to freely explore the full extent of the CPP apparatus for 30 min. There were no group differences in bias for either chamber, and conditioning groups were balanced in an unbiased fashion to account for start side preference. The acquisition phase consisted of two successive days with two conditioning trials each day for a total of four acquisition trials. Morning trials (between 8:00 AM and 10:00 AM) and afternoon trials (between 3:00 PM and 5:00 PM) consisted of CD-1 mice confined to one chamber for 20 min paired with an intraperitoneal injection of cocaine (10 mg kg⁻¹). Afternoon sessions were paired with saline injections. All groups were counterbalanced for conditioning chamber. On the test day, CD-1 mice were placed into the middle arena of the CPP apparatus and allowed to freely explore both chambers for 30 min. Analysis of duration spent within either context was used to identify a CPP or CPA to the cocaine-paired context. For optogenetic experiments, stimulation was performed during the full duration of the test phase. Total locomotor responses were also assessed to ensure equal exploratory behaviour between groups.

Sucrose preference. Sucrose preference was performed as previously described. One week after the final screening session, AGG and NON mice had their standard water bottle removed and replaced with two 50-ml conical tubes with sipper tops filled with water. After a 24-h habituation period, water from one 50-ml conical tube was replaced with 1% sucrose. All tubes were weighed, and mice were allowed 24 h to drink. Tubes were then weighed, and their locations in the wire tops were switched before a second 24-h period of drinking. At the end of the second day of sucrose testing, preference was calculated as the total amount of sucrose consumption divided by the total amount of fluid consumed over the 2 days of sucrose availability.

Elevated plus maze. The elevated plus maze was performed as previously described. One week after the final screening session, AGG and NON mice were acclimated to the testing facility for 1 h before testing and then placed in the elevated plus maze under red light conditions for 5 min. Each arm of the maze measured 12 × 50 cm. The Plexiglas cross-shaped maze consisted of two open arms with no walls and two closed arms (40-cm-high walls) and was on a pedestal 1 m above floor level. Behaviour was tracked using an automated system (Noldus Ethovision; Noldus Interactive technologies). Behaviour was measured as total time in open and closed arms.

Open field and locomotor measures. Open field was performed as previously described. One week after the final screening session, AGG and NON mice were acclimated to the testing facility for 1 h before testing. Open-field testing was performed in a black Plexiglass arena (42 × 42 × 42 cm; Nationwide Plastics) under red light conditions. Testing sessions were 10 min long. Behaviour was tracked using an automated system (Noldus Ethovision; Noldus Interactive technologies) to record the total distance moved and time spent in the total arena and a delineated ‘centre zone’ (24 cm × 24 cm).

Forced-swim test. The forced-swim test was performed as previously described. One week after the final screening session, AGG and NON mice were placed in the test room for an hour before behavioural testing for habituation. Mice were tested in the forced-swim test on the day of sucrose testing. The test consisted of placing a mouse into a glass beaker, containing 2 litres of water at 23 ± 1 °C for 6 min. Behaviour was videotaped (Noldus Ethovision; Noldus Interactive technologies) and analysed for duration immobile, duration mobile and total movement.

Social interaction (approach). Social approach testing was performed as previously described. One week after the final screening session, AGG and NON mice were acclimated to the testing facility for 1 h before testing, and all testing was performed under red light conditions. Mice were placed in an open field black Plexiglass arena (42 × 42 × 42 cm; Nationwide Plastics) with a small animal cage placed at one end. Their movements were then automatically monitored and recorded (Ethovision 3.6; Noldus Information Technology) for 2.5 min in the presence of a novel C57BL/6j mouse in the absence phase of a social target. This phase is used to determine baseline exploratory behaviour. We then immediately measured 2.5 min of exploratory behaviour in the presence of a caged novel CD-1 or C57BL/6j mouse (target present phase), again recording total distance travelled and duration of time spent in the interaction and corner zones. Social interaction behaviour is determined by the total time spent in each zone.
Novel object versus social target preference. The novel object versus social target test consisted of two phases: pre-test and test on consecutive days, as previously described35. One week after the final screening session, AGG and NON mice were acclimated to the testing facility for 1 h before both sessions. All phases were run under red-light and sound-attenuated conditions. The testing apparatus (Med Associates) consisted of two identical chambers, with a neutral middle zone that allowed for unbiased entry into either chamber at the initiation of each trial. All sessions were video recorded from above (Noldus EthoVision 3.0, Noldus Information Technology, Netherlands) for later analysis. Briefly, during the pre-test phase, mice were placed into the middle chamber of the apparatus and allowed to freely explore all zones for 5 min. There were no group differences in pre-test preference for either chamber. Conditioning groups were then balanced in an unbiased way to account for individual animals’ preference. On the test day, mice were placed back into the apparatus in the presence of both a novel object (an upside-down steel-bar pencil holder) on one side and a social target (identical pencil holder containing either a novel CD-1 or a C57BL/6J mouse) on the other. Test mice were allowed to freely explore the apparatus for 5 min. The time spent in each chamber was recorded and used for analysis. The subtracted social score is derived by subtracting time in social-paired chamber from time in novel-object-paired chamber during the test phase. Normalized social score is the ratio of time spent in the chamber of interest (social target or novel object) during the test phase over the pre-test phase.

Blood sampling and testosterone/corticosterone ELISA. Submandibular vein bleeds were taken from mice 4–24 h after the final screening session as previously described26. Serum testosterone (RND Systems, Testosterone Parameter Assay Kit) and corticosterone (Immunodiagnostic Systems, IDS Corticosterone EIA Kit) levels were assessed via ELISA according to manufacturer specifications. Briefly, blood was collected in a serum separator tube, allowed to clot for 30 min at room temperature, and centrifuged at 1,000 g for 15 min and stored frozen (−20 °C) until analysis. The sensitivity of the testosterone assay (minimum detectable range: 0.02 ng ml⁻¹) falls well below the ranges detected experimentally within our cohort (lowest serum testosterone concentration of 2.58 ng ml⁻¹).

Perfusion and brain tissue processing. For immunohistochemistry and histology, mice were given a euthanizing dose of 15% chloral hydrate and transcardially perfused with cold 1% paraformaldehyde in PBS (pH 7.4) followed by fixation with cold 4% paraformaldehyde in PBS. Brains were dissected and post-fixed for 18 h in the same fixative. Coronal sections were prepared on a vibratome (Leica) at 50 μm to assess visual placement and immunohistochemistry. For in situ hybridization, mice brains were rapidly removed and flash frozen in −80 °C isopentane for 60 s and then kept at −80 °C until sectioning.

Immunohistochemistry, in situ hybridization and confocal microscopy. For c-Fos experiments, sections were infiltrated overnight in blocking solution (3% normal donkey serum, 0.3% Triton X-100 in PBS), washed in PBS for 2 h, then incubated for 48 h in primary antibody (rabbit anti-c-Fos (Santa Cruz Biotechnology, SC-42) 1:2,000). Slices were then washed in PBS for 2 h, incubated in secondary antibody for 2 h (donkey anti-rabbit Cy2 1:200 (Jackson ImmunoResearch)), then washed in PBS for 30 min before staining with 1 μg ml⁻¹ DAPI (Sigma) for 20 min. Sections were then mounted, air-dried overnight and coverslipped with hard-set Vectashield (Jackson ImmunoResearch). All slices were images using a Zeiss LSM 780. For c-Fos analysis, all images were taken with 20 magnification for both the BF and IHb, using the tile-scan function to span the entire region of interest. Analysis of c-Fos-positive nuclei was performed using NIH Image in conjunction with the ‘analyze particle’ function on single images. For representative images demonstrating the areas of viral infection, images were acquired at ×10 magnification using the tile-scan function.

For all other immunohistochemistry, coronal sections (50 μm) were used for all immunofluorescence experiments. Sections were incubated in blocking solution (3% normal donkey serum, 0.3% Triton X-100 in PBS) for 1 h. Sections were then incubated in blocking solution overnight with the primary antibody overnight at 4 °C. Sections were then incubated in primary antibody overnight at 4 °C (VGAT 1:500 (Synaptic Systems); GAD67 (Channel 2) were hybridized at 40 °C for 2 h, and then subjected to a series of amplification steps at 40 °C (1-FL: 30 min; 2-FL: 15 min; 3-FL: 30 min; 4-FL: 15 min). For the fourth amplification step, Reagent Alt-A was used, corresponding with Channel 1 visualization at 488 nm and Channel 2 at 550 nm. Finally slides were treated for 2 min with DAPI, an immediately coverslipped with EcoMount. For confocal microscopy, all immunofluorescence experiments. Sections were incubated in blocking solution (3% normal donkey serum, 0.3% Triton X-100 in PBS) for 1 h. Sections were then incubated in primary antibody overnight at 4 °C. Sections were then transferred into a recording chamber fitted with a constant flow rate of ACSF equilibrated with 95%/5% O₂/C₅O₂ (2.5 min ml⁻¹) maintained at 35 °C. Cell-attached recording mode was used to measure the firing rates of IHB neurons. In these recording experiments, glass recording pipettes (7–10 MΩ) were filled with an internal solution composed of (in mM): 115 potassium gluconate, 20 KCl, 1.5 MgCl₂, 10 phosphocreatine, 10 HEPES, 2 magnesium ATP and 0.5 GTP (pH 7.2, 285 mOsm). For the experiments to measure inhibitory postynaptic currents, whole-cell recordings were performed under voltage-clamp mode (holding at −70 mV) in the presence of kynurenic acid (1 mM) with or without gabazine (2 μM) in ACSF. Glass recording pipettes (3–4 MΩ) for these whole-cell studies were filled with the internal solution composed of (mM): 120 CsCl, 10 phosphocreatine, Na 10 HEPES, 10 EGTA, 2 ATP-Mg, 0.3 GTP-Tris (pH 7.2, 285 mOsm). Data acquisition was conducted using a Digidata 1440A digitizer and pClamp 10.2 (Axon Instruments).

Stereotaxic surgery and viral gene transfer. All surgeries were performed under aseptic conditions using anaesthetic. Briefly, mice were anesthetized with a mixture of ketamine (100 mg per kg body weight) and xylazine (10 mg per kg body weight) and positioned in a small-animal stereotaxic instrument (David Kopf Instruments) and the skull surface was exposed. Thirty-three-gauge syringe needles (Hamilton Co.) were used to bilaterally inject either 0.5 μl (BF) or 0.4 μl (IHb) of virus over a 5 min period and the needle was removed after 5 min. NAc shell–septum transition zone BF stereotactic coordinates taken from bregma (anteroposterior +1.5 mm; mediolateral, +1.6 mm; dorsoventral, −4.4 mm; angle 10°). IHb stereotactic coordinates taken from bregma (anteroposterior, −1.7 mm; mediolateral, +0.4 mm; dorsoventral, −2.5 mm; angle 0°). For IHB optogenetic experiments, animals were implanted with an optical fibre at the same time as viral injection (dorsoventral, −2.0 mm). For secure fixture of the implantable fibre to the skull, the skull was dried and then industrial-strength dental cement (Grip cement; Dentply) was added to the base of the implantable fibre and the skull. For non-conditional axonal tract tracing, 0.5 μl AAV2-hSyn-eYFP (1.5 × 10¹³ infectious units per ml, UNC Vector Core) was injected bilaterally into the BF. For retrograde tracing, 0.4 μl G-deleted-rabies-eYFP (1.3 × 10¹³ infectious units per ml, Salk Gene Transfer Targeting and Therapeutics Core) was injected into the IHB. For behavioural optogenetic experiments, 0.5 μl of non-conditional AAV2-hSyn-eYFP, AAV2-hSyn-HChR2(H1.4R)-eYFP or AAV-hSyn-eNhHR3.0-eYFP (1.5 × 10¹¹–13 infectious units per ml, UNC Vector Core) were injected into the BF (na) or IHB (cell body stimulation). All non-rabies AAV injections were performed between 4–6 weeks before tracing or behavioural experiments; rabies-infected brains were collected 7 days after injection.

Blue light stimulation. Optical fibres (Thor Labs, BFL37–200) were connected to a 10 mW diode laser (Crystal Laser, BCL-473-050-M) and a stimulator (Agilent Technologies, no. 33220A) was used to generate blue light pulses. For all in vivo behavioural experiments and optrode recordings, mice were given 40 Hz 5 ms light stimulations. Intensity of light delivered to ferule was ~10 mW. These parameters are consistent with previously validated and published protocols for NAc medium spiny neurons29,30 and IHB neurons5,34.

Yellow light stimulation. Optical fibres (Thor Labs, SFS200/220) were connected using an FC/PC adaptor to a 561-nm yellow laser diode (Crystal Laser, CBL-473-050-M) and a stimulator (Agilent Technologies, no. 33220A) was used to generate yellow light pulses. For in vivo optrode recordings we tested a protocol of 8 s of light stimulation, followed by 2 s of light off. Intensity of light delivered to ferule was ~10 mW. These parameters are consistent with previously validated and published protocols for NAc MSNs and IHB neurons5,34.

In vivo recordings. Optrode construction and implantation. An optrode was constructed by gluing four tetrodes to an optical fibre. Four tetrodes spun of 12.7-μm-diameter nichrome wire
(California Fine Wire) were glued to a 200-μm-diameter optical fibre (Thor Labs, SFS200/220Y) and cut so that they extended between 750 and 250 μm beyond the end of the fibre. The tetrodes were pinned into an electrode interface board (EIB; Neuralynx) and the tips were plated by passing 0.2 μA current pulses through the individual wires and a gold solution until the impedance reached 150–200 kOhm. The optrode was mounted on a stereotax arm (Kopf Instruments) and then lowered into the brain during surgery. Two small holes were drilled anterior and posterior to the recording site to serve as sites for ground screws. The ground screws were constructed by soldering stainless steel self-tapping screws to 3 mm stainless steel wire secured to the EIB. Screws were inserted far enough to come in contact with dura.

**Recording.** Recordings were carried out using a Digital Lynx 16SX recording system and Cheetah data acquisition software (Neuralynx). Signals from the tetrodes were bandpass filtered between 600 and 9,000 Hz and digitized at 32 kHz. Spike detection was performed in real time using a thresholding procedure: when the filtered signal reached threshold amplitude on any wire, a sweep including 8 data points before the crossing and 24 points after (32 points, or 1 ms) were saved as a putative spike event. Spike sorting and noise filtering was performed online. The laser intensity was adjusted to ~5 mW at the tip of the optrode before implantation. The optrode was lowered using the stereotax arm until the tetrode tips reached the dorsal extent of the LHb. Once the tissue and recordings stabilized, the optrode was slowly advanced until spikes were observed on at least one of the tetrodes. Spike amplitude and firing rate were allowed to stabilize and observed for several minutes before recording. For all trials a 30 s baseline recording was acquired, followed by 1 min of stimulation and ending with a 30 s post-stimulation baseline. The optrode was then stepped forward and this procedure repeated until the inferior extent of the LHb was reached.

**Analysis.** Data were analysed using custom scripts written in Matlab (MathWorks). A first round of preliminary spike sorting was carried out using spike waveforms as parameters in KlustaKwik. The output from KlustaKwik was then imported into Matlab and clusters were manually edited using custom spike sorting software. Clearly separated clusters of spikes were assigned to functional units and entered into further analysis; noise spikes (for example, from spurious threshold crossings) and units that fired fewer than 100 spikes during recording were discarded. Spike rates were calculated in 2-s non-overlapping bins across the baseline and stimulation epochs. The resulting functions were smoothed using a Gaussian window with a standard deviation of 10 s. The rate function for each unit was then z-scored across all three epochs. For statistical analyses, rates were calculated in either 15-s bins or bins encompassing the entire baseline and stimulation periods. No smoothing was applied. The rate functions for each unit were z-scored across all three epochs and the z-scored rate functions were used to assess statistical significance.

**Randomization and blinding.** All experimenters were blinded to experimental condition. Mice were first screened to determine whether they were aggressive or non-aggressive and then randomly assigned to optogenetic viral conditions for further behavioural analysis. For behavioural studies in Fig. 1 and slice physiology and c-Fos mapping studies in Fig. 2, AGGs and NONs were pre-screened for aggression and assigned to groups on the basis of their behavioural profile.

**Statistical analysis.** Sample size was calculated based on previous studies using Stattmate from Graphpad prism (Graphpad Software). All t-tests, one-way ANOVA, two-way ANOVAs and chi-squared tests were performed using Graph Pad Prism software (Graphpad Software Inc.). Bonferroni was used as a post-hoc test when appropriate for one-way and two-way ANOVA. Normality was determined by D’Agostino–Pearson, Shapiro–Wilk and Kolmogorov–Smirnov normality tests. Statistical significance was set at $P < 0.05$.

31. Kudryavtseva, N. N., Bondar, N. P. & Avgustinovich, D. F. Association between experience of aggression and anxiety in male mice. Behav. Brain Res. 133, 83–93 (2002).
32. Russo, S. J. et al. Nuclear factor kappa B signaling regulates neuronal morphology and cocaine reward. J. Neurosci. 29, 3529–3537 (2009).
33. Golden, S. A. et al. Epigenetic regulation of RAC1 induces synaptic remodeling in stress disorders and depression. Nature Med. 19, 337–344 (2013).
34. Krishnan, V. et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131, 391–404 (2007).
35. Yang, M., Silverman, J. L. & Crawley, J. N. Automated three-chambered social approach task for mice. Curr. Protoc. Neurosci. Chapter 8, Unit 8 & 26 (2011).
36. Golde, W. T., Gollobin, P. & Rodriguez, L. L. A rapid, simple, and humane method for submandibular bleeding of mice using a lancet. Lab Anim. (NY) 34, 39–43 (2002).
37. Chaudhury, D. et al. Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. Nature 493, 532–536 (2013).
38. Friedman, A. K. et al. Enhancing depression mechanisms in midbrain dopamine neurons achieves homeostatic resilience. Science 344, 313–319 (2014).
39. Lobo, M. K. et al. A FosB induction in striatal medium spiny neuron subtypes in response to chronic pharmacological, emotional, and optogenetic stimuli. J. Neurosci. 33, 16381–16395 (2013).
40. Lobo, M. K. et al. Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. Science 330, 385–390 (2010).
41. Starmatikas, A. M. & Stuber, G. D. Activation of lateral habenula inputs to the ventral midbrain promotes behavioral avoidance. Nature Neurosci. 15, 1105–1107 (2012).
42. Chandra, R. et al. Optogenetic inhibition of D1R containing nucleus accumbens neurons alters cocaine-mediated regulation of Tiaml1. Front. Mol. Neurosci. 6, 13 (2013).
43. Aquili, L., Liu, A. W., Shindou, M., Shindou, T. & Wickens, J. R. Behavioral flexibility is increased by optogenetic inhibition of neurons in the nucleus accumbens shell during specific time segments. Learn. Mem. 21, 223–231 (2014).
44. Kadir, S. N., Goodman, D. F. M. & Harris, K. D. High-dimensional cluster analysis with the masked EM algorithm. Neural Comput. 26, 2379–2394 (2014).
Extended Data Figure 1 | Social behaviours exhibited by resident CD-1 and intruder C57 mice during aggression screening. a, Experimental schematic of aggression screening procedure used in a subset (40 residents and 40 intruders) of mice to quantify social behaviours. b–e, Bouts of attacks ($F_{2,156} = 13.10$, two-way ANOVA $***P < 0.0001$; post-hoc test $***P < 0.001$; $n = 40$ per group) (b), pursuits (c), withdrawals (d) and non-aggressive social approaches (e). f–i, Duration of attacks ($F_{2,156} = 5.745$, two-way repeated measures ANOVA $**P < 0.001$; post-hoc test $***P < 0.001$; $n = 40$ per group) (f), pursuits (g), withdrawals (h) and non-aggressive social approaches (e). All data are presented as mean ± s.e.m.
**Extended Data Figure 2 | Detailed ethological analysis of AGG aggression-related behaviours.**

a. Experimental schematic of aggression screening procedure used in a sample (448 mice total; 138 NON and 310 AGG) of mice. b. Histogram of attack latency frequency using 10-s bins. c–e. Mean distribution across screening sessions (left) and individual screening sessions (right) for latency to aggression ($F_{2,1326} = 49.37$, two-way repeated measures ANOVA $P < 0.001$; post-hoc test, *$P < 0.001$; $n = 138–310$) (c). Number of attack bouts ($F_{2,1326} = 21.03$, two-way repeated measures ANOVA $P < 0.001$; post-hoc test, *$P < 0.001$; $n = 138–310$) (d). and mean attack duration ($F_{2,1324} = 11.96$, two-way repeated measures ANOVA $P < 0.001$; post-hoc test, *$P < 0.001$; $n = 138–310$) (e). f, g. Correlation of mean latency to initial aggression with mean attack bouts ($r = -0.78, P < 0.0001$) (f) and mean duration of attack bouts ($r = -0.40, P < 0.0001$) (g). Distribution plots are presented as the median with interquartile range and normality determined by D’Agostino–Pearson, Shapiro–Wilk and Kolmogorov–Smirnov normality tests ($P < 0.0001$). Summary data are represented as mean ± s.e.m.
Extended Data Figure 3 | Aggression CPP behaviour. a, Experimental schematic of aggression CPP procedure. b, c, Individual duration spent in the intruder-paired context for AGG ($t = 3.106, *P < 0.05$; two-tailed paired $t$-test, $n = 8$ per group) (b) and NON ($t = 2.918, *P < 0.05$; two-tailed paired $t$-test, $n = 8$ per group) (c). d, Duration spent in the middle neutral chamber during pre-test and test sessions. e, Experimental schematic of sensory CPP procedure. f, g, Individual duration spent in the intruder-paired context for AGG (f) and NON (g). h, Duration spent in the middle neutral chamber during pre-test and test sessions. Summary data are represented as mean ± s.e.m.
Extended Data Figure 4 | BF–lHb circuit tracing and GABAergic cell-type specificity. a, Schematic of viral tracing strategy. b, Representative BF viral infection with AAV2-hSyn-eYFP. Scale bar: 500 μm. c, Histological analysis of viral infection with AAV2-hSyn-eYFP ($F_{2,11} = 223.0$, one-way ANOVA ***$P < 0.0001$, post-hoc test, ***$P < 0.0001$; $n = 3$ mice, 3 slices per mouse) across adjacent anatomical regions. d, e, Whole-cell electrophysiological recordings (d) and representative traces of lHb neurons photostimulated with AAV2-hSyn-ChR2.0 in the absence or presence of bath-applied GABA$_A$ receptor antagonist gabazine (2 μm; $F_{2,7} = 220$, one-way ANOVA $P < 0.05$; post-hoc test, ***$P < 0.001$, n = 4, 2, 2 cells from 2 mice) (e). f, Optically evoked IPSC response delay ($n = 21$ oIPSC events, 2 mice). g, Representative images of eYFP BF→lHb terminal colocalization between vesicular GABA transporter (top), and not vesicular glutamate transporter 1 (bottom). Scale bars: 10 μm; white arrows indicate colocalization within insets. MS, medial septum; pLS, posterior lateral septum. Summary data are represented as mean ± s.e.m.
Extended Data Figure 5 | Multiunit anaesthetized optrode recordings.

**a** | Schematic of in vivo anaesthetized multi-unit optrode recording procedure (left) and representative optrode placement in lHb (right; scale bar: 200 μm).

**b**, **c** | Heatmaps of normalized firing rates for lHb neurons in response to BF terminal stimulation with ChR2 BF → lHb (**b**) or NpHR3BF → lHb (**c**) and averaged spike wave-form shown below for pre-stimulation, stimulation and post-stimulation epochs.

**d** | Percentage of cells by firing response (top) and average normalized lHb firing rate (bottom) after BF–lHb terminal stimulation with ChR2 BF → lHb for all identified cells ($F_{2,134} = 8.249$, one-way repeated-measure ANOVA $P < 0.001$; post-hoc test, *$P < 0.05$; $n = 68$ cells from 3 mice) and cells that significantly decreased firing during the stimulation epoch ($F_{7,105} = 8.868$, one-way repeated-measure ANOVA $P < 0.0001$; post-hoc test, *$P < 0.05$; $n = 16/68$ cells from 3 mice).

**e** | Percentage of cells by firing response (top) and average normalized lHb firing rate (bottom) after BF–lHb terminal stimulation with NpHR3BF → lHb for all identified cells ($F_{2,128} = 10.32$, one-way repeated-measure ANOVA $P < 0.0001$; post-hoc test, *$P < 0.05$; $n = 65/65$ cells from 3 mice) and cells that significantly increased firing during the stimulation epoch ($F_{7,203} = 17.58$, one-way repeated-measure ANOVA $P < 0.0001$; post-hoc test, *$P < 0.05$; $n = 30/65$ cells from 3 mice).

mHb, medial habenula. Summary data are represented as mean ± s.e.m.
Extended Data Figure 6 | BF–lHb AAV infection and CPP locomotor behaviour. **a**, Schematic of BF coronal slice (left), alongside representative AAV-ChR2-eYFP (top) and AAV-NpHR3.0-eYFP (bottom) infections. Scale bar: 500 μm. **b**, Schematic of lHb coronal slice (left), alongside representative images of BF terminal infection by AAV-ChR2-eYFP (middle top) and AAV-NpHR3.0-eYFP (middle bottom) within the lHb. Representative close-ups of terminal regions shown in insets on right. Scale bar: 50 μm. All representative images counterstained with DAPI. **c, d**, Histological analysis of BF infection in NON (c) and AGG (d) mice. **e, f**, Histological analysis of habenular viral infection in NON (e) and AGG mice (f). **g–j**, Total distance travelled (g, h) and mean velocity (i, j) between NON and AGG during the CPP pre-test and test phase. All data are presented as mean ± s.e.m., and are not significant as determined by two-way ANOVA. P < 0.05. dStr, dorsal striatum; mHb, medial habenula; MS, medial septum; pLS, posterior lateral septum.
Extended Data Figure 7 | Direct lHb stimulation bi-directionally modulates aggression reward. **a**, Schematic of viral infection strategy. **b, c**, Representative images of lHb cell body infection in NON (b) and AGG (c). Scale bar: 200 μm. **d**, Histological analysis of lHb viral infection. **f**, Representative CPP traces of NON. NON::NpHR lHb cell body infection mimics the physiological effect of NON::ChR2 BF → lHb terminal stimulation. **g**, Individual duration spent in the intruder-paired context for NON::eYFP lHb and NON::NpHR lHb. **i**, Representative CPP traces of AGG::eYFP and AGG::ChR2 lHb. **j**, Individual duration spent in the intruder-paired context for AGG::eYFP and AGG::ChR2 lHb. Summary data are represented as mean ± s.e.m. dStr, dorsal striatum; mHb, medial habenula.
Extended Data Figure 8  |  BF–lHb stimulation modulates cocaine CPP.  
a, Experimental timeline of general anxiety and cocaine CPP testing. 
b–e, BF–lHb stimulation during open field testing (b, c) and elevated plus maze testing (d, e). 
f, Subthreshold cocaine (10 mg kg⁻¹, intraperitoneal) CPP procedure with BF–lHb stimulation during CPP test ($t_\text{0} = 2.403$, $P < 0.05$; two-tailed unpaired $t$-test, $n = 5–6$ per group).
Extended Data Table 1 | Stress and anxiety behaviours in AGG and NON

**Elevated plus maze**

|         | Duration (s) | Latency to enter (s) | Distance (cm) | Velocity (cm/s) |
|---------|--------------|----------------------|---------------|-----------------|
|         | Closed arms  | Open arms            | Closed arms   | Open arms       |                 |
| NON     | Mean 136.53  | 58.85                | 13.51         | 9.06            | 2412.14         | 8.16            |
|         | SEM 11.71    | 8.55                 | 9.22          | 2.37            | 114.19          | 0.38            |
| AGG     | Mean 137.39  | 65.07                | 5.11          | 5.50            | 2515.77         | 8.67            |
|         | SEM 6.51     | 10.48                | 2.21          | 1.79            | 136.55          | 0.46            |
| P value | 0.95         | 0.65                 | 0.39          | 0.24            | 0.57            | 0.40            |
| n       | 12           | 12                   | 12            | 12              | 12              | 12              |

**Open field and locomotion**

|         | Duration in (s) | Latency (s) | Distance (cm) | Velocity (cm/s) |
|---------|-----------------|-------------|---------------|-----------------|
|         | Center          | Middle      | Periphery     | Center          | Distance       | Velocity       |
| NON     | Mean 18.80      | 126.63      | 373.76        | 24.35           | 4909.51        | 8.77           |
|         | SEM 3.28        | 28.87       | 58.22         | 7.78            | 374.32         | 0.59           |
| AGG     | Mean 15.59      | 138.70      | 445.82        | 31.31           | 4335.25        | 8.90           |
|         | SEM 1.77        | 11.63       | 12.20         | 7.22            | 211.61         | 0.36           |
| P value | 0.36            | 0.82        | 0.48          | 0.53            | 0.73           | 0.84           |
| n       | 7-11            | 7-11        | 7-11          | 7-11            | 7-11           | 7-11           |

**Forced-swim test**

|         | Duration (s) | Distance (cm) | Velocity (cm/s) | Percent immobile |
|---------|--------------|---------------|-----------------|------------------|
|         | Mobile       | Immobile     |                 |                  |
| NON     | Mean 260.96  | 175.76       | 2041.17         | 11.40            | 0.40            |
|         | SEM 7.78     | 8.21         | 101.09          | 0.58             | 0.02            |
| AGG     | Mean 275.40  | 164.19       | 2076.95         | 11.57            | 0.37            |
|         | SEM 8.20     | 8.92         | 70.68           | 0.39             | 0.02            |
| P value | 0.22         | 0.35         | 0.78            | 0.81             | 0.29            |
| n       | 10           | 10           | 10              | 10               | 10              |

**Sucrose preference**

|         | Volume consumed (ml) |          |                |                |
|---------|----------------------|----------|-----------------|-----------------|
|         | Day 1                | Day 2    | Day 1 + Day 2   | Sucrose preference |
|         | Water                | Sucrose  | Water          | Sucrose         | Sucrese / (Sucrose + Water) |
| NON     | Mean 2.44            | 5.74     | 1.54           | 5.74            | 3.98             | 11.48           | 0.71            | 0.76            | 0.75 |
|         | SEM 0.65             | 0.66     | 0.26           | 0.69            | 0.79             | 0.86            | 0.07            | 0.05            | 0.04 |
| AGG     | Mean 2.39            | 5.15     | 2.03           | 7.50            | 4.42             | 12.65           | 0.69            | 0.78            | 0.74 |
|         | SEM 0.58             | 0.58     | 0.59           | 0.77            | 0.70             | 0.10            | 0.06            | 0.06            | 0.04 |
| P value | 0.95                 | 0.51     | 0.47           | 0.11            | 0.68             | 0.41            | 0.82            | 0.77            | 0.84 |
| n       | 10-11                | 10-11    | 10-11          | 10-11           | 10-11            | 10-11           | 10-11           | 10-11           | 10-11 |

The behavioural data are shown as mean ± s.e.m. and analysed by unpaired Student’s t-test. Significance at *P < 0.05.
Extended Data Table 2 | Social approach behaviours in AGG and NON

### Social interaction test

| Approach | No target interaction zone (s) | Target interaction zone (s) | No target corner zone (s) | Target corner zone (s) |
|----------|--------------------------------|-----------------------------|--------------------------|------------------------|
|          | CD-1 | C57 | CD-1 | C57 | CD-1 | C57 | CD-1 | C57 | CD-1 | C57 |
| NON      | Mean  | 56.62 | 51.55 | 77.49 | 76.42 | 34.77 | 32.53 | 21.54 | 25.03 |
|          | SEM   | 7.20  | 7.66  | 5.25  | 6.71  | 5.64  | 6.23  | 4.76  | 2.80  |
| AGG      | Mean  | 55.71 | 54.17 | 82.44 | 79.37 | 28.24 | 29.83 | 20.79 | 23.20 |
|          | SEM   | 4.67  | 6.07  | 6.13  | 8.91  | 2.52  | 5.71  | 2.84  | 4.95  |
| P value  | n     | 0.67  | 0.80  | 0.53  | 0.85  | 0.56  | 0.55  | 0.81  | 0.82  |

### Locomotion

|          | No target distance (cm) | Target distance (cm) | No target velocity (cm/s) | Target velocity (cm/s) |
|----------|-------------------------|----------------------|---------------------------|------------------------|
|          | CD-1 | C57 | CD-1 | C57 | CD-1 | C57 | CD-1 | C57 |
| NON      | Mean  | 1501.64 | 1715.50 | 1344.34 | 1284.00 | 10.01 | 11.44 | 8.96  | 9.65  |
|          | SEM   | 198.09  | 183.30  | 153.22  | 94.24  | 1.32  | 0.56  | 1.02  | 0.59  |
| AGG      | Mean  | 1544.73 | 1699.40 | 1284.39 | 1474.84 | 10.01 | 11.39 | 8.57  | 9.87  |
|          | SEM   | 31.04  | 121.71  | 41.37  | 97.81  | 0.20  | 0.83  | 0.28  | 0.63  |
| P value  | n     | 0.83  | 0.92  | 0.72  | 0.84  | 0.82  | 0.96  | 0.72  | 0.81  |

### Normalized social score

|          | CD-1 Target | C57BL6/J Target |
|----------|-------------|-----------------|
|          | Duration (s) | Normalized social score | Subtracted social score |
| NON      | Mean  | 114.42 | 94.84 | - | - | 19.59 | 114.91 | 95.51 | - | - | 19.40 |
|          | SEM   | 5.30  | 8.12  | - | - | 11.78 | 7.96  | 7.70  | - | - | 15.22 |
| AGG      | Mean  | 121.48 | 97.66 | - | - | 23.82 | 106.41 | 95.02 | - | - | 11.38 |
|          | SEM   | 6.40  | 8.34  | - | - | 12.70 | 10.22 | 10.81 | - | - | 18.66 |
| P value  | n     | 0.41  | 0.81  | - | - | 0.81 | 0.52  | 0.97  | - | - | 0.74 |
|          | Social | Novel | Social | Novel | Social | Novel |

### Pretest phase

|          | CD-1 Target | C57BL6/J Target |
|----------|-------------|-----------------|
|          | Duration (s) | Normalized social score | Subtracted social score |
| NON      | Mean  | 166.49 | 93.31 | 1.50 | 1.08 | 73.17 | 153.47 | 95.64 | 1.41 | 1.04 | 57.83 |
|          | SEM   | 12.49 | 10.70 | 0.16 | 0.19 | 22.88 | 6.41  | 4.87  | 0.16 | 0.09 | 9.14 |
| AGG      | Mean  | 154.89 | 100.56 | 1.30 | 1.08 | 54.33 | 148.77 | 98.80 | 1.50 | 1.13 | 49.97 |
|          | SEM   | 5.03  | 7.04  | 0.09 | 0.12 | 10.61 | 7.60  | 8.42  | 0.16 | 0.14 | 14.21 |
| P value  | n     | 0.41  | 0.59  | 0.32 | 0.98 | 0.49 | 0.55  | 0.48  | 0.85 | 0.32 | 0.83 |
|          | Social | Novel | Social | Novel | Social | Novel |

### Test phase

The behavioural data are shown as mean ± s.e.m. and analysed by unpaired Student’s t-test. Significance at *P < 0.05. The subtracted social score was derived by subtracting time in the social-paired chamber from the novel object-paired chamber during the test phase. Normalized social score is the ratio of time spent in the chamber of interest (social target or novel object) during the test phase over the pre-test phase.